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.

Edited by

F. JEFFREY BELL, M.A.,

One of the Secretaries of the Society,
and Professor of Comparative Anatomy and Zoology in King's College;

WITH THE ASSISTANCE OF THE PUBLICATION COMMITTEE AND

A. W. BENNETT. M.A., B.Sc., F.L.S.,
Lecturer on Botany at St. Thomas's Hospital,
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J. ARTHUR THOMSON, M.A.,
Lecturer on Zoology in the School of Medicine, Edinburgh,

FELLOWS OF THE SOCIETY.

FOR THE YEAR

1891.

Part 1.

PUBLISHED FOR THE SOCIETY BY

WILLIAMS & NORGATE,

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MICROSCOPY.

a. Instruments, Accessories, &c.*

(1) Stands.

Report of the Committee of the American Society of Microscopists on Uniformity of Tube-length.†—The following Report has been issued by the American Society of Microscopists :—"Believing in the desirability of a uniform tube-length we unanimously recommend :—

(1) That the parts of the Microscope included in the tube-length should be the same by all opticians, and that the parts included should be those between the upper end of the tube where the ocular is inserted and the lower end of the tube where the objective is inserted.

(2) That the actual extent of tube-length as defined in section 1—Be, for the short or Continental tube, 160 mm. or 6·3 in., and 8½ in. or 216 mm. for the long tube, and that the draw-tube of the Microscope possess two special marks indicating these standard lengths.

(3) That oculars be made par-focal, and that the par-focal plane be coincident with that of the upper end of the tube.

(4) That the mounting of all objectives of 1/4 in. and shorter focus should be such as to bring the optical centre of the objective 1½ in. below the shoulder; and that all objectives be marked with the tube-length for which they are corrected.

(5) That non-adjustable objectives be corrected for cover-glass from 15/100 to 20/100 mm. (1/130 to 1/170 in.) in thickness.

These recommendations give a distance of 10 in. (254 mm.) between the par-focal plane of the ocular and the optical centre of the objective for the long tube, and are essentially in accord with the actual practice of opticians.

At the request of the committee, a joint conference was held with the opticians belonging to the society and present at the meeting. They expressed their belief in the entire practicability of the above recommendations, and a willingness to adopt them.—Signed, Simon H. Gage, A. Clifford Mercer, Prof. Barr."

Swift and Son's Improved Student's Microscope.—At the October meeting of the Society, Mr. G. C. Karop exhibited and described this instrument (fig. 1), which he said had been brought out by Messrs. Swift at his suggestion. The aim was to produce a Student's Microscope of a superior design, with which high-class optical appliances could be used.

The body-tube is made to take the full-size eye-pieces in general use, and short enough to work with objectives adjusted to the Continental tube-length.

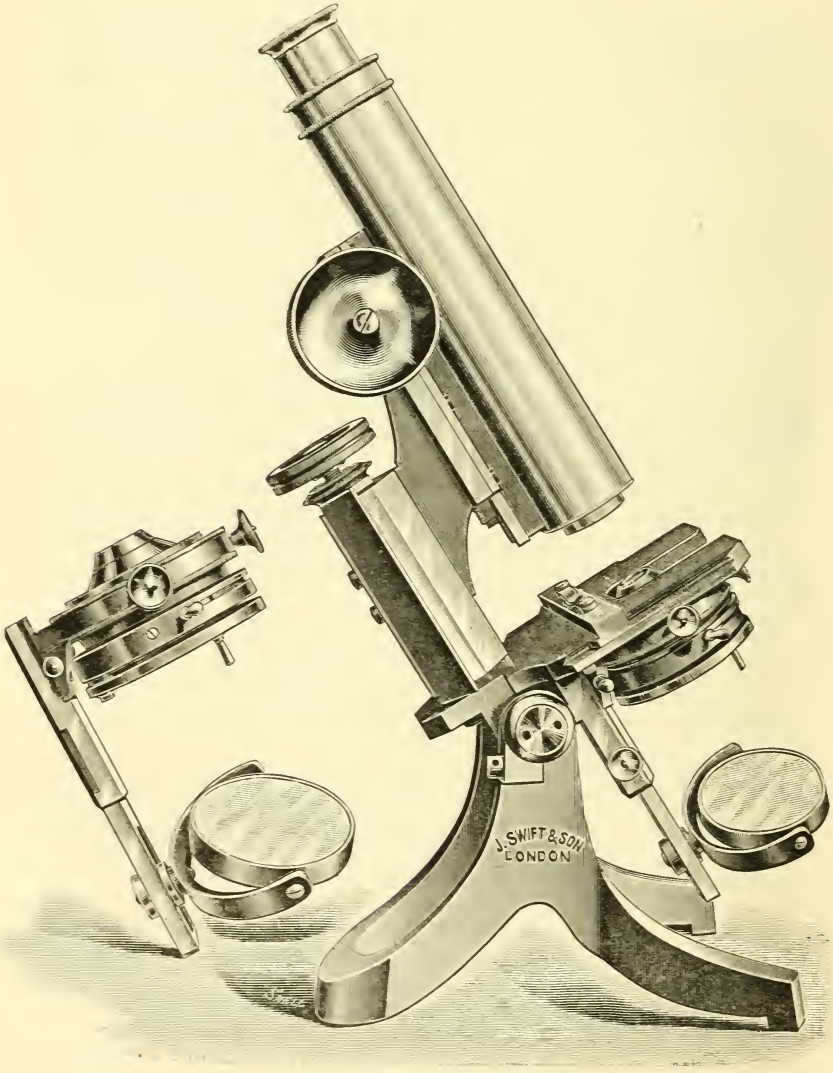
A draw-tube lengthens to the English standard of 10 in. The bearing carrying the body is made longer than usual in students' instruments, so as to give greater firmness with low-power objectives. The fine-adjustment was that known as Campbell's Differential Screw

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

† Microscope, x. (1890) p. 297.

system, and is arranged for very delicate focusing. Both the coarse and fine adjustments are provided with extra large milled heads to afford a firm grasp. The stage is of the Nelson horse-shoe shape, and

Fig. 1.



large enough to take culture-plates ; this form is adopted for lightness and for the facility it gives in feeling the working distance of the objective. Instead of the usual spring clips, a sliding frame is provided

with sprung guides moving in grooves at the sides of the stage; small clips are applied for use in the horizontal position. The Mayall mechanical stage can be applied if required. The sliding-bar carrying the substage is specially well fitted so that a condenser of fairly large aperture may be focused, and a clamping screw fixes it in position. The substage has mechanical centering movements, and an iris-diaphragm. The mirror is removable in case it may be desired to work with direct light from the lamp.

We are requested by Messrs. Swift to note that at a small additional cost they can apply a rack-and-pinion instead of the sliding movement to the substage.

Mason's Improvements in Oxy-hydrogen Microscopes.*—Mr. R. G. Mason, of 69, Clapham Park Road, Clapham, S.W., has introduced the above form of lantern and table Microscope, a patent for which has been applied for. Until the present time the lantern Microscope has been a distinct instrument from the table form of stand. By the union of the above parts an instrument is obtained that, when not in use for

Fig. 2.

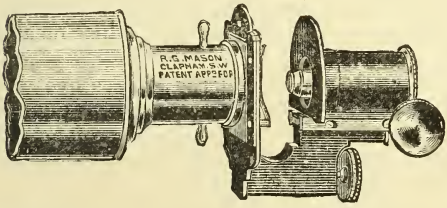
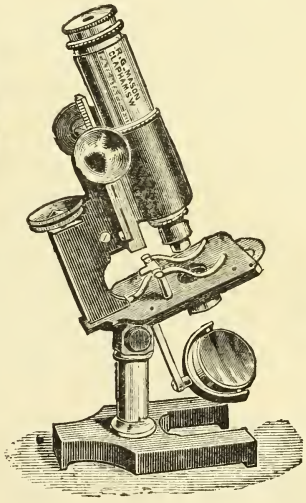


Fig. 3.



screen projections, can be easily altered, as shown by fig. 3. No unscrewing is required, and there are no loose parts. Fig. 2 shows the instrument as used on the lantern. It is very convenient for the science teacher or general lecturer, as a demonstration may be made to either a small or large audience with equal facility. The lower part, which carries the joint for inclining the instrument at any angle, is fitted with concave and flat mirrors on swinging arm, also with the universal size substage fitting tube for apparatus. The body and draw, which fits into the upper part, is of large diameter, and is screwed with the Society's size screw, thus enabling any ordinary microscopic objective to be used with it. It is fitted with a first-class rack, and also screw fine motion working steadily under high powers. This fine motion is especially useful in photo-micrography. The stage being of the usual form, both object and objective are in view, and easily manipulated while in use, thus doing away with an objection that is often present in the older forms of lantern

* Engl. Mech., lii. (1890) pp. 306-7.

Microscope. A further improvement is a spring clip, enabling the object to be easily changed without scratching the labels, &c., its construction admitting of either a deep zoophyte trough or the thinnest 3 × 1 slip being held gently but firmly. The parts are supplied separately, so that any one needing only the lantern arrangement can add the other at any future time.

(3) Illuminating and other Apparatus.

The Substage Condenser: its History, Construction, and Management; and its effect theoretically considered.*—Mr. E. M. Nelson remarks—"The substage condenser is nearly as old as the compound Microscope itself. The first microscopical objects were opaque, and in very early times a lens was employed to condense light upon them. It was an easy step to place the lens below the stage when transparent objects were examined.

Coming to more modern times we find that the culminating type of non-achromatic Microscope was fitted with a substage condenser, but it had a very brief existence, not being able to hold its own against the recently introduced achromatic. Had the invention of achromatism been delayed, it would, I have no doubt, have had enormous popularity for those times. I allude to the Wollaston doublet with its substage condensing lens, particularly that form designed by Mr. Valentine and made by Andrew Ross in 1831.

Before proceeding we must remember by whom the Microscope was used at that time. As far as this country was concerned, it was merely looked upon as a philosophical toy. It was principally to be found in the hands of a few dilettanti; science of every kind was tabooed, the Microscope being placed at the lowest end of the scale.

Now, the Microscope of the dilettanti is usually a perfect instrument of its kind, fully supplied with apparatus, the greater part of which is absolutely useless, but among this apparatus there would always have been a substage condenser. One of the principal things the dilettanti have done for us is the keeping up through early achromatic days of the continuity of the condenser.

On the Continent, where science held a much more important place, the real value of the Microscope was better understood, and it at once took an important place in the medical schools. But the increase of light due to the more perfect concentration of rays by achromatism enabled objects to be sufficiently illuminated by the concave mirror to meet their purposes. Therefore, we find that on the Continent the Microscope had no condenser. Of course there were isolated exceptions, Amici's for example; but I think we may safely say that for every Hartnack made with a substage condenser there were upwards of one thousand made without.

England followed the Continental lead, and now the "foolish philosophical toy" has entirely displaced in our medical schools the dog-Latin text-book with its *ordo verborum*. But the kind of Microscope adopted was not that of the English dilettanti, but the condenserless Continental. It may be said that the Microscope for forty years—that

* Journ. Quek. Micr. Club, iv. (1890) pp. 116–36. For the use of the accompanying plate we are indebted to the kindness of Mr. Nelson and the Quekett Microscopical Club.

is, from the time it was established in the schools in, say, 1840 to 1880, has been without a condenser. Not only did those who used the condenserless Microscope consider the condenser an unnecessary appendage, but they looked down upon it and regarded it in the same category as one of the multitudinous appliances that are packed in such a wonderful manner in the apparatus cabinet of a Microscope made for exhibition.

In 1880 a change came from two separate causes—first, the rise of bacteriology; secondly, the introduction of a cheap chromatic condenser by Abbe in 1873.

Taken by itself, the introduction of the Abbe condenser had not much effect, but as Zeiss's Microscopes had for some time been displacing the older forms, and when the study of bacteriology arose, oil-immersion objectives of greater aperture than the old dry objectives (especially those of the histological series) were used, illumination by the mirror was soon discovered to be inefficient, so a condenser became a necessity. The cheap Abbe condenser was the exact thing to meet the case.

Since 1880 the percentage of educational Microscopes, medical or otherwise, without condensers, has been daily on the decrease. There has not been, during the past history of the Microscope, a more marked change of opinion with regard to any apparatus than that which has taken place in connection with the condenser.

It is worthy of notice that this change of opinion has been so complete that those who formerly condemned all condensers now look upon the Abbe chromatic (probably the worst condenser ever constructed) as a distinct advance in microscopy!

It must be remembered that the end of an educational Microscope is not to discover anything new, but to follow the figures given in the text-books, and when the text-books kept on the level of the larger objects any tube with a piece of glass at either end was sufficient for the purpose; but as the text-books improved and went deeper into the structure of things it was necessary that the student's Microscope should be of a better description. For example, as long as the text-books wrote about and figured the spiral vessels in the blowfly's tongue, so long the student did not require a Microscope capable of showing the cut suctorial tubes.

As I mentioned above, the "few," principally dilettanti, had all along used a condenser. I myself had not long entered the microscopical world as a member of the latter class before I found out that a condenser was a necessity. Now, as I have used all the kinds of condensers that have been introduced, I will give my own history in connection with them, as it will be the history of the condenser.

My first condenser was a Gillett; this was in power a 1/4, and it had 80° of aperture. The Gillett is practically the first achromatic condenser really constructed as such; before that time objectives were used, the rule being to select that objective which was next lower in power to the objective on the nose-piece. The manner of centering—for centering was duly insisted upon even in those early times—was so funny that I must recall it. Vertical movement was performed by the substage, but the horizontal movement by the Microscope body!

The Gillett was an elaborate instrument; it was supplied with a

correctional lens adjustment for the aberrations arising from the thickness of the slip. I have a distinct affection for the Gillett, for it was with that condenser I taught myself what a critical image was. In 1874, however, I purchased a P. and L. new formula water-imm. 1/8 of N.A. 1·21. These and similar lenses by Tolles far surpassed anything at that day. There was a greater difference between these lenses and their cotemporaries than there was between the homogeneous immersion and these same lenses four years later. I can only liken the improvement which those lenses ushered in to that which has lately been achieved by Abbe's apochromatics. It was the possession of this lens (P. and L. new formula 1/8) that first made the inadequacy of the Gillett apparent to me. This led me to get P. and L. dry achromatic condenser, which I still have. This condenser was designed by Powell in 1857; it is a 1/5 in power, and ·99 N.A. in aperture, and is the best ever introduced.

I must now say a word or two on low power condensers. Low power objectives had, somehow or other, been left out in the cold, no condenser having been provided for them. A sop, in the shape of a paraboloid or spot lens, was every now and then thrown to them, but, as far as I know, the first low power condenser we hear of is Webster's, in 1860.

The next was Abbe's chromatic,* 1873; Swift's achromatic, 1874; Abbe's achromatic, 1888, and Powell's new one, last year.

On the Continent the Microscope may be said to have remained condenserless until the rise of bacteriology compelled the general adoption of the Abbe in 1880. I will now give a parallel table showing the data and form of various condensers that have been introduced since the days of achromatism:—

England.	Continent.
1826. Single lens, Tulley.	1827. Single lens, Amici.
1840. Objective.	1833. Chromatic, Chevalier.
1850. Gillett, three pairs, N.A. ·65.	1839. Objective, Dujardin
1857. Powell, two pairs and single, anterior middle concave, N.A. ·99.	
1865. Webster, single front, achromatic back.	
1874. Swift, two pairs and single front, N.A. ·9.	1873. Abbe, chromatic, N.A. 1·2, hemispherical front, crossed back.
1878. P. and L. achromatic, improved anterior middle plane, ·99 N.A.	(? date) Another form, N.A. 1·4, single front, Herschelian doublet back.
1881. P. and L. oil chromatic, same as Abbe only higher power, N.A. 1·3. Ditto, truncated, N.A. 1·4.	
1887. P. and L. oil achromatic, N.A. 1·4, three pairs and single front.	1888. Abbe achromatic, two pairs and a single front, N.A. 1·0.
1889. P. and L. low power achromatic, N.A. 1·0, one pair and two singles.	

* Abbe's chromatic stopped down makes a far better low power condenser than it does a high power, as the stop reduces the abnormal amount of spherical aberration.

This table shows that here, at least, there was an activity with regard to the condenser that was totally absent abroad. It must, moreover, be remembered that the list gives only types of condensers: Ross, for instance, made improvements on the Gillett, and Smith and Beck made numerous forms of condensers which have not been mentioned, simply because they were not typical. Messrs. Crouch and Collins made numerous condensers, mostly after the Webster type. So also, on the other side, Nachet and Hartnack fitted object-glasses as condensers, only to a much more limited extent. My impression is that, if statistical tables were available, it would be found that up to 1880 there were more condensers turned out by any one well-known English maker than by all the Continental firms put together.

We now come to the use of the condenser, and the first question that arises is with regard to the nature of the source of light: Is daylight or lamplight to be used?

I find with low and medium powers, the condenser being centered to the optic axis, the plane mirror used, and a window-bar focused on the object, that daylight gives very good results, especially if a brightly illuminated white cloud is the illuminating source; but when the white cloud has blown across the field, leaving only blue sky, the illumination becomes poor. My complaint against daylight illumination for low power work is that I believe it not only to be always changing, but also very injurious to the eyesight. When I began Microscopic work the white cloud was everything, but on account of the above-mentioned drawbacks I adopted artificial illumination. The most extraordinary ideas prevailed respecting artificial illumination. The history is as follows:—Brewster wrote a treatise on the Microscope in 1837, and in it explained his method of illumination. He was very keen on monochromatic illumination; this he obtained from some chemical substances flaming in a saucer, without any wick or chimney; light from this was parallelized by a bull's-eye formed by a Herschelian doublet, and this brought to a focus by another exactly similar lens. He is very particular to enforce that the image of a diaphragm placed between the source of illumination and the bull's-eye should be focused on the object. This was in preachromatic days, and the kind of Microscope he experimented upon was the simple Microscope, the lenses being jewel singles, doublets, triplets, Coddingtons, which last were his own invention, &c.

With such a source of illumination, unless his object had been in rays considerably condensed, he would not have seen anything at all. Be that as it may, the fact is that the rule of having the source of light in focus has been handed down by the text-books all along, only with this curious proviso, viz. that each author had his own particular directions for disregarding the rule.

Taking Andrew Ross first, whose directions are considered so admirable that Quekett says he will quote them at length, we find that after he has given instructions with regard to centering, he says that delicate objects are best seen by racking the condenser within, and objects having some little thickness without the focus. Further on he says that slight obliquity of the illumination subdues the glare attendant upon perfectly central and full illumination by lamplight; he then goes on to say how this slight obliquity may be secured. The above words

form the keynote for artificial illumination in every subsequent text-book. They are repeated by Carpenter, who, after giving directions as to centering and focusing the image of the lamp-flame on the object, says that "the direction of the mirror should then be sufficiently changed to displace the image and to substitute for it the clearest light that can be obtained." Further, he recommends that while with daylight the condenser should be used in focus, with lamplight it should be somewhat racked down. From this I gather that Dr. Carpenter's best artificial illumination is oblique light out of focus. Of course the actual fact is that daylight focus is not nearly so important as lamplight. In illustration of another kind of mistake, as late as ten years ago it was recommended that the diaphragm be placed above the condenser as giving a better result than when placed below.

Of course the optical effect is precisely the same, the only thing is that the diaphragm below the condenser is much more readily manipulated and is much more likely to be accurate in centering, unless the one above be of the cap form. To change a cap diaphragm necessitates either the removal of the slide or the condenser, and all for no purpose. The next idea was worse, viz. the calotte diaphragm. This being fixed to the stage and not to the substage, gave as often as not excentric pencils. Whatever diaphragm is used it obviously must be centered to the condenser and must move with it, otherwise it will be put out of centre during the operation of centering the condenser to the optic axis of the objective.

Further, the calotte diaphragm is useless for ordinary illumination without a condenser, as the apex is not the proper place to cut the illuminating cone. The proper place, therefore, for a diaphragm, when no condenser is used, is some distance from the object, and when a condenser is used, is at the back of the combination. Further, when a diaphragm is above the condenser the apertures become almost microscopic in size, and a very small difference between them will make a considerable difference in their effect; but when they are placed behind the combination they may be larger, and it becomes more easy to graduate them in accordance to any desired effect.

Again, it is a fallacy to suppose that a Kelner eye-piece is superior to a condenser as an illuminator for high powers.

A Kelner eye-piece, if a C, is only 1 in. in power, and has a small angular aperture somewhat less than 45°, therefore it cannot possibly give a cone at all comparable with that from a most elementary condenser. It might be used as a substage condenser for low powers, but from its small aperture it would hardly give a good dark-ground illumination for a 1-in. objective.

With regard to low power condensers, the Webster (as designed by Webster) is the proper form. There are many so-called Webster condensers in existence which are on a totally wrong principle. The right kind of Webster has a single front lens and a back lens composed of a plano-concave flint and a crossed convex crown, the cemented surfaces having a deep curve to overcorrect the lens. The other kind, which is quite wrong, has an achromatized front and a single back; it is merely done for cheapness, as small achromatic pairs are not so expensive as large ones, and the back lens of a condenser is always larger than the front.

Another mistake is that direct light is more critical than indirect, which means, in other words, that illumination without a mirror is more critical than illumination with a mirror. Presupposing the same conditions, viz. the same condenser with the same stop, the centering and focus being precise, the optical conditions must be identical and the result the same. The ground is entirely cut away from the one only thing which could possibly affect the result—I allude to the loss of light by reflection at the mirror, by the fact that you have, with merely a 1/2-in. paraffin wick, more light than you know what to do with. So much is this the case that in my own practice I am in the habit of using a double cobalt pot-glass screen to reduce the intensity.

I am aware that direct illumination is a most convenient and time-saving method, especially when the instrument is well tucked up on its trunnions, but that it makes any perceptible difference in the criticalness of the image I am not prepared to admit.

With regard to mirrors, a good deal of misapprehension exists. It matters little whether the mirror be dusty or scratched, or the silver in bad condition; the only effect these will have will be to cause a little less light to fall on the back lens of the condenser, a matter supremely unimportant. An old scratched dull mirror will yield as critical an image as the finest worked up silver on glass Newtonian flat.

The three things that are of paramount importance are the direction of the light, the angle of the cone, and the spherical aberration of the condenser. Mirrors which yield secondary reflections are to be avoided, but if they can be turned round in their cells the secondary images can be easily eliminated.

Having touched upon the errors in the use of the substage condenser, let me say a few words with a view of clearing up some strange notions that are held with regard to its office. The original prevailing idea with regard to the office of a substage condenser was, I believe, in the first instance, that of a contrivance by which more light could be secured; afterwards it became chiefly important as an oblique illuminator; but its true function as that of a cone-producer was not generally recognized. As this view of mine will probably be met by the criticism that in the text-books, both ancient and modern, we read "that the condenser must be accurately focused," that the use of the diaphragm is for the purpose of contracting the cone of illumination" (many similar passages might be quoted), I nevertheless contend that there are other passages which conclusively prove that the writers were ignorant of the true function of the condenser.

The following is an example :—"If the cone of rays should come to a focus in the object, the field is not unlikely to be crossed (in the daytime) by the images of window-bars or chimneys, or (at night) the form of the lamp-flame may be distinguished upon it; the former must be got rid of by a slight change in the inclination of the mirror; and if the latter cannot be dissipated in the same way, the lamp should be brought a little nearer."

This passage proves that the end-all and be-all in the writer's mind was the agreeableness of the illumination; when the glare of the lamp-flame becomes unpleasant, the cone may go to the wall.

If the importance of the cone had been paramount in the mind of

the writer. he would have certainly suggested the obvious method of softening down the intensity of the flame-image by interposing coloured screens. Taking the whole tenor of the passage, there cannot be the least doubt that the ends sought for were suitable intensity of light and equable illumination of field; the frequent mention of the word cone being more accidental than insisted on for the sake of the cone itself.

It is as a cone-producer wherein the efficacy of the condenser lies. If, as is implied in the text-books, it were only light-intensity which gave criticalness to the image, that could be secured by exchanging the light from the 1/2-in. paraffin wick for that from the electric arc, but such an exchange would cause no alteration in the character of the image so long as the aperture of the cone remained the same.

The real office of the substage condenser being a cone-producer, the first question that arises is, What ought to be the angle of the cone?

This is really the most important question that can be raised with regard to microscopical manipulation. To this I reply that a 3/4 cone is the perfection of illumination for the Microscope of the present day.* By this I mean that the cone from the condenser should be of such a size as to fill 3/4 of the back of the objective with light, thus N.A. 1·0 is a suitable illuminating cone for an objective of 1·4 N.A. (dark grounds are not at present under consideration). This opinion is in direct opposition to that of Prof. Abbe in his last paper on the subject in the December number of the R. M. S. Journal for 1889, where he says: —"The resulting image produced by means of a broad illuminating beam is always a mixture of a multitude of partial images, which are more or less different (and dissimilar to the object itself). There is not the least rational ground—nor any experimental proof—for the expectation that this mixture should come nearer to a strictly correct projection of the object (be less dissimilar to the latter) than that image which is projected by means of a narrow axial illuminating pencil." †

This paper I consider to be the most dangerous paper ever published, and unless a warning is sounded it will inevitably lead to erroneous manipulation, which is inseparably connected with erroneous interpretation of structure.

If you intend to carry out his views and use narrow-angled cones, you do not need a condenser at all—more than this, a condenser is absolutely injurious, because it affords you the possibility of using a large cone, which, according to Prof. Abbe, yields an image dissimilar to the object. If there is the slightest foundation for Prof. Abbe's conclusion, then a condenser is to be avoided, and when a mirror is used with low powers care must be exercised to cut the cone well down by the diaphragm.

In the opening sentence of the paper Prof. Abbe says, "The diffraction theory leads to the following conclusions in regard to the mode of illumination in question." We are, therefore, thrown back on the diffraction theory, for the discussion of which I must ask your kind

* Mr. Comber (R. M. S., May 21st, 1890) states that in practice he finds a 2/3 cone best for photomicrography. A 2/3 cone (photographically) is to a 3/4 cone (visually) as 10/12 is to 9/12. Mr. Comber's experience is therefore in accordance with this statement.

† R.M.S. Journal, 1889, Part 6, p. 723.

indulgence, as the only other avenue for such a purpose has been closed to those who do not accept Prof. Abbe's theory in its entirety.

The diffraction theory has been likened, as you are aware, to the theory of gravitation. Let us, therefore, compare them. The theory of gravitation may be said to rest on three points—viz. mathematical proof, physical law, and experimental proof;—moreover, it is not afraid of criticism.

The diffraction theory rests on no mathematical proof—in the main it accepts the physical law of diffraction; but on experiment it utterly breaks down, all criticism is stopped, and everything connected with it has to be treated in a diplomatic kind of way.

Both theories may be said to resemble an arch, being built up on theory and experiment, and held in equipoise by a keystone at the top. The diffraction arch, after being built up on theory and experiment, culminates with the calculation of the Eichorn intercostals as its keystone. The discovery of these intercostals on the *P. angulatum* (which has been likened to the discovery of the planet Neptune) was arrived at by "a mathematical student, who had never seen a diatom, and who worked the purely mathematical result of the interference of the six spectra."

In the same way the discovery of Neptune may be called *a* keystone of the gravitation theory. It would be incorrect in this connection to say *the* keystone, because the gravitation theory has many keystones, while the diffraction theory has only the one, viz. the Eichorn intercostals. If, for instance, one could prove that the planet Neptune had no objective reality, but was a mere optical ghost, the gravitation theory would be seriously compromised. If, this evening, I can prove that the Eichorn intercostals are ghosts, then I maintain that I have taken the only keystone from the diffraction theory arch, and the conclusions which Prof. Abbe has arrived at in consequence of that theory, with regard to illumination by means of the wide-angled cone, are fallacious.

Let me at this place state that I wish it to be distinctly understood that I am not, in this paper, attacking Prof. Abbe's brilliant discovery that the image in the Microscope is caused by the reunion of rays which have been scattered by diffraction, neither do I question what I venture to think is his far more brilliant experiment, which exhibits the duplication of structure, when the spectra of the second order are admitted, while those of the first are stopped out. I regard these facts as fundamental truths of microscopy. The thanks of all true microscopists are due to Prof. Abbe for giving them to us. It will be then asked, how can you disagree with that which you admit? The point is, that it is in the meaning of the word "diffraction image" that the difficulty lies. Let me explain. There are in reality three kinds of diffraction images, for which I will now substitute the following names, "true diffraction image," "true diffraction ghost," and "false diffraction ghost," in place of those I used in my previous paper.* Now I maintain that both Prof. Abbe and his exponents at the R.M.S. have fallen into the grievous error of not distinguishing between these

* Q.M.J., Ser. II., vol. iv., No. 25, p. 17, "true, true false, and false."

three images, viz. the true diffraction image, and the true and false diffraction ghosts. You will naturally ask, how do you distinguish between these three images? A true diffraction image goes in and out of focus like a daisy under a 4 in. In other words, a true diffraction image is one out of which it is impossible to make another image by focal adjustment. A diffraction ghost, on the other hand, is one which changes into other images on focal adjustment, a false diffraction ghost being an image which is dissimilar to the original, and a true diffraction ghost one in which it is fairly in accordance with the original.

A true diffraction image is produced by a large cone of illumination, except in those cases where the structure is so fine, in relation to the aperture of the objective, that the large cone does not cause the spectra to overlap one another and the dioptric beam.

True and false diffraction ghosts are produced by small cones, except in those cases where the structure is either so coarse that the spectra overlap, even with the small cone, or so fine that only spectra of the first order are taken up by the objective; in this latter case a false diffraction ghost becomes impossible. Taking the ghosts first, the reason why there is a change of image on alteration of focus may be seen on reference to plate II. fig. 3. Let O be an object having about 20,000 interference elements per inch, let DD be an infinitely thin dioptric beam in the optic axis, then S and M will be the spectra of the first order, and T and N those of the second. If the object be examined by an objective whose aperture is greater than the angle T O N, i. e. upwards of 100°, a diffraction ghost will be seen, because at the longer focus the spectra S and M will be united with D, and a representation similar to the true structure will be produced; but on shortening the focus the spectra T and N will be united with D, and a picture having double the fineness of the original structure will be seen. (You require no stop at the back of your objective to perform this experiment; the spherical aberration, which is always present, even in the best corrected lenses, will be sufficient to prevent the union of S and M with T and N. See Mr. Leroy's results on applying the Foucault test to Microscope objectives, R.M.S.J., 1890, p. 224: the spherical aberration varied from tenths of mm. to several mm.) It is therefore a diffraction ghost, because the image alters on focal adjustment; it is a true ghost at the upper focus and a false ghost at the lower focus.

Let us now see what takes place when a large cone is used. Let PP be an isolated pencil of such a cone, then HQ will be spectra of the first order, and R a spectrum of the second, and K one of the third order. These dotted lines are drawn at a little distance from the others for the sake of clearness, but they are supposed to be either coincident with or very near the others. Here we see at the upper focus that a spectrum of the second order R is combined with a dioptric beam P, and a first diffraction spectrum Q, and this takes place in addition to the combination of S and M with D mentioned above. Bringing in a diffraction spectrum of the second order will tend to improve the image. At the lower focus even now there will be a first diffraction spectrum H, combined with a third order spectrum K, together with the combination of H and N with D as above. This combination would give a confusion of images, so it comes to pass that images with

a wide angled cone at the lower focus are blotted out. To state the combinations more concisely at the upper focus, we have two first order spectra and a dioptric beam; and a first and second order spectrum and a dioptric beam.

At the lower focus you have two second order spectra and a dioptric beam; and two first orders and a third order.

It may be as well to explain to those not acquainted with optics, that these combinations are caused by the spectra T N and H R passing through the same zone of the objective. The union of a set of spectra such as S D M makes a certain kind of image, and the union of P Q R will make a very similar image, not absolutely similar, but so similar that it would be difficult to tell the difference between them. So it comes to pass that the superposition of a number of very similar images strengthens the picture and gives a resultant image very close to the original structure. But the image caused by the union of T D N is totally dissimilar to the original, and H Q K would also be very dissimilar and the superposition of a number of these can only make a stronger dissimilar picture, or if the pictures, which are superposed, differ widely from one another, then the superposition of them will produce a fog. By way of illustration, suppose I made a large number of photomicrographic lantern slides, using certain spectra, which gave an image closely resembling an original known structure, and suppose each lantern slide to be a picture, resulting from a different narrow dioptric beam, such as D and P in our diagram, and others lying between them, we should then have a number of lantern slides, all very similar to the original and consequently to one another. Now suppose we had a number of lanterns and projected these several images at once on the screen, the several images would combine to form a strong image closely resembling the original structure. If, however, we make other lantern slides, using spectra, such as T N, which double the original structure, and if these are projected on the screen in place of the others, we shall get a strong image of a structure altogether dissimilar to the original. But if we increase the number of our lanterns, and project the other images as well, we shall have a confused image on the screen, or fog. Another illustration may help to simplify the matter. Suppose it were possible in photographing a dog with an ordinary camera, by manipulations at the back of the objective, to obtain, either an image only very slightly dissimilar to the real dog (such as an image slightly out of focus), or with other manipulations to obtain a picture of a hayrick. If a number of these slightly dissimilar images of the dog were projected on the screen, we should still have the image of a dog, and one that we could readily recognize. But if we projected the images of the hayrick, we should not have the slightest idea that the original object was a dog, and further, if the images of the hayrick were projected at the same time as those of the dog, the result would be a confused mass of light in which it would be impossible to recognize any image. Whether any particular lantern slide turn out a dog or a hay-rick, depends on the physical union of various other oscillations, but whether the image of either the dog or hayrick be a strong one, or a mass of fog, depends on the mechanical combination of similar or dissimilar images.

We must now return to the Eichorn intercostals; the history regarding these is as follows:—

The six spectra of the first order of *P. angulatum* (fig. 1) were set to a student who had never seen a diatom, and he calculated the presence of an intercostal. These intercostals were afterwards seen by Mr. Stephenson, and the student's discovery was likened to that of Neptune. There is a double error here. The first is that the intercostal is a function of the spectra of the second order, and can neither be calculated, originated, nor seen by those of the first order.

Secondly, the intercostal is not a true diffraction image, but is a false diffraction ghost, and is caused by the reunion of the spectra of the second order, and the exclusion of the first order.

The very data given to the student have to be excluded before an intercostal can appear or be calculated!

The error in connection with the exhibition of the intercostals of the *P. angulatum* is that no sufficient checks were imposed to render it absolutely certain that no spectra of the second order were present at the time the intercostals were seen. The intercostals have also been accounted for by a fallacious geometrical picture. Thus, the six spectra (fig. 1) account for three sets of lines ruled at 60° to each other. Now, as I pointed out in the 'English Mechanic,' vol. xliii., No. 1108, p. 337, two very different pictures are produced according to whether the third line be ruled through the apices of the rhombs (fig. 4) or not. It is for those who uphold the truth of the intercostals to show which spectrum or what arrangement of the six spectra determines that the third line does not pass through the apices of the rhombs (fig. 6). The contrary is really the fact, viz. that if there is any truth at all in the diffraction theory, then with a spectral arrangement as set to the mathematical student the third line must pass through the apices of the rhombs (fig. 5). Figs. 4, 5, and 6 show the rhombs and the formation of the two kinds of pictures. In passing, it is as well to note that objectives being for the most part spherically undercorrected, generally show the intercostals at the lower focus. In other words, you have to lower your objective in order to obtain the reunion of the spectra of the second order by means of the outer zone of the objective. Intercostals are due to illumination by means of a narrow cone, which allows and aids zonal differences to operate on the spectra, uniting those of the second order, whilst excluding those of the first.

Illumination by a large cone neutralizes the effect of these zonal differences, and intercostals disappear.

I have given much attention to diffraction ghosts, and have made several photographs of them for your inspection. Instead of confining my investigations to *P. angulatum*, as has been usually done, I thought it better to select very coarse structures, concerning the true appearance of which all microscopists are entirely agreed. In the first instance, I experimented with the coarse hexagonal structure of a *Triceratium*, which measured 1/3600 in. A photograph × 387 taken with a large cone I will now project (fig. 7). The illuminating cone was now cut down by closing the iris diaphragm, and the aperture of the objective stopped down until the spectra at the back of the objective appeared as in fig. 2. The next photograph (fig. 8) shows the image due to those

spectra; this shows the intercostals, and is what I term a false diffraction ghost. You will observe that the objective has been placed at a lower focus. If the same, i. e. the upper focus, had been used, then a picture similar to the true image taken with the large cone would be seen, except that the walls of the hexagons would be considerably thicker, and in the centre of each areolation there would be a dark spot.

If the illuminating cone be enlarged to a 3/4 cone the image will closely resemble the critical image (fig. 7) already shown, and moreover will be a true diffraction image, because it will go in and out of focus as a daisy under a 4-in. In examining the various images presented by a hexagonal grating in focal alteration, when a small cone of illumination is used, I found that these false diffraction ghosts followed a certain sequence, and might be grouped in three classes, which I term degrees. The false diffraction ghost of the first degree requires spectra of the second order (fig. 2), for its production. It is the Eichorn intercostal image.

The next experiment was performed with the narrow cone as before, but with the aperture of the objective reduced so that the second order of spectra (fig. 1), were cut out; according to my theory no intercostals should now be visible; on taking the photograph, however, a trace of them could be distinguished (fig. 9). This is such an interesting result that I have printed the negative. The fact was that I had cut out the second order spectra visually but not photographically. On further cutting down the aperture quite up to the end of the spectra of the first order, no intercostals could either be seen or photographed (fig. 10).

This is an additional proof that the intercostal image is a function of the spectra of the second order. Further, if an intercostal on *P. angulatum* is resolvable by means of spectra of the first order, which diverge about ·5 N.A. from the central dioptric beam, as affirmed by Eichorn, Abbe, and the anonymous writer of the article on microscopic vision in the R. M. S. Journal,* then the theoretical limit tables at the back of the Journal had better be torn up. The intercostals would count about 95,000 per in., and according to those tables they would cause the spectra of the first order to diverge about ·99 N.A. from the dioptric beam. So it would require an aperture of nearly 2·0 N.A. to grasp all the six. Therefore all these years the tables at the back of the R. M. S. Journal, and the anonymous article on microscopic vision, which is a condensed summary of all their and Prof. Abbe's teaching on the subject, are, as I have often pointed out, contradictory. This last experiment on the *Triceratium* with only the spectra of the first order admitted, shows that on focal alteration only a change from positive to negative diffraction images takes place, i. e. black to white dots; in other words, a black hexagon with a white centre changes to a white hexagon with a black centre and *vice versa*. The word hexagon is here incorrect; the pattern strictly speaking under these conditions is black or white circular dots arranged in a quincunx form. This experiment is most important, because it shows that when a small cone of illumination is used a more truthful image is secured by

* R.M.S.J., Ser. 2, vol. i. pp. 354.

reducing the aperture of your objective until all spectra are cut out, except those of the first order. The reasoning is as follows: With a small cone and an aperture sufficient to take in many orders of spectra on focal alteration, you obtain a series of changing images similar to those seen in a kaleidoscope. Without *à priori* conclusions you do not know your focus, consequently you cannot select the true diffraction ghosts from among the false diffraction ghosts.

But the moment the aperture of the lens is contracted so that only the spectra of the first order are admitted, one image and one image only is possible. This image is certainly not a very good image of the structure, nevertheless it cannot be very dissimilar.

In the case before us, instead of getting well-defined hexagons like those of a bee's honeycomb, we have in place of them circular bright spots, spaced correctly and in arrangement precisely similar to the original.

But it may be urged that all this only applies to diatom work, and has nothing whatever to do with ordinary microscopical objects. If you will pardon me for a moment I will endeavour to prove to you that it is of the highest importance with regard to almost every microscopical object, But first let me draw your attention, before leaving the *Triceratium*, to a false diffraction ghost of the second degree (fig. 11). This picture is only possible when four orders of spectra are admitted. Here you will notice that each bar of the hexagon is broken up into three dots, and six spots with a central one are imaged in each areolation. This is a difficult one to photograph on account of the great brightness of the areolations, which accounts for the images in those parts being weak. To show that this is a subject not at all confined to diatomic structures, the next experiments will be performed on the eye of a fly.

The spectra arising from this structure are identical with those from similar diatomic structures, only they are not so widely spread out, the intervals being 1/800 in. This proves that diffraction does not begin at 1/2500 in. I will first project the critical image (fig. 12) taken with a 3 4 cone × 165. The illuminating cone was now reduced, and the spectra, as in the next picture, allowed to pass into the objective (fig. 13). We now get the Eichorn intercostals. This shows that the diffraction theory has just as important a bearing in connection with a common entomological object as with a diatom. The next picture (fig. 14) was taken with a large cone, but the aperture of the lens was reduced so that it should bear the same proportion to the eye of a fly as an oil-imm. of 1·4 N.A. does to the *P. angulatum*. Here you will notice that the hexagon runs into a kind of square shape. A similar appearance can be obtained with a *P. angulatum*.

The structure of the eye of the fly being very coarse it is possible to pick up the whole of the diffracted fan; this, as seen at the back of the objective, is in itself such a beautiful object that I have endeavoured to produce it, but as yet without success. It is a beautiful star with hyperbolic edges, and is, as far as I am aware, unknown, nor figured anywhere. If this whole diffraction fan be admitted to the objective, then we get a false diffraction ghost of the third degree (fig. 15), and this is the last and most complicated ghost you can have. The founda-

tion of the picture is composed of three lines drawn at 60° to one another, the third line passing through the apices of the rhombs. I will next project a false diffraction ghost of *P. formosum*, showing intercostal dots (fig. 16). These were produced in precisely the same manner as the others. The focus, you will notice, is only slightly within the true focus. The greater the aperture of the objective used the less out of focus the object requires to be in order to produce the intercostals. Now I have shown you the three degrees of diffraction ghosts; these are all produced, and can only be produced by the small cone. It cannot be wondered that Prof. Abbe and his exponents say that "whether for example, *P. angulatum* possesses two or three sets of striæ, whether striation exists at all, whether the visible delineation is caused by isolated prominences, or depressions, &c., no Microscope, however perfect, no amplification, however magnified, can inform us."*

Again, we read "that every attempt to discover the structure of finely organized objects – as, for instance, diatom-valves— by the mere observation of their microscopic images, must be characterized as wholly mistaken." And again, "The interference images of minute structure, however, stand in no direct relation to the nature of the object, so that the visible indications of structure in a microscopical image are not always or necessarily conformable to the actual nature of the object examined."

The explanation of all this is that Prof. Abbe takes cognizance of one kind of image, and that one a diffraction ghost, and it is perfectly true that so long as you are dealing with diffraction ghosts you cannot, for certain, determine the nature of the structure you are observing.

At different foci when a small cone is used there are different images, and without *à priori* knowledge it is impossible to determine the correct focus, and consequently the true diffraction ghost. Now it is the function of the condenser to put an end to all these difficulties; it enables you to illuminate by means of a wide-angled cone, and then you have a true image at one definite focus, and at any other focus there is no image at all to confuse you.

Of course it must be understood that when the structure is very fine, and the spectra are diffracted through great angles, your widest-angled cone really becomes a narrow one in relation to that structure; and then you are obliged to make the best you can with diffraction ghosts. But there is, on that account, not the least reason why, for all coarser structures, you should not have a true diffraction image by means of a large cone instead of either a true or false diffraction ghost by a small cone.

Eventually our diffraction ghosts with very fine structures and wide-angled cones may through increase in the apertures of our objectives and improvements in our condensers, be changed to true diffraction images.

Prof. Abbe's last paper takes account only of small differences between very similar images, and ignores altogether the enormous differences due to the union of different orders of spectra and the exclusion of others. He is in fact straining at the gnat and swallowing the camel. In his paper he disregards the possibility of getting (to

* M.M.J., xiv. (1875) p. 220.

return to our former simile) a picture of a hayrick instead of a dog, while he insists that a small cone is preferable to a large one lest the dog appear foggy. To which I reply that a foggy dog is preferable to a hayrick, however sharp.

When the illuminating cone is enlarged so that it fills about 3/4 of the back of the objective, one image, and one image only, can be produced, which, as I have said, goes in and out of focus as a daisy under a 4-in. There can be now no doubling of the structure, and no multiple images are produced. Spherical aberration in the lens merely veils the image under an appearance of fog or mist. The clearness and distinctness of the image may be marred by its means, but the image cannot be altered in form.

I have only one more point to bring to your kind notice, and that is the statement that the wide-angled cone, by means of the superposision of dissimilar images, obliterates uncoloured histological tissues.*

The truth regarding this is that the wide-angled cone gives you a faithful representation of uncoloured histological tissues (very likely not the preconceived images regarding them), blotting out all those parts which are out of focus. In other words, it gives you a truthful picture of a definite plane in the structure. To illustrate this I have selected the thinnest and most transparent histological object, and one which would be more likely to be blotted out than any other with which I am acquainted. I have photographed this both with a wide and narrow cone, and you shall judge for yourselves which is the more faithful picture. The object is cartilage in a young rat's tail, of which I will project a low power view, × 8, in order that you may identify it. I now show you an image (fig. 17), × 390 diams., taken with a small cone. The most prominent features in this image are the parts which are out of focus. I wish to draw your particular attention to a cell-wall seen end-on running nearly in a vertical direction in the centre of the slide. The focus was adjusted precisely on that point, and I would like you to notice the apparent thickness of that line.

I will now show you the same object (fig. 18) taken by a large cone, and you will at once understand the extreme tenuity of that particular cell-wall which in the previous picture was so thickened by false diffraction ghosts. This picture, I maintain, is a true representation of an exquisitely thin cell-wall; there is no blotting out of any structure in focus, only a removal of false diffraction ghosts. Of course it may be useful to produce a false image for the purpose of obtaining an idea as to the relative position of the part in focus to those parts out of focus. But this has nothing to do with the bare fact of the obliteration of structure by means of a wide cone.

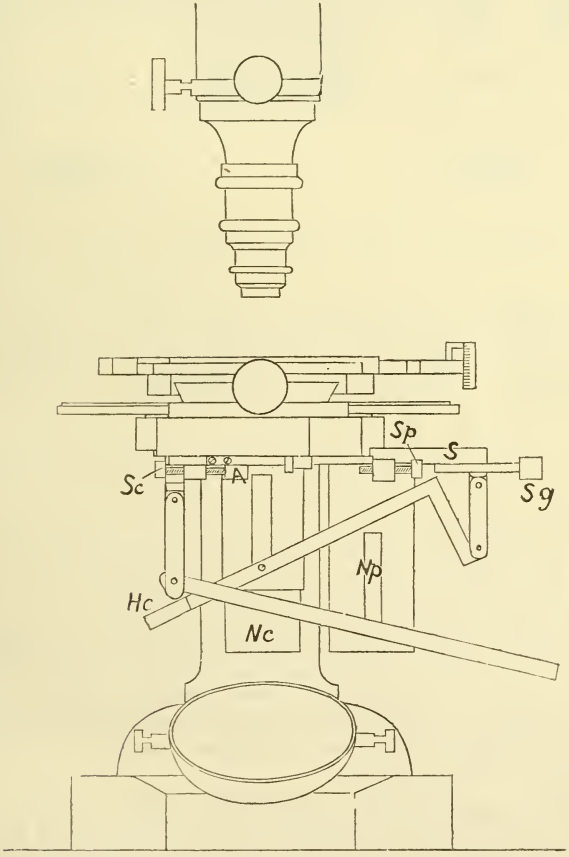
In conclusion, I believe the objection to the use of a narrow-angled cone to be due to the fact that it emphasizes zonal differences, and the efficacy of the wide-angled cone that it as far as possible neutralizes the effect of those differences. Prof. Abbe states (p. 724) "there is not the least rational ground, nor any experimental proof, for the expectation that this mixture [he is alluding to the mixture of slightly dissimilar images in consequence of the employment of a wide cone] should come nearer to a strictly correct projection of the object than that image which

* R.M.S.J., 1889, Part 6, p. 723.

is projected by means of a narrow axial illuminating pencil." Now, I take it that I have proved to you this evening one thing at least, that there is rational ground and experimental proof for the expectation that this mixture does come very much nearer to a strictly correct projection of the object than that image which is projected by means of a narrow-angled illuminating pencil. Finally, I am of opinion that a correct understanding of diffractional effects will, more than anything else, tend to produce in the minds of microscopists a true appreciation of the importance of the achromatic condenser."

Apparatus for the rapid change from parallel to convergent polarized light in connection with the Microscope.*—Dr. E. A. Wülfing gives a description of an apparatus invented by himself,

FIG. 4.



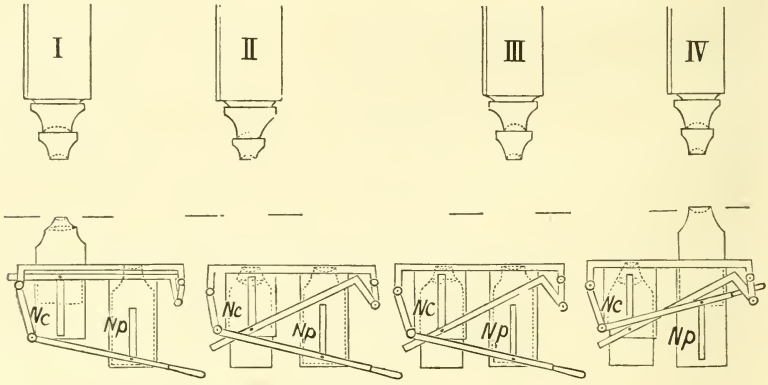
intended to save time in studying mineral or rock sections by polarized light. A plate S (fig. 4), sliding in a groove beneath the stage of the

* Neues Jahrb. f. Mineralogie, 1889, ii. p. 199–202.

Microscope, bears on its under surface two vertical tubes containing two Nicol prisms N *c* and N *p*, one of which N *c* is permanently arranged for observation by convergent light, i. e. with the usual two convergent lenses, whilst the other N *p* bears the usual lens employed in observations by so-called parallel light. Both of these polarizers, together with their lenses, can be raised or lowered independently of one another by means of suitable forked two-pronged levers H *c* and H *p*.

When changing from one form of illumination to the other, the Nicol prism last in use is pushed down, the plate S is slid in its groove so as to bring the other Nicol to the centre of the stage, and this second Nicol is then raised by its lever into position beneath the mineral section. These four stages in the process are shown in the diagrams I. to IV. (fig. 5), where I. shows Nicol N *c* in its highest position,

Fig. 5.



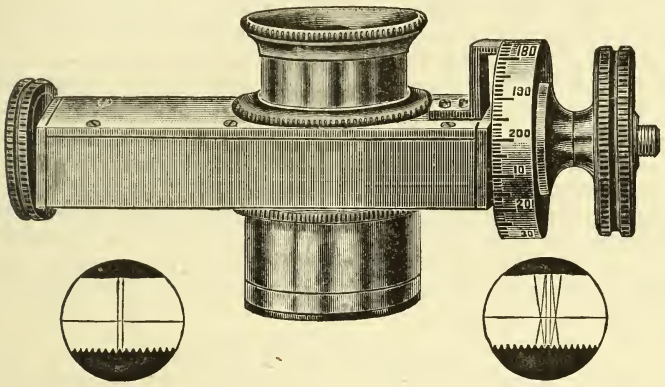
beneath the section; Nicol N *p* in its lowest position on one side, not in use. II. Nicol N *c* in its lowest position beneath the section; Nicol N *p* in its lowest position on one side, as before. III. Nicol N *c* in its lowest position, pushed aside; Nicol N *p* in its lowest position beneath the section. IV. Nicol N *c* in its lowest position, pushed aside as before; Nicol N *p* in its highest position beneath the section.

The catch or stop at A can be turned aside to permit of both polarizers being pushed aside for observation by ordinary light. Dr. Wülfing has the apparatus attached to a Fuess No. 1 large Microscope. It would require special fitting to other patterns. The apparatus is made by Zimmermann, Mechaniker, Hauptstrasse, Heidelberg, and costs, without Nicol prisms and lenses, 60 marks. It will be observed that two polarizers are required.

Bulloch's improved Filar Micrometer.—Fig. 6 shows this instrument, of which a short description was given in this Journal for 1886, p. 132, in which we described it as having a second screw, worked by a milled head at the opposite end to the micrometer-screw, which moves both sets of lines together, so that it is possible to set the graduated screw-head at zero for any particular measurement.

Mr. Bulloch now describes it as follows:—"The improvement consists in the secondary slide, by which the whole micrometer is movable; avoiding the uncertainty of adjustment made by the ordinary micrometer in getting contact with the cross-hair at zero; as the amount of

Fig. 6.



spring with the best mechanical stage (excepting micrometer stages) prevents the cross-hairs being brought gently and perfectly in contact with the object to be measured."

Mr. Bulloch writes as follows with regard to the additional diagonal lines shown in one of the small figs.:—"From my experience in comparing micrometers and getting the value of divisions of the screw, much better results can be reached by intersecting the line on the micrometer with the cross line in the eye-piece micrometer; for when using the ordinary filar micrometer it is almost impossible to judge the amount of space left between the line on the micrometer and the spider line."

(4) Photomicrography.

Photomicrographs and Enlarged Photographs.—At the December meeting of the Society, Mr. J. Mayall, jun., read the following note:—

The application of photography to microscopy has received in recent years so great an impulse from the introduction of the dry-plate processes, that the Society has received very large accessions to its collection of photographs—especially of such as have been produced with a view to illustrating various theories of diatom structure.

Apart from the question how far it is legitimate to infer the structure of such diatom valves either from the images seen in the Microscope or from those produced by photomicrography—a question which Prof. Abbe's researches appear to answer in the negative—it appears to me that, unless great discretion is used, the after-processes of enlarging from photomicrographs may easily lead microscopists astray in giving fictitious appearances of contrast in the structure, leading to the belief that such strong images must necessarily represent what was visible in the Microscope. It is well known that photomicrographs frequently give a very erroneous rendering of the different tints seen on delicate

and transparent objects in the Microscope; and when this erroneous rendering is supplemented by the artificial contrasts due to chemical intensification of the original negatives, or to the after-processes of copying and enlarging, it becomes of the first importance in cases where photographs are brought forward in illustration of special points, that either the original negatives, or reproductions of them as exact as possible, should be exhibited. When, as in many cases—notably by Dr. Van Heurck—enlarged photographs are brought forward without any precise description of the process of their production, and without the original photomicrographs for comparison, useful criticism is difficult if not impossible. All that can be said about them amounts to expressions of vague astonishment that the image should look so strong and so highly magnified. I think it would be advisable in all cases to distinguish between photomicrographs produced with the Microscope, and enlarged photographs from photomicrographs: the former can only be usefully criticized by one who is familiar with the object as seen in the Microscope; the latter need other criteria whence their utility may be estimated, and, above all, they need the presence of the originals from which they were enlarged.

(5) Microscopical Optics and Manipulation.

The full Utilization of the Capacity of the Microscope, and means for obtaining the same.*—In a paper read before the American Society of Microscopists, Mr. E. Bausch said:—The cover-glass may truly be called a necessary evil, for while absolutely required in microscopical investigations, there is no adjunct to the Microscope that has been and is productive of so much evil, and has so retarded the utilization of benefits made possible by the advance in the construction of objectives. This fact was appreciated as early as 1837, when the angular apertures were what would now be considered extremely limited, and the appreciable effect of variations in thickness of cover-glass was not then nearly so pronounced as it is at the present time, even in modern objectives of a narrow angle.

The accommodation for the different thicknesses was obtained by varying the distance between the systems of objectives, and has been followed with modifications in the mode of obtaining the necessary motion up to the present day. While open to some objection, it accomplishes the purpose quite satisfactorily, and must continue to be used until something better is suggested.

One of the purposes of the homogeneous immersion is, as we know, the avoidance of the necessity of the cover-correction, in that the cover-glass, immersion fluid and front of objectives are to be one homogeneous mass, but even under these conditions, which in practice were found to be not constant, it has been found advisable to provide cover-correction to obtain the highest possible results. However, even should this not be found necessary in the development of improvements in this class of objectives, it must be remembered that the majority of objectives will always be dry, and especially so when such improvements, which we hope are still to be made, are accomplished. It is an unfortunate circumstance that with this class of objectives the influence of variation

* Microscope, x. (1890) pp. 289–96.

in thickness of cover-glasses is most apparent; but since it is so, we should, if possible, provide an agency which, eliminating the personal factor of efficiency, will give under all conditions, results closely equal to those under which the objectives were originally corrected.

It is surprising to see how little attention is paid to this subject in the large majority of standard works on the Microscope. Almost all books give carefully prepared illustrations and descriptions showing the effect on the course of light by the interposition of the cover-glass, and after giving conclusive evidence of its disturbing influence, still, in a general way, say it is of little moment. Thus, in a German work of the highest standing, which has also been translated into the English language, is found the following utterance, freely translated:—

"In regard to modern Microscopes, which we have had opportunity to examine, we have not found the differences in thickness such as occur in commercial cover-glass, when, for instance, three to six are equal to a mm., have any noticeable influence on the microscopical image."

In another work of great popularity are found the following quotations:—"That the effect of thickness of cover-glass has a great influence on the perfection of the microscopical image is beyond the slightest question, and certainly deserves the most careful attention of the optician as well as the observer, but whether the devices for its removal are of such great importance and so absolutely necessary as it is claimed, is another question."

"On the other side, the difference in the cover-glass used in different directions for the most delicate preparations is hardly of any account. I, at least, possess, besides my individual preparations covered with glass of about 1/5 mm. thickness, a collection of objects which I obtained from London and Paris, in which there is such a slight difference of cover-glass thickness that I can observe them all with my objectives of powers from 2 to 1·3 mm. (equivalent to about 1/12 to 1/20 in.) without showing the slightest difference in optical qualities, and in the definition and clearness of the image under the same illumination, as I have convinced myself by careful comparative tests."

With such statements to guide the microscopists, it is not surprising that the subject should have received so little attention, and that any efforts to lead to improved methods of manipulating objectives should have almost completely failed because of a lack of the true understanding of their need and consequent failure to create interest. The belief is quite general that any time devoted to this subject is wasted, and might better be utilized in other directions. I hope to be able to show that this is entirely wrong, and may here say that while I may be considered an extremist in the other direction, my efforts emanate from the desire to put it in the power of every microscopist to obtain the highest possible results from his optical battery, and equal to those obtainable by the optician.

When, in 1887, Prof. S. H. Gage addressed a circular letter to all opticians in the world inquiring for the dimensions of their standard tube-length, as well as for the thickness of cover-glass which they used as a standard in the correction of objectives, I looked forward to the result with considerable interest, as it would bring together data which it was impossible otherwise to obtain.

At the meeting of this Society in 1887 at Pittsburgh, he gave the results of his efforts, which show some astonishing facts. I would here say that while for a long time I had felt that a system that would permit the full utilization of the optical capacity of objectives of different makers under varying conditions of cover-glasses was desirable, I was then forcibly impressed with the absolute necessity of a plan which would offer this advantage. One is as much surprised by the differences in cover-glasses used by various makers in correcting non-adjustable objectives, as by the great differences in the length of tube, which influences so considerably the spherical aberration of the objectives. With a thickness of 0·1 mm. for the thinnest, and 0·25 mm. for the thickest, it is only too apparent that with the additional variation in lengths of tubes, it is beyond the power of the microscopist to obtain even approximately the best results from his objectives. More than this, a large quota of the advance made in recent years in the capacity of objectives has been lost.

As Prof. Gage states, "A uniform thickness for cover-glass for unadjustable objectives seems also desirable," and this would be the easiest solution of the question, but while, on the one hand, the makers of objectives have not yet agreed to use one standard on account of the technical difficulties involved in departing from their established precedent, on the other, the microscopist would hardly be willing to bear the expense which would be occasioned by the loss of cover-glass not conforming to the standard, in order to use those of one thickness. This expense might be greatly reduced by using selected covers of one standard on objects for all medium and high-power objectives, and the balance on all other preparations, on which only low powers would be used, but this would of course be of little avail in face of the fact that manufacturers follow no standard.

The greatest difficulty is met with non-adjustable objectives. As is well known, compensation for thickness may be obtained in the proper adjustment of tube-length, but while not all Microscopes are suitably provided with draw-tubes, the requisite experience and skill are lacking with a large number of microscopists to make the correction properly in this manner, as well as in objectives especially provided with collar correction. I am sure that microcopists of long experience will bear me out in the statement that results with adjustable objectives depend upon individual skill, and that many such objectives now in use fail to give results corresponding to their capacity. It would seem, therefore, that any system to permit the full utilization of the capacity of objectives should depend on no personal factor, in fact, should be mechanical, and this I have followed out in the system that I shall explain.

In an objective corrected for normal thickness of cover-glass there will be spherical over-correction with thick covers and under-correction with thin covers, the amount of correction varying in a different ratio to the amount of variation from the normal thickness. The chromatic correction will also lose correspondingly, but not to so high a degree. While a deviation of a few hundredths of millimeter in either direction will not signify, that which occurs in covers classified in price lists under one number is sufficient seriously to affect, and in the higher powers totally obliterate the definition, which under normal conditions it may possess.

The microscopist is therefore not obtaining such results as his objectives should enable him to obtain, and the efforts of the conscientious optician to provide classified objectives of reliability and similar performance are almost entirely nullified. In making the necessary experiments some astonishing results appear. With a non-adjustable dry 1/5 corrected for a cover-glass of 0·16 mm., employing the extremes of cover-glass

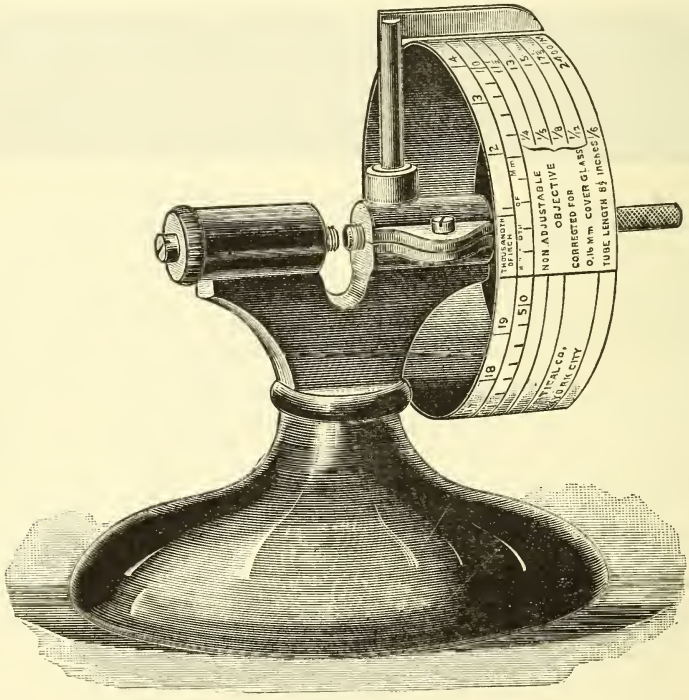
Fig. 7.

which are used by the various manufacturers as standard as obtained by efforts of Prof. Gage, I found that for 0·25 mm. a tube-length of 6 in. is required to obtain the proper correction, while for a thickness of 0·1 mm. 13 in. of the tube-length are necessary. In a 1/8 objective adjusted under the same conditions, 4½ in. are the requisite for a cover of 0·25 mm., and for 0·1 mm. 15 in. The further fact is shown that with a 1/5, which under conditions of tube-length and cover-glass given above shows certain structure well defined, absolutely fails to show anything of it under a cover-glass of 0·1 mm. on one side and 0·25 mm. on the other, and further a marked chromatic over or under correction. With a cover of 0·14, which would seem but a slight variation from the standard, the objective is spherically highly under-corrected, and with 0·18 highly over-corrected. With objectives of high power the difference is still more marked. For these experiments I have had Mr. J. D. Möller, of Germany, mount a series of *Pleurosigma angulatum* dry and *Amphipleura pellucida* in balsam under a series of covers varying from 0·1 mm. to 0·34 mm. each carefully measured and marked. I have used these objects because they are my favourite tests, and it goes without argument in saying that any preparation showing structure under the above objectives will be affected to the same extent by the varying conditions of cover-glass as these objects, and in objects of still finer structure the limit of visibility will be reached correspondingly sooner.

The system which I have devised to aid in overcoming these difficulties depends in the first instance upon a micrometer for measuring the thickness of cover-glass. While the delicate instruments made by M. Grossman, of Germany, are excellently suited for this purpose, they are expensive. I have endeavoured to overcome this objection by constructing a plain screw which, while not so sensitive to the touch, is sufficiently so for all practical purposes. The instrument is provided with a stand of japanned iron. Cut horizontally through the top is a thread of 1/50 in. pitch, and 3/16 in. outside diameter; a recess is cut on the top below the line of the screw, and at right angles to it for placing the covers. The one-half of the top of the stand which receives the micrometer screw is slotted longitudinally to the depth of the screw, and is provided with a set screw to take up wear. The other half has the

fixed screw, adjustable, however, for final adjustment. The end of the micrometer screw is milled, but of a small diameter, so that no force can be exerted to endanger the cover-glass. Fixed on the screw between two nuts is a brass drum with a 1/2 in. face; a knife-edge index-finger is fixed to the top of the stand, and projects over the top of the drum; to the outside diameter of the drum is fixed a strip of glazed paper

Fig. 8.



Actual size.

provided with a series of divisions. The first gives the thickness of cover-glass in thousandths of in., the second in hundredths of mm. The third indicates the proper tube-length with various thicknesses of cover-glass with a non-adjustable 1/4 corrected under a tube-length of $8\frac{1}{2}$ in., and cover thickness of 0·16 mm.; the fourth gives the tube-length of a 1/5 in. objective under the same conditions; the fifth for a 1/8, and the sixth for a 1/12 for same conditions of tube-length and cover; the seventh is for a 1/6 with the same cover, and tube-length of 160 mm.

In objectives provided with cover correction the graduation is so arranged as to read to 0·01 mm. No matter what the power of objective or whether dry or water-immersion, the number gives proper correction for a thickness corresponding to it. Thus, with a cover-glass of

·20 mm. the collar of such an objective needs merely to be set at 20 to give the proper correction, and consequently the best results. On the other hand, with an objective which is graduated on this system, the correct thickness of cover-glasses can be determined by obtaining the proper correction on preparations previously made, but on which the thickness of cover-glass is not noted, and the thickness may be marked on them for future convenience. To do this successfully, however, considerable experience is requisite. All the other scales give the correct tube-length in inches and millimetres for covers corresponding to them, and in this manner offer a ready and definite means of correction. The tube-lengths required for the thinnest and thickest covers are so extreme that probably no convenient means for obtaining them can be practically arranged, but they can be so approximately if not entirely. At any rate, the micrometer will detect the requirements before using the covers, and those deviating considerably from the normal can be used on objects for use with low powers only, in which case the effect will not be very appreciable.

In this system I do not overlook the fact that variation in tube-length involves a variation in magnifying power, but except in cases when micrometers are used, I consider this of secondary importance, as it always is in comparison with the results obtained in resolving and defining power.

This system involves four conditions :—

First. That all cover-glass be measured before using, and that the thickness be noted on the preparation.

Second. That for convenience all draw-tubes be marked in inches or millimetres, or both.

Third. That adjustable objectives be corrected according to this scale.

Fourth. That the same tube-length and cover-glass thickness be used in all original corrections of objectives.

As regards the first condition, there are many microscopists now who measure all their covers before using them, but the mere knowledge of thickness has been of no value up to the present time, because this in itself has been no guide in obtaining better results except by approximation. My aim in connection with this system has been to devise an instrument which shall possess a high degree of accuracy, and shall still be so inexpensive that its price should be no obstacle to its general use.

The celebrated preparer of objects, Mr. J. D. Möller, and others, have kindly agreed to mark the thickness of covers on their objects, so as to aid in the introduction of this system, and other preparers can no doubt be induced to do so if its advantages can be proved.

As regards the second point, many manufacturers now graduate their tubes, and modern requirements demand that this should be more generally done. Our company intends, as soon as it can possibly arrange to do so, to graduate the tubes of all its instruments.

As to the third and fourth conditions, I cannot, of course, presume to ask manufacturers to adapt their standards to this system. While it will be a convenience to a large number of microscopists, I must leave it to the merits that this system may possess, to exert their influence in this direction.

On the Amplifying Power of Objectives and Oculars in the Compound Microscope.*—Dr. G. E. Blackham writes:—"A great deal has been said and written on this subject, and still the matter is not as clear and accurate as could be desired.

The European opticians usually name their objectives and oculars in an arbitrary manner, as No. I., No. II., No. III., &c., or A, B, C, &c., but these designations give no clue to the amplifying powers, except that the lower numbers or earlier letters usually indicate the lower magnifying powers. The No. I. objective of one maker does not, however, necessarily correspond with the No. I. of another maker, and the No. I. objective does not necessarily correspond in amplifying power with the No. I. ocular of the same maker.

The English makers have attempted to avoid this confusion and, to introduce a degree of uniformity, have long adopted a system of nomenclature based upon the amplifying power; that is, if a combination of lenses magnifies equal to a single convex lens of one-inch focus, the combination is *called* a one-inch; if the same as a single lens of one-quarter inch focus, it is *called* a quarter-inch, &c., &c. This system has long been in use in England and this country for objectives, and more recently has been extended to oculars (or eye-pieces, as they are commonly called). This was supposed to give a very simple and accurate means for determining the power of any objective or ocular or combination of objective and ocular, provided only that they were correctly named by the maker and were used on a tube of the standard length. The rule commonly in use is based upon the assumption of the arbitrary distance of ten inches as the distance of distinct vision, and that the number of times the focal length is contained in ten inches is the amplifying power; so that a *one*-inch lens would magnify ten times, a one-fourth inch forty times, a one-tenth inch one hundred times, &c., &c. The image of the object projected by the objective being again magnified by the ocular, it was further assumed that the same rule would apply, and therefore that the amplification produced by the combination of a 1/10 objective with a one-inch ocular would be found by multiplying the assumed power of the 1/10 objective (100) by the assumed power of the one-inch ocular (10) = 1000 diameters. And so, by the application of this simple rule, every owner or user of objectives and oculars of the new nomenclature could calculate correctly the theoretical power of each and of any combination, with the understanding that, if the distance between the optical centre of the objective and that of the ocular varied, the amplifying power would vary in proportion.

The object of the present paper is, 1st, to show the incorrectness of this rule, in that the real image projected by a simple convex ten inches from its optical centre is *not* amplified the number of times the focal length is contained in ten inches, and that the same rule of amplification that is true and correct for the objective that projects a *real* image cannot be true and correct for the ocular, which projects a *virtual* image; and, 2nd, to present a correct method of determining the (linear) amplifying power of any objective or ocular, correctly named on the equivalent focal length system, and of any combination of such objective and ocular at any given distance between their optical centres.

* Proc. Amer. Soc. Microscopists, xi. (1889) pp. 22–31.

Now, in so far as the amplification of the image projected by the objective is concerned, the distance of distinct vision is of no consequence whatever, but the result is governed solely by the well-known optical law that, "The linear dimensions of object and image are directly as their distances from the optical centre of the lens." The correctness of this can be demonstrated by actual measurement, for the image is as real as the object, and its distance from the optical centre of the lens and its dimensions can as easily be measured.

Before proceeding to the further discussion of this subject it may be well to define some of the optical terms which I shall be obliged to use. I am quite aware that, for the majority of my hearers, this is a work of supererogation that almost savours of impertinence; but there are always beginners amongst us, and it is for their sakes that I insert these elementary definitions.

Definitions.—Optical Centre.—The point through which all rays traversing a lens with parallel directions at incidence and emergence must pass. In double convex or double concave lenses it lies in the interior of the lens; in plano-convex or plano-concave lenses it lies on the curved surface; while in a meniscus of either kind it lies outside the lens altogether.

Principal Axis.—The straight line passing through the centres of curvature of both faces of a double convex, a double concave, or a meniscus lens, or passing through the centre of curvature of the curved face and cutting at right angles the plane face of a plano-convex or a plano-concave lens, is called the Principal Axis; the optical centre is always in this line.

Secondary Axes.—All other straight lines passing through the optical centre are called "Secondary Axes."

Principal Focus.—The point at which rays originally parallel to the principal axis are made to converge (approximately) to one point.

Focal Length.—The distance from the optical centre to the principal focus.

Conjugate Foci.—Rays emerging from a point more distant than the principal focus on one side of a convex lens and passing through the lens will be brought to a focus at a point on the other side of the lens, and the points thus related are called conjugate foci.

As one conjugate focus advances from infinite distance (parallel rays) to the principal focus, the other recedes from the principal focus to infinite distance, the most distant focus always moving most rapidly, and the least distance between them is therefore attained when they are equidistant from the optical centre, in which case the distance of each from the optical centre is $2f$, and their distance from each other $4f$. If either is less than the principal focus, then the other becomes negative; that is, the rays are no longer brought to a focus on the *opposite* side of the lens, but are only rendered less divergent, as if coming from a more distant point on the *same* side, and this point from which they *appear* to come (the more distant of the two) is called the virtual conjugate focus. In this case, as one conjugate focus advances towards the optical centre, the other advances in the *same* direction till they become coincident.

Secondary principal and conjugate foci exist in each of the secondary

axes of a convex lens, and are under the same laws as the primary foci.

Each point in an object has its conjugate point in the image of it formed by a lens, and this image, if on the opposite side of the lens, is real and inverted; if on the same side, is virtual and erect. The linear dimensions of the object and image are directly as their distances from the optical centre of the lens; so that, if the object be nearer than the image, then the image is magnified, and *vice versa*.

Formulæ.—The formulæ for the determination of the conjugate foci when

f = principal focus (or focal length);
p = one conjugate focus;
p' = the other conjugate focus.

When the conjugate foci are on opposite sides of the lens (real image):

$$\frac{1}{p} + \frac{1}{p'} = \frac{1}{f}.$$

This formula suffices for the determination of either of the conjugate foci, the other conjugate focus and the focal length being given; or of the focal length, the two conjugate foci being given; and as it applies equally well to points in the secondary axes, it suffices equally to determine the distances of the object and image (and thence their relative linear dimensions), if one of these distances and the focal length of the lens be given.

When the conjugate foci are on the same side of the lens (virtual image) the formula becomes

$$\frac{1}{p} - \frac{1}{p'} = \frac{1}{f}.$$

The plus sign here becomes minus, or, to express it in other terms, as the two conjugate foci are now on the same side of the lens, it is the difference instead of the sum of their reciprocals that equals the reciprocal of the focal length. This formula is as applicable as that for real conjugate foci to the determination of the places, and therefore of the relative linear dimensions, of image and object; but, of course, the change of sign produces marked differences in the results when the given quantities are the same; that is to say, with a given focal length and image distance, the distance of the object, and therefore the ratio between its linear dimensions and those of the image, will vary according as the image is real or virtual.

In the compound Microscope we have to deal with both real and virtual images; the real produced by the objective, and the virtual by the ocular.

The real and inverted image produced by the objective becomes in its turn the object of which the ocular produces a virtual image; erect so far as it is concerned, but, of course, still inverted as regards the original object.

The degree of amplification of the real image produced by the objective depends upon two factors: 1st, the focal length of the objective, and, 2nd, the distance from its optical centre at which the image is

formed. It can be formed at any distance from the focal length of the objective up to infinity.

In most Microscopes of the English and American model the tube is of such length that the image is formed at a distance of about ten inches, and that distance is therefore taken as the basis of calculation, and the formula then is

$$\frac{1}{p}\ \frac{1}{\text{(object distance)}} + \frac{1}{p'}\ \frac{1}{\text{(image distance)}} = \frac{1}{f}\ \frac{1}{\text{(focal length)}};$$

or, substituting the image distance 10 for p':

$$\frac{1}{p} + \frac{1}{10} = \frac{1}{f}.$$

With this formula let us work out the case of a 5-in. objective; then

$$\frac{1}{p} + \frac{1}{10} = \frac{1}{5};$$

$$\frac{1}{p} = \frac{1}{5} - \frac{1}{10} = \frac{1}{10};$$

$$\frac{p}{1} = \frac{10}{1};$$

$$p = 10;$$

that is, the object and image are equidistant from the optical centre, and therefore of equal size, and there is no amplification. Of course, it can be assumed that the image distance p' is greater than 10 in., as in case the draw-tube is used, when the formula will show that, with a 5-in. objective, the image is larger than the object, or p' can be taken as less than 10 in., when the formula will show that, with a 5-in. objective, the image is less than the object.

Keeping 10 in. for our image distance, let us take the case of a 1/5 in. objective, then $f = \frac{1}{5}$.

$$\frac{1}{p} + \frac{1}{10} = \frac{1}{\frac{1}{5}}$$

$$\frac{1}{p} + \frac{1}{\frac{1}{5}} - \frac{1}{10} = \frac{50}{10} - \frac{1}{10} = \frac{49}{10}$$

$$p = \frac{10}{49}$$

$$a\ \text{(amplification)} = \frac{10}{\frac{10}{49}} = 49\ \text{times.}$$

By this formula we can easily calculate the amplification of the real image projected at 10 in. by any simple-convex lens, if the focal length

of the lens be known; and I present herewith a table so calculated for most of the focal lengths used for Microscope objectives. (Table A.)

It will be noted that the amplifications obtained are, in every case, less than those obtained by the number of times the focal length is contained in 10 in., and the reason is that one conjugate focus (the image distance) being at less than infinite distance, the other conjugate focus (the object distance) *must* be at a greater distance than the focal length, and therefore a quantity greater than the focal length must be used for the divisor, and the quotient (the amplification) must be less.

As a 1-in. simple-convex lens amplifies the image projected by it at 10 in. from its optical centre 9 times, a 1-in. objective should do the same (without reference to its actual focal length). If it fails to do so, if the image projected by it at 10 in. from its optical centre is amplified more or less than 9 times, then the objective has been incorrectly named; it is not a 1-in. objective, but something else.

The Ocular.—Having disposed of the real image projected by the objective, we come to the virtual image projected by the ocular; here the formula is

$$\frac{1}{p} - \frac{1}{p'} = \frac{1}{f};$$

substituting the image distance 10 for p' we have

$$\frac{1}{p} - \frac{1}{10} = \frac{1}{f}.$$

With this formula let us work out the case of a 5-in. ocular:

$$\frac{1}{p} - \frac{1}{10} = \frac{1}{5}$$

$$\frac{1}{p} = \frac{1}{5} + \frac{1}{10} = \frac{3}{10}$$

$$p = \frac{10}{3}$$

$$a = \frac{10}{\frac{10}{3}} = \frac{30}{10} = 3 \text{ times.}$$

The wide difference of this result from that obtained for a lens of the same focal length used as an objective shows very plainly the absurdity of using, as many of us have done, and as many of the books teach, the same general rule for determining the amplifying power of objective and ocular, viz. to divide 10 in. by the focal length expressed in inches.

I present herewith a table of amplifications of virtual images projected at 10 in. by simple lenses corresponding to the most commonly used oculars. (Table B.)

The total amplifying power of any combination of objective and ocular is obtained by multiplying the amplifying power of one by that of the other.

For instance, the total amplifying power of a Microscope with tube of standard length carrying a 1-in. objective and a 2-in. ocular should be $9 \times 6 = 54$, instead of $10 \times 5 = 50$, as per the usual rule.

It is to be noted, however, that while the formulæ here given are theoretically correct for the objective and ocular respectively, and are applicable to any image distances by substituting the desired image distance for p', as 10 was substituted for it in the examples given, yet there are many complications in the practical application of any formula to the determination of the actual amplification obtained by the modern compound Microscope; among these complications are,

1st. The highly complex construction of many objectives, making it very difficult to ascertain with any degree of accuracy the position of the optical centre, which difficulty is still further increased when the objective under consideration is furnished with a correction arrangement for various thicknesses of cover-glass, which, by varying the relative positions of its component lenses, varies its actual and nominal focal length and the position of its optical centre. The exact position of the optical centre of the ocular is also, at times, difficult of determination.

2nd. The refractive condition of the observer's eye is also a factor in the amplification under which the image is finally *seen*, for the reason that the dioptric system of the observing eye becomes, in fact, a part of the ocular, and any difference of its refractive power greater or less than that required to focus on the retina rays proceeding from a radiant situated at the given image distance, must be added to or subtracted from the refractive power of the ocular, and thus decrease or increase its focal length. That is to say, a person who can and does accommodate for precisely 10 in. while looking through the Microscope will, if all the other conditions are rigidly complied with, see the image under the exact amplification indicated by the formula, while one, who by reason of myopia or of excessive use of the muscle of accommodation accommodates for a less distance, will see it under a greater amplification, and the emmetrope or hyperope who relaxes his accommodation to less than that required to bring rays from a radiant at 10 in. to a focus on his retina, will see it under a less amplification than that indicated by the formula. For instance, let us take the case of the combination of 1-in. objective and 2-in. ocular for which we have found the total amplication, when image distances are taken as 10 in. in case of both objective and ocular, to be $9 \times 6 = 54$. If the observing eye be accommodated for just 10 in., the image will be seen clearly and under an amplification of $\times$ 54. If, however, the eye is accommodated for any other distance, then the image will not be clearly seen and a change must be made in the adjustment of the Microscope to make it clear. The reason is that the excess or defect of the refraction of the eye above or below what is required to accommodate it to 10 in. has, in effect, been added to or taken from the refractive power of the ocular.

Suppose an observer, as the result of myopia or from spasm of, or voluntary action of the muscle of accommodation, accommodates for a distance of 5 in. instead of 10 in.; he has, in effect, added to the refractive power of the ocular the refractive power of the lens which represents the difference between a refractive power of 10 in. and of

5 in. The refractive power of a lens is the reciprocal of its focal length. Hence the equation is

$$\frac{1}{5} - \frac{1}{10} = \frac{1}{10}.$$

Then refractive power of ocular + excess of ref. of eye = ref. power of eye and ocular taken as one, or

$$\frac{1}{2} + \frac{1}{10} = \frac{6}{10} = \frac{1}{1{\cdot}66}.$$

Hence the resulting amplification will be as if the ocular had a focal length of $1{\cdot}66$ instead of 2, and the formula will be

$$\frac{1}{p} - \frac{1}{10} = \frac{1}{1{\cdot}66}$$

$$\frac{1}{p} = \frac{1}{1{\cdot}66} + \frac{1}{10} = \frac{11{\cdot}66}{16{\cdot}6}$$

$$p = \frac{16{\cdot}6}{11{\cdot}66}$$

$$a = \frac{10}{\frac{16{\cdot}6}{11{\cdot}66}} = \frac{1166}{16{\cdot}6} = 7.$$

Hence for the observer whose eye is accommodated for 5 in., the expression for total amplification of the 1-in. objective and the 2-in. ocular will be $9 \times 7 = 63$.

On the other hand, let us take a case much more unlikely to occur in practice, that of a person who by reason of excessive hypermetropia or paralysis of accommodation, is unable to focus any but parallel rays upon his retina, or, in other words, to accommodate for any point nearer than infinite distance. If such an observer make use of the same combination of objective, he has in effect subtracted from the refractive power of the ocular the refractive power of the lens, which represents the difference between a refractive power 0 and of 10 in. = 1/10; then

$$\frac{1}{2} - \frac{1}{10} = \frac{4}{10} = \frac{1}{2{\cdot}5}.$$

Hence the resulting amplification will be as if the ocular had a focal length of $2{\cdot}5$ instead of 2, and the formula will be

$$\frac{1}{p} - \frac{1}{10} = \frac{1}{2{\cdot}5}$$

$$\frac{1}{p} = \frac{10}{25} + \frac{1}{10} = \frac{125}{250} = \frac{1}{2}$$

$$p = 2$$

$$a = \frac{10}{2} = 5.$$

Hence, for the emmetropic observer whose accommodation is entirely relaxed, or for any observer whose eye is accommodated for parallel rays, the total amplification of the 1-in. objective and the 2-in. ocular will be $9 \times 5 = 45$.

For strict accuracy, the change in the position of the image produced by the objective, if the adjustment for the different eyes be produced in the usual way by means of the fine or coarse adjustment moving the objective to or from the object, should be taken into consideration, but the amount is so small, about $2\frac{1}{2}$ per cent. in the first case, and 5 per cent. in the second, that it may be neglected without seriously impairing the practical accuracy of the general result, while if the adjustment for different eyes be made with the draw-tube moving the ocular only, the position of the image produced by the objective is not changed, and therefore, so far as it is concerned, the original formula remains strictly correct."

"TABLE A.

"Amplification (linear) of Real Images projected at 10 in. from optical centre by simple bi-convex lenses.

Focal Length of Lens in inches.	Linear Amplification of Image.	Focal Length of Lens in inches.	Linear Amplification of Image.
5	1	1/4	39
4	1·5	1/5	49
3	2·33	1/6	59
2	4	1/7	69
1	9	1/8	79
3/4	12·33	1/9	89
2/3	14	1/10	99
1/2	19	1/12	119
4/10	24	1/16	159
1/3	29	1/25	249

TABLE B.

"Amplification (linear) of Virtual Images projected at 10 in. from optical centre by simple bi-convex lenses.

Focal Length of Lens in inches.	Linear Amplification of Image.	Focal Length of Lens in inches.	Linear Amplification of Image.
5	3	3/4	14
4	3·5	1/2	21
3	4·33	4/10	26
2	6	1/3	31
$1\frac{1}{2}$	7·73	1/4	41
1	11		

"NOTE.—In the Huyghenian ocular (the form most commonly in use) the field-lens, while mechanically part of the ocular, is optically part of the objective, in that it contributes to the formation, not of the virtual image projected by the ocular, but of the real image projected by the objective, upon which it acts negatively, diminishing its size while increasing the superficial area brought into view at one time. So that, in this form of ocular it is the eye-lens alone that contributes to the reamplification of the image, but the negative action of the field-lens must, of course, always be taken into consideration when attempting to determine the amplifying power of a Huyghenian ocular by calculation."

New Method for Constructing and Calculating the Place, Position, and Size of Images formed by Lenses or Compound Optical Systems.* —The late Prof. G. Govi wrote:—"The theory of lenses and of compound systems has taken a new form, and reached far greater perfection since Moebius, Gauss, Listing, and others have introduced the consideration of certain planes and cardinal points, which simplify the construction of the place, position, and size of the images, allowing account to be taken of the thickness of the refractive medium traversed by the light. But the preparatory operations, either as constructions or calculations, by which we succeed in determining the place of the points and cardinal planes in lenses or systems, are long and wearisome, and often out of proportion to the importance of the result we hope to obtain; and, above all, it is always most difficult to determine by experiment the place of these planes and points in lenses already worked or in optical systems already constructed.

Physicists, therefore, in spite of the practical methods and instruments proposed for the purpose by Cornu, Gariel, and others, are for the most part limited to considering the lens as having no thickness, and to calculate directly and for every limiting surface the path taken by the rays in traversing the given media, thus sacrificing a part (and at times not a small one) of the necessary precision, or increasing the fatigue of the calculations when many determinations of the same optical system are in question.

The suggestion, therefore, of a quicker method for constructing and calculating the images given by thick lenses will not be unwelcome to students, the same method being also applicable to any optical system whatever.

This method requires the determination of two points which, very probably, have not until now been taken into consideration by physicists or mathematicians who have treated of these matters; probably they passed them unawares, because if any one had pointed out their importance and usefulness they would at once have been recognized, and the very latest treatises on optics would have recalled them.

The two new points, by which the theory of lenses is very much simplified, and which are easily determined by observation, are the images of the centres of curvature of the two faces, anterior and posterior, of the lens, seen through that one of the two faces to which they do not belong. In order to obtain them it is necessary to suppose that the luminous rays diverging from the centre of curvature of one face, or converging towards it, meet the second face of the lens where by refraction they are made to converge towards the image of this same centre, or diverge from that image when it becomes virtual. We thus have on the axis of the lens the places of the two images q and q_1, (fig. 9), of the centres c and c_1, and of the curvature of the two faces al and bl_1.

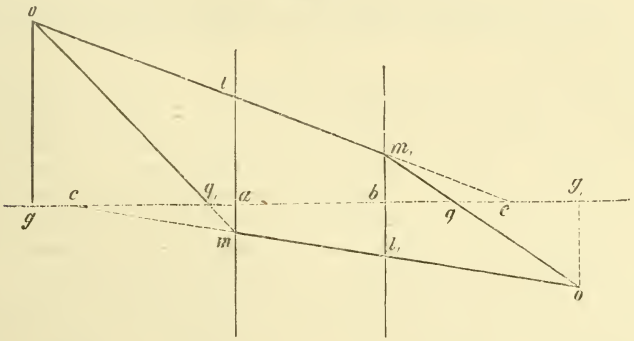
Having fixed the position of these two points, which we may call the centric points of the given lenticular system, nothing else is needed to determine any conjugate focus of a point situated upon the axis or outside the principal axis of the system, and to obtain the size and position

* Rend. R. Accad. Lincei, iv. (1888) pp. 655–60 (3 figs.).

of the real or virtual images which may be produced by the system itself.

The determination *à priori* of these points (like the determination of the points and planes of Gauss and Listing), demands the knowledge of the length and sign of the radii of curvature of the two surfaces of the lens, that of the thickness of the lens, or the axial distance of the two refracting surfaces, and finally that of the relative velocity of light in the three successive media—that is to say, of their relative indices of refraction. We can with these data alone construct or calculate the

Fig. 9.



place of the centric points q and q_1, without first determining the principal foci and the principal distances or anterior foci of the two surfaces of the lens; we can also, if we wish, determine these quantities which, introduced in successive calculations or in ulterior constructions, abbreviate or simplify the work.

In any case, having obtained the two centric points, we have no further need either of the optic centre, or of its two images, or the nodal points of Listing, or Gauss' principal planes, or the principal foci of the whole lens, to construct or calculate the places, positions, and size of the images. And as such constructions are made very quickly, we may use them in order to find the final effect of any series whatever of surfaces and of different refracting media, centered on the same axis.

It is not, therefore, necessary in the case of optic systems to have recourse to the laborious process of construction or calculation by means of successive images, for there can always be determined in every optic system (however complex) the images of the centres of curvature of its first and of its last surface, seen successively through the whole of the rest of the system, observing the image of the centre of the first surface through the second, then the image of this image through the third, and so on to the image of all the preceding images, seen through the last surface, and repeating the same operation in the opposite direction for the centre of the last surface and for its successive images up to the last, which is seen through the first surface. In this way the centric points of the whole system are obtained, by means of which we

can construct afterwards or can calculate with great rapidity the image of any point placed at any distance whatever from the system.

The greater simplicity of the new method arises from considering those rays which undergo neither deviation nor displacement either at the entrance into or exit from the different media, so that the faces of the lens or the external surfaces of the system perform the function of the principal planes of Gauss, the centres of curvature of these surfaces that of the nodal points of Listing, and their images or centric points that of the principal foci of the optic system.

Without now entering into minute details of the new method, it will be sufficient to show how, by having recourse to it, we can easily find the centric points of a given lens, and how, once these points are found, we can easily construct the image of any object seen through the lens. We shall thus see whether the proposed method deserves or not to be preferred to others.

In order to find practically the position of the centric points of a given lens, we measure its thickness γ, and with the spherometer, or by reflection or otherwise, the radii of curvature r and r_1 of its first and second surfaces. Having obtained these quantities we place normally to the axis of the lens an object of a known size $o\,g$, at a determinate distance $a\,g$ from one of the faces, and we find the image $o_1\,g_1$ either real or virtual of the object, seen through the lens, measuring this image, and determining its distance $b\,g$ from the other surface.

Then by drawing a straight line from the extremity o of the object to the centre c of curvature of the first face of the lens, this straight line will cut the last face in a certain point m_1; by drawing a straight line from the extremity o_1 of the image to the centre of curvature c_1 of the last face, we mark by m the point in which this straight line cuts the first face of the lens. Join o_1 to m_1, the point q, in which the straight line $o_1\,m_1$ cuts the axis of the lens, will be the first centric point, that is, the place of the image of the centre c of the first face seen through the second. Let o be similarly joined to m, the point g_1, in which the line $o\,m$ cuts the axis, will be the second centric point, that is, the image of the centre c_1 of the second face seen through the first. Having thus obtained the points q and q_1, the construction of the principal or conjugate foci of the system and that of all the images which it can give, can be made exceedingly rapidly, and we can then deduce very easily the places of the principal planes, the nodal points, the optic centre, &c., if we wish to treat the problems relating to the given lens by the methods of Gauss, Listing, or other mathematicians.

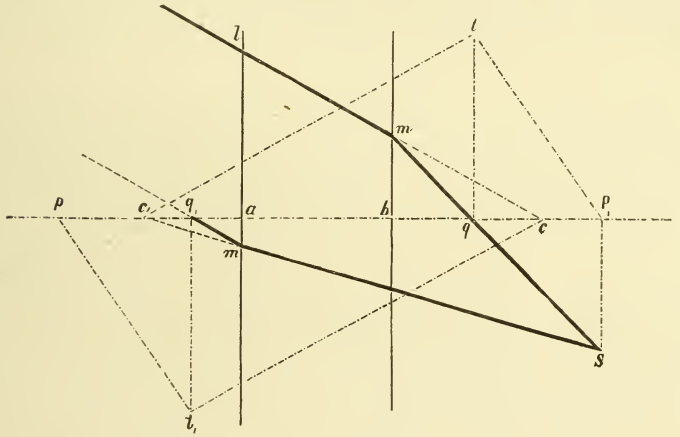
The preceding diagram shows at once how we may obtain the image of a point o placed outside the axis of the lens. (If the point given were on the axis, we might raise from it a perpendicular to the axis, and determine the image of any point on this perpendicular, drawing from the image obtained a normal to the axis itself. The meeting point of this normal and the axis would be the place of the image of the given point.) Let a straight line be drawn from the point o to the centre c of the face through which it is intended the light should pass; such a straight line will represent a luminous ray, which starting from o will pass, neither deviating nor displaced through the lens, until it meets in m, the second face. The ray having reached m, will deviate towards the

point q, the image of c; draw the line $m_1\ q$ on which prolonged will be found the image of o. From the point o draw through q_1 the line $o\ q$, until it meets the first face of the lens in m. Through m and c_1 draw the line $c_1\ m$, which prolonged will pass without deviation out of the lens, and will meet $m_1\ q$ in a point o_1; the point o_1 will be the image of o.

If from the point o the perpendicular $o\ g$ be let fall on the axis, and from o_1 the line $o_1\ g_1$, the point g_1 will be the place of the image of the point g seen through the lens.

In order to obtain the principal foci of a given lens, draw a radius $l\ c$ (fig. 10) to the centre of its first face, and draw its corresponding refracted ray $m_1\ q$, then through the point q_1 draw $q_1\ m$ parallel to $l\ c$, drawing $m\ c_1$ and prolonging it till it meets $m_1\ q_2$ prolonged in S.

FIG. 10.



The point S will be the image of a point situated at an infinite distance in the direction of $c\ m\ l$. Drawing from S a normal to the axis we obtain in p_1 a principal focus of the lens. The same construction repeated for the other face will give the second principal focus p, or the point of the principal distance of the lens.

We can obtain the second focus more quickly when once the first is known, profiting by a very simple relation which exists between the two distances $q\ p_1$ and $q_1\ p$ of the two principal foci from the centric points.

Representing by r the radius of curvature $a\ c_1$ of the first face of the lens l_1, by r the radius $b\ c_1$ of the other face, by x the distance $b\ q$ of the centric point q from the second face of the lens, by x_1 the distance $a\ q_1$ of q_1 from the first face, and denoting by F the distance $q\ p_1$ and by F_1 the line $q_1\ p$ we readily obtain the following relation :—

$$\frac{F - r_1 + x}{F_1 - r + x_1},$$

which gives directly F, if we know F_1, or F_1 when F is known.

The construction of this formula is very simple. From the points q and q_1 let two normals be drawn to the axis; through the centre c draw $c\ t_1$ until it meets in t_1 the normal passing through q_1; let $c_1\ t$ be drawn through the centre c_1 parallel to it, until it meets in t, the other normal $q\ t$. Having then joined the principal focus p (which we suppose to be known) with t, let a parallel to $p\ t$ be drawn through t_1; the point p_1, where it cuts the axis, will be the other focus, or the principal distance of the lens.

The same graphic process, and therefore the formulas derived from it, are very easily applied also to optic systems composed of lenses without thickness. In this case we first determine the successive images of the centre of the first and of the last lens seen through all the others; then, considering the centres of the lenses as we just before considered the centres of curvature (since we suppose the rays to pass through these centres without deviation and without displacement) we make relatively to them and to their images the constructions already indicated, and so we solve with rapidity all problems relating to optic instruments composed of thin lenses."

(6) Miscellaneous.

"**New Inventions.**"—"Her Majesty's Royal Letters Patent have been granted to the inventor of a wonderful as well as useful little appliance. This is a Pocket Microscope and Floriscope combined, about 3 in. in length and $1\frac{1}{4}$ in. square. It is constructed upon an entirely new principle, and has a magnifying power and definement superior to some of the most elaborate and expensive instruments, and yet so simple that any schoolboy or girl can use it. Its magnifying power is registered as 150 diameters, or 22,500 surfaces, and distinctly shows all the thousands of different kinds of animalcula in water, &c., or any other microscopic objects. This new patent was sealed by the Comptroller-General of Patents on the 13th of August last, and is now offered to the public at the nominal price of 1*s.* each, and sent free by parcel post upon receipt of postal order value 1*s.*—stamps not taken—with a 12-page pamphlet of instructions for use, and a large double sheet of engravings in black and gold (with key) free. The inventors and manufacturers also guarantee that, in any case where the instrument is not approved of and returned within reasonable time, a postal order value 1*s.* will be forwarded by return of post. The medical profession, chemists, schoolmasters, teachers and students, as well as parents and guardians, should send for one on approval. This is no foreign rubbish, but of good English workmanship throughout. Address, Conway Rae & Company, The Premier Patent Microscope Depôt, Stafford Street, Birmingham."

Upon reading the above advertisement in 'Nature' * we applied for one of the Microscopes, and were informed that for an additional remittance of 6*d.* we should receive an instrument of superior make, giving "better definement," with four extra "object-glasses," and a larger pamphlet of instructions. As we were desirous of comparing the two qualities of Microscopes, we requested both to be forwarded.

The lower priced one consists of a tin tube of square section, having a tin diaphragm with square aperture in the middle. At one end is

* October 11th, 1890.

another similar diaphragm of stamped brass fitting after the manner of a cap, but with internal flange; a similar cap, but with deeper flange, is applied at the other end, and this has a circular hole in the centre, against which a blown-glass spherical lens of about 1/4 in. diameter is pressed on the inner side by a tin plate with corresponding central hole. The object is placed between two square plates of glass and thrust up against the lens, a tin diaphragm follows, and these are held in position by a roughly bent piece of tin serving as a spring. The ends of the caps are stamped with an inscription and lacquered; the tin tube is also lacquered.

The higher priced Microscope differs from the other, (1) in having the tin tube coloured in addition to being lacquered; (2) it has four extra pairs of glass plates termed "object-glasses"; and (3) a fuller pamphlet accompanies it.

Whilst wholly disclaiming any desire to depreciate the quality of these Microscopes, we are compelled to state that the whole manufacture suggests that of common toys of tin. And as it would be obviously unfair to compare their optical quality with that of more expensive instruments, we have compared it with a Stanhope lens, such as is commonly sold in the London streets at the price of 1*d.* each, including wire and tin mounting and a pair of glass plates for clipping objects, and our impression is that the latter is not inferior.

The late Mr. Brady, Hon. F.R.M.S.*—We give, almost verbatim, a copy of the best of the notices we have seen of our deceased Fellow. As it is from the pen of Prof. M. Foster, Sec. R.S., it is written by one who knew him well.

Henry Bowman Brady was born on February 23rd, 1835, at Gateshead. His father, an esteemed medical practitioner of that place, belonged to the Society of Friends, and retained to the end the dress and manner of conversation of that body. The father's house, for many years the home of the son, was one of those charming Quaker abodes where strength and quietude sit side by side, and where homely plenty and orderly preciseness hide, for a moment, from the stranger the intellectual activity which is filling the place. Though the son, when I knew him, had abandoned the characteristic dress and speech of the society, without, however, withdrawing from the body, the influences of his surroundings moulded his character, making him singularly straightforward and free from any manner of guile.

After an ordinary school career spent in Yorkshire and Lancashire, and an apprenticeship under the late Mr. T. Harvey, of Leeds, and some further study at Newcastle in the laboratory of Dr. T. Richardson, which may be considered as the forerunner of the present Newcastle College of Science, he started in business in that city as a pharmaceutical chemist in 1855, while yet a minor. That business he conducted with such ability that in 1876 he felt able to resign it to Mr. N. H. Martin, and to devote the whole of his time to scientific work. He contributed to science in two ways—one direct, the other indirect. Of the many scientific movements of the last thirty years or so, one, not of the least remarkable, has been the scientific development of the pharmaceutical

* Nature, xliii. (1891) p. 299.

chemist. Into that movement Brady threw himself with great vigour, especially in his earlier years. He was for many years on the Council of the Pharmaceutical Society, and the progress of that body was greatly helped by his wide knowledge of science and of scientific men and things, as well as by his calm and unprejudiced judgment.

His more direct contributions to science were in form of researches in natural history, more especially on the Foraminifera. His first publication seems to have been a contribution, in 1863, to the British Association as a report on the dredging of the Northumberland coast and Dogger Bank; his last was a paper which appeared in the October number of our Journal. Between these two he published a large number of researches, including a monograph on Carboniferous and Permian Foraminifera, an exhaustive report on the Foraminifera of the 'Challenger' expedition, as well as monographs on *Parkeria*, *Loftusia*, and *Polymorphina*, in which he was joint author.

By these works he not only established a position, both in this country and abroad, as one of the highest authorities on the subject, but, what is of more importance, largely advanced our knowledge. Every one of his papers is characterized by the most conscientious accuracy and justice; and though his attention was largely directed to classification, and to the morphological points therein involved, his mind, as several of his papers indicate, was also occupied with the wider problems of morphological and biological interest which the study of these lowly forms suggests. I have myself often profited by his wide knowledge and power of accurate observation in discussing with him questions of this kind arising out of his studies, and learning from him views and opinions which, to his critical mind, were not as yet ripe enough for publication.

The leisure of the last fifteen years gave him opportunity for travel, and he visited various parts of the world, utilizing many of his journeys —notably one to the Pacific Ocean—in the collection and study of Foraminifera. Some of these travels were undertaken on the score of health, to avoid the evils of an English winter, for he was during many years subject to chronic pulmonary mischief.

During his last journey for this purpose—one to the Nile in the winter of 1889-90—he met with difficulties, and failed to receive the benefit from the change which he had secured on former occasions. During the last two or three years, and especially during the last year, his condition gave increasing anxiety to his friends; the malady against which he had so long struggled seemed to be beating him at last; and we heard with sorrow rather than with surprise that the fierceness of the recent cold had conquered him. Settled for the winter at Bournemouth, and full of cheerful hopes for the coming summer, he succumbed to a sudden attack of inflammation of the lungs, and died on January 10th, 1891.

Science has lost a steady and fruitful worker, and many men of science have lost a friend and a helpmate whose place they feel no one else can fill. His wide knowledge of many branches of scientific inquiry, and his large acquaintance with scientific men, made the hours spent with him always profitable; his sympathy with art and literature, and that special knowledge of men and things which belongs only to the travelled man, made him welcome where science was unknown; while the brave patience with which he bore the many troubles of enfeebled health, his

unselfish thoughtfulness for interests other than his own, and a sense of humour which, when needed, led him to desert his usual staid demeanour for the merriment of the moment, endeared him to all his friends.

Angling and Microscopy.—A "microscopical evening" could, we should have thought, hardly be looked for at an angling society, but the following appears in 'Flood and Field' of the 29th November, 1890:—

"Gresham Angling Society.—There was a good attendance again on Tuesday, with Mr. Vail in the chair. This being a 'Microscopical Evening,' Dr. Brunton and Messrs. Norman, Parker, and Bentley showed a number of interesting subjects. Among other objects, Dr. Brunton exhibited a hank of so-called *silk*, sold by City houses for fly-tying, &c. Under the Microscope this proved to be nothing but *jute*, a fact which explains the frequent breaking away of large fish, and the consequent loss of tackle, temper, &c."

The Microscope and the McKinley Tariff.—Among numerous examples of the mischievous working of the McKinley tariff, the New York 'Nation' cites the instance of Microscopes. Since the branch of medical science known as bacteriology assumed so much prominence, these articles have risen in the United States from the rank of a toy to that of the most valuable and important of all medical instruments. Meanwhile a foolish legislature has been doing its best to make Microscopes artificially dear, and more and more difficult to procure. It was bad enough before the new tariff; but it is now worse. In spite of the touching appeals of eminent medical men, a Microscope which could be bought in Germany for 94 dollars now costs in America over 150 dollars. This is but one of many examples given of how the tariff is felt to be affecting the vital interests of the American people.

β. Technique.*

(1) Collecting Objects, including Culture Processes.

Experiments on Cultivation Media for Infusoria and Bacteria.†—In his experiments with anthrax, Dr. Hafkine obtained varying results; thus when cultivated in the aqueous humour of rabbits, guinea-pigs, or dogs, sometimes copious development occurred, but sometimes it altogether failed. When sown with typhoid bacillus the inhibitive action of the humour was very manifest, reducing the number of viable bacilli from 1880 to 7 in four hours. This result is explained by the author on the supposition that the bacilli, which had been cultivated for a long time in pepton bouillon, had not yet become acclimatized to the new medium. For by gradually adding an increasing amount of aqueous humour to the pepton bouillon, in twelve successive generations a strong increase in fresh humour was eventually obtained, indeed it was greater than in the bouillon. Control experiments made with bacilli obtained directly from a typhoid patient, behaved in a manner analogous to the artificial

* 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.

† Annales de l'Institut Pasteur, iv. (1890) p. 363. See Centralbl. f. Bakteriol. u. Parasitenk., viii. (1890) p. 435.

cultivations in aqueous humour. The germicidal action of the humor aqueus is explained conformably to the ideas of Metschnikoff, with whom the author is working, as being entirely due to an imperfect adaptation to the new medium.

Silicic Acid as a Basis for Nutrient Media.*—Prof. W. Kühne employs silicic acid as a basis for nutritive media which will bear prolonged exposure to high temperatures, and which have the further advantages of resisting the action of organisms and reagents. To make the compound, the author mixes, with frequent shaking, three parts of commercial silicate of soda (sp. gr. 1·08) and one part dilute hydrochloric acid (HCl sp. gr. 1·17 one part, and water two parts). The mixture is then freed, in a dialyser, from free acid and from sodium chloride, by suspending the dialyser for four days in a stream of running water. The pure solution is then condensed to a specific gravity of 1·02 by heating it in a platinum vessel. In this condition it contains 3·4 per cent. pure acid, is as thin as water, can be boiled, is miscible with alcohol, and only coagulates on addition of neutral salts. The nutrient addendum employed by the author was meat-extract: a piece of Liebig's extract about the size of a bean is dissolved in 22 ccm. of water, and of this solution 0·5 to 1 ccm. is added to 4 ccm. of silicic acid. If it be desired to set it quickly some cooking salt must be added. Thus obtained the jelly is of the proper consistence, transparent as glass, and scarcely coloured by the meat-extract. It bears the addition of sugar, glycerin, &c.

Pure Cultivations of Green Unicellular Algæ.†—M. W. Beyerinck has obtained pure cultivations from two species, *Chlorococcum protogenitum* Rabenh. and *Rhaphidium naviculare* sp. n., which are frequent in stagnating water near Delft. The author succeeded in getting rid of the numerous water bacteria by the following method:—Ditch water was boiled up with 10 per cent. gelatin, and before setting was mixed with a drop of the water coloured green by the algæ. In this mixture only those bacteria which liquefy gelatin could develope. The number of such colonies may be few enough not to liquefy the whole of the gelatin in two or three weeks. With a hand-lens the algar colonies may then be recognized as dark green points. These can then be distributed to fresh gelatin and so pure cultivations obtained. *Rhaphidium* was found to excrete a trypsinoid ferment which liquefied gelatin. It multiplied by fission. *Chlorococcum* does not liquefy gelatin, and was cultivated on seven different nutritive media with a neutral or slightly acid reaction. Development in all the seven media proceeded at about the same pace, but the colour of stroke cultivations was very different.

In sterilized ditch water with 1 per cent. gelatin previously liquefied by pancreas, the growth advances well, and after three or four weeks there results a yellow fluid with a dark green sediment of *Chlorococcum*.

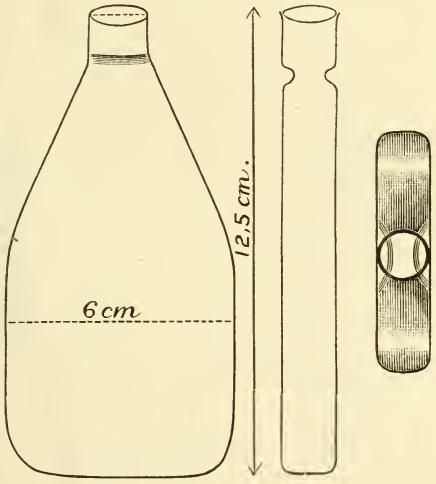
* Zeitschr. f. Biologie, xxvii. n.s. ix. (1890) No. 1. Cf. Centralbl. f. Bakteriol. u. Parasitenk., viii. (1890) pp. 410–11.

† 'Aanteekeningen van het verhandelde in de sectie-vergaderingen van het Provinciaal Utrechtsch Genootschap voor kunsten en wetenschappen gehouden den 25 Juni 1889,' pp. 35–52. Cf. Centralbl. f. Bakteriol. u. Parasitenk., viii. (1890) pp. 460–2.

By mixing this sediment with liquefied gelatin, and pouring it into test-tubes, or flattening it out between two glass plates, an equally coloured green cast or plate is obtained which serves excellently for studying the action of light on chlorophyll and the excretion of oxygen.

Flat Flask for cultivating Micro-organisms.*—Dr. J. Petruschky has devised a convenient apparatus for cultivating micro-organisms on the plate or surface principle. It is merely a flat flask, and is made in two shapes. Shape A is made of thin lamp-glass, and the B shape of thick or plate glass. Both have pretty much the same form; that is, they are flat and somewhat triangular, or rather like a flat worm. Their general aspect is seen from the illustrations, which give a front and side view, and also the view down the neck when looked at from above. There is a slight difference in the measurements; those of the A pattern being, height 10–11 cm., breadth 5½–6 cm., width (same measurement as neck) about 1½ cm. In the neck there is a circular indentation. The measurements of the B pattern are, height 12·5 cm., breadth 6 cm., width (same as neck) 2 cm. In this pattern the indentation is confined to the broad side of the neck.

Fig. 11.



The A pattern is more suitable for delicate work, such as the differentiation of typhoid colonies, while the B form suffices for isolation, enumeration, and inoculation purposes.

These flat flasks are specially adapted for the bacteriological examination of water, and for the cultivation of anaerobic microbes in hydrogen.

Apparatus for filtering perfectly clear Agar.†—Dr. J. Karlinski has invented an apparatus for filtering agar, and though it agrees with that devised by Jakobi, differs from the latter in that its intention is, besides obtaining a perfectly clear solution, to prevent the too quick cooling and setting of the medium.

The apparatus, seen in section, fig. 12, consists of a tin vessel *a*, the upper end of which is closed with a perforated caoutchouc plug, and its bottom ends in a tube fitted with a stopcock.

The vessel *a* is surrounded by the vessel *b*, made of similar material, and from near the bottom passes out a short closed pipe. The space *b*

* Centralbl. f. Bakteriol. u. Parasitenk., viii. (1890) pp. 609–14 (3 figs.).
† T. c., pp. 643–5 (2 figs.).

is intended to contain hot water, which is heated by means of a spirit-lamp. In the vessel *a* is placed a layer of cotton-wool 10 cm. thick, and this is, before using, damped with hot water.

The agar solution, made according to Jakobi's formula, is then poured into *a*, and the aperture closed with the caoutchouc plug, to which is attached the hand-bellows. The agar solution is thus made

Fig. 12.

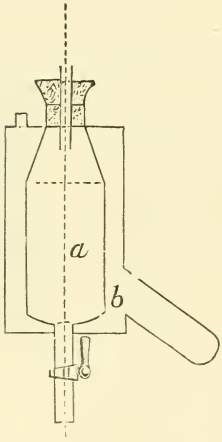
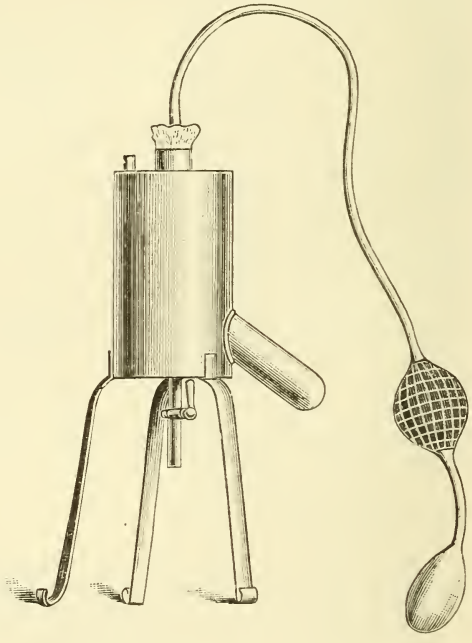


Fig. 13.



to run out through the pipe at the bottom by means of compressed air, and is allowed to flow into sterilized vessels. The hot-water jacket prevents the agar from cooling too quickly, so that many test-tubes may be filled very easily. Fig. 13 gives an outside view of the whole apparatus.

Pure Cultivations of Gonococcus.*—Herren H. von Schrötter and F. Winkler recommend the albumen of plover's eggs as an excellent nutritive medium for easily obtaining pure cultivations of Neisser's gonococcus. The medium is prepared after the method of Dal Pozzo.†

* Mittheil. aus d. Wiener Embryol. Institut, 1890, pp. 29–34.

† See this Journal, 1888, p. 1037.

(2) Preparing Objects.

Methods for the Preservation of Marine Organisms employed at the Naples Zoological Station.*—Prof. Playfair McMurrich writes, "Unfortunately for our students, especially those living inland and depending largely for their knowledge of marine forms upon dried or preserved specimens in museums, the old-fashioned methods of throwing any material which the collector may find into a jar of alcohol without further attention, or else drying it in the sun, are still almost the only ones made use of for the preservation of museum specimens. The result is that the majority of forms which the student has for study are either dried skeletons, or shrivelled up monstrosities giving no idea whatever of the actual appearance of the creatures supposed to be represented by them. How many college museums possess a specimen of coral showing in any recognizable form the polyps by which the skeleton coral was formed? Or how many have even a satisfactorily prepared Lamellibranch?

There are, however, in this country, a few collections which show a marvellous improvement in their manner of preparation, and which have been purchased from the Naples Zoological Station, whose conservator, Salvator Lo Bianco, has for several years been devoting himself to the discovery of the best methods for the preservation of the form and colour of the marine animals occurring in the Mediterranean. Until the present, however, his discoveries have not been made common property, except in the few cases where most successful methods for preserving certain forms have been published in connection with accounts of their structure. The last number of the Naples 'Mittheilungen,'† however, contains a full description, by Lo Bianco, of the methods found most successful for the preservation of the various forms which occur at Naples, and which are undoubtedly applicable to the similar forms found upon our own coast. An abstract of these methods is given in the following pages, in the hope that they may be found useful by the museum curators of this country, and that their application may result in the much-needed improvement of the appearance of the specimens found in the majority of the college museums.

It must be fully understood, however, that much depends upon the skill of the preparator, and that want of care and patience will frequently counteract all the advantages to be derived from a good method. All who have had the opportunity of examining specimens prepared by Lo Bianco can appreciate readily the great advantages which may result from the careful application of his methods, and can perceive how greatly we are indebted to him and to Prof. Dohrn for their publication.

Alcohol is, of course, indispensable as preservative fluid, but certain precautions are necessary in its use. Except in a very few cases it is unnecessary to use it in its full strength, 70 per cent. being quite sufficient for preservation, and producing much less contraction and fragility in delicate organisms. Strong alcohol should be reduced with distilled water to the desired strength, ordinary spring water frequently contain-

* Amer. Natural., xxiv. (1890) pp. 856–65.

† See Mittheil. Zool. Stat. Neapel, ix. (1890) pp. 435–74.

ing a sufficient amount of carbonate of lime and other substances in solution to give a cloudy precipitate, after a time, which may effectually destroy the appearance of a specimen.

Furthermore, delicate organisms should first be placed in weak alcohol (35 to 50 per cent.) for from two to six hours, the changing of the fluids being effected by a siphon, a small quantity of the weak alcohol being withdrawn and stronger added, until finally the desired strength is obtained. With delicate gelatinous structures the increase in the strength of the alcohol should be as gradual as possible. In many cases it is necessary to use a hardening or fixing reagent before the final consignment to alcohol, which is principally useful as a preservative. The most fixing reagents, according to Lo Bianco, are the following :—

Chromic acid.—1 per cent. in fresh water. Objects should not remain in the fluid longer than is necessary to fix them, as they are apt to become brittle. Subsequently they should be well washed with distilled water to prevent the formation of a precipitate when placed in alcohol, and also to prevent their taking on too green a tinge from the reduction of the acid.

Acetic acid, concentrated, kills rapidly contractile animals, but must be used with caution, as it produces a softening of the tissues if they are subjected for too long a time to its action.

Osmic acid, 1 per cent. solution, hardens gelatinous forms well, and preserves their transparency, but its prolonged action renders the objects brittle and gives a dark brown tint. Objects hardened in it should be well washed in distillled water before being placed in alcohol.

Lactic acid.—1 part to 1000 parts sea-water fixes larvæ and gelatinous forms well.

Corrosive sublimate.—Saturated solution in fresh or sea-water; may be used either hot or cold. It acts quickly, and preserves admirably for histological purposes. It is especially good combined with copper sulphate, acetic acid, or chromic acid. Objects hardened in it should be subsequently well washed in distilled water and in iodized alcohol (the recipe for which is given below), to remove all traces of the sublimate, which in alcohol crystallizes out in the tissues of the organisms and so injures the preparation.

Bichromate of potassium.—5 per cent. solution in distilled water hardens gelatinous organisms slowly, without rendering them fragile. It gives, however, a precipitate in alcohol, and discolours the specimen. The discoloration, however, may be removed by adding to the alcohol a few drops of concentrated sulphuric acid.

Copper sulphate.—5 per cent, or 10 per cent. solution in distilled water, used either alone or in combination with corrosive sublimate, kills larvæ and delicate animals without distortion. The objects should be subsequently repeatedly washed with water to remove all traces of the salt, otherwise crystals will form when the object is placed in alcohol.

Various combinations of these reagents are especially useful, and some of those most serviceable are given here :—

Alcohol and chromic acid.—70 per cent. alcohol, 1 per cent. chromic acid, equal parts.

Alcohol and hydrochloric acid.—50 per cent. alcohol, 100 ccm.; Hydrochloric acid, concentrated, 5 ccm.

Iodized alcohol.—35 per cent. or 70 per cent. alcohol, 100 ccm.; Tincture of iodine, 2·5 ccm.

Chrom-acetic acid, No. 1.—1 per cent. chromic acid, 100 ccm.; Concentrated acetic acid, 5 ccm.

Chrom-acetic acid, No. 2.—Concentrated acetic acid, 100 ccm.; 1 per cent. chromic acid, 10 ccm.

Chrom-osmic acid.—1 per cent. chromic acid, 100 ccm.; 1 per cent. osmic acid, 2 ccm.

Chrom-picric acid.—1 per cent. chromic acid, Kleinenberg's picro-sulphuric acid, equal parts.

Copper sulphate and corrosive sublimate.—10 per cent. solution of copper sulphate, 100 ccm.; saturated solution of corrosive sublimate, 10 ccm.

Potassium bichromate and osmic acid.—5 per cent. solution of potassium bichromate, 100 ccm.; 1 per cent. osmic acid, 2 ccm.

Corrosive sublimate and acetic acid.—Saturated solution of corrosive sublimate, 100 ccm.; concentrated acetic acid, 50 ccm.

Corrosive sublimate and chromic acid.—Saturated solution of chromic sublimate, 100 ccm.; 1 per cent. chromic acid, 50 ccm.

Frequently great difficulty is experienced in killing an animal without producing a considerable amount of contraction, and in the case of elongated forms, such as Nemerteans and other worms, without causing them to coil up or become twisted, To avoid this, it is expedient to narcotize the animals before killing them, and for this purpose Lo Bianco recommends immersion in weak alcohol. He uses generally a mixture of sea-water 100 ccm. and absolute alcohol 5 ccm. In other cases 70 per cent. alcohol may be carefully poured upon water in which the specimen lies, so that it forms a layer at the surface. It will gradually mix with the subjacent water, and in the course of a few hours will narcotize the animal, so that it may be treated with fixing reagents without fear of contraction.

Chloral hydrate, 1 to two parts sea-water, is also efficient as a narcotizing agent, and has the advantage of allowing a recovery of the animal, if there should be necessity for it, by placing it in fresh sea-water. For some sea-anemones tobacco smoke is useful, the smoke being conducted by a V-shaped tube into a bell-jar covering the vessel of sea-water in which is the anemone. Certain of these reagents will prove most satisfactory with some animals, others with others. Lo Bianco details the best method for treating the various forms in a second portion of his paper, and an account of some of his methods of procedure, so far as they concern forms which resemble those found upon our coast, may now be presented.

Sponges.—Direct immersion in 70 per cent. alcohol, with subsequent renewal of the fluid, is recommended for the majority of forms. To avoid contraction in the case of the Halisarcidæ, they should be left for half an hour in 1 per cent chromic acid, or in concentrated solution of corrosive sublimate for fifteen minutes. To prepare dried specimens the sponges should be washed in fresh water for a few hours, and then allowed to remain in ordinary alcohol for a day, after which they may be dried in the sun.

Anthozoa.—The first care must be to place the forms belonging to this group in fresh salt water, to allow them to expand, a result which may not be obtained until the following day in some cases. Alcyonarians should be killed with chrom-acetic solution No. 2, withdrawing the water in which they lie, until there is left just enough to cover them, and then adding a volume of the chrom-acetic solution double that of the sea-water. The animals should be removed from this mixture the moment they are killed, since the acid will quickly attack the calcareous spicules, which are important for the identification of the Alcyonaria, and placed in 35 per cent. or 50 per cent. alcohol, it being well to inject the alcohol into the mouths of the polyps to keep them freely expanded. The preparation should finally be preserved in 70 per cent. alcohol.

Regarding the Actinians no definite rule for preservation can be given. Much of the success of the preparation depends on the form employed, some species contracting much less readily and less perfectly than others. Some may be killed in a fair condition by pouring over them boiling corrosive sublimate, and then, before consigning them to alcohol, treating for a few minutes with one-half per cent. chromic acid. This method may be employed with small forms such as *Aiptasia*. Narcotization may be tried with others. For this purpose, remove from the vessel in which the animals are contained, two-thirds of sea-water, and replace it with a 2 per cent. solution of chloral hydrate. After a few minutes the fluid is again removed, and cold concentrated sublimate solution is poured in. Tobacco smoke in some cases, as with *Adamsia*, will act satisfactorily, if followed with vapour of chloroform for two to three hours, after which the animals may be killed in chrom-acetic solution No. 2, and hardened in one-half per cent. chromic acid.

Edwardsia may be narcotized by gradually adding 70 per cent. alcohol to the sea-water in which they are, and subsequently may be killed with hot corrosive sublimate.

Cerianthus should be killed with concentrated acetic acid, placing it as soon as possible in weak alcohol, in which it should be suspended, so that the tentacles may float freely—if necessary, disentangling them.

Corals should be allowed to expand fully, and should then be killed with boiling solution of corrosive sublimate and acetic acid used in volume equal to that of sea-water containing the coral. The colony should then be transferred to 35 per cent. alcohol, some of this fluid being injected into the mouth of each polyp. The injection should be repeated at every change of the alcohol, and the specimens should be preserved in 70 per cent. alcohol, after washing them well in iodized alcohol.

Hydromedusæ.—For the hydroid colonies the best fixing reagent is hot corrosive sublimate. The smaller Tubularian medusæ should be killed either in the mixture of corrosive sublimate and acetic acid, or in Kleinenberg's picrosulphuric acid. Larger forms may be fixed with concentrated acetic acid, and then allowed to fall imto a tube containing the alcohol and chromic acid mixture, in which they are gently agitated and allowed to remain for fifteen minutes, after which they should be transferred to 35 per cent. alcohol, and gradually carried to 70 per cent.

Small Campanularian medusæ, e. g. *Eucope* and *Obelia*, may be killed in the mixture of copper sulphate and corrosive sublimate. *Æquorea*

should be killed with concentrated acetic acid, and immediately transferred to chrom-osmic mixture for fifteen to thirty minutes. The same method answers for *Cunina*, while *Liriope* should be treated at once with chrom-osmic from five to twenty minutes.

Scyphomedusæ are the best fixed with 1 per cent. osmic acid, to the action of which they are subjected until they assume a pale brown tint. They should then be thoroughly washed with fresh water before being placed in 35 per cent. alcohol, and should be finally preserved in 70 per cent.

Siphonophores.—The forms of this group should be preserved soon after capture, and specimens in good condition should be selected.

Agalma and similar forms should be killed in the mixture of copper sulphate and sublimate, which should be used in volume equal to or double that of the sea-water in which the animal floats. The mixture should be poured in rapidly, and not over the animal. When killed, the specimen should be carefully lifted upon a large horn spatula, and transferred to 35 per cent. alcohol for a few hours, and then placed in 70 per cent. It is recommended to preserve the animals in tubes just large enough to contain the specimens, and placed within a second larger tube. In this way evaporation of the alcohol is prevented, and also injury of the specimen from movements of liquid is avoided.

Physalia should be placed in a cylinder filled with sea-water, the animal being lifted by the pneumatophore. When well expanded, it is killed by pouring over it the sublimate and acetic acid mixture (one-quarter the volume of the sea-water), and when dead, is transferred to a cylinder containing one-half per cent. chromic acid, and then after twenty minutes to 50 per cent. alcohol, and finally to 70 per cent.

Velella may be killed with chrom-picric or sublimate and chromic acid mixture, and after a few minutes should be transferred to weak alcohol. *Porpita* may be fixed by dropping Kleinenberg's picro-sulphuric acid into the vessel in which it is contained, and when the blue colour commences to change to red it should be transferred to Kleinenberg's fluid, and after fifteen minutes to weak alcohol.

Diphyes may be killed expanded by hot corrosive sublimate.

Ctenophora may be killed by throwing them into the chrom-osmic mixture, where they should remain for fifteen to sixteen minutes, according to the size, and then gradually passing them through alcohol to 70 per cent. A mixture composed of pyroligneous acid, concentrated, 1 vol.; corrosive sublimate solution, 2 vol.; one-half per cent. chromic acid, 1 vol., is also recommended as a fixative.

Echinodermata.—Starfish may be prepared with the ambulacral feet in full distension by allowing them to die in 20 to 30 per cent. alcohol. Echinoids should be placed in a small quantity of water, and killed with chrom-acetic mixture No. 2, being removed from it as quickly as possible, as the acid corrodes the test. To preserve the internal parts it is necessary to make two opposite openings in the test, so that the alcohol may penetrate the interior readily.

Holothurians, such as *Thyone* and *Cucumaria*, after the tentacles are fully expanded, should be seized a little below the bases of the tentacles by forceps, using a slight pressure, and the anterior portion of the body should then be immersed in concentrated acetic acid. Alcohol (90 per

cent.) should then be injected into the mouth, and the specimens placed in 70 per cent. alcohol. The injection should be repeated each time the alcohol is changed.

Synapta should be fixed by immersion in a tube containing a mixture of equal parts of sea-water and ether (or chloroform), where they remain completely expanded. They should then be washed for a short time in fresh water, and passed into alcohol, taking care to increase the strength of this very gradually.

Vermes.—Cestodes, Trematodes, Turbellaria, as well as Nemathelminths, are most satisfactorily killed with corrosive sublimate, either cold or hot. *Sagitta*, however, succeeds best in copper sulphate and sublimate or chrom-osmic mixture.

Nemerteans should be narcotized in a solution of chloral hydrate in sea-water 1 per cent., where they should remain for six to twelve hours. They are then to be hardened in alcohol. Gephyreans may be narcotized with 1 per cent. solution of chloral hydrate in sea-water, or in alcoholized sea-water, three to six hours; or may be killed at once in one-half per cent. chromic acid, which last method may be also applied to Hirudinea.

Chætopods are best narcotized in sea-water containing 5 per cent. of absolute alcohol, or by adding gradually to the surface of the sea-water in which they are contained a mixture of glycerin 1 part, 70 per cent. alcohol 2 parts, and sea-water 2 parts, hardening them subsequently in alcohol. *Chætopterus* is best killed with 1 per cent. chromic acid, in which they should remain for half an hour; while the Hermellidæ, Aphroditidæ, and the Eunicinæ may be killed in cold corrosive sublimate. Some of these, such as *Diopatra*, may, however, be narcotized in alcoholized sea-water.

Serpulidæ, before treatment with corrosive sublimate, should be narcotized in 1 per cent. chloral hydrate, which causes them to protrude wholly or partly from their tubes.

Crustacea.—Cladocera, Copepods, and Schizopods may be killed in corrosive sublimate dissolved in sea-water. Ostracods may be thrown at once into 70 per cent. alcohol. Cirripeds die expanded in 35 per cent. alcohol, and if some specimens contract it is easy to draw out the cirri with forceps. Amphipods and Isopods may pass directly into 70 per cent. alcohol, except the Bopyrids and Entoniscids, which should be killed in the mixture of equal parts of 90 per cent. alcohol and sublimate solution.

To avoid the casting-off of the appendages of the Decapods they should be allowed to die in fresh water, care being taken not to allow them to remain in it longer than is necessary, as it causes a distortion of the membranous appendages.

Pycnogonids will die in one-half per cent. chromic acid, with the appendages fully extended.

Mollusca.—Lamellibranchs, Prosobranchs, and Heteropods should be narcotized in alcoholized sea-water. To avoid the closure of the valves of Lamellibranchs on immersion in 70 per cent. alcohol, little plugs of wood should be placed between the margins of the valves. The same result may be effected in the case of Prosobranchs by tying the internal edge of the operculum to the shell.

Of the Opisthobranchs the Æolidæ may be the best preserved by

pouring over them concentrated acetic acid in volumes equal to or double that of the sea-water containing them. Dorids should first be narcotized by gradually adding 70 per cent. alcohol to their sea-water, and then killed with concentrated acetic acid or boiling sublimate. The larger forms may be killed in 1 to 5 per cent. chromic acid.

Pteropods are preserved well in Perenyi's fluid for 15 minutes, whence they are passed to 50 per cent. alcohol. Gymnosomatous forms should be first narcotized with 1 per cent. chloral hydrate, and then killed in acetic acid or sublimate.

Decapod Cephalopods may be fixed directly in 70 per cent. alcohol, making an opening on the ventral surface to allow the alcohol to reach the internal parts.

Bryozoa.—The genera *Pedicellina* and *Loxosoma* may be left for an hour in 1 per cent. chloral hydrate, and then killed with cold corrosive sublimate, washing them immediately afterwards. Some species of *Bugula* give good results when the expanded animals are suddenly killed by pouring over them hot corrosive sublimate. With other forms it is sometimes possible to preserve them well expanded by adding 70 per cent. alcohol gradually to the surface of the water in which they are, or by narcotizing first in weak chloral hydrate or in alcoholized sea-water. The results are, however, uncertain, and depend largely on the skill of the preparator. Brachiopoda may be treated in the same manner as Lamellibranchs.

Tunicates.—*Clavellina*, *Perophora*, and *Molgula* may be killed with the orifices expanded by immersing them in 1 per cent. chloral hydrate for 6 to 12 hours. They should then be killed in chromic-acetic mixture No. 2, and quickly transferred to 1 per cent. chromic acid, injecting some of the fluid into the body. After half an hour they should be transferred to 35 per cent. alcohol, the injection being repeated, and finally to 70 per cent. Other simple forms may be treated in the same manner, or may require the 2 per cent. solution of chloral hydrate, or may be killed by pouring a little 1 per cent. chromic acid on the surface of the water in which they are, subsequently hardening in 1 per cent. chromic acid. The method recommended for *Perophora* may be employed for compound Ascidians, using, however, corrosive sublimate instead of the chrom-acetic mixture.

Salpæ vary considerably in consistency, according to the species, and different methods are consequently required. The denser forms, such as *S. zonaria*, should be placed in a mixture of 100 ccm. fresh water and 10 ccm. concentrated acetic acid, where they should remain for 15 minutes. They should then be washed in fresh water for 10 minutes, and pass gradually into alcohol. Less dense forms such as *S. democratica mucronata*, may be fixed in chrom-acetic mixture No. 1, and then passed directly into fresh alcohol; while the soft forms such as *S. pinnata* and *maxima*, should be placed in chrom-osmic mixture for 15 to 60 minutes, then washed in fresh water, and transferred to weak alcohol.

Fishes.—*Amphioxus* will die with the buccal cirri distended in sea-water alcoholized to 10 per cent. They should then be transferred to 50 per cent. alcohol, and gradually to 70 per cent.

Other forms may be preserved in alcohol (70 per cent.), taking care to make a ventral incision, and also to inject the alcohol and renew it

frequently at first. If it is wished to preserve some of the larger Selachians for some months in order to prepare at leisure the skeleton, the intestines should be removed, and the animals placed in a 10 per cent. solution of salt.

Elasmobranch embryos may be fixed in corrosive sublimate, leaving them in the solution for 5 to 15 minutes, afterwards washing well in iodized alcohol. Embryos of *Torpedo* with the yolk were preserved by immersing them in a mixture of equal parts of 1 per cent. chromic acid and corrosive sublimate for 15 minutes, and then transferring to alcohol. Transparent fish-eggs may be preserved for the purpose of demonstration by subjecting them for a few minutes to the action of the alcohol and hydrochloric acid mixture, and then transferring them to pure alcohol.

Some Hints on the Preparation of Delicate Organisms for the Microscope.*—Mr. E. Lovett observes that such organisms as the ova of Mollusca, Crustacea, fishes, &c., are often of such a nature as to be very difficult of permanent preservation, but he has succeeded in overcoming the difficulty satisfactorily by means of a fluid, the density of which he modifies in accordance with the organism about to be mounted. The fluid was composed as follows:—Three parts pure alcohol, two parts pure glycerin, and one part distilled water. This strength was suitable for young crustaceans, the ova of the fishes, and for the tougher ova-sacs of the Mollusca. For the ova of crustaceans and insects, and for those of very small fishes, one or two parts more of distilled water may be added; whilst for such exceedingly delicate substances as the ova of the nudibranchiate Mollusca, zoophytes extended from their capsules, and for various delicate fresh-water forms, a weaker formula than this is necessary; but as practice is the best instructor, he recommends students to be guided by what they find to be the best proportions.

This fluid should be put into small glass tubes, with corks bearing numbers corresponding to those in a note-book, so that full details of the contents may be recorded. These tubes should be taken down to the shore by the collector, and the organisms should be placed therein alive, direct from the sea. The length of time required for the preservation of the object by the fluid varies, according to the organism, from a week to a year, but some of Mr. Lovett's best preparations had been soaking, before being mounted, for five or seven years; and as a proof of the value of the preservative fluid, he cites the mucus-like ova mass of an *Eolis*, which was in quite its natural condition, although eight years of age as a micro-slide. The cement used by Mr. Lovett for fixing cells for this fluid, for fixing the cover-glasses to the cell-wall, or for covering sunk cells, is composed of equal parts of red lead, white lead, and brown litharge, pounded to a powder and kept dry. When wanted for use, a little is mixed with japanner's gold size as thick as required, and it must be used with great care to insure success; but in this case also practice is the best way to satisfactory results.

Improved Method of preparing Ascidian Ova.†—Dr. T. H. Morgan found that the ordinary methods of preparation do not show the boundaries of the cells of the follicle in sections of young ova. He made,

* Trans. Croydon Micr. and Nat. Hist. Club, 1889–90, pp. 203–4.
† Journal of Morphology, iv. (1890) p. 198.

therefore, various experiments, and found the following method satisfactory. The fresh ovaries were teased apart in very dilute osmic acid, washed in distilled water, and placed in a 1 per cent. solution of silver nitrate, where they remained for half an hour; they were then put into acetic acid for the same length of time, and placed in the sunlight. On examination under the Microscope the cell-boundaries were distinctly seen.

Simple Method of examining living Infusoria.*—Herr J. Eismond has discovered a method of slowing those rapid movements of Infusoria which make the examination of these objects during life so difficult. The method is based on that of crystallographers, who retard the formation of crystals by the addition of a colloidal material. He added a drop of thick watery solution of cherry-gum, and obtained the desired effect. In a very short time the Ciliata were seen to be imprisoned, with all their cilia moving actively, but effecting no change in position. All the vital processes can be most satisfactorily observed in Infusoria so treated, and a certain amount of locomotion can be allowed by using a less dense solution. Small Crustacea, Worms and Flagellata, and other marine animals, may be well studied by this method. It may be added that gum-arabic and other fixing materials are useless.

New Method for demonstrating Tubercle Bacilli in Sputum.†—Dr. E. Czaplewski recommends the following method which he says gives ideal pictures in about three minutes of tubercle bacilli in sputum. Three solutions are required :—(1) The Ziehl-Neelsen carbolic-fuchsin. (2) Saturated alcoholic solution of yellow fluorescin to which methylen-blue is added to excess. (3) Saturated alcoholic solution of methylen-blue.

A very thin layer of sputum must be fixed on the cover-glass in the usual manner. On the cover-glass held in a pair of forceps, sputum side upwards, is then let drop sufficient of the fuchsin solution to form a complete layer. It is then held over the flame of a spirit-lamp until it vaporizes or begins to boil. The fuchsin is then run off and the cover-glass waved to and fro in the fluorescin solution six to ten times, and after this in the methylen-blue solution ten to twelve times. The cover-glass is next quickly washed in pure water and then at once laid with the prepared surface upon a clean slide. The superfluous water is then expressed by means of a piece of blotting-paper placed on the top, and any deposit of pigment removed with a moist cloth. Finally, a drop of cedar oil is laid on the back. The preparation is then ready for examination. Hence it will be seen that the organisms are observed in water, but the preparation may of course be mounted in the usual manner.

Method for Differential Diagnosis of Bacilli of Typhoid (Eberth).‡—The procedure consists in a modification by J. Gasser of Noeggerath's method for recognizing the typhoid bacillus. To a test-tube full of nutrient agar twenty drops of a saturated aqueous solution of fuchsin are added, the mixture sterilized and poured into a Petri's

* Zool. Anzeig., xiii. (1890) pp. 723–4.

† Centralbl. f. Bacteriol. u. Parasitenk., viii. (1890) pp. 685–94.

‡ La Semaine Méd., 1890, No. 31. Cf. Bakteriol. u. Parasitenk., viii. (1890) p. 411.

capsule. When set the surface is scratched with the bacillus and then incubated at 37°. In four hours the cultivation has developed, the agar round about it being decolorized. The whole plate has lost its colour in six to eight days, but the cultivation itself is quite red.

Control experiments with numerous other micro-organisms showed that typhus bacillus and *B. coli communis* were the only two which decolorized the medium. It is said that the two may be distinguished by the fact that *B. coli comm.* does not exceed the inoculation track, while typhus bacillus forms a broader strip with irregular margins.

New Criterion for distinguishing between Bacillus Choleræ Asiaticæ and the Finkler-Prior Bacillus.*—If these two bacilli, say Herren O. von Hovorka and F. Winkler, be cultivated on plover's egg albumen they may easily be distinguished. The Finkler-Prior bacillus rapidly liquefies, and imparts a yellow colour to the medium, while Koch's comma bacillus neither liquefies nor stains it. This difference is clearly distinguishable in the first six days of the cultivation.

Reference Tables for Microscopical Work.†—Professor A. B. Aubert has compiled the following tables which have been in great part translated and adapted from Dr. Behrens' 'Tabellen zum Gebrauch bei Mikroskopischen Arbeiten.' They address themselves especially to workers in the various departments of microscopy where such aids to the memory may be helpful in everyday work. The methods given are such as have received the approval of many of the best workers at home and abroad. A glance at the tables will generally give all the information necessary to any one fairly familiar with micro-manipulation, and while they do not aim at replacing the larger and more complete works, it is hoped that they will prove useful on the work-table of microscopists generally.

Preservative and Mounting Media :—Alcohol-glycerin.—Glycerin, 1 part; alcohol (96 per cent.), 1 part; water, 1 part. Specially recommended for plants, entire or in parts.

Canada balsam in alcohol, chloroform, benzol, turpentine, xylol.—The balsam is hardened by low heat until brittle when cold, broken up or pulverized, dissolved in the solvents, filtered through paper, and evaporated until of the thickness of syrup.

Boroglyceride.—Dissolve as much boracic acid in warm glycerin as possible. The solution is thick when cold; use for mounting some animal or plant preparations in the same way as balsam.

Canada balsam :—The thick balsam is heated, and the mounting done on the warm table; the object must first be soaked in absolute alcohol, then in oil of cloves.

Glycerin and carbolic acid :—Glycerin, 100 grm.; absolute alcohol, 50 grm.; water, 50 grm.; carbolic acid, 3 grm. For plant sections, &c.

Chloride of calcium concentrated, or 33, 25, 12 per cent. For vegetable preparations, &c.

Dammar :—Dissolve gum dammar in equal parts of benzol and turpentine; the solution is filtered and evaporated to syrupy thickness.

* Mittheil. aus d. Embryol. Institute der K. K. Univ. Wien, 1890, pp. 10–14.

† Micr. Bull. and Sci. News, vii. (1890) pp. 35–6.

Farrant's medium:—Gum arabic, 1 ounce; glycerin, 1 ounce; water, 1 ounce; arsenious oxide, 1½ grains. Dissolve the oxide in water, then the gum, without heat; when entirely dissolved add the glycerin, take care not to form bubbles; can be filtered through fine flannel. Specially recommended for delicate plant or animal tissues.

Glycerin:—Concentrated or diluted with water, to which may be added a few drops of acetic or carbolic acid. For vegetable and animal preparations.

Glycerin-jelly:—Glycerin, 120 grm.; water, 60 grm.; gelatin, 30 grm. Dissolve the gelatin in warm water, add the glycerin, filter, if necessary, through flannel. All forms of glycerin-jelly must be used warm. For vegetable and animal tissues.

Deane's medium:—Similar to glycerin-jelly but with the addition of honey and a small quantity of alcohol. Used in place of glycerin-jelly.

Glycerin-salicylic vinegar:—Glycerin, 1 vol.; water, 4 vol; salicylic vinegar, 0·1 vol. For Infusoria.

Glycerin-salicylic vinegar for larvæ, *Hydra*, Nematodes, &c.:—Glycerin, 1 vol.; water, 2 vol.; salicylic vinegar, 0·1 vol. Salicylic vinegar is made by dissolving 1 part salicylic acid in 100 parts pyroligneous acid, sp. gr. 1·04.

Goadby's medium:—Corrosive sublimate, 0·25 grm.; alum, 60 grm.; boiling water, 2300 grm.

(3) Cutting, including Imbedding and Microtomes.

Imbedding Seeds by the Paraffin Method.*—Mr. W. W. Rowlee writes:—"The modifications that may be made of the paraffin method of imbedding objects for sectioning are very many. There is always, however, some danger of shrinking delicate and very soft plant tissue. This is due to the use of heat in the process of infiltration; and probably some of the non-heat-employing methods will be found preferable where such delicate tissue is to be imbedded. But for objects that will withstand this process of infiltration, the paraffin method has many advantages over others. Imbedded in paraffin, objects are held firmly, and may be preserved as long as desired without further attention.

For imbedding mature seeds I have found nothing equal to paraffin. The texture of the seed is often very dense, and offers much resistance to the knife. For this reason I found it better to use the harder grade of paraffin. A second serious difficulty that was met with in imbedding seeds was the fact that there was little, if any tissue connecting the embryo † with the seed-coats. Thus it would happen too often that just as the sections were being taken through the middle of the seed—and the most valuable ones are those near the centre—the embryo would leave the coats and the whole series would be spoiled. The inner surface of the inner coat in many seeds is highly polished, and as soon as there is nothing to retain the embryo but its adhesion to the coat, it will loosen. The paraffin does not hold the two together as would be expected. It was suggested that, in order to soften the tissue

* Amer. Mon. Micr. Journ., xi. (1890) pp. 228-30.

† The term "embryo" is used here where on some accounts it would be better to use the word "nucleus." The embryo is often but a very small part of the substance contained within the seed-coats.

and thereby make it more susceptible of infiltration, it would be well to thoroughly soak the seeds in water before hardening in alcohol. This was tried, and there was a great improvement in the results. Fewer of the sections went to pieces after they were transferred to the slide, and the parts of the seed kept their respective positions much better.

In order to study the microscopic structure of seeds, much more satisfactory results can be obtained if the sections are kept in series. It is often necessary to have two or more successive sections before a correct idea of the seed can be obtained.

The method is a modification of the one used and taught in the histological laboratories of Cornell University. In its practical application it is as follows:—In choosing seeds to section, great care is taken to get those which are well filled. This precaution is especially important, as many seeds, for various reasons, never develope more than the coats or the enveloping ovary coats. If a seed has a straight embryo, or even a bent or curved one, it is better to determine by dissection just how the parts of the embryo are arranged with reference to the external parts of the seed. Thus, the seeds of *Helianthus tuberosus* are flattened, and slightly wedge-shaped. The embryo within is straight, and the upper or inner surface of the cotyledons lie in a plane parallel to the plane in which the seed is flattened. Moreover, the cotyledons are in the upper broader end of the seed. Where the seed has no external character, as in a *Eupatorium*, by which the position of its internal parts may be located, one has either to take the chances of getting the sections in the right plane, or open the coats enough to see how the parts are arranged, and then mark the seed in some way. Having selected a well-filled seed, I next put them in water at the ordinary temperature of the laboratory from 24 to 36 hours. From the water they are transferred to weak alcohol (40 per cent.), and gradually hardened by transferring to stronger until they are in 95 per cent. alcohol. Schultze's apparatus may be used to advantage in hardening. Next transfer to equal parts of alcohol and chloroform for from 4 to 8 hours, the time depending on the size of the seeds. Then in pure chloroform for the same length of time. Then for 24 hours into chloroform with as much paraffin in it as it will dissolve at the ordinary temperature. From this into paraffin softened with chloroform, the melting-point of which is about 36° C. The specimens are kept in this melted paraffin 24 hours. I have always been careful not to let the temperature go above 47° C., although I think it probable that a somewhat higher temperature would not injure the tissue of a seed. From this the seed may be imbedded in hard paraffin, and will be found to be thoroughly infiltrated.

The seeds may be sectioned in the paraffin blocks either free-hand or with a microtome. It is highly essential that the sections be kept in series, and that none be missing. The texture of a seed is so fragile that when cut in thin sections the least carelessness may spoil a section. A very effectual way to keep sections intact when they are cut in paraffin is that proposed by Dr. Mark.* It consists in collodionizing the object as the sections are taken. Very thin collodion should be used, and applied to the cut surface after the section is taken, Lee† recommends that 'the collodion be of such a consistency that,

* Amer. Nat., 1885, p. 628. † 'Vade-mecum,' 2nd ed., p. 150.

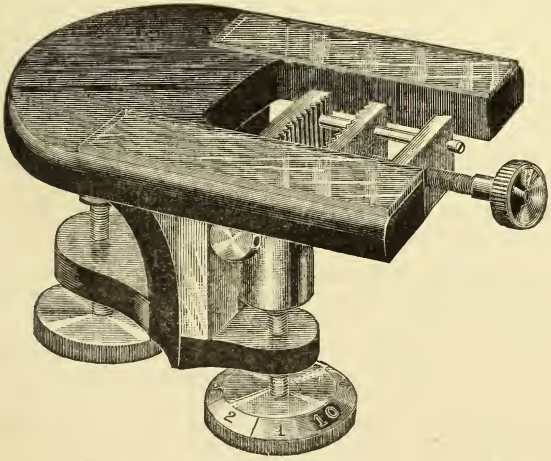
when applied to a surface of paraffin, it dries in two or three seconds. This has no tendency to cause the sections to roll. . . . As soon as the collodion is dry, which ought to be in two or three seconds, cut the section, withdraw the knife, and pass the collodionized brush over the newly exposed surface of paraffin.' The sections are placed collodion side down on the slide. They may be fastened by first painting the slide with a few drops of clove-oil collodion, placing them in it, and then evaporating off the clove oil.

The sections are then placed in xylol for 15 minutes. This removes the paraffin. They are then washed in alcohol, afterwards with water, and stained. I have found no stain that was as effective in staining seeds as hæmatoxylin. They should be stained from 3 to 5 minutes. After washing the staining agent away with water, dehydrate with alcohol, and clear. Three parts of turpentine and two parts of carbolic acid make a very good clearing mixture. Canada balsam dissolved in xylol is used for mounting. In sections thus prepared one can distinguish without difficulty in shepherd's purse, golden-rod, or any endospermous seed, the coats, the plumule composed, as is the lower tip of the radicle, of small thin-walled nucleus-bearing cells. These two regions of growth are connected by slightly elongated cells, which are also thin-walled. The larger cells making up the tissue of the cotyledons are stored with food. In many seeds a trace of a fibrovascular system may be seen; also the peculiar arrangement and markings of the cells composing the coats.

Seeds differ so much, that one would need to make many variations in method to suit different cases; but as a general method I have found this to be a success, and I believe the histology of any seed may be demonstrated by applying it."

Microtome.*—Messrs. Bausch and Lomb write:—"We have found that the section-cutters formerly made by us and other manufacturers

Fig. 14.



* Proc. Amer. Soc. Micr., xi. (1889) pp. 133-4.

are in some respects not suited to modern requirements. We have therefore ceased to make such, and have replaced them by new instruments, which we shall hereafter class under the head of microtomes.

The instrument presented here is dissimilar from the Laboratory and Student microtomes of our manufacture in not having mechanical movement for the knife; it is intended to be fastened to the table-top by means of thumb-screw. The cutting-plate of the instrument is inlaid with glass to obtain perfect smoothness. To the carriage are directly fitted the micrometer-screw with graduated disc, and a section-clamp which is acted upon by the former. The pitch of the screw is 1/50 in., graduation on disc 10, and the finest degree of feed 1/500 in. The regular section-knives as well as the ordinary razors can be used with the instrument."

(4) Staining and Injecting.

Brown-staining Bacillus.* — Herr D. Scheibenzuber describes a bacillus which he has isolated from rotten plover's eggs, and of which the chief characteristic is that it stains the gelatin in the immediate vicinity of the colonies of a brownish colour. The colonies when grown on plates are stated to consist of a central area, which is surrounded by a radiately striated zone. The gelatin surrounding the colonies is not liquefied; when cultivated in test-tubes (puncture cultivation), the inoculation track becomes characteristically serrated, and produces a brown pigment.

When examined with 1/20 oil-immersion the micro-organism is found to be a short bacillus pointed at both ends.

New Method for Staining and Mounting Tubercle Bacilli.†— Dr. H. Kühne recommends the following method for staining tubercle bacilli:—

After the cover-glasses have been prepared, that is, coated with sputum and dried in the flame, they are stained in carbolic fuchsin for five minutes. They are then thoroughly decolorized in 30 per cent. nitric or sulphuric acid, and subsequently washed in water and dried. After this they are examined in a drop of anilin oil stained slightly yellow with picric acid. This mixture is best made by adding 2 to 3 drops of concentrated solution of picric acid in anilin oil to a capsule full of anilin oil.

Preparations obtained in this way will remain fit for examination for at least a week. If permanent preparations are desired, the cover-glass, after it has been decolorized by the mineral acid, is placed for some minutes in an aqueous solution of picric acid, then dried and mounted in the usual manner.

Staining Flagella of Spirilla and Bacilli.‡—Dr. Trenkmann finds that the flagella of bacteria may be stained with very satisfactory results in the following manner:—

The cover-glass having been prepared from a cultivation in the usual manner, is immersed for 6 to 12 hours in a solution of 2 per cent.

* Mittheil. aus d. Embryol. Institute d. K. K. Univ. Wien, 1890, pp. 1–9 (4 figs.).
† Centralbl. f. Bakteriol. u. Parasitenk., viii. (1890) pp. 293–7.
‡ T. c., pp. 385–9.

tannin and 1/2 to 1/4 per cent. HCl. The preparations are next carefully washed and placed in iodine water. Gram's iodine or one drop of iodine tincture to 10 ccm. water does very well, but iodine mixed with water and allowed to stand for 24 hours (shaking frequently) answers better.

In the iodine solution the covers remain for about one hour; they are then washed in water, and stained with gentian-violet. The violet solution is made in a 25 ccm. test-tube. One drop of a saturated alcoholic solution of gentian-violet solution is mixed with 10 ccm. distilled water. Half of this is poured away and the test-tube filled up with anilin water. In this solution the covers remain for about 30 minutes.

Afterwards the author advises using a less quantity of hydrochloric acid, and to have three different solutions, viz.:—Two per cent. tannin, with 1, 2, and 3 per 1000 HCl. The 1 per 1000 may be made by mixing 10 grm. of a 2 per cent. tannin solution and 2 drops of 8 per cent. HCl.

Impregnation of Bone Sections with Anilin Dyes.*—Herr N. Matschinsky finds that saturated aqueous solutions of anilin pigments are excellent for demonstrating the growth-appearances of bone. The pigments used were eosin, safranin, gentian-violet, methylen-blue, methyl-green, iodide-green, and fuchsin, and though all were satisfactory, eosin and safranin gave the best results.

The bones examined were sectioned transversely and longitudinally, and were both macerated and fresh. If fresh, the fat was removed by immersing the sections for half an hour in ether, and after having been polished up, the dust removed, and washed in water, they were transferred to the staining solution.

Macerated bones were allowed to remain for about 48 hours, but if kept at a temperature of 40° C., the staining was more rapid. Sections of fresh bone stained more slowly.

When removed from the staining solution the sections were dried, and having been again carefully polished up, were examined in air or in Canada balsam.

From examination of different bones and bones of different ages (young, adult, old), it was found that the staining was proportionate to the changes going on. Thus, in young bone the staining was more pronounced in the subperiosteal and subendosteal regions than in adult bones, and much more than in old osseous tissue.

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

To rectify Turpentine for Microscopical Use.†—Mr. Charles C. Faris writes:—As it is difficult to obtain nice, clear turpentine for microscopical purposes, I want to give other workers the benefit of my experience in rectifying the ordinary fluid. I proceed as follows:—

Take one pint of the common turpentine and mix in a quart bottle with 4 oz. of 98 per cent. alcohol. Agitate well, and let stand until the two fluids separate. Decant the turpentine (which will form the lower layer) from the alcohol, and mix it with one pint of clear water.

* Anat. Anzeig., x. (1890) pp. 325–36. † The Microscope, x. (1890) p. 179.

Agitate thoroughly, and let stand until these two fluids separate, then from the water decant the turpentine (which this time will form the upper layer), and finally, mix with the turpentine about 1 oz. of powdered starch, and filter through paper.

By pursuing the foregoing plan any one may secure a pure, limpid, and brilliant turpentine. The alcohol used in rectifying it need not be wasted, as it will do to burn, to clean slides, or for other purposes. I usually make a large quantity, and recover the alcohol by distillation.

(6) Miscellaneous.

Biological Examination of Potable Water.*—Mr. G. W. Rafter describes a modification of Prof. W. T. Sedgwick's method of determining the number of organisms in drinking water. The water is filtered through a short column of fine sand in the stem of a funnel, the sand being supported on a plug of wire-cloth placed beneath it. The sand retains the whole of the organisms contained in the water. After the completion of the filtration, the sand is washed with distilled water into a test-tube, and shaken, when all the sand falls to the bottom and the organisms remain uniformly distributed through the water. A definite quantity of this is taken out by a pipette and placed in a cell of known dimensions. The enumeration of the organisms is accomplished by transferring the cell to the stage of the Microscope and examining with the aid of the micrometer.

Tests for Glucosides and Alkaloids.† — Herr A. Rosoll gives the following tests for berberin and cytisin:—Berberin dissolves in concentrated nitric acid with a reddish-brown colour, and may then be precipitated in star-like groups of crystals of berberin nitrate by the successive action of alcohol and nitric acid; or it can be precipitated as characteristic green capilliform crystals by potassium iod-iodide from the alcoholic solution; the crystals being again soluble in sodium hyposulphate. It occurs in all the organs of mature plants of *Berberis vulgaris*. Cytisin occurs in all parts of the laburnum, but there are only traces in the leaves or flowers. It gives a red-brown precipitate with potassium iod-iodide, leaf-like groups of crystals with picric acid; a light reddish-yellow solution with sulphuric acid, which becomes yellow, brown, and finally green, on addition of a small piece of potassium bichromate; a yellow turbidity with phosphor-molybdic acid. Tests are also given for coniferin, phloroglucin, vanillin, salicin, syringin, hesperidin, solanin, saponin, tannin, veratrine, strychnine, brucine, colchicine, nicotine, aconitine, and atropine. The author asserts that strychnine occurs in solution in the drops of oil held in solution in the endosperm-cells, and not, as sometimes stated, in the thickenings of the cell-walls.

Materials of the Microbe-Raiser.‡—Dr. S. Hart makes the following somewhat amusing remarks:—"Some of the means and methods

* Proc. Rochester (N.Y.) Acad. Sci., 1890, 10 pp. and 4 figs.

† 'Ueb. d. mikrochemischen Nachweis d. Glycoside u. Alkaloide,' Stockerau, 1890, 25 pp. See Bot. Centralbl., xliv. (1890) p. 44.

‡ "Invisible Assailants of Health," 'Popular Science Monthly.' See Amer. Mon. Micr. Journ., xi. (1890) p. 232.

of the micrologist in his researches must be mentioned. His outfit is extensive and novel. It includes the best known Microscopes and a well-constructed incubator with heater and thermometer, numerous test-glasses, beakers, filters, acids, alkalies, deep-coloured dyes, and a good supply of prepared cotton.

In studying the life-history of his microbes he will require a well-supplied commissariat. He must be a professional caterer and a bountiful feeder. He must have fluids, semi-fluids, and solids, broths of various meats, peptonized food, the serum of blood *à la Koch*, and Pasteur's favourite recipe with the French refinement: Recipe, 100 parts distilled water, 10 parts pure cane-sugar, 1 part tartrate of ammonium, and the *ash* of 1 part of yeast. Among the substantials must be found boiled white of egg, starch, gelatin, Japan isinglass, and potato, the last from South as well as North America."

A Query.—As "Novice" will perhaps get the best advice by means of our Journal we hasten to give his questions the widest publicity we can:—"I am thinking of starting a street exhibition with four Microscopes (two by Beck and two by Watson). Will some kind reader please tell me which objectives I should use to please the public most —1/4, 1/2, 1, 2, or 3 in.? Also please tell me of a few good mounted objects that will please them as well; and which objectives I should use to get the best result when examining a frog's foot. And do you think there is a living of, say, 35*s.* per week by going from town to town? Any information on the above will be gladly received by—Novice."

* Eng. Mech., lii. (1891) p. 471.

MICROSCOPY.

a. Instruments, Accessories, &c.*

(2) Eye-pieces and Objectives.

"On a new System of Erecting and Long Focus Objectives."† — M. L. Malassez, after referring to the advantage of erect images and long focal lengths, when delicate dissections have to be made, exact measurements determined, &c., writes:—

"For these purposes we have already at our disposal the simple lens or the doublet, the Brücke lens, and the ordinary compound Microscope furnished with erecting apparatus. These instruments are excellent in certain cases, but are certainly unsatisfactory in many others. Thus, the simple lens and the doublet do not give sufficiently strong magnifications with foci sufficiently long, and, in making use of them, it is necessary to bend over the object to be examined in a very uncomfortable way. The Brücke lens possesses the advantage of having a very long focus, but the magnification which it affords is not very considerable. The Microscope itself gives all the magnification desired, but as soon as this becomes at all considerable, the focus is very short, and there is no room for manipulation.

I have devised a new system of objectives, which gives the best results. Adapted to the ordinary Microscope, the objective gives at once, without erecting apparatus, an erect image of the object examined. Its focus is very long, as long as could be wished. One of them has a focus of 7 cm., while it gives a true magnification of 30 diameters with a No. 2 eye-piece of Vérick, and a tube-length of 16 cm. I have made some which had foci much longer, reckoned by metres instead of centimetres. With these it was possible to see with the Microscope objects placed at the other end of the work-room, or even objects more distant still, such as houses and monuments at a distance from the window. However, as we lose in magnification and light what we gain in length of focus, it is of advantage to limit this as much as possible.

These new objectives possess the further advantage of considerable penetrating power, i. e. it is possible to vary the focus without losing the object. The one mentioned above has, for instance, a penetration of 2 to 3 millimetres. It is possible to get more, but it is necessary to limit it, for it would be at the expense of the defining power, i. e. at the expense of the clearness of the images.

The field of view is sufficiently large; that of the objective already taken as an example is from 8–10 mm. in diameter. With it microscopic images are obtained perfectly plane. The field is, of course, enlarged as the magnification is reduced. The device by which I have obtained the two principal properties characteristic of this new system of objectives, viz. the erection of the images and the indefinite length of the foci, is as follows:—

The different lenses composing the objectives really form two distinct

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

† Arch. de Med. Expér., i. (1889) pp. 449-54.

optical systems, each acting as a single convergent lens. One, called for convenience of description the first lens, occupies the lower part of the objective next the object to be examined; while the other, called the second lens, occupies the upper part in connection with the Microscope-tube.

Matters are so disposed that the first lens gives behind it and in front of the second an inverted image of the object, and the second then gives behind it an inverted image of this first. It follows from this that this second image, inverted in relation to the first, is really erect in respect to the object. As this is the image examined by the eye-piece which does not invert, it accordingly remains erect with respect to the object. In other words, the aim of the first lens is to give an inverted image of the object; while the second acts as an ordinary objective, and with the eye-piece constitutes a compound Microscope; so that we examine with this Microscope, not the object itself, but an inverted image of it produced by a lens placed in front of the objective, between it and the object. The Microscope, as it inverts anew this inverted image, gives a final image, which is erect with respect to the object examined.

The possibility of obtaining with these new objectives very long foci, and of any length desired, is explained very easily. With convergent lenses, the farther the object seen, the nearer to the principal focus is the image on the other side of the lens; so that if it is wished to receive it on a screen or examine it with an optical apparatus, it is necessary to approach the nearer to the principal focus. Reciprocally, when very near the lens, it is only possible to see the images of very distant objects; and, on the other hand, when receding from it, only those of objects very near. Similarly, with this new system of objectives, if the second lens is brought near the first, only very distant objects can be seen, and accordingly the focal length of the whole system will be augmented; while by separating the lenses the focal length will be diminished, and only nearer objects can be seen. I have made one of these objectives in which the two lenses can be approached or separated at will, so as to vary at pleasure the length of the foci, and to see with the Microscope objects more or less distant. In practice, however, I think that it is better to use objectives with fixed focus.

The idea of erecting microscopic images by means of the objective is not new. Strauss-Durkheim says, in his treatise on Comparative Anatomy (I. p. 81), published in 1842, that he succeeded twenty-five years previously in erecting the images of compound Microscopes by placing an additional objective below the ordinary objective, and he describes and figures the arrangement which he adopted. He would seem to have shown this improvement to Trécourt and Oberhauser, and it was probably their Microscope thus modified which they presented in 1839 to the Academy of Sciences (ix. p. 322, "Microscope achromatique à tous grossissements"). It gave very variable magnifications, had foci greater as the magnification was weaker, and gave erect images. Fischer de Waldheim, of Moscow, had the same idea about the same time, and constructed a Microscope to which he gave the name of pancratic.

These Microscopes did not appear to have any success. Robin, in his 'Traité du Microscope' (1871, p. 162), states that their images were

wanting in clearness, and that for a long time he had given up their use "pour quelque genre de travail que ce soit." Now they are forgotten, very few treatises on the Microscope mention them, nor are they referred to in catalogues of makers.

In my preliminary trials I contented myself with adding an objective to the existing one, thus making unconsciously a pancratic Microscope. But although by this arrangement very curious optical effects could be obtained, it was not advantageous for the special end in view, viz. convenience of manipulation under the Microscope; for that purpose what could be the use of these foci of indefinite length, or these high magnifications which are only obtained by reducing the working distance and losing light? I then conceived the idea of replacing the single lens system, giving by simple changes of position all kinds of foci and magnification by a series of special systems, each composed of fixed lenses, and consequently giving a definite focal length and magnification, each system being specially combined in order to produce a definite optical effect, and presumably giving more perfect results. Thence followed the erecting objectives of long focal length described above. If the principle on which they depend is already known, they may at least be considered as a new and more practical application.

The first of these new objectives was constructed by me eleven years ago, and was shown then to many persons, amongst whom was M. Vérick, who undertook to make similar ones. He did not do so, but his successor has been engaged under my direction in this new work. Any maker will be able easily to do the same after some trials."

The New Apochromatic Objective.*—Dr. J. D. Cox writes:—"In the February number of the Royal Microscopical Society's Journal we find a synopsis of work done with the new apochromatic objective of 1·63 N.A. by Dr. Van Heurck, the distinguished director of the Antwerp Botanical Garden. Some references to the same appear in a late bulletin of the Belgian Microscopical Society. The results mark a positive advance in the perfection of objectives, though, as Prof. Abbe warned us when announcing the apochromatic lenses which the new Jena glass made possible, each step must be a small one in the present state of the art, and there are apparently but few more within the range of the knowledge and the means possessed by us.

Now, as heretofore, the study of the diatoms gives the means of testing the progress in lens making, and gives the chief stimulus to scientific opticians. Dr. Abbe, who has become personally interested in the Zeiss optical establishment at Jena, is uniting all the resources of scientific formulæ with the skill of an almost perfect mechanical *atelier* to produce wider angled objectives; whilst Dr. Van Heurck, stepping into the place so long occupied in the microscopical world by our lamented Dr. Woodward, of the Army Medical Museum, is, with his dark-room and heliostat, demonstrating what the new lenses will do upon the old familiar tests of *Pleurosigma angulatum*, the small *Navicula rhomboides* (*Frustulia saxonica*), and *Amphipleura pellucida*. When, under his skilful manipulation, real progress is recorded, the improved lens quickly finds its way into the hands of the enthusiasts of the school

* Microscope, x. (1890) pp. 164-8.

of Koch in the rival department of investigation of the infinitely little, to determine what it can tell us in regard to the structure and growth of bacteria.

The photographs which illustrate Dr. Van Heurck's latest work with the new lens seem to show two things: first, that he exhibits the areolation on the valve of *Amphipleura* with a distinctness of definite resolution beyond anything heretofore published; second, that in regard to the less finely marked shells, no perceptible advance upon work done with glasses of narrower angle is apparent. A word further as to each of these points.

In the resolution of *Amphipleura*, as in regard to other tests, there has been a regular progress, partly dependent on real improvement in lenses, and partly upon the use of better methods of manipulation. The mounting of the specimen has also been an element of no small importance. Everybody knows that a diatom mounted dry is much more boldly visible than one mounted in balsam. Striæ are shown, in this case, with much less oblique light, and may be resolved by a glass which quite breaks down when used on the balsam mounted specimen. This is consistent with the fundamental principle that the angle of aperture of a lens determines the possibilities of its work in the resolution of fine details in all microscopic objects. A well corrected glass will do more than a badly corrected one made on the same formula and with the same aperture. No glass of high power has ever been made so perfect as to perform all the theoretic possibilities for a glass of its angle. But however well made the lens may be, it is a mere waste of time and eye-sight to try to make it show details too fine for the theoretic capability of its angle of aperture.

The average fineness of striation of *Amphipleura pellucida* is for the transverse lines about 90,000 to the inch, and of the longitudinal (by Dr. Van Heurck's measurement) about 125,000. But the finest of these are (by the R. M. S. tables) theoretically resolvable in photography by a dry glass of 180° aperture, by a water-immersion glass of 100° water angle, or by a homogeneous immersion glass of 83° balsam angle, all being of a numerical aperture 1, substantially. It thus appears that the angle of 1·63 N.A. is ·63 in excess of what is theoretically required to do the work which Dr. Van Heurck has accomplished with it, or, in other words, that our high power glasses do less than two-thirds of what perfect glasses of their aperture might do, if the tables are correct. Here, then, is a large margin for the improvement of the whole series of immersion lenses, since almost any lenses of first class makers found in the market, have angle enough, in the high powers, to do all that the new apochromatic has done.

But before we can decide how far the new lens is superior to older ones of less aperture, we must have the latter tested under equal conditions, and it is to be hoped that Dr. Van Heurck will add this to his useful labours. The new photographs are from objects mounted in a medium of refractive index 2·4, with both slide and cover-glass closely approximating the same index. They are also taken with monochromatic sunlight. Ever since Prof. H. L. Smith introduced the highly refractive media, it has been well known to microscopists that a very thin and finely areolated shell like *Amphipleura pellucida* is so much

more easily resolved in them that the test when so mounted loses very much of its difficulty. If we make the slide and cover-glass nearly or quite homogeneous with the medium, and in addition to this increase considerably the aperture of the substage condenser, and connect it with the slide by a highly refractive immersion medium, it needs no telling that the difficulties of resolution have been still further and very greatly diminished. Prof. Abbe and Dr. Van Heurck deserve great praise for devising and putting to use the means of effecting such favourable conditions; but the difference between these and the conditions under which other glasses are used must be eliminated before we can tell how much of the improvement in work is due to the lens, and how much to the conditions.

In regard to my second point, viz. that as to the less finely marked shells, no perceptible advance in the character of work is apparent, I will only say that Dr. Woodward's photographs, exhibited at the Centennial Exposition at Philadelphia in 1876, must remain the standard of comparison when the work of the older lenses is brought into question. He worked with a Powell and Lealand water-immersion of 1/16 in. of 1869, and Tolles's 1/10 and 1/18 of similar construction. A little later he used also Spencer's glycerin immersion 1/6 and 1/10, Zeiss's homogeneous immersion 1/8 and 1/12, and a homogeneous 1/10 by Tolles. I think none of these glasses had an aperture greater than 1·25 N.A. Whilst writing this paper I have examined those photographs afresh, and am entirely sure that for the exhibition of the areolation and structure of *Navicula rhomboides*, both the coarser and finer forms (including *Frustulia saxonica* or *Navicula crassinervis*) *Surirella gemma*, *Amphipleura Lindheimerii*, *Pleurosigma angulatum*, and for the transverse striation of *Amphipleura pellucida*, they are fully equal to anything that has been done with the most recent and widest angled glasses, not merely as photographs, but as conclusive evidence of the quality of the glasses he used and their satisfactory work within the limits named.

Dr. Van Heurck has taken one step in advance (and it is a real one), which shows with what labour each step is now gained. The new glass has cost Prof. Abbe months of labour, as is reported; and no inconsiderable expense in money, as well as time, has been lavished upon it. The result, to put it in its most general form, is that where we could distinguish objects in the approximate form of circles or squares of a diameter of 1/100,000 in., we may now (under exceptional conditions) distinguish them if 125,000 to the inch. Yet this may make the difference between tracing definitely some part of the life-history of a bacillus or failing to trace it.

The apochromatic system is by no means synonymous with increase of angular aperture, though it adapts itself readily to the widening of angles. It is distinctively a step in the reduction of the conflict between the chromatic and spherical corrections, by the aid of the wider range in refraction and dispersion which the newly-invented Jena glass possesses. It is therefore directly aimed at the problem stated before, viz. the bringing of the practical performance of our lenses more nearly to the standard of their theoretic possibilities. The effort to do this by using material of higher refractive index for lenses is an old one. Even diamonds have been used for experiment in this direction. The solution

of the problem has been sought also in the identical direction in which Dr. Abbe is working. More than a dozen years ago Charles A. Spencer told me of his own efforts, in the earlier part of his life, to manufacture new varieties of glass with the qualities now found in the Jena glass, but abandoned it because his pecuniary means were wholly inadequate to that sort of experiment. The liberality of the German Government, backing up the combination of high scientific acquirements of Dr. Abbe and his associates in the directions of physics and chemistry, has produced the valuable results we see. A single consideration still holds back many investigators on this side the ocean from giving implicit faith to the new system, and that is the fear as to the durability and chemical stability of the new glass. There is, whether rightly or not, a strong impression that a too large proportion of the apochromatic lenses have been short lived, and some of the failures have been in the hands of such careful and skilful manipulators that careless handling cannot be assumed. To have a costly lens fail on one's hands when the maker, who alone can be properly trusted to repair it, is on the other side of the globe, and custom house regulations are a practical veto on sending it back and forth, makes an earnest student of Nature pause. The same doubt seems to make American opticians cautious in using the new material, and it is hardly to be regretted that they should first exhaust the means of perfecting objectives made of the "old reliable" flint and crown glass. In the hands of the average manipulator the new lenses do not show superiority over high-class American ones. The art of manipulating them (for it is an art) may well occupy some of the hours of the student, with the assurance that till he has acquired some skill in that way, he will not be able to detect the difference between tools having so nice shades of merit. And even then he may console himself that many experts agree with the opinion of Dr. Detmers, that, angle for angle, it cannot yet be said that the best European lenses excel the best American."

Ancient Lenses.*—Mr. Henry G. Hanks calls attention to a very old reference to lenses, or magnifying glasses, which he recently found in an old work, 'The Vanity of Arts and Sciences,' by Henry Cornelius Agrippa. The edition shown was an English translation, published in 1676, from the original Latin edition, published in 1527. The reference alluded to reads thus:—

"So we read, as Celius in his ancient writings relates, that one Hostius, a person of an obscene life, made a sort of glasses, that made the object seem greater than it was, so that one finger should seem to exceed the whole arm, both in bigness and thickness."

It was found that Cælius Antipater (to whom Agrippa probably refers) was a Roman historian who lived 125 years B.C. He wrote a history of the first Punic War, only parts of which were extant. So far as known, this was the first account of magnifying glasses in history. Henry Cornelius Agrippa, the author of this curious old book, was born at Cologne in 1486, and was a man of talents, learning, and eccentricity. In his youth he was secretary to the Emperor Maximilian, and was knighted for bravery in Italy. On quitting the army he devoted himself

* Amer. Mon. Micr. Journ., xi. (1890) p. 243.

to science, and made pretensions to an acquaintance with magic. In 1530 he wrote his treatise 'On the Vanity of the Sciences,' which was a caustic satire upon the inefficiency of the common modes of instruction. After an active, varied, and eventful life, he died at Grenoble in 1539.

(3) Illuminating and other Apparatus.

New Measuring Apparatus for Microscopical Purposes.*—Dr. G. Lindau remarks that of all the pieces of apparatus which have been proposed for the measurement of small objects under the Microscope, the screw and glass micrometer in combination with objective or eye-piece has proved the best. Of these the eye-piece micrometer is by far the most convenient, and is to be preferred to all other micrometers, especially where a mean of several observations is taken. Cases however occur in which the eye-piece micrometer fails to be of service, as in the measurement of thin membranes or threads and in physical investigations on wave-lengths of light, &c. A micrometer constructed by Dr. V. Wellmann may replace it with advantage in these cases. It was originally intended for astronomical purposes, but forms a very useful micrometer for the Microscope. It is especially serviceable for measuring very small objects not exceeding a few μ in size. It differs in principle from all other micrometers in depending on the double refraction of light in certain crystals. It is well known that on looking at a point through a prism of rock-crystal two images, the ordinary and the extraordinary, are seen. As the prism is rotated about the optic axis, the extraordinary image rotates about the ordinary. Consequently, if such a prism is fitted over a microscopic eye-piece in whose focus a thread is stretched, two images of the thread are seen. On rotating the prism the apparent distance of these two images for a certain position becomes zero (the images coincide), and on rotating through 90° from this position it reaches a maximum. On continuing the rotation up to 180° the images again coincide. In the rotation from 180° to 360° the images behave in a similar way, except that the movable one changes over to the other side.

In the new micrometer these two images are used in precisely the same way as the threads of a screw micrometer, for by suitable rotation of the prism their distance is made equal to the image of the object to be measured. The distance of the two images is given by

$$\Delta = m \sin \phi,$$

where ϕ is the angle through which the prism is rotated, and m is the apparent maximum distance of the images for a given magnification v. This constant m is easily determined by measuring objects of known size.

Now the apparent magnitude of an object, of which the actual size d is to be determined, is given by

$$\Delta' = d \,.\, v.$$

Consequently, when by rotating the prism

$$\Delta' \text{ is made } = \Delta = m \sin \phi,$$

* Naturwissensch. Wochenschr., iv. (1889) pp. 185-6 (1 fig.)

we have

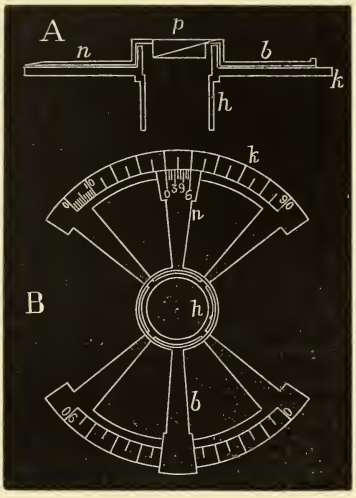
$$d = \frac{m \sin \phi}{v}.$$

To avoid calculations during the observation, Dr. Wellmann has prepared tables of the value of d for different values of ϕ and v.

$\frac{m}{v}$ is usually a very small quantity, which for the prism and lenses used by the author amounted only to 9 μ. Accordingly, to pass from 0 μ to 9 μ the prism must be turned through 90°, so that great exactness is obtained for even a comparatively rough reading. Thus a reading of 1/10 degree on the circle gives an exactness of

$$\frac{9}{90 \cdot 10} = 0 \cdot 01 \ \mu.$$

Fig. 15.



The apparatus itself, as constructed by Schmidt and Hänsch, consists of two parts (fig. 15 A and B). The divided circle k is, by means of the socket h, passed over the body-tube of the Microscope, and is fastened by three screws. Only two opposite quadrants are used, and the rest of the circle, together with the middle portion of the quadrants, is cut away to diminish the weight of the apparatus. The eye-piece is then inserted in the body-tube. Above the socket of the divided circle, which projects upwards, another is fitted which easily turns about it. This carries in its upper part the prism p of rock-crystal, with refracting angle of 70°. Beneath are two projecting arms, one of which n serves as vernier reading to 1/10 degree, and the other b to balance and turn the apparatus.

Polarizing Prisms.*—Dr. W. Grosse calls attention to the important part played by calc-spar prisms in so many physical instruments, and regrets the high price of the material and the great loss which takes place in the course of preparation of the prisms. The various forms of prism are classified as follows :—

I. Prisms in which both rays wholly or partially occupy the field of view. Besides the older well-known forms of Wollaston, Senarmont, and Rochon, there are the more recent prisms of Dove and Abbe, the latter of which consists of an equilateral prism of calc-spar, with wedges of crown glass on the sides.

II. Prisms in which the central zone of the field of view is occupied only by one ray (the extraordinary).

* Zeitschr. f. Instrumentenk., x. (1890) p. 445.

1. With one diagonal slit, and between the two faces
   a. Canada balsam or linseed oil;
   b. Air.
2. With two diagonal slits intersecting
   a. In the middle of a basal plane (Ahrens);
   b. In the middle of the prism (Bertrand).

III. Prisms which contain only the lamella of a doubly refracting medium.

The requirements of an ideal prism are:—Plane polarized field, largest possible field of view, slightest possible refraction of the ray, smallest possible ratio of length and breadth, and least possible waste of material.

In the following table the numbers 1 to 5 serve to estimate the value of the prism for the specific property indicated in each of the horizontal rows. The last column gives for each of these properties the most advantageous forms—viz. those marked with 4 or 5.

	Nicol Group.					Air Prisms.		Double Slit.		Double Slit Air Prism.	Plate Prisms.	Advantageous Form.
	Nicol.	Hartnack.	Thompson.	Dove.	Abbe.	Glan.	Foucault.	Bertrand.	Ahrens.			
1. Plane polarized field	3	4	5	2	2	2	2	2	3 (1)	2	1	Thompson, Hartnack.
2. Field of view ..	3	3	3	2	2	1	1	4	4	1	5	Plate prism, Bertrand, Ahrens.
3. Loss of light ..	4	5	5	5	3	2	2	3	5	2	1	Dove, Hartnack, Thompson, Ahrens.
4. Displacement of ray	2	5	5	5 (1)	5	3	1	3	5	3	4	Hartnack. Thompson, Abrens (Dove).
5. Ratio of length and breadth ..	1	1	1	3	3	4	4	1	3	5	2	Air-prism with double slit, Glan, Foucault.
6. Waste of material	4 (3)	2	1	4	4	2	4	3	3	4	5	Plate-prism, Dove, Abbe, air-prism.
Total.. ..	17 (16)	22	20	17(+4)	19	14	14	17	21 (+2)	17	18	

At the author's suggestion, Herr Halle, of Potsdam, has undertaken the preparation of the air-prism with double slit, referred to in the above table. It is not half so thick as broad and long, and would be useful as a polarizer. Another form suggested by the author may be described as a Bertrand prism with air slit. It is rather thicker than broad and long; but as the field of view amounts to 15° it would be very serviceable as an analyser.

A new Camera Lucida.*—Herr G. Govi describes a new camera lucida, which consists of two rectangular equal-sided prisms of the same glass, one of which is smaller than the other. The hypothenuse face of the smaller, which is coated with thin gold-leaf, must be as large as the side face of the larger, on which it is fastened with Canada balsam or some other substance with refractive index as near as possible to that of the glass.

The hypothenuse face of the larger prism is turned at 45° to the

* Central-Ztg. f. Optik u. Mechanik, x. No. 22, p. 260.

horizontal, and lies above the eye-piece of the Microscope. The eye sees from above through one side face, at the same time, the microscopic object and the paper on which the drawing is to be made. The rays proceeding from the latter fall on the other side face of the small prism, are refracted into this, and so reflected on the gold-leaf that they reach the eye in the direction of the rays coming from the Microscope.

A new Ocular Diaphragm.*—Prof. Wm. Lighton writes:—In a paper read before the American Society of Microscopists at Indianapolis in 1878, I described a new dark-field eye-piece which was the result of experiments begun in 1863, and which was also described and illustrated in the first number of the 'American Quarterly Microscopical Journal,' published in 1878 by Prof. Romyn Hitchcock.

There also appeared in the 'American Monthly Microscopical Journal' for June 1887 a description of an analysing diaphragm for an eye-piece, to be used with the polariscope.

These two pieces of apparatus were to be used above the eye-piece, and were designed for a special kind of work. That now to be described is also to be used above the ocular, but for work of another sort. Its aim is to intensify the image of a certain class of objects, notably the Diatomaceæ, and its construction is shown in the accompanying figures, fig. 16 being a top view, fig. 17 an end view, fig. 18 an inside view, and fig. 19 is a sectional side view of the cap. The same letters in the different figures always refer to the same parts.

A, fig. 19, represents the axis of the Microscope; B, the eye-piece. G, fig. 16, the top of the eye-piece in which is a groove J. D is a sliding diaphragm moving in the groove from right to left and the reverse, by means of the screw F and spring I. Fig. 17 shows the manner of fitting the diaphragm in the groove.

To the under side of the diaphragm is fastened a square post H, by means of the screw L. This post gives motion to the diaphragm by the use of the screw F, and in the opposite direction by the spring I, which is supported by studs K.

It is very important that a proper adjustment of the diaphragm be made.

C, fig. 19, is the image of the mirror brought to a focal point through the eye-lens. It is at this point that the knife-edge of the diaphragm should be placed; the field will then have a subdued tint, and the object an exceedingly clear definition. Covering the point C with the diaphragm gives a *brilliant image of the object on a dark field*, and withdrawing the diaphragm from all contact with this point, the object will appear as ordinarily seen in the Microscope. I have obtained the best resolution of diatoms by the use of an achromatic eye-piece. I have also used Steinheil's 1 in. and 1/2 in. lenses as eye-pieces with good results.

The field under these eye-pieces assumes a soft grey tint the instant the diaphragm touches the point C. I do not find the use of the Huyghenian eye-piece to be satisfactory. I would strongly advise the use of the Nelson ocular.

* Microscope, x. (1890) pp. 8–10.

When using oblique light it is important that the diaphragm be placed on the side of the eye-piece nearest the mirror. For example, if the mirror is placed at the left of the stage, the screw F should be at

Fig. 18.

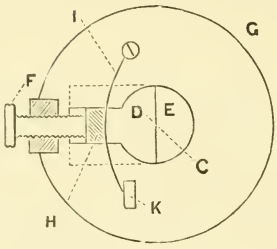
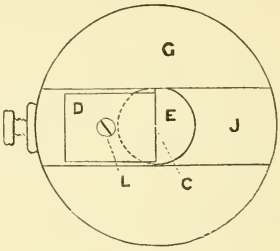


Fig. 16.



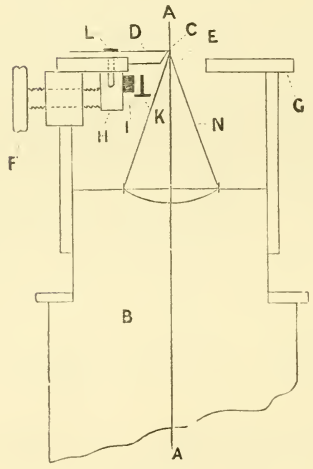


Fig. 19.

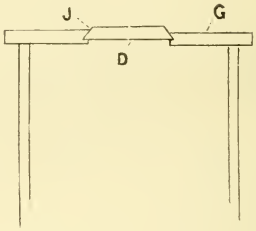


Fig. 17.

the left of the Microscope-tube. The eye-piece can be revolved to bring the diaphragm into the required position, and this revolving motion will also give a variety of beautiful effects.

The diaphragm works equally well with all objectives which I have used, and will, I think, repay all workers with the Microscope for the practice necessary to become familiar with its use.

Substage Condenser.* —Mr. Hyatt stated that the condenser which he exhibited to the New York Microscopical Society was constructed by himself on the principle announced some time since by Prof. Alfred M. Mayer—three plano-convex lenses, the largest one, of 2 in. focal distance, with a central stop, placed below, and two smaller lenses, paired, with their convex surfaces opposed to each other, and placed

* Journ. New York Micr. Soc., vii. (1891) p. 54.

near the under surface of the stage. The combination gives an excellent dark-ground illumination.

New cheap Centering Substage.—Mr. E. M. Nelson's substage, exhibited at the last November meeting,* is made thus:—The substage tube is made 1/4 in. larger in diameter than usual; a smaller tube, which holds the condenser, fits in this; this second tube has a large flange on the top, which prevents it passing through the large substage tube. A screw is cut on the bottom of the inner tube, and a flange similar to the upper one screws on. Obviously, therefore, by screwing the lower flange tight, the inner tube may be secured in any desired position.

(4) Photomicrography.

Handy Photomicrographic Camera.†—Mr. W. H. Walmsley writes: —Although photography in conjunction with ordinary microscopical observations (in other words, photomicrography) has undoubtedly grown in usefulness and popularity among workers with the Microscope during the past five years, there can be no doubt that its aid is very sparingly employed—a fact greatly to be regretted. For it is quite self-evident that the value of any microscopical research would be greatly enhanced, not only to the observer himself, but to his readers (in the event of his work being published), by full and accurate illustrations. Very few microscopists are competent draughtsmen, or capable of making drawings of objects under the lens at all correctly, or even presentable as illustrations thereof. And a drawing thus made is always permeated more or less by the imagination of the artist; so that the greater his skill in that direction the more likely is he to introduce features, not as rendered by the tube, but as he thinks he sees them. To be sure, photographic reproductions of microscopic objects are in a majority of cases not by any means perfect, or what one could desire, but they are vastly superior to almost any drawings in their accurate delineation of the various features of the specimen. The saving of time is another most important feature, as a dozen negatives may be taken in less time than that required to make a single careful drawing.

In the days of the old "wet-plate," the comparative insensitiveness of which precluded the use of a lamp as the illuminator, only those possessing a well-filled pocket-book or having access to the resources of a governmental or college laboratory could avail themselves of the aid of photography in connection with the Microscope. But the modern gelatin "dry plate" has placed in the hands of every one a cheap and efficient means of doing the highest class of work readily and perfectly. The very highest powers may be used with the light from an ordinary petroleum lamp. I have a print from a negative of *Pleurosigma angulatum* magnified 2400 diameters, by Spencer's 1/10 homogeneous objective; the illuminant being an ordinary single wick coal-oil lamp. It is the work of Dr. J. E. Baker, of Wyoming, Ohio, and is fully equal to the best work given to the microscopic world during the past six months.

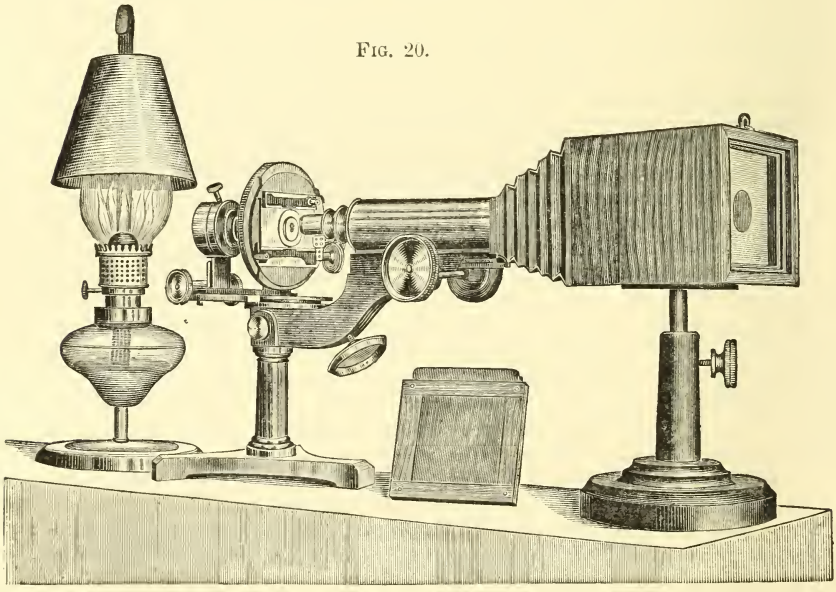
Why, then, has the use of photography not become more general

* See this Journal, 1890, p. 838.

† Amer. Mon. Micr. Journ., xi. (1890) pp. 257-61; and Proc. Amer. Soc. Micr., xii. (1890) pp. 69-74.

among microscopists? Simply from the fancied difficulties of the necessary but simple manipulations required; and from the real one of the absence of any form of camera which could find a regular and permanent home upon the work-table, occupying no more space than the Microscope itself, and always ready for immediate use. The latter is a most important requisite. How frequently does the student find in the course of his observations upon living and other tissues, features that are vital toward proving the truth of his researches, but so evanescent that the lapse of even a few minutes may suffice to obliterate them? If, then, there be at his elbow a small, simple camera which can be at once applied to the Microscope without the slightest alteration of the latter, save placing the body in a horizontal position, using the same source of illumination, be it diffused daylight or that of the ordinary lamp, has he not a boon within his reach, which a few years since would

Fig. 20.



have been deemed impossible? And are not his thanks due to the fellow-worker, whose own wants found expression in the original of the "Handy" photomicro camera?

My friend Mr. H. Wingate, of Philadelphia, has long been an ardent worker with the Microscope, his studies being almost exclusively confined to the minute fungi belonging to the family of Myxogastres. He is exceedingly skilful with the pencil, and his drawings of these minute organisms, their spores, &c., are at least equal to any that have ever come under my observation. But, being actively engaged in business, the time wasted in making these drawings was a large tax, and he determined upon calling in the aid of photography; and there being

absolutely no camera in the market to meet his requirements, he proceeded to construct one. Procuring a plate-holder of the proper size, he built the camera to suit it after the plan of the man who carried the bung-hole to a cooper shop to have a barrel made for it. His material was some heavy blackened cardboard, and an old piece of a steam-fitting some 4 in. long; his tools, a pocket-knife and a glue-pot, with the brains to use them. With these crude appliances he produced a camera, adapted to his Microscope, and capable of doing the highest class of work. He uses a Zeiss's 1/18 homogeneous lens constantly; and frequently makes a dozen or more negatives of an evening therewith.

Upon seeing this little affair, I was at once struck with the conviction that if it could be produced in a form adaptable to any Microscope, it would fully meet the long-felt want of just such an instrument. The result was the construction of the "Handy" camera, which has already been supplied to many institutions of learning and to private workers.

The camera consists of a mahogany box about $2\frac{5}{8}$ in. square, corrugated and blackened on the inside to prevent any reflections of light. A solid cone of some 4 in. in length, tapering to receive the tube of the Microscope, is attached to the front of the box. Preferably, this cone front should be in a bellows form, as in the sample sent, but this being rather more costly than the solid cone, many will be satisfied with the latter. In one case the bellows responds readily to the movements of the Microscope tube in focusing; in the other the tube must slide readily into and out of the solid cone. At the opposite end of the box is a groove, in which the plate-holder and frame containing the focusing screen slide. The former carries two plates $2\frac{1}{2}$ in. square, amply large for all ordinary illustrations. Should larger sized pictures be required they can be made by enlarging upon bromide paper.

The focusing screen is made of very thin crystal glass, most carefully ground by hand, presenting the smoothest surface obtainable by this means, but still quite too coarse for the exact focusing of delicately marked objects. In fact the focusing screen is mainly useful in procuring even and full illumination of the field, and in properly centering the object. The final fixing of the exact focus is done by means of a focusing glass used in conjunction with a disc of thin cover-glass attached to the ground surface of the screen by means of Canada balsam.

The camera is mounted upon a stout metal rod, which slides into the upright shaft of a very heavy japanned base, and can be secured at any height to suit that of the Microscope (when the latter is placed in a horizontal position) by means of a milled head. The base is shod with thick felt cloth, so that it may be placed upon any polished table-top without scratching the latter, and at the same time remain firmly fixed in the position it may be placed in.

And this is all there is of it: simple, compact, always ready for immediate service, and occupying no appreciable space upon the work-table. Although primarily intended for use with the Microscope-body inclined in a horizontal position, it may be as readily adapted to the latter in a vertical one, when the character of the objects (as those mounted in fluids) may require. My own method has been to remove the camera from its base and mount it upon the top of an open box con-

taining the Microscope. An opening in the top of the box allows the cone to be slipped over the tube of the Microscope, and in this manner I have made very successful negatives of blood-corpuscles in rouleaux in their own serum, yeast spores in fluid, &c. A correspondent in Boston writes me that he has mounted the camera upon a firm retort-stand for the same purpose. Many methods of using the instrument in an upright position will doubtless present themselves to the worker therewith.

The illumination may be by reflection from the mirror as in ordinary work, or by removing the latter and placing the lamp behind the stage in a direct line with the optic axis. It must be carefully centered in order to illuminate alike in all portions. Condensers of various kinds, bull's-eye, achromatic, Abbe, &c., can be used as desired, but only with moderate powers. The best results will be obtained by the employment of simple diaphragms of various sizes to suit, and so placed as to come as close as possible to the under surface of the slide upon which the object is mounted. All extraneous light should be excluded and none be allowed to enter the objective other than the rays which illuminate the specimen. Opaque objects may be photographed quite as successfully as transparent ones, but the time of exposure would be very greatly shortened by employing direct sunlight.

The eye-piece may be removed or not, as the observer may elect. Following the teachings and practice of the late Dr. J. J. Woodward, I have almost invariably worked without it, using an amplifier where sufficient magnification could not be obtained with the objective alone. In using medium and high powers, I have not found the eye-piece objectionable, but with low powers it certainly detracts from sharpness of definition, so that my preference is decidedly in favour of the amplifier where an increase of power beyond that obtainable with the unaided objective becomes necessary. If possible, however, always use the latter alone. The short tube-length, alone possible (when using the "Handy" camera), renders the employment of amplifier or ocular necessary, if enlargement beyond three or four hundred diameters is to be made, since the limit of a 1/18 used direct is less than 350°.

The corrections of most modern objectives as to visual and actinic foci, are so nearly identical that no difficulty will be experienced in obtaining sharp definition of any subject if a little care be used. But it may not be amiss to say the student's series of Bausch and Lomb are the best, by all odds, of any I have ever seen or used at all approaching them in moderation of cost. I have numerous remarkable examples of their work which I have never seen excelled by lenses of equal powers, no matter what their cost. It certainly is not necessary to go abroad in these latter days to get the best in the optical as well as in many other directions.

The dry plates for the "Handy" camera are furnished by the makers in two degrees of sensitiveness to suit every variety of subject. They are readily developed by any of the methods used for gelatin plates, my own preference being given to hydroquinone or a mixture of that with cikonogen, as giving the clearest results, clearest details, and sharpest contrasts with any desired amount of density. Their cost is but 25 cents per dozen, certainly cheap enough to tempt any one to their use.

In conclusion, a few words upon various printing methods. Pre-

suming that every microscopist who ventures into the realms of photography will do his own printing, a few hints may prove useful. There can be no doubt of the beauty and perfection of a good, properly toned, and finished print upon albumenized paper. This is conceded. But comparatively few amateurs will ever succeed perfectly in the operation of sensitizing the paper and toning the print, whilst most of the "ready sensitized" papers on the market are an abomination and a snare. Therefore avoid this method of printing, unless prepared to do first class work.

Passing by platinum as being both expensive and uncertain, excepting in the hands of an expert (although its beauty and perfection cannof be too highly extolled), let us consider for a moment the decided claim of bromide paper, as being the best material for printing in our class of work. Using the smooth surface paper and developing with ferrous oxalate, we get a perfect print rendering the most delicate details with the crispness and clearness of a steel plate engraving, which indeed it most closely resembles in very many instances. The exposure is made by lamplight, so that one is entirely independent of time or weather, and the finished print is quite permanent; as much so, it is reasonable to believe, as a carbon print. If the sheet be allowed to dry by itself, it will present the appearance of an ordinary plate engraving. If a polished surface be desired, all that is necessary will be to float the paper, print side down, upon a sheet of polished hard rubber; to squeeze it into optical contact, removing all superfluous moisture, and when quite dry it will peel off the rubber plate with a beautful polished surface, greatly increasing the delicacy of detail in many subjects, especially diatoms. Most decidedly my preference is given to this form of printing.

But there is another method which, at the risk of being laughed at, I am inclined to gently urge. I refer to the ferro-prussiate, or more commonly named "blue prints." This method of printing is tabooed in many instance, "blue prints" being rigorously proscribed in the albums of the Postal Photographic Club, but for all that it has decided advantages and merits for the work we are considering. It is cheap, as the paper may be purchased ready sensitized, at very trifling cost, and it requires no skill or experience in the using. It is merely necessary to expose to bright sunlight until sufficiently printed (a few experiments will determine this), and then to wash in several changes of water; the result being a bright permanent blue print upon a clear white ground, with excellent detail, excepting in the most delicate structures.

The negatives made with the "Handy" camera are of a convenient size for printing lantern-slides by contact. A print on glass is certainly the most perfect of any that possibly can be made, and the importance of this method of demonstration has long since been conceded. Gelatin plates coated on thin glass with special slow emulsions are furnished by several makers, and microscopists can readily make their own lantern slides with a little expenditure of time and patience.

On some Processes of Photomicrography.*—Dr. S. Capranica gives an account of the processes and apparatus which he employed to establish the results given in this Journal, 1888, p. 651.

* Zeitschr. f. Wiss. Mikr., vi. (1889) pp. 1–18.

(1) Apparatus for instantaneous photomicrography.—The author strongly recommends the use of the finder, with which it is possible to view simultaneously the preparation on the stage of the Microscope, and the projection of the image on the ground glass of the camera. The apparatus employed is a modified form of the Bourmans system. The camera is of wood, 30 cm. by 30 cm. and 12 cm. deep. On the front is a board, sliding in grooves, on which a metal flange with circular aperture of 5 cm. is screwed. A short tube carrying a shutter is attached to the flange. On this tube is screwed a totally reflecting prism in a circular metal box, having a right-angled tube of less diameter, to which is applied a stereoscopic binocular eye-piece of Abbe-Zeiss. The two eye-pieces of the latter are unscrewed and replaced by two other tubes. The tube replacing the straight eye-piece is of the same length, and can be fitted with slight friction into the socket of the vertical tube of the prism-box. The other tube replacing the inclined eye-piece has a rack and pinion motion, and can be lengthened by the addition of other pieces of tube of the same diameter. The finder having been arranged, the tube of the stereoscopic eye-piece is placed in that of the Microscope, and by a strong binding screw the foot of the instrument is fixed to the work-table. A special slide, which serves the triple purpose of plate-holder, support for the ground glass, and shutter, consists of a square box, provided with two cylinders, on which is rolled a band of black sheet indiarubber completely light-proof. A screw button presses on the spring, which by a pneumatic release sets the cylinders in motion. On drawing a silk thread, the indiarubber sheet unrolls itself on the cylinders and, traversing with great rapidity the free surface of the slide where the sensitive plate is exposed, returns instantly to its first position. The maximum rate of passage of the indiarubber band was 1/20 of a second. Since the shutter is placed at the back of the camera, the shock of its release can only very slightly affect the Microscope.

The author in all his experiments made use of the large Microscope of Koritska, and considers that the apochromatic objectives supplied to him by this maker are in many respects superior to those of Zeiss. The polarization apparatus is disposed as in the Nachet petrographical model. The micrometer screw has a divided head and reads to the 500th mm. with great exactness. For very delicate work the author used the stand (large model) of Powell and Lealand, or a Ross stand with swinging tail-piece for oblique light. When powerful condensing systems were used, a special cell containing a saturated solution of alum was placed beneath the stage, and the rise of temperature was noted by a thermometer.

The indispensable condition for success with a high-power objective, as e.g. a 1/25 immersion, is to have a sufficiently strong illumination. To a bad illumination of the image the author attributes most of the faults usually noticeable in photomicrographs.

(2) Apparatus for the reproduction of consecutive movements of microscopic creatures.—Experiments made by the author with one of the first photographic cameras of Stirn succeeded sufficiently well to induce him to make it the basis of the apparatus which he subsequently employed. This camera consists of a circular box, 2 cm. thick,

having a shutter, with a continuously intermittent release, each movement of which simultaneously caused a circular sensitive plate to advance by a sector. Of this camera only the shutter in the front of the apparatus was retained. This was fixed to the plate of a camera similar to that described in (1). The two special slides designed by the author have a clockwork movement. The one for use with a glass sensitive plate consists of a rectangular box 20 cm. by 20 cm. and 5 cm. deep. At the centre of the box is a metal wheel, put in motion by a clockwork arrangement at the back of the slide, which is provided with an escapement pneumatically regulated. In the metal wheel are fixed the sensitive plates, of the same size as those used in the Stirn camera. This part of the slide is protected from light by a screen, which is placed on a frame in such a way as not to interfere with the movement of the wheel. The screen is pierced with a circular aperture of 5 cm., placed in a line with the aperture of the shutter. The focusing is effected either by Moitessier's method, or by the substitution of a ground glass for the slide. When the slide has been placed in position, the finder, previously described, is fixed by a screw tube in the shutter. As soon as focusing is finished, the slide is charged with the sensitive plate, and successive photographs are taken of the movements of the object on the stage, while it is kept under observation the whole time with the finder. The disadvantage of this slide is that with it only a very limited number of proofs can be obtained. The apparatus devised by the author to remedy this defect can give theoretically 250 impressions of 9 cm. by 9 cm. in a minute. This second slide is a modified form of that of Eastman.* A band of very sensitive negative paper is rolled in turn on two cylinders. A powerful clockwork arrangement placed at the top of the slide effects the movement of the sensitive paper. A lever, pneumatically regulated by a caoutchouc ball, communicates with the spring which sets the clockwork in motion. The rest of the apparatus is in all respects similar to that first described.

The first of the two slides was constructed at Milan by Mr. Oscar Pettozzi, and the second at Genoa by Mr. Ettore Guelfi.

New Flash-light for Photography.†—Dr. Thomas Taylor made an exhibition of his new discovery before the Washington Chemical Society, which, it is believed, will supersede several now in use for photographing at night. The composition consists largely of charcoal made from the silky down of the milk-weed—a form of carbon which he prefers to all others, because of its freedom from ash. A few grains being placed on tissue paper and ignited by a punk match, produces a prompt and blinding flash, while it was observed that the paper on which the powder rested was not even scorched, thus demonstrating the greater security from accidents.

Notes on Photomicrographic Prints exhibited at Meeting of R.M.S. on 19th November, 1890.—Mr. A. Pringle writes as follows regarding the remarks made on his prints by Dr. Dallinger and Mr. E. M. Nelson.‡

(1) Photographs of *B. termo.*—Mr. G. F. Dowdeswell, F.R.M.S., is

* M. A. Londe's 'La Photographie Moderne,' 1888, p. 22.

† Microscope, x. (1890) p. 190. ‡ See this Journal, 1890, pp. 836-7.

responsible for the nomenclature *B. termo*. The organism corresponds with sufficient accuracy—allowance being made for the somewhat vigorous treatment in staining—with the measurements, and also with the behaviour in cultivation, of Cohn. The staining was effected by the method of Loeffler—modified, I understand, by Mr. Dowdeswell—tannin and iron sulphate being used as a mordant. The photographs exhibited, and about ten others not shown, were obtained with an apochromatic 2 mm. glass by Zeiss, projection ocular, and a dry condenser, nominally of N.A. 1, also by Zeiss.

The preparation viewed with the named objective and compensating oculars Nos. 8 and 12, shows the flagella apparently as long and almost as wide as they are shown in the photographs. That is to say, that the flagellum so viewed varies from twice to six times as long as the "body" of the organism, and in some cases the flagella seem to be even longer in proportion to the bodies than six times.

I agree with Dr. Dallinger that, as a rule, *B. termo*, unstained, or slightly stained, or stained without the use of Loeffler's mordant, shows a flagellum about one and a half times the length of the body. Until I saw late preparations by Mr. Dowdeswell, I had never seen flagella nearly so long attached to *B. termo*.

Dr. Dallinger appears to attribute the great prolongation as well as an "extremely rotten" appearance, or "imperfectly defined edge" to imperfections inherent in, or frequently found in, photographic representations of microscopic images. It is difficult to conceive the operations or the optics concerned in photomicrography producing such a very remarkable prolongation of a flagellum as that with which we are dealing. But in respect of the width of the flagellum, much may be said on a certain shortcoming of photography. The phenomenon known as "lateral development" has a marked bearing on photographic images of very minute objects, such as the case in point. The silver is not reduced, I believe, precisely at right angles to the surface of the vehicle-film, there is a certain amount, varying no doubt with the nature and properties of the vehicle, of "spreading" of the silver image through the menstruum containing it, hence (among other reasons) a negative image is never quite so "sharp" as the image projected on the film. This action of lateral development takes place twice in the production of a print; first, in the production of a negative; second, in the operation of printing. Further, I believe that this lateral reduction takes place to a greater extent in printing processes where the image is revealed by development, and I believe that the gelatin processes of photography are more apt to favour this phenomenon than, for instance, albumen processes.

The negatives and the prints exhibited were produced by development processes on gelatin films, and, moreover, the prints were left with surfaces more or less "matt"; and it is probably not stretching any point to say that the fact of the width of the flagellum on the print being at least 50 per cent. greater than if accurately represented it would be, is accounted for by the photographic imperfections I have named. The inaccuracy in the length of the flagella due to these causes is so slight as to be negligible.

Photomicrography in Space.—Dr. Fayel, President of the Société Linnéenne de Normandie, communicated to that Society * a note on this subject which we translate:—"Under the designation of *Photography in space*, Dr. Fayel records a process of his invention which facilitates the observation of opaque objects by the Microscope, even with powerful objectives, and which he thinks will hence render important service. Instead of focusing directly upon the object, Dr. Fayel allows the image to be projected on the ground glass of the photographic camera, and then removes the ground glass and examines the aerial image with a Microscope. In order to reduce the labour of adjusting the Microscope, it should first be focused very near the plane of the ground glass. The image appears so sharp that the minutest relief-forms of the opaque object may be observed by manipulating with the fine-adjustment screw."

(6) Miscellaneous.

Liquid Crystals.†—Prof. O. Lehmann has been able to demonstrate the remarkable fact that three organic substances at certain temperatures, although actually in a liquid state, show strong doubly refracting power, and may therefore be regarded as anisotropic crystals.

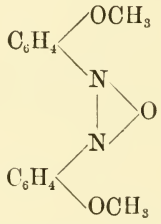
All crystals hitherto known consist of solid aggregates. The author has previously shown, however,‡ that some crystals can be made to flow when subjected to pressure exceeding the limit of elasticity. This has been long known with respect to amorphous bodies like sealing-wax and soft glass. Bodies like these pass, by increase of temperature, continuously out of the solid into the liquid state, i. e. the limit of elasticity gradually diminishes until at a certain critical temperature its value is zero. Beyond this temperature the body is liquid, and the smallest force is capable of causing it to flow. If a crystal then possesses a very low limit of elasticity, it can be made to flow, just as a liquid can, by means of a very slight force. The question therefore arises whether a crystal could not have an elasticity limit zero, and thus be referred no longer to solid but to liquid bodies.

According to the prevailing ideas, which receive their most perfect development in the theory of Soncke, this should be impossible; for this theory supposes that the molecules of crystals form regular point systems, held in position by the elastic force. The author, however, considers that this theory is unsatisfactory when the physical instead of the purely geometrical relations of crystals are considered. If the existence of a crystal as such depend on a regular distribution of the molecules, long continued deformation should at length lead to the production of a body not possessing this regular arrangement, i. e. to an amorphous substance. Experiments, however, made by the author showed that no amount of deformation was capable of converting a crystal into anything resembling in any way an amorphous body. Having regard to this slight correspondence of the theory with fact, the idea of a liquid crystal appeared to be justifiable. One distinction between crystalline and amorphous bodies is the capacity possessed by the former alone of growth in a supersatu-

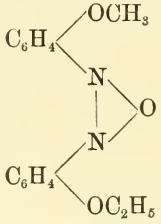
* Bull. Soc. Linnéenne de Normandie, iii. (1888–9), p. 13.
† Pogg. Ann., xl. (1890) No. 7, pp. 401-23.
‡ Zeitschr. f. Phys. Chem., iv. (1889) p. 462.

rated solution. Now almost all known liquids possess this property, so that, to be consistent, they should be considered as crystallized, and, since they are isotropic, as belonging to the regular system. The three organic substances which form the subject of the paper are examples (at present the only ones known) of liquid crystals which are anisotropic. They are:—

1. Azoxyphenetol.
2. Azoxyanisol.



3. Compound of the composition



The following observations apply to all three alike.

The normal form of crystal drops.—Crystals of the substance under examination, placed on the stage of the Microscope under a cover-glass, were melted and then allowed to cool again to the point of crystallization. On again warming and examining between crossed nicols, at a certain temperature (134° for azoxyphenetol, 116° for azoxyanisol, and 87° for the third substance), a sudden transformation into strongly doubly refracting crystals took place. These newly formed crystals preserved the same form as the first, but were seen by pressing down the cover-glass to be not really solid, but liquid. By warming still further, at a definite temperature (165° for azoxyphenetol, 134° for azoxyanisol, and 140° for the third substance), they passed into the ordinary isotropic liquid modification. In order to isolate the liquid crystals a solvent in the shape of Canada balsam was used. By then heating to a temperature above their point of fusion, and subsequently cooling, the crystal drops separated out from the solvent in perfect spheres, which in ordinary light showed peculiar shading effects, most of them like fig. 1 or fig. 2 (Plate V.). These figures really represent the same object in different positions. The shading effect is due to irregularities of refraction, like the "Schlieren" seen in amorphous bodies when examined under oblique illumination by Töpler's "Schlieren-apparatus." *

* Pogg. Ann., 127 (1866) p. 556.

Between crossed nicols fig. 1 appears as fig. 3 * with a black cross, fig. 2 as fig. 4*a* or 4*b*, dark or clear, according as the long "Schliere" is parallel or inclined to one of the directions of vibration of the nicols.

The distribution of the directions of extinction for the individual points of the drop is given by the curves represented in figs. 5 and 6. They correspond to the electric level surfaces and lines of flow in a conducting sphere in which the current enters and emerges at the extremities of a diameter. Regarding a drop as composed of uniaxial crystal particles, the optic axes must be considered as arranged in positions corresponding to the lines of flow, and the equatorial planes as representing the level surfaces. An optically uniaxial crystal in which the limit of elasticity is zero must assume such a form. For the surface tension between crystal and liquid will possess different values on the different crystal faces; but since there is no counteracting elastic force, the molecules will change their positions until the surface tension has everywhere the same value, and that the smallest possible, since the potential surface energy tends to a minimum. The molecules will accordingly arrange themselves so that they all present the same side outwards.

By the use of the polarizer alone indications of dichroism are obtained. Thus, when the long "Schliere" is parallel to the short diagonal of the nicol, the crystals appear colourless and with faint outline; when at right angles, yellow and sharply defined.

Deformation of crystal drops.—By pressing the drop between stage and cover-glass the principal form (fig. 1) changes to that shown in fig. 7. In this the "Schlieren" have given place to a sharp nucleus at the centre and another around the circumference of the disc. Between crossed nicols the appearance is the same as before, except that the quadrants show colour differences. By suitable pressure on the cover-glass the central nucleus can be made to approach the edge and finally to take up a position between the broken ends of the marginal nucleus (fig. 8). By further deformation the two nuclei pass through various transitions until the symmetrical form of fig. 9 is obtained. By then diminishing the pressure until the drop once more assumes the spherical shape, the simple form of fig. 2 is obtained. Thus the total effect of the series of changes has been to turn the drop through 90° about a horizontal axis.

More exact observation with greater magnifying power showed the peculiarity in the spaces between the two nuclei seen in fig. 10. This was found to be due to a rotation of the whole drop in a direction contrary to the hands of a watch. The rotation was so much more rapid, the greater the difference in temperature between stage and cover-glass. On account of the friction of the glass and because the rotating force mainly affected the circumference, the outer layers became distorted with respect to the inner. Fig. 11 represents a much twisted drop produced in this way. Between crossed nicols it showed concentric dark rings with alternating white and yellow ones.

With very rapid rotation the ends of the nuclei bend towards the

* The punctuation in the figures denotes, according to thickness, pale to dark yellow.

centre (fig. 12), and finally a double spiral filling the whole space may result (fig. 13).

Another effect of heat is the production of local rotations about horizontal axes. A portion of the marginal nucleus may thus be drawn into the interior of the drop (fig. 14); a fresh nucleus then forms on the edge, and may follow the first, and so on until the whole surface becomes covered with parallel nuclei (fig. 15).

When a flattened-out drop is very strongly heated, it is gradually dissolved, and, in consequence of the above movements, the thickness diminishes most quickly at the centre. Accordingly a hole is soon formed, and the drop is changed into a ring. At the moment of production of the hole, one or two nuclei form on its edge. The same thing happens when there is an air-bubble in the interior of the mass (fig. 16). For this case the structure lines are represented in fig. 17. They correspond exactly to the electric lines of force in a dielectric, into which an insulated conducting sphere is brought. Effects similar to those produced by heat can be brought about by mechanical means. Fig. 18 represents the effect of the passage of an air-bubble into a large mass, by which the nuclei are drawn out into parallel threads, like the "oligen Streifen" observed with cholesteryl benzoate.

Division of crystal drops.—When an ordinary liquid crystal is cut into two parts, each part forms a new individual with spherical form and nucleus like the original. Fig. 19 *a*, *b*, *c*, shows the division of a crystal by means of an air-bubble; the crystal is first deformed, and then cut into two parts.

Copulation of crystal drops.—In the case of two drops with central nuclei the union of two into one may take place in two ways, either by the two nuclei closing up to the double nucleus in the centre of the compound drop, or by one nucleus being driven off to the edge, while the one-half of the compound drop grows at the expense of the other. The first process is represented in figs. 20 *a*, *b*, *c*, to 23 *a*, *b*, *c*. One of the processes by which two drops with marginal nuclei may unite is explained by figs. 24 *a*, *b*, *c*, 25 *a*, *b*, *c*, and 26 *a*, *b*, *c*. The ultimate form taken by the complete union of two drops need not be the only possible stable one. Quite stable intermediate forms may occur, especially where several drops are united. Figs. 27, 28, and 29 show some examples of the copulation of drops with central nuclei.

With regard to the copulation of *dissimilar* crystals, it was found that any one of the three organic substances could unite with any other. Their properties, however, were so similar that no striking result was obtained.

In support of the contention that optically isotropic liquids should be considered as liquid crystals belonging to the regular system, the author brings forward the following considerations. In the conversion of a substance into an allotropic modification the newly formed crystals generally occur regularly orientated with respect to the earlier ones. Now suppose that in a fused mass of regular crystals this orientating effect acts upon the crystallized modification resulting from solidification, and the latter upon the liquid crystals formed by the fusion. Then by repeated fusion and solidification the visible solid crystals, as well as the invisible liquid ones, should always occupy the same positions,

and be of the same size, provided that internal currents are guarded against.

Some such effect the author obtained, not, however, by conversion of a fused mass into a solid modification, but into a liquid crystallized one. To avoid currents in the fused mass, special care was taken to have everything perfectly clean, and only very slight thickness was given to the liquid layer between stage and cover-glass. In this capillary space one of the three substances was heated until the solid crystals were converted into the doubly refracting liquid modification. This occurred in regular orientation with respect to the solid crystals, i.e. a copy of each in its outline was obtained, but with other interference colours and different directions of extinction. On heating still further, until the doubly refracting liquid was converted into the singly refracting, the field of view between crossed nicols became dark by the widening out of dark circular spots which formed in the doubly refracting mass. The fused mass was then cooled down again, when the doubly refracting liquid crystals again appeared with precisely the same outline and directions of extinction as before. The author considers that this experiment serves to strongly support the idea that non-doubly refracting fused masses are regularly crystallized enantiotropic modifications. That doubly refracting liquids are so rare as to have hitherto escaped discovery, receives some explanation from the fact that with substances having many enantiotropic modifications, the crystal system, with increased temperature, tends to a higher degree of symmetry, and thus finally to the regular system.

On the History of the Invention of Spectacles, Microscope, and Telescope.*—Herr C. Landsberg shows on what uncertain grounds it was that the year 1890 was regarded as the 600th anniversary of the invention of spectacles, and the 300th of that of the Microscope. It is impossible either to fix a precise date for these inventions, or to give with certainty the names of the inventors. The art of cutting and polishing precious stones was known to the ancients, and among the relics of this art we posses lenses, both convex and concave, which are at least 3000 years old, e.g. the plano-convex lens of rock-crystal discovered by Layard in the ruins of Nineveh. It can scarcely be doubted that the men who made these lenses were acquainted with their magnifying power, and in fact made use of it in the execution of those delicate engravings on gems which have been handed down to us. An exact description of the effect of spherically-cut glass is, however, not to be found in ancient literature; but Pliny mentions that the near-sighted Nero looked at the gladiatorial games through a smaragd. The Arabian physician Alhazen (about 1100 A.D.), who was the first to give an exact anatomical description of the eye, showed by his writings that he knew the magnifying effect of a segment of a sphere made of a denser material than the air. Later writers on optics refer to the observations of Alhazen, but add nothing to them. To Roger Bacon (1216–1294), however, much more extensive knowledge is ascribed. He is often credited with the invention of eye-glasses and the telescope. All that can be gathered from his writings

* Central-Ztg. f. Optik u. Mechanik, xi. (1890) pp. 265, 277.

is that he possessed plano-convex lenses and knew their magnifying power ; that he attributed this power to the fact that the lenses made it possible to see objects under a greater angle ; and that he perceived how useful such lenses might be for people with weak sight. There is no evidence, however, to show that he was actually the inventor of spectacles. That honour, it appears, must be divided between Alexander de Spina of Pisa, and Salvino degli Armati of Florence, for an old chronicle of the monastery of St. Katharina, in Pisa, ascribes it to the first. while an inscription discovered on a tombstone in the church of Maria Maggiore, in Florence, gives it to the second. The first authenticated notice of the use of glasses for weak sight is contained in a letter dated 1299, and Jordan di Rivalto, in a speech made in the year 1305, refers to the invention of spectacles as being then scarcely twenty years old. Thus the date of the invention was close at the end of the thirteenth century, but no precise year can be given. For the next three centuries no advance in the theory of optics appears to have been made, and it was not until the beginning of the 17th century that the Microscope and telescope were invented. Italy and Holland both claim the honour of the invention, and each of these nations brings forward different names. There is very little doubt that the honour belongs to Hans and Zacharias Jansen, father and son, glass-cutters of Middelburg. Evidence in support of their claim by the son and sister of Zacharias Jansen, and by Wilh. Borell his friend, is contained in a paper by Pet. Borelius on the invention of the telescope which appeared in 1655. According to the description given by Borell, the short-tube telescopes (Microscopes) made by the Jansens were about $1\frac{1}{2}$ ft. long. The tube, which was about 2 in. in diameter, was supported by three brass dolphins, and had a base of ebony on which the small objects to be examined were laid. The long telescope, or telescope proper, was not made by the Jansens until some time after the Microscope. A rival claimant for the honour of this invention is Lepreg, or Lipperstey, or Lipperheim, another glass-cutter of Middelburg. He certainly did construct a telescope, and was able to exhibit it to a stranger who came to Middelburg (probably about 1608) in order to make inquiries about the new invention ; but whether his instrument was made independently or only in imitation of that of the Jansens it is impossible to say. In Italy, Galileo is generally accepted as the inventor of the telescope, but, as he himself allows, it was not until after he had heard of the Dutch invention that he attempted to construct an instrument for himself. To him is due the credit of being the first to direct the telescope to the heavens; and with its aid in 1610 he made the discovery of Jupiter's satellites. Although the earlier discovered, the Microscope was almost unknown beyond its birthplace at the time when the telescope was in all hands. Thus Cornelius Drebbel, who exhibited an instrument in London in 1621, was looked upon as the inventor, and is so described by Huyghens and many others.

Similarly, in Italy, the Microscope was unknown until about 1624. One explanation of this may be found in the fact that the instruments were then very incomplete. For the long series of improvements, both in the optical and mechanical parts, which has led to the perfection of the instruments of to-day, the present century is mainly responsible.

Microscopes, Microtomes, and Accessory Apparatus exhibited at the Tenth International Medical Congress at Berlin.*—At this exhibition there appears to have been a very fair show of instruments specially adapted for medical and pathological work. Most of the chief firms were represented, the greater number, of course, being German. Novelties were apparently conspicuous by their absence, the exhibitors' claims to inspection being chiefly for thoroughness and effectiveness, such as a Microscope with movable stage and nose-piece with four objectives, and a similar instrument fitted with Mayall's stage.

Microtomes were in full force: besides the commonly used sliding microtomes and freezers, the less known instruments for cutting under water, automatic microtomes, and Altmann's "Support-Mikrotom," were exhibited.

International Exhibition at Antwerp.—A circular letter has been issued regarding the "Exposition de Microscopie Générale et Rétrospective," to be held at Antwerp in August and September next.

The Executive Committee consists of M. Charles de Bosschere, President, Dr. Henri Van Heurck, Vice-President, MM. Edmond Grandgaignage and Gustave Royers; M. Charles Van Geert, junr., is General Secretary, and M. Ferdinand Van Heurck is Secretary.

The Honorary Presidents are Prof. Abbe, Mr. F. Crisp, and M. Nachet. Among others of the Honorary Committee are Prof. Strassburger, Dr. W. J. Behrens, Dr. E. Hartnack, Dr. Rod. Zeiss, and Dr. Sieg. Czapski, from Germany; Sir Joseph D. Hooker, Mr. John Mayall, junr., Mr. Julien Deby, Dr. Maddox, and the Rev. Dr. Dallinger, from England; Dr. Cox, Dr. H. Ward, and Prof. Hamilton L. Smith, from the United States; Dr. J. Pelletan and Dr. P. Miquel, from France; Dr. Engelmann, from Holland; the Abbé de Castracane, from Italy; and Dr. P. T. Clève, from Sweden.

The following is the "Programme de l'Exposition de Microscopie":—

Classe I. *Microscopes pour toutes les recherches courantes.*—A. Microscopes à platine et à sous-platine ("substage") à mouvements mécaniques. Modèles à tube anglais et à tube continental. Microscopes ordinaires pour recherches usuelles. Microscopes à bon marché pour les études élémentaires. B. *Microscopes spéciaux.* — Microscopes binoculaires. Microscopes pour la minéralogie et la pétrographie. Microscopes comparateurs. Microscopes spéciaux pour la photographie. Microscopes renversés. Microscopes de voyage. Microscopes de poche. Microscopes de démonstration. Microscopes à deux ou plusieurs corps. Microscopes pour musées à platine portant de nombreuses préparations etc. Microscopes de projection. Objectifs et oculaires. Objectifs achromatiques et apochromatiques. Objectifs à sec, à immersion dans l'eau, à immersion homogène, etc. Oculaires: de Huygens, de Ramsden, holostériques, compensateurs, à projection. Appareils optiques pour l'éclairage. Condenseurs achromatiques et non-achromatiques.

Classe II. *Appareils d'éclairage.*—Lampes à pétrole. Lampes à gaz. Appareils pour la lumière oxyhydrique. Appareils pour l'éclairage électrique à arc, à incandescence. Piles électriques spéciales.

* Central-Ztg. f. Optik u Mechanik, Oct. 15, 1890.

Classe III. *Appareils pour la photomicrographie.* — Microscopes spéciaux. Chambres photographiques diverses. Photomicrogrammes.

Classe IV. *Appareils divers.*—Appareils binoculaires ajustables à volonté sur des microscopes quelconques. Revolvers; adapteurs; spectroscopes-microspectromètres. Appareils de polarisation. Chambres claires: pour microscope vertical, pour microscope incliné, pour microscope horizontal. Goniomètres, hématimètres, chromomètres. Chambres de culture ("Growing-cells"). Compresseurs. Platines à chariot indépendantes du microscope. Prismes redresseurs, oculaires redresseurs, oculaires binoculaires, oculaires stéréoscopiques. Plaque de diffraction d'Abbe. Appareil à échauffer l'objet sous le microscope. Appareils divers non mentionnés.

Classe V. *Appareils de mensuration* pour l'oculaire, pour la platine; appareils de mensuration pour les couvre-objet.

Classe VI. *Microtomes.*—A mouvements mécaniques, à main. Appareil à diviser pour tracer les micromètres et les tests dites de Nobert.

Classe VII. *Appareils et accessoires pour les préparations microscopiques et les dissections.*—Microscopes simples, doublets, loupes montées.

Classe VIII. *Préparations microscopiques.*—Préparations de toute espèce. Préparations simples. Préparations systématiques. Typen-Platten et Test-Platten.

Classe IX. *Appareils pour la bactériologie.*—Étuves à culture. Étuves à températures basses et constantes. Étuves à stériliser par l'air sec et par la vapeur. Appareils pour la coagulation du sang. Appareils pour la stérilisation des sérums. Boîtes pour désinfecter les instruments et pour stériliser les plaques à gélatine. Régulateurs pour la pression du gaz. Lampes inextinguibles et lampes se fermant automatiquement lorsque la flamme s'éteint. Appareils pour les recherches des microbes dans l'air et dans l'eau. Verrerie pour bactériologie (ballons, tubes, billots, plaques, entonnoirs à eau chaude, crochets, etc.).

Classe X. *Ouvrages de microscopie.*—Traités de micrographie. Ouvrages traitant de toutes les applications du microscope.

Prof. Gilberto Govi.—He was born at Mantua in 1834, and was educated at Turin and Florence, subsequently taking the professorship of physics at the University of Naples. He died on 29th June, 1889. At his funeral the President Brioschi, of the Accademia Reale dei Lincei, referred in high terms to the great capacity of Govi, and to his ardour in historical research in difficult points connected with scientific discovery. He was a frequent contributor to the transactions of the learned societies of Italy, and was particularly versed in the literature of electricity and optics. He made a special study of the labours of Volta, and threw much new light on the varied attainments of Leonardo da Vinci, to whose manuscripts he had access at the Biblioteca Ambrosiana of Milan. Govi's contributions to microscopy, theoretical, practical, and historical, were numerous. Most of his devices were carried out in conjunction with M. Alfred Nachet, the optician, of Paris. His latest historical research was an elaborate paper communicated to the Reale Accademia dei Lincei, in which he sought to establish the invention of the compound Microscope by Galileo; this paper we

translated and published in this Journal, 1889. Govi was elected an Honorary Fellow in 1888.

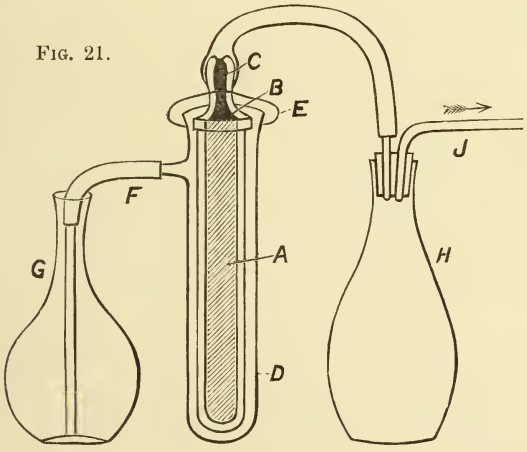
Mr. A. P. Schulze.*—Our readers will regret to see the announcement of the death of Mr. Adolf Paul Schulze, F.R.S.E. and F.R.M.S. Mr. Schulze was a yarn merchant in Glasgow, and made the study of microphotography, microscopy, and optics, the special pleasure of his spare time. Born in 1840 at Crimmitschau, Saxony, he was educated at the Polytechnic of Chemnitz, where he studied engineering, and came to England in 1864, ultimately settling in Glasgow in 1869. He made the subjects above named his special study, and was known from his scientific work to the leading men in all that is comprised in the term "optics," Prof. Abbe, of Jena, being in regular correspondence with him. Perhaps it would be too much to say that Adolf Schulze's life was lived in the wrong place; but for a busy man, in the commercial sense, he did much in the interests of science—so much as to give an idea of what he might have done had it been possible for him to have devoted himself to research.

β. Technique.†

(1) Collecting Objects, including Culture Processes.

Simple Apparatus for filtering Sterilized Fluids.‡—The apparatus invented by Dr. O. Bujwid consists of a Chamberland bougie A, about

Fig. 21.



15 cm. long and 2–3 cm. thick. Upon the top is placed a sort of cover B, through which runs a hole C. These two parts are very easily sterilized with steam or hot air. When required for use, the arrange-

* Engl. Mech., lii. (1891) p. 440.

† 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., ix. (1891) pp. 4–5 (1 fig.).

ment is as seen in the illustration. The filter is placed in a test-tube D, and the air therefrom is exhausted by means of an air-pump. Hence the fluid to be filtered flows from the flask G, through D and C to H. Between the bottom of the porcelain filter and the test-tube, a piece of cotton-wool is placed.

Apparatus for cultivating Anaerobic Microbes.*—Dr. C. Brantz has invented an apparatus for the better cultivation of anaerobic micro-organisms. The apparatus, which is depicted but not described, consists of a holder or receiver for the solution of pyrogallic acid. It is placed beneath the slide and has two apertures, one of which opens into the chamber where the organisms are being cultivated in hanging drops, while the other is for connection by means of a caoutchouc tube with an apparatus for developing hydrogen gas. The receiver is capable of containing 5 grm. of the pyrogallic acid solution.

(2) Preparing Objects.

Method of investigating Development of Limax maximus.†—Miss Annie P. Henchman found that the best way of obtaining embryos was to keep adults, say twenty-five or thirty, in a large tin pail, the cover of which was perforated with small holes. It is best to feed them on cabbage, which affords them a sufficient protection against desiccation, and a place where they may lay their eggs. Care must be taken to keep the vessel clean. Eggs were generally found in the morning, in bunches of from thirty to forty. As they are more abundant in the early stages of confinement, it is better to obtain a few slugs often than many at once. The eggs must be carefully protected from drying. In a moderately warm room hatching occurs between the twenty-second and the twenty-seventh day.

The best agents for killing embryos are either 0·33 per cent. chromic acid or Perenyi's fluid. The chromic material, when well stained with alcoholic borax-carmine, shows the differentiation of nerve-cells and nuclei excellently. Good results for the study of cell-division have also been obtained by staining with Czokor's cochineal. Picro-carminate of lithium is valuable in later stages, as it brings out nerve-fibres, which are stained yellow, while the ganglionic cells are coloured red.

It is best to remove only the outer envelope before killing the embryos, as they are thus less likely to be injured. The inner membrane may be removed with needles after the eggs have been dropped into water to which a few drops of acid have been added. The embryos will be found to be very delicate, and must be handled with great care through every step of the process. Miss Henchman employed only the chloroform method of imbedding in paraffin. The embryo should be carried through the period of heating as quickly as possible, for the embryos are very apt to become brittle if subjected to the heat too long. They should be imbedded within an hour, or an hour and a half, from the time they are first put upon the bath in the chloroform. Paraffin

* Centralbl. f. Bakteriol. u. Parasitenk., viii. (1890) pp. 520–1 (1 fig.).
† Bull. Mus. Comp. Zool., xx. (1890) pp. 171–5.

which melts between 50° and 52° C. is better for imbedding than that which is harder, for in the latter the embryo may be cracked.

Sections from 10 to 15 μ thick, and in the oldest stages even thicker, are better than those that are very thin.

Method of observing Asexual Reproduction of Microstoma.*—Dr. F. von Wagner kept his living specimens of *Microstoma* in small breeding aquaria, 15 cm. long, 10 cm. broad, and 6 cm. high; and he did his best to reproduce the natural conditions of their existence without diminishing the opportunities for observation. A thin layer of mud was spread on the floors of the vessels, and food was provided in the shape of abundance of Daphnids of various sizes. Only a few plants were admitted, and they were, therefore, renewed completely every week. Care was taken to prevent the entrance of any other animals. Notwithstanding all this care, the specimens did not live for more than two or three weeks.

The animals, when required for measurement, must be carefully drawn out with a pipette, placed in a small watch-glass, and measured with an eye-piece micrometer. Great patience is needed.

Various preservative reagents were tried, and a concentrated watery solution of sublimate was found the best. Lang's fluid and a half per cent. osmic acid solution often gave good results. Weigert's picro-carmine was used for staining, and sections 1/100 to 1/500 mm. in thickness were cut.

Examining Bone Marrow for developing Red Corpuscles†—Herr E. Neumann says that phases of the development of the red blood-corpuscles may be observed by obtaining bone marrow in the following manner:—The marrow is squeezed out of some cancellated bone by means of a vice, and a small quantity of this taken up in a capillary tube and placed on a slide. Having been covered, it is examined directly without any addition. By this means good results can be obtained from ribs of human bodies which have been dead for some days.

Study of Contraction of Living Muscular Fibres.‡—M. L. Ranvier studied the appearances of living striated muscular fibres during stimulation by an electric current in the following manner:—The retro-lingual membrane of the frog is stretched over the platinum ring devised by the author, and placed in some indifferent fluid in a moist chamber. Before putting on the cover-glass and closing it down with paraffin, two strips of tinfoil are placed on the slide in such a way that they may serve as electrodes. These movable electrodes receive the current from a bichromate battery, the ends of the wires of which are surrounded by flat lumps of lead. These rest on the tinfoil.

Observations carried out in this way show that when a striated muscular fibre is stimulated, the striation is present during all stages of contraction, and that the contractility of muscle is invariably associated with the contraction of the thick discs, which assume a somewhat spheroidal shape, the thin discs on the clear spaces being unaffected.

In a similar way the contraction of unstriped muscular fibre is observed.

* Zool. Jahrb., iv. (Abth. f. Anat. u. Ontog.) pp. 420–1.

† Virchow's Archiv, cxix. (1890) pp. 385–98. See Zeitschr. f. Wiss. Mikr., vii. (1890) p. 364.

‡ Comptes Rendus, cx. (1890) pp. 613–7 (2 figs.).

And for this purpose the mesentery of *Triton cristatus* is recommended. The smooth fibre requires a greater stimulus than the striated muscle. The difference between the contraction of the two varieties of muscle is merely one of manner and not of kind; the striated muscle contracts quickly, the unstriped slowly.

Examining the Endbulbs of the Frog.*—M. J. Fajerstajn demonstrates the termination of the nerves in the tongue and palate of the frog as follows.

The fixatives used were chromic acid 1 to 400, sublimate 5 to 100, Kleinenberg's solution, Flemming's chrom-osmium-acetic acid, and Carnoy's fluid (alcohol 6 vols., glacial acetic acid 1 vol., chloroform 3 vols.). The sublimate and Flemming's and Carnoy's fluids were the best. The preparations were hardened in alcohol, and imbedded in celloidin or paraffin.

For isolating the cylinder-cells the following method gave the best results: a mixture of 4 per cent. bichromate of potash and 1 per cent. chloral hydrate is made, and in it is placed either a piece of the palate mucosa, or the whole tongue, for 12 to 60 hours. The preparation is then placed under a dissecting Microscope, and teased out in a very weak solution of iodine-green.

For staining sections, several procedures were followed, e. g. sublimate 5 per cent., alcohol, paraffin, alum-carmine, with acetic acid and anilin-blue; or Flemming's mixture, alcohol, paraffin, methyl-green, metanil-yellow or the latter, preceded by safranin, Carnoy's fluid, alcohol, paraffin, dahlia.

Methylen-blue was used very satisfactorily for demonstrating the course of nerves. For this purpose it is advised to inject through the abdominal veins, as thereby the circulation is least interfered with. The injection must be done slowly, after paralysing with curara or anæsthetizing with ether. The solution used by the author was 1 part methylen-blue to 800 parts of a 0·6 per cent. chloride of soda solution.

Preparing Retrolingual Membrane of the Frog to show the junction of Muscular and Elastic Elements, and the natural termination of Muscle Fibre.†—M. L. Ranvier was enabled to demonstrate the connection between the elastic and the muscular elements of the retrolingual membrane in frogs by the following method. It was thereby found that the elastic fibres are attached to the sarcolemma, the two structures being welded together so intimately that mechanical means fail to break the continuity.

The membrane taken from a pithed or decapitated frog is placed for 24 to 48 hours in one-third alcohol. The epi- and endothelia are then removed with a brush; after this the membrane is immersed for 24 hours in a weak solution of methyl-violet, 5B. The preparation is again washed and then mounted and examined in glycerin.

Another histological problem was also resolved from this membrane. What is the natural termination of a muscular fibre? Does it end in a thick disc, a thin disc, or in a clear space? By means of the following

* Arch. de Zool. Expér. et Gén., vii. (1889) pp. 705–50 (1 pl.). See Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 357 9. † Comptes Rendus, cx. (1890) pp. 504–8.

method, the author demonstrated that the ending was in the thick disc. A frog is curarized. The lymphatic sacs are injected to distension with 2 per cent. bichromate of potash or ammonia. Eight or ten days afterwards, the retrolingual membrane is detached, and placed in water until it is decolorized. The epithelium is removed with a brush, and the preparation then stained with fresh hæmatoxylin and alcoholic eosin. The membrane is then dehydrated in alcohol, cleared up in oil of cloves, and mounted in balsam. Thus prepared, the thick discs are stained a bright red, the thin discs present a yellowish-rose colour, while the clear spaces are absolutely uncoloured.

In this way the succession of discs and clear spaces is easily followed right up into the tendon, where the muscle is seen to end as a rose-coloured hemispherical mass, which seems to correspond with a thick disc.

Hence, the author concludes that muscle fibrillæ end at the thick disc.

Examining the histolytic phenomena occurring in the tail of Batrachian Larvæ.*—For paralysing batrachian larvæ, Herr A. Looss prefers the use of an electric current (see this Journal, 1886, p. 700) to curara solutions, or to the pressure of a cover-glass. This method does not affect the histolytic processes which are taking place in the tail of the larva, and the only impediment to observation is the increasing pigmentation. The best fixative was found to be a mixture of sublimate and acetic acid (saturated aqueous solution of sublimate 150 ccm., distilled water 150 ccm., acetic acid 3–4 ccm.). After long washing in water, and having been tested with iodine alcohol to detect any remains of sublimate, the preparations are carefully hardened. For this, Fol's modification of Flemming's chrom-osmium acetic acid is recommended, but Müller's fluid, chromic acid, picric acid, and the mixture of chromic acid and platinum chloride are condemned. Staining was done in toto, in order to avoid damaging the preparation. Picrocarmine gave the best results, but acid-borax, alum and indigo-carmine, hæmatoxylin and anilin dyes were also employed. The paraffin imbedded sections (0·01 to 0·0075 mm. thick) were stuck on with glycerin albumen, and finally mounted in balsam.

For examining the so-called sarcolytes, decomposition-derivatives of striated muscle, Paneth's method was used. This consists in overstaining with picrocarmine, and then, after extraction of the excess of pigment, with hæmatoxylin and then dehydrating. After the sections have been freed from paraffin and stuck on the slide, they are washed with undiluted alcohol (96 per cent. spirit and 2·5 to 3 per cent. HCl). This leaves the hæmatoxylin only in the nucleus, and after thoroughly washing with slightly ammoniacal spirit, in order to remove all trace of acid, the nuclei are seen clearly defined, of a pure blue colour, and lying in a more or less red mass of protoplasm.

Examining the Blood for the Hæmatozoon of Malaria.†—M. Laveran states that the best time for examining malarious blood is

* Preisschr. d. Fürstl. Jablonowski'schen Gesellschaft zu Leipzig . . . d. Math.-Naturw. Section, 1889, 116 pp. and 4 pls. See Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 352–4.

† La Semaine Méd., x. (1890) No. 53. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1890) pp. 15–6.

during the height of the paroxysm, and the patient should not have taken any quinine for some time. The tip of the finger, having been properly cleaned, is pricked with a lancet, and the drop of blood is then placed between two cover-glasses. Fresh blood is best examined by daylight and with high dry powers. The flagella will be seen most frequently on the edges of the pigmented round free corpuscles. If a dry preparation is to be examined, the cover-glasses are drawn apart, the blood allowed to dry, and then the cover-glass is drawn thrice through the flame.

The preparations may be examined unstained, but the author prefers to stain with a saturated aqueous solution of methylen-blue, before using which the cover-glass must be washed with equal parts of alcohol and ether. In this way the nuclei of the white corpuscles are stained dark blue, while the round bodies, either free or adhering to the red corpuscles, are pale blue, and the growing corpuscles scarcely at all coloured.

Hydroxylamin as a Paralysing Agent, or Prefixative, for small animals.*—Dr. B. Hofer recommends hydroxylamin for paralysing small animals, as this substance and its hydrochlorate or sulphate possess a well marked paralysing action on contractile elements.

In commerce it is obtained as the crystalline hydrochlorate; of this a 1 per cent. solution in water is made, and this is then rendered neutral by the addition of carbonate of soda. For dissolving the salt, spring, pond, or sea water must be used, and not distilled water. It is not advisable to have excess of the carbonate of soda, as this renders the solution too strongly basic and also less stable.

The animals having been palsied in this neutral solution of hydroxylamin, the next step is to fix them: for this purpose alcohol, picric and acetic acid, or a mixture of these acids, are recommended, as osmic and chromic acid, sublimate, the chlorides of gold and platinum are too easily reduced. The author gives several special examples of the action of this fluid. It is sufficient to state that it is used in 0·1 to 1 per cent. solution, the most useful strength being 0·25 per cent. From the examples quoted, e. g. *Stentor cæruleus*, *Spirostomum teres*, *Carchesium polypinum*, *Hydra grisea*, *Bunodes gemmacea*, *Dendrocœlum lacteum*, *Hirudo medicinalis*, Rotatoria and Mollusca, it is obvious that this reagent possesses a specific paralysing action on the contractile elements of the lower animals, and that its use as a preliminary to the permanent fixative is a distinct advantage. The length of time needed to produce the paralysing action of course varies with the size of the animal and the strength of the solution.

Preparation of Aleurone-grains.†—M. V. A. Poulsen calls attention to Overton's method of preparing and fixing the aleurone-grains in the endosperm of *Ricinus*. By plunging an absolute alcohol section in an aqueous solution of gallo-tannic acid, crystalloids are made to imbibe the acid and take a brown colour; they are then placed in a 1 per cent. solution of osmic acid, washed in distilled water, and preserved in glycerin. This method depends on the production of metallic osmium

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 318–26.
† Rev. Gén. de Bot. (Bonnier), ii. (1890) pp. 547–8.

in the crystalloids. M. Poulsen recommends also the two following processes:—

(1) Very thin sections of the endosperm are first placed in absolute alcohol for twenty-four hours, and, as soon as they are hard, transferred for an hour to a 25 per cent. aqueous solution of tannic acid, and then washed with distilled water. They are then plunged in an aqueous solution of potassium bichromate until they become brown or yellow. The sections thus made are preserved in glycerin, and show the transparent aleurone-grains with great clearness.

(2) After being hardened as before, the sections are made to imbibe tannin and washed; they are then placed for an hour or less in a 10–20 per cent. aqueous solution of iron sulphate, which brings out a very dark-blue or almost black colour. The sections are then washed and dehydrated in absolute alcohol; and the preparations thus made are placed first of all in essence of clove, and finally in Canada balsam. They are beautifully clear, and very durable.

Reference Tables for Microscopical Work.*—The following continues Prof. A. B. Aubert's reference tables:†—

Gum with chloral hydrate:—Gum arabic, chloral hydrate, water. A cylinder, 60 ccm. contents, is filled two-thirds with gum arabic in pieces; to this is added a solution of chloral hydrate (several per cent.) containing 5–10 per cent. of glycerin; shake often; in a few days the gum will dissolve; the syrupy liquid is filtered. Carmine and hæmatoxylin stained objects can be mounted in this medium.

Gum and acetate of potash or of ammonia:—Gum arabic, acetate of potash or of ammonia, glycerin, water. Made as the preceding medium, only a solution of potassic or ammonic acetate is used instead of a solution of chloral. Anilin-stained objects can be mounted in this.

Iodized serum, artificial (Ranvier):—(1) distilled water, 135 grm.; (2) egg albumen, 15 grm.; (3) common salt, 0·2 grm.; (4) tincture of iodine, 3 grm. Mix 1, 2, and 3, and filter; add 4, and filter again. Used for examinations, not for mounting.

Potassio-mercuric iodide (Stephenson):—Biniodide of mercury, iodide of potassium, water. To the water add an excess of each salt, and filter. This gives a very dense liquid of high refractive index (3·02). For diatoms, &c., may be used diluted.

Monobromide of naphthalin.—High refractive index; for diatoms, &c.

Monobromide balsam:—Solution of hardened Canada balsam in monobromide of naphthalin. Refractive index high, 1·6; shows finer structure of diatoms, &c.

Monobromide tolu. Weir's medium:—Solution of balsam tolu in monobromide of naphthalin. Refractive index, 1·73; may prove very valuable as a medium for diatoms. *Preparation.*—Dissolve 3 oz. of balsam of tolu in 4 fluid drams of benzol, add 4 fluid oz. carbon disulphide; renew this treatment with more carbon disulphide; pour it off again; evaporate the benzol from the balsam tolu. The tolu will now be free from cinnamic acid; put 1 fluid dram of monobromide of naphthalin in 1/2 oz. vial; add enough of the purified tolu to make a stiff mixture or solution when cold. Heat to 104° or 122° F. when using.

* Microscope, xi. (1891) pp. 12–14.

† See *ante*, p. 142.

Pacini's solution:—Sodium chloride, 1 part; corrosive sublimate, 2 parts; water, 113 parts; glycerin, 13 parts. Let it stand three months, then use 1 part with 3 of water; filter before using. Recommended as a preservative of delicate tissues.

Phosphorus (Stephenson):—Concentrated solution in carbon disulphide. High refractive index; difficult and dangerous to use; takes fire spontaneously in the air.

Ripart's solution:—Camphor water, 75 parts; distilled water, 75 parts; glacial acetic acid, 1 part; copper acetate, 0·3 part; copper chloride, 0·3 part. Useful for delicate vegetable tissues, desmids, Confervæ, &c.

Styrax:—Chloroform solution. For diatoms; high refractive index.

American styrax:—Chloroform solution filtered and hardened, Colour as light as that of good balsam; high refractive index; for diatoms and fine tissues.

Harting's corrosive sublimate solution:—Corrosive sublimate, 1 part; water 200 to 500 parts. For blood-corpuscles, &c.

Williams' solution:—Saltpetre, 2 oz.; sal-ammoniac, 2 drams; corrosive sublimate, 1 dram; glycerin, 2 oz.; alcohol, 1 pint; water, 2 quarts. Let stand for several days; filter. More properly a preservative for large anatomical and other specimens.

Wickersheim's solution:—Alum, 100 grm.; saltpetre, 12 grm.; potash, 60 grm.; arsenious oxide, 20 grm.; boiled water, 3000 grm. A preservative of large anatomical and other specimens.

Virodtzeff's solution:—Glycerin, 2160 parts; water, 1080 parts; alcohol, 45 parts; thymol, 5 parts. A preservative of large anatomical and other specimens.

Use of Gelatin in fixing Museum Specimens.*—Herr E. Schmidt recommends the use of gelatin- instead of glass-plates as a basis on which to fix small animals for demonstration. The spirit-specimen is laid on a moistened portion of the gelatin-plate, and is fixed as the gelatin dries, or it is attached by silver thread. Herr E. Weltner describes how small and delicate specimens may be attached to glass plates by means of concentrated (aqueous) solution of fine French gelatin. The spirit-specimens are as far as possible dried from the involved alcohol, and then fixed by the gelatin on a warm glass plate. Sponges, Hydroids, Anthozoa, Ctenophores, Bryozoa, Tunicates, and such delicate animals as *Salpa*, *Ophrydium*, and *Collozoum*, are in this way successfully prepared. The gelatin solution must be concentrated, else it turns white when put into alcohol. For Medusæ and similar organisms, Weltner has adopted the glycerin and gelatin method recommended by List. Gelatin is dissolved in equal parts of glycerin and water; the cold mixture is again dissolved by boiling with about three times as much glycerin and water (again in equal parts); the almost cooled result is spread on a glass plate; on this the spirit-specimen with the alcohol dried off is then laid. A douche of absolute alcohol will hasten the fixing. The objection to the method seems to be that the cementing material turns white when the specimen is returned to alcohol. For the closure of glass vessels, Herr Weltner finds the use of gutta-percha most effective.

* SB. Gesell. Naturf. Freunde, 1890, pp. 95–8.

(3) Cutting, including Imbedding and Microtomes.

Strasser's Ribbon Microtome for Serial Sections.* — Prof. H. Strasser describes a microtome upon which he appears to have expended considerable pains in order to make the sections adhere to the under surface of a specially prepared roll of paper. Hence he calls it the "Schnitt-Aufklebe" microtome.

In the microtome proper there does not appear to be anything new,

FIG. 22.

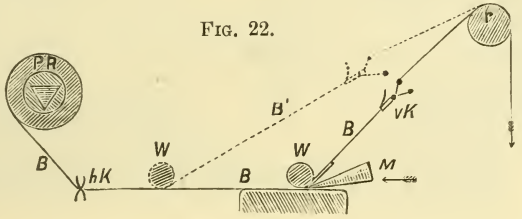
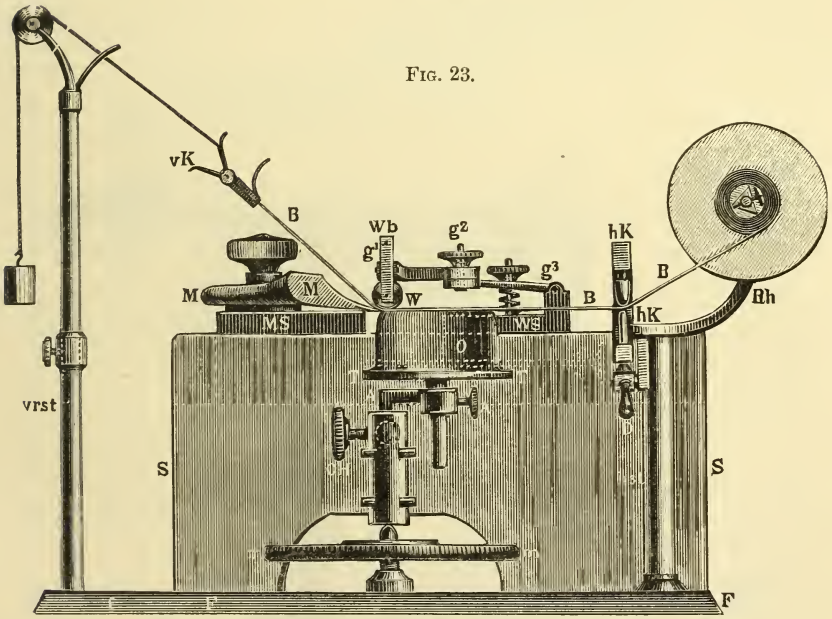


FIG. 23.



as it merely consists of the usual arrangements; that is, there is an object-holder raised by the micrometer-screw, and a knife-holder running on a heavy block in a V-shaped slide-way. The novel details consist in the apparatus for receiving the sections as they are cut off, and the

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 289–304 (5 figs.).

Fig. 24.

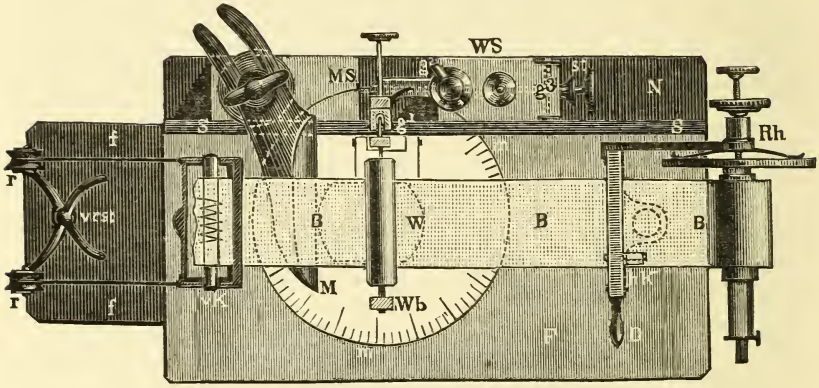
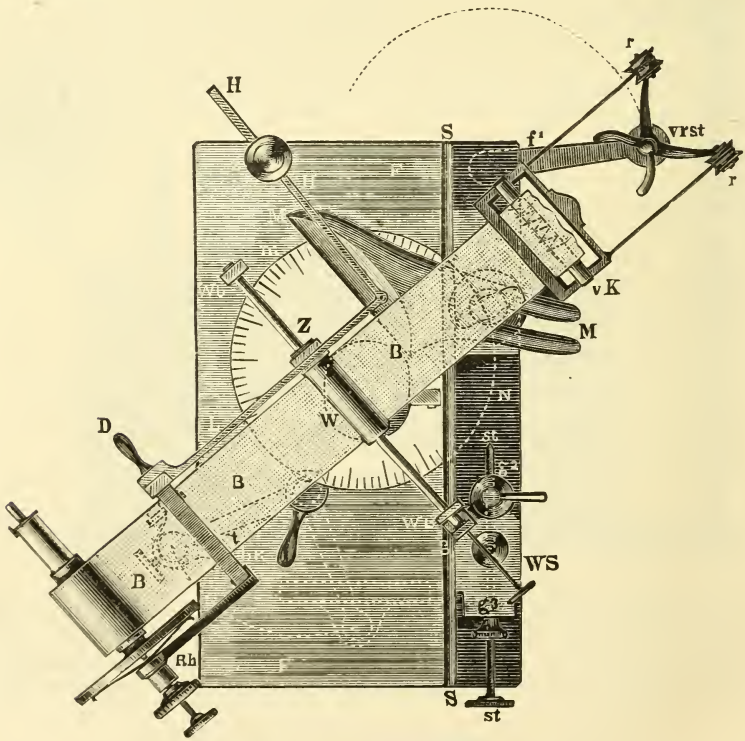
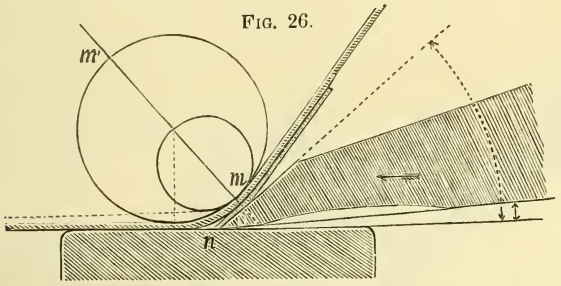


Fig. 25.



knife. The band arrangement is a roll of paper kept taut (figs. 22–25), and passing close to the edge of the knife. The roll P R passes first through a guiding loop *h* K, which gives it its direction parallel to the object. It is kept in this position, and at the same time applied to the edge of the knife M, by the roller W, the diameter of which is 8 mm. The band, after leaving the space between roller and knife-edge, is directed upwards to the clamp *v* K, and there passes over another roller *r*, and to its end is attached a weight for the purpose of keeping the whole quite taut (fig. 23). In order to reduce the friction between the knife, the roller, and the paper band to the practical minimum, the roller W is made as small as possible (8 mm.), and the upper surface of the knife-

FIG. 26.



edge is ground to an angle of 20°–25°. (See fig. 26.) The desired effect is thus obtained, and by using a paraffin of medium softness for imbedding the specimen, the sections show little tendency to break or curl up.

When in actual use, the paper band requires to be removed from the surface of the paraffin block in order to let the knife be put into position for cutting again. (See figs. 22 and 23.) This done, the roller is replaced, and receives after each alteration the necessary tension from a spring. As the sections are cut they adhere to the under-surface of the band. The adhesion is effected by smearing the block surface after every section with a mixture of castor oil 3 parts, and 1 part collodion of double strength. The microtome is made in two forms, as shown in figs. 23, 24, and 25.

In fig. 24 is seen a view from above of the simple construction for the cross position of the knife. In fig. 25 is a similar view of the more complicated apparatus, which allows the knife to be used in any position.

Miehe's Improved Lever Microtome.*—The lever microtome of Gustav Miehe, so called because the knife-carrier is fitted with a handle so that this piece may be easily worked, has been improved by the addition of a spring catch to the microtome screw-plate, so that every division of the plate, and therefore, of course, the rising or descent of the screw, is audibly clicked.

The mechanism of the recent addition is simple. It consists in a catch *m*, held in its place by the spring *n*, which is fitted on the end of an arm *o*, locking in the teeth of the microtome plate. As the pitch of the

* Preis-Verzeichniss von G. Miehe, 1889; Miehe's Catalogue of Microtomes.

microtome screw is 0·5 mm., and as there are 100 teeth on the edge of the plate, one turn equals 0·005 mm. If a section-thickness of 0·03 mm. be desired, the screw *l* is undone, and the circle segment *c* pushed back until the mark 3 corresponds with that on the vernier, after which it is tightened up. The handle *a* is then pushed from the upright *b* to the upright *d*. By this action the catch *m* pushes the micrometer

Fig. 27.

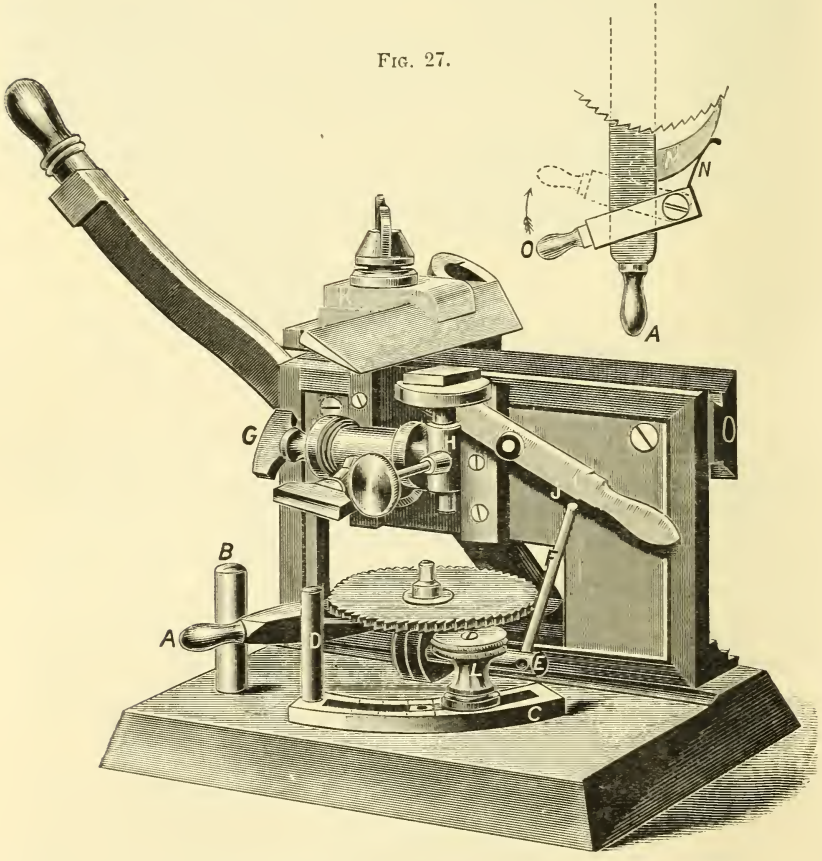


plate round, and the object-holder is thereby raised 0·03 mm. The handle is then pushed back again to *b* and the section made.

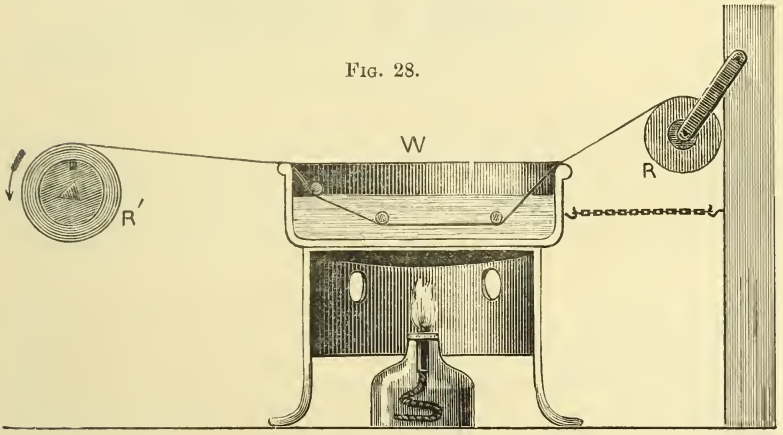
If the preparation has been raised too high, the object-holder is lowered in the following manner:—The screw *e* is unloosed by means of the rod *f*, and then the handle *o* is pushed in the direction of the arrow. This action sets free the catch *m*, so that the preparation-holder is easily lowered by screwing down the micrometer plate, and then pushing down the preparation-holder until its lowest part is in contact with

the uppermost part of the micrometer screw. The screw *e* is then tightened up.

With this instrument the knife may be used either in the cross or in the oblique position. The latter is shown in the illustration; the object-holder is moved to either position by means of the screw *g*.

Treatment and Manipulation of Paraffin-imbedded Sections.*— The principal advantage that celloidin possesses over paraffin is that it is more suitable for the manipulation of large sections. Prof. Strasser has laid himself out to devise means whereby this reproach may be

Fig. 28.



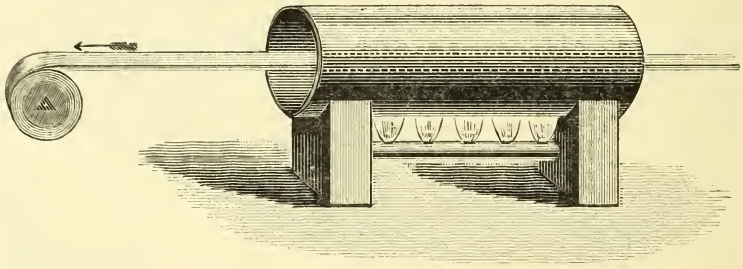
taken away from paraffin. This end may be attained by the adoption of the provisional slide (paper), by using a special form of microtome in which the sections are made to adhere to the provisional slide at the time of sectioning, and by leaving the sections on the provisional slide as long as possible. The provisional slide, which must necessarily be a roll of paper, is prepared either with wax or gum. In fig. 28 is shown the method of saturating the roll with Japanese wax. The illustration perfectly explains the method, and it is only necessary to point out that the roller R' is so far from the immersion tank that the wax is dry in the band before it reaches the roller. The rolls of gummed paper are made by passing the roll through a tank containing in solution gum arabic 50, glycerin 20, and water 100 parts, and then the band is dried by passing it through a tube heated underneath by a series of gas-jets (fig. 29).

The sections are then stuck on by means of an adhesive made of collodion and castor oil. This procedure is facilitated by the use of Strasser's "Schnitt-Aufklebe" microtome. After the bands or sections have been carefully numbered, they are covered with an adhesive composed of 2 parts collodion and 1 part castor oil, after which they are deposited in a turpentine bath, in order to dissolve the paraffin, and at

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 304–17 (2 figs.).

the same time to harden the collodion. From this point we found it somewhat difficult to follow accurately the author's diffuse directions,

Fig. 29.



but it seems that the continuation of the procedure is as follows:—Immersion in pure benzin, then in 95 per cent. spirit, then in thick collodion. After this they are stained, and thereupon cleared up, first in 70–80 per cent. spirit, and finally in carbolxylol. The sections are in the end mounted definitely in balsam, or provisionally in paraffin.

(4) Staining and Injecting.

Metallic Impregnation of the Cornea.*—Prof. F. Tartuferi says that the fixed cells of the cornea, even to their most delicate prolongations, may be deeply stained by immersing the cornea of some adult animal (ox) in a solution of hyposulphite of soda (15 grm. to 100 of distilled water) for three days or longer, and keeping it at a temperature of about 26°. The preparation is then placed in a vessel containing finely powdered chloride of silver and a little pure water, for two days or longer.

If the adult cornea be treated in this manner for a still longer period, or if the cornea of a young animal be used, these fixed elements are but imperfectly visible, but other details are brought out, for example, numerous elastic fibrillæ; while by further variations of the foregoing method the isolated elastic fibrillæ of the cornea may be obtained. The preparations are quite permanent.

Staining Medullated Nerve-fibres with Hæmatoxylin and Carmine.†—Prof. N. Kultschitzky now gives more complete details of his method for staining sections of the central nervous system.‡ The material is hardened in Erlitzki's fluid for one or two months, and is then placed in running water for one or two days. It is next hardened in alcohol and imbedded in celloidin. The sections obtained in this way are stained with the hæmatoxylin solution (1 grm. hæmatoxylin dissolved in a small quantity of C_2H_6O and 100 grm. of 2 per cent. acetic acid). The staining is effected in from one to three hours. The sections are then placed in a mixture of 100 ccm. of saturated solution of lithium carbonate, and 10 ccm. of 1 per cent. solution of red prussiate of potash.

* Anat. Anzeig., v. (1890) pp. 524-6. † T. c., pp. 519-24.
‡ See this Journal, 1890, p. 115.

When sufficiently decolorized (two to three hours) the sections are thoroughly washed and then mounted in balsam.

For staining sections with carmine, the author uses a stain made as follows:—Powdered carmine is boiled for two to four hours in 10 per cent. acetic acid; for every 100 ccm. of acetic acid, 2 grm. of carmine are required. After cooling, the solution is filtered. In this acetic carmine the sections are immersed for twenty-four hours, after which they are decolorized in the lithium and prussiate of potash solution. As this is rapidly effected, the sections must be, at the proper moment, removed to distilled water and thoroughly washed therein, after which they are mounted in the usual manner.

Kultschitzky's Nerve-stain.*—Dr. J. Schaffer relates his experience of this method and his improvement thereon. This consisted in removing some of the stain from sections over-coloured in acetic-hæmatoxylin by means of borax-ferridcyanide of potassium solution. As to the previous preparation of the tissue by means of chromic acid, Erlicki's or Müller's fluid, Schaffer explains that the myelin of the medullary nerves has the strongest affinity for chromic acid and its salts, that in washing out there is a stage at which the chromic acid or salt has been removed from all the tissues except the medullary sheaths of the nerves, and that this is the moment for staining with hæmatoxylin.

Staining the Motor Nerve-cells of Torpedo.†—G. Magini, in studying the different positions of the caryoplasma and of the nucleolus in motor nerve-cells, obtained the best results in an examination of the electric lobes of the Torpedo by staining with Weigert's hæmatoxylin, after-staining with safranin, and then decolorizing with ferrocyanide of potash, and also by staining with methylen-blue in 1/10,000 KHO, and after-staining with safranin. The latter method produced especially fine preparations, the body of the cell being violet, the caryoplasma red, and the nucleolus blue.

Fixing and Staining Glands of Triton helveticus.‡—M. Heidenhain, in studying the histology of the cloaca and its glandular adnexa in the Triton, proceeded as follows:—Fixing was best done in picric acid or concentrated sublimate solution. For hardening, alcohol gradually increasing in strength until it became absolute. The specimens were stained with alum-carmine, and then treated with picric acid-alcohol, or aqueous solution of pure hæmatoxylin, and then mordanted with 1/2 to 1 per cent. alum solution.

When stained in sections stuck on with alcohol, or by Schällibaum's method, anilin dyes, acid fuchsin, methyl-green, orange were used, and these combinations with sublimate fixation produced excellent results.

Fixing, Staining, and Preserving the Cell-elements of Blood.§—Dr. H. Griesbach deals chiefly with the blood of Mollusca, although a few remarks are devoted to the blood-corpuscles of Vertebrata. As a fixative, the author has the highest opinion of the value of osmic acid.

* Anat. Anzeig., v. (1890) pp. 643–5.

† Atti R. Accad. dei Lincei Roma, vi. (1890) pp. 466–7 (2 figs.). See Zeitschr. f. Wiss. Mikr., vii. (1890) p. 356.

‡ Arch. f. Mikr. Anat., xxxv. (1890) pp. 173–274 (4 pls.). See Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 356–7.

§ Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 326–32.

The blood on the cover-glass may be exposed to the action of the vapour, or the acid (1 per cent. solution) may be mixed with it thereon, or it may be dropped into a watch-glass full of the acid. If the last method be adopted, the procedure may further be improved and simplified by mixing some pigment with it, so that the blood is at once stained and fixed. For this purpose the most useful dyes are methyl-green, methyl-violet, crystal violet, safranin, eosin, acid fuchsin, and rhodanin. In all cases a saturated aqueous solution of the pigment is mixed with a 1 per cent. solution of osmic acid.

For fixing and imparting a double stain good results were obtained from rhodanin and methyl-green. These pigments are to be dissolved separately and then added to the osmic acid. A blue-red fluid results, which stains the cell-body red and the nucleus green. Besides osmic acid, picrosulphuric acid, chrom-osmium-acetic acid, and gold chloride are favourably alluded to as fixatives.

For preserving specimens of fixed molluscan blood, resinous media are not suitable, the best material for the purpose being glycerin, which mixes easily with the before-mentioned fluids, and also keeps the colour fairly well. Permanent preparations are made by running a thin border to the cover-glass with some oil-colour (Cremser white), so as to prevent any pressure on the cell-elements, and also to keep the glycerin from exuding. Some glycerin is placed on the middle of the cover-glass, and to this is added the mixture of the blood and fixative and the whole carefully mixed. The cover-glass is then carefully laid upon a slide and ringed round.

Staining Terminations of Tracheæ and Nerves in Insect Wing Muscles by Golgi's Method.*—By the application of Golgi's method to the muscles of insects, Prof. S. R. Cajal obtained some unexpected results. It was found that the tracheæ in the feet and wings (the non-dissociable muscles) terminate in two horizontal networks, while in the dissociable muscles only one such network was demonstrable. The method also showed the termination of the nerves in the muscle-fibres as a system of delicate filaments, some of which were disposed upon and others beneath the sarcolemma. The technique is as follows:—Pieces 3–4 mm. thick are cut from the wing muscles and immersed for 12–24 hours in a mixture of osmic acid and potassium bichromate (1 per cent. osmic acid 5 parts, 3 per cent. bichromate of potash 20 parts). They are next placed for 24 hours in 0·75 per cent. nitrate of silver solution, after which they are treated with 40 per cent. alcohol. Thus prepared, the pieces are teased out and then again washed several times in spirit; after this they are cleared up in oil of cloves, passed through oil of turpentine, and then mounted in the usual way. For obtaining transverse sections, the muscle may be placed in elder-pith and so cut up, or it may be imbedded in paraffin and sectioned.

The black reaction in the tracheæ is always constant, but the staining of the nervous tissue is less certain, so that it is advisable to make a good number of preparations.

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 332–42 (1 pl. and 3 figs.).

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

Can mounting media be improved for high powers by increasing the index of refraction?*—In answer to this question, Mr. J. D. Beck writes:—"It has been the aim of the microscopist to increase the refractive power of mounting media for diatoms, bacteria, biological and other specimens requiring a high amplification and the best resolution. Whether better results are attainable in this direction I am unable to say. All my diatoms, slides from J. D. Möller and others, are mounted dry or in balsam; I have never tried Prof. Smith's medium. If the increase in refraction is an improvement, would it not be a desideratum to attain still more satisfactory results, which perhaps might be accomplished by increasing the index of refraction of mounting media? The desideratum is to see what exists, and to secure for that the most favourable means, bearing in mind that we must not expect too much from the best lenses under unfavourable conditions or circumstances. A certain quack condemned my Beck's 1/6 in. objective because with it and a Beck's No. 2 ocular he could not see bacteria in spring water, when in fact the water, which was cold as ice, came out of a mountain of rocks so free of vegetable and organic matter that no organisms could live in it, while a drop of water from a rivulet showed thousands of bacteria under the same lens.

Insomuch as a large majority of microscopists cannot afford to buy the new Zeiss apochromatic objectives, they may perhaps increase the resolving or defining powers of the lenses of a cheap grade by improving the refractive properties of mounting media. While the philosophy of the Irishman, that "if a little is good, more is better," when he imbibed the second glass, may be rather extravagant in such cases, yet it may be solid philosophy for practical purposes in other directions; so then, may we not continue to experiment on media to increase the refractive power until we find still more satisfactory results?

A friend of mine copied and sent me a list of refractive indices. The highest index of fifty substances given is that of chromate of lead at 2·50 to 2·97. It would appear that all the salts of lead and zinc have a high index of refraction, which seems to be very much increased by the action of chromic acid, which probably exists in the metal chromium in a higher degree than in lead or zinc. I do not believe that nitre, which combines with chromium to form chromate of potassium, afterwards changed to bichromate of potassium through the action of sulphuric acid when exposed to acetate of lead, really increases the refraction of chromate of lead. I have my doubts whether the acetate of lead adds any refractive power to the bichromate of potassium. Native sulphur is given at 2·115, but when distilled with charcoal and reduced to a volatile spirit by adding one atom of carbon to two atoms of sulphur, forming bisulphide of carbon, the index is reduced from 2·115 to 1·678. This is what the carbon has done, and yet diamond, which is carbon crystallized, is way up to 2·47 to 2·75. I suppose it would be impossible to bleach and to reduce the chromate of lead to a colourless medium without destroying its high refraction. We might expose colourless linseed oil to the action of chromate of lead by heat,

* Microscope, x. (1891) pp. 18–20.

and when well settled filter a number of times, or clarify it as varnish is clarified. This would become a rapid drying medium *per se*. Resins might be treated with chromate of lead in the same manner. Whether this suggestion is practical I will leave for others to decide who have more experience and skill in chemistry than I.

What can be done with sulphur and phosphorus? Can we dissolve sulphur in oil and make a transparent medium of it?

There are phosphorus, 2·224; carbonate of lead, 1·866; oil of anise seed, 1·111; bisulphide of carbon, 1·678—all pretty high—what can be done with them? There may be other substances higher and better than those mentioned. How many will act in this important matter?"

Useful Mounting Menstruum.*—Dr. Alfred C. Stokes writes:—"In a recent number of 'Malpighia' M. Aser Poli called attention to the oil of cajeput as a valuable medium in which to place objects before their permanent mounting in Canada balsam, it being used as a clearing agent instead of the oil of cloves. He states that it is soluble in dilute alcohol, and thus permits of the direct transfer of the object to it, thereby avoiding the use of absolute alcohol. He also remarks that trials with the oil have been followed by beautiful results, the preparations being perfectly clear, and that delicate objects such as the marine Algæ, which are among the most difficult to preserve in a satisfactory way, are, when treated with the oil of cajeput, almost entirely free from the ordinary obnoxious shrinkage.

These qualities are all excellent ones, and by the microscopist that does but little work in mounting, the chance to simplify the operation should be hailed with joy. To do away with one of the processes that modern methods seem to consider necessary will be a boon. By the use of the oil of cajeput the worker can simplify his methods by discarding the absolute alcohol, and thus not only save himself considerable trouble and some time, but some expense, as an object cleared or soaked in oil of cloves cannot well be transferred from it to balsam without the intervention of absolute alcohol.

After having been cleared or soaked in the cajeput oil, the object may at once be mounted in the ordinary balsam, or in that dissolved in benzol or in chloroform. Absolute alcohol must be kept in a specially prepared bottle, as it evaporates rapidly and absorbs water greedily. To avoid its use is pleasant indeed.

Since reading M. Poli's account of the action of the oil I have been making a few experiments, and refer to them here in the hope that some that in their microscopical work have more need of mounting than I, will take the subject, continue the experiments, and report the results.

In my limited experience I have been pleased with the oil. It has a pleasantly aromatic odour and pale-green colour that are in no way objectionable.

Placed on a glass slip it evaporates, but not with such haste that the microscopist must hurry his movement to do as he would before it is gone; it evaporates somewhat slowly, and leaves no trace on the glass.

* Microscope, xi. (1891) pp. 4-6.

It is soluble in carbolic acid, or the commercial liquid acid as obtained of the druggist is soluble in it. With old benzol balsam that had become so hard and so nearly dry in the bottle that it had to be dug out with a knife in a stringy mass, the oil mixed perfectly, making the old material fluid and easily worked. What its action would be with benzol itself I can only infer from this experiment. In dilute alcohol it is, as M. Poli has said, perfectly soluble.

After evaporating Canada balsam to glassy hardness in the ordinary way before dissolving it in benzol or in chloroform, I dissolved it in the oil of cajeput, to learn what would be the result. This I found to be excellent. The hard balsam dissolves readily in the oil, and makes as thick or as thin a fluid as may be wanted. The solution, however, although readily effected, appears to take place with rather less facility than with benzol or chloroform. Still, it is accomplished by leaving the mixture to itself, the solution being made without attention on the part of the microscopist.

The results of mounting in the cajeput balsam justify all the good words that M. Poli has spoken of the oil as a clearing medium. After the object has been soaked in dilute alcohol for a convenient time, it is transferred to the oil of cajeput for as long as the microscopist may wish, and thence to the cajeput balsam in which it is to be mounted.

Under the cover-glass drying seems to be as rapid as with benzol balsam; the little that is unavoidably spread on the slip appears, however, to harden rather more slowly, yet I have made no comparative test. The effects of the mounting medium are excellent; as far as I can perceive, quite as good as those from benzol or chloroform balsam; and the simplifying of the process should be greatly in its favour with those that are not professional preparers, and are therefore not ready to give any amount of time and attention to their special work.

I have not tried it with staining fluids. This I must leave to others. M. Poli, however, in the note already referred to, says that objects treated with it, stained green and then mounted in Canada balsam, retain their colour. Further than that nothing is known about this part of the subject.

The reader will perceive that my experiments have been few and of little importance. I mention the matter only because I believe the menstruum will prove to be an exceedingly useful one, especially to the amateur, to whom the simplifying of the process and the avoidance of the use of absolute alcohol should certainly make it acceptable. The suggestion is not original with M. Poli, as the oil has been used by others and referred to in print, but has never come into general use as it should.

(6) Miscellaneous.

Desk for Microscopical Drawing.*—Dr. Giesenhagen has devised a desk or framework for microscopical drawing which is very easy to manage. The construction of the apparatus is easily understood

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 169–72 (2 figs.).

from the accompanying illustrations. It is made of wood, and the drawing surface can be altered to and fixed in any desired position with ease. It is scarcely necessary to observe that it is intended for camera drawing.

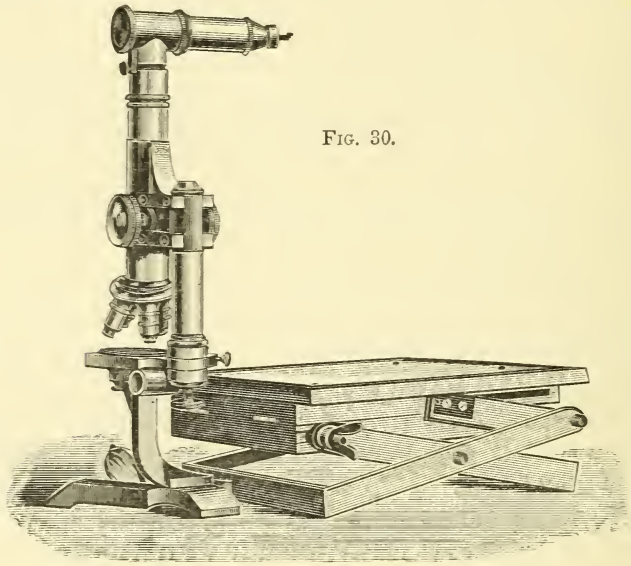


Fig. 30.

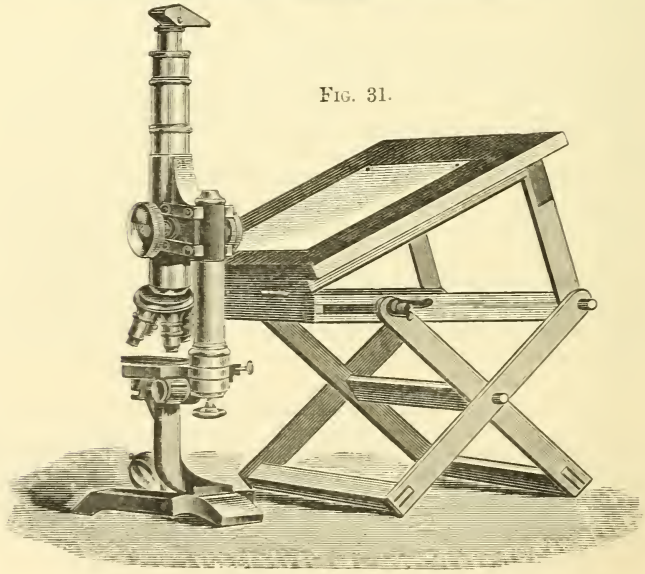


Fig. 31.

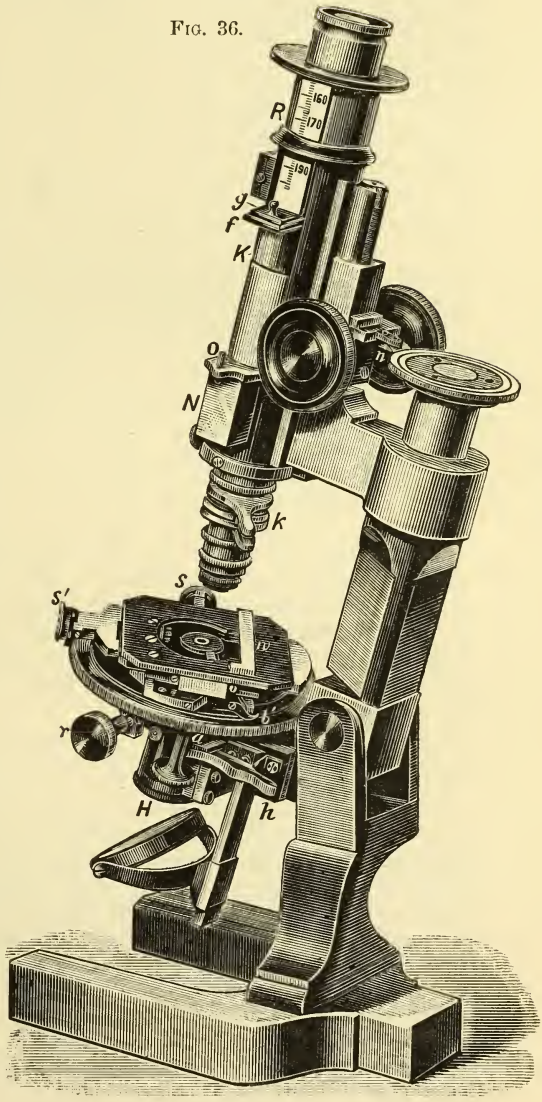
MICROSCOPY.

a. Instruments, Accessories, &c.*

(1) Stands.

Fuess's Petrological and Crystallographic Microscopes.† — Herr R. Fuess has introduced several improvements into his Petrological Microscope, which now has the form given in fig. 36.

Fig. 36.



The object-stage is fitted for fine measurements. The scale is graduated in half degrees and two verniers read to minutes. The stage-plate has a toothed edge and is rotated by means of a pinion *a*, which can be thrown out of gear by the lever *h*. A mechanical stage is applied on the rotating stage-plate. By one of the rectangular movements effected by the screw S an interval of 0·01 mm. can be indicated. The other screw S′ has a more rapid thread to enable the preparation to be quickly passed across the field of view. The mirror slides vertically on an arm which can be rotated to one side. The polarizer has a rack-and-pinion movement. A conical stop fitting into corresponding slots in the socket determines the position of the nicol for 0°, 45°, and 90°. An iris-diaphragm can be inserted beneath the nicol. A condensing lens of great focal length atttached to the polarizer serves for 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.

† Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 177–87.

illumination of the preparation when the lower objectives are used. This lens forms the lower member of the condensing system, which consists of three lenses, the other two being connected together but detached from the polarizer. The common socket of the two upper lenses is supported in a ring which forms the end of the arm *b* of a rotating plate fitted in the object-stage. By means of a weak spiral spring in the ring-holder, the socket follows the movement of the polarizer, so that the whole condensing system can be adjusted by the pinion which effects the movement of the polarizer. By a second arm *b'* from the rotating plate of the lens-holder, the upper pair of lenses can be moved to one side beneath the mechanical stage, so that the change from convergent to parallel light and *vice versâ* can be rapidly effected without moving the preparation.

The coarse-adjustment of the Microscope is by rack and pinion. The fine-adjustment screw has a pitch of 0·5 mm. The head is divided into 100 parts, and a vernier reads to a fifth of a division, i. e. to 0·001 mm. The end of the body-tube carrying the objective is movable by two fine screws for centering. To facilitate the change of objectives, the latter are not screwed on, but held by the clamp *k*. Immediately above the clamp is a slit for the introduction of a Klein's plate, quarter-wave plate, &c. The analysing nicol N is inserted in a wider opening at the lower end of the body-tube. Another opening K serves for the introduction of the auxiliary objective into the draw-tube R. The lens is fastened in the slide *f*, and forms with the Ramsden eye-piece a complete Microscope, with a magnification of about five times. This constant magnification is advantageous for measuring the apparent optic axial angle. The draw-tube carries a millimetre scale which gives the distance of the eye-piece from the objective. Among the accessories of the Microscope are the illuminating apparatus and spectropolarizer of Abbe, the twin-nicol for stauroscopic measurements, and the illuminating arrangement of Sorby which serves for the observation of the internal and external conical refraction.

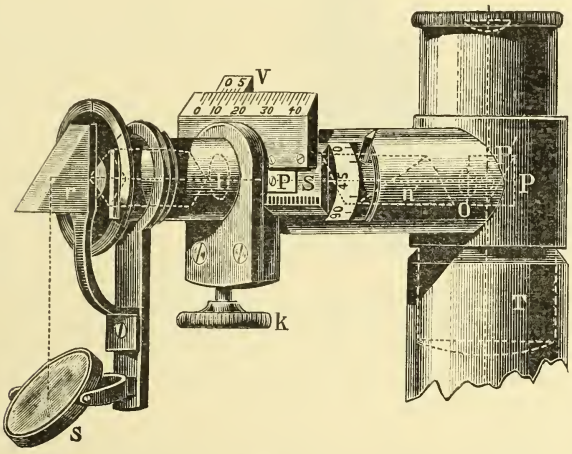
Of the special eye-pieces, the goniometer eye-piece consists of a Ramsden eye-piece which is directed upon cross wires centered by four adjusting screws exactly in the axis of a divided circle.

The quartz-wedge comparator shown in fig. 37 is a modified form of that of Michel Levy. It slides in the tube T of the Microscope, and in this part consists of an ordinary weak eye-piece, in which, in the place of the usual diaphragm, is a double prism of glass P P', with the hypothenuse faces cemented together. The hypothenuse P' is silvered with the exception of a small circle in the centre, through which the polarization tint of the preparation is seen. The main part of the comparator is contained in the side tube. Rays from the mirror *s* are diverted at right angles by the prism *r* into the polarizer *n*, the rotation of which can be measured on a divided circle. The lens *l* concentrates the light upon the quartz wedge *q*, behind which is a diaphragm with a very small aperture. The quartz wedge is fastened in the slide S', which is moved by rack and pinion, and its position is given by the vernier V. The light passes through the analyser *n'* to the lens *o*, and is reflected from the hypothenuse of the prism P' in the direction of the axis of the eye-piece. The polarization tint of the preparation is thus seen

surrounded by that of the quartz wedge, and a ready comparison can be made.

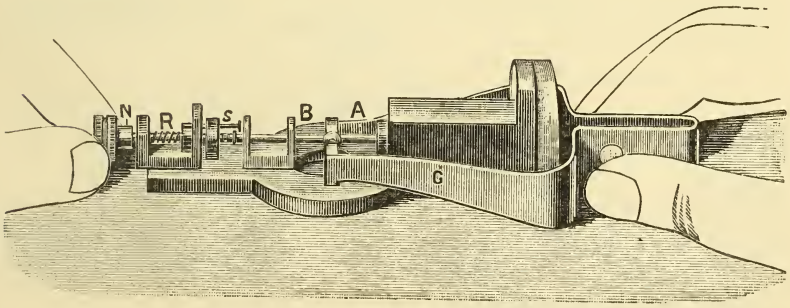
The axial angle apparatus for very small plates, shown in fig. 38, is similar to the Schneider-Adams apparatus, in which two small hemispherical lenses inclosing the plate can be rotated between condenser and

Fig. 37.



objective. The base-plate is held on the stage by spring clips. Above this plate is the horizontal axis, carrying at its outer end a divided circle, with vernier reading to five minutes. The spindle at the other end of the axis A reaches nearly to the middle of the apparatus, and

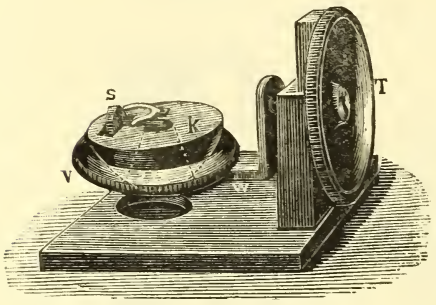
Fig. 38.



is funnel-shaped at its end. The cylindrical bolt B, also funnel-shaped at the end, is movable by the screw R in the direction of the axis. By left-turning of the mother screw N, a spiral spring drives forward the screw shaft, whose end has a funnel-shaped depression in which the end of the bolt fits. By right-turning of the screw the shaft is drawn

back and with it the bolt, by the pin *s* coming in contact with the small projecting plate on the bolt. The two hemispherical lenses are held between the funnel-shaped depressions of bolt and axis. By pressure of the spiral spring the bolt is kept centered with respect to the axis, while it can move freely at both ends so that it rotates with the axis when the latter is turned.

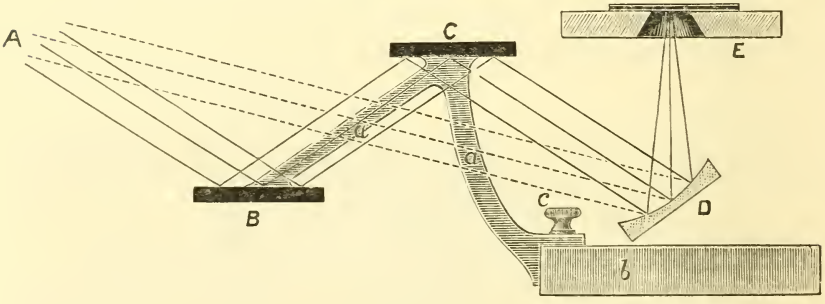
Fig. 39.



The goniometer for microscopic crystals (fig. 39) consists of a base-plate, on which is an upright carrying the divided circle T movable about a horizontal axis. The angle arm *w* ends in a ring which is parallel to the axis of the divided circle, and supports a second ring *v* movable in it. The hemisphere *k*, movable with friction in the ring *v*, has a conical opening bored through it, with the narrow central aperture in the flat surface. Along a radial groove runs the needle, at the point of which the crystal to be measured is fixed. It is kept in position by a spring, and is rotated by the milled head S.

Some Improvements in the Crystallization Microscope.*—Prof. O. Lehmann remarks that the old form of crystallization Microscope, described in Zeitschr. f. Instrumentenk., 1886, p. 325, suffers from the disadvantage that it is impossible to observe the preparation between crossed nicols during the heating. The method which first suggests

Fig. 40.



itself for obviating this difficulty, that of placing the polarizing nicol before the mirror, is unsatisfactory, owing to the large size of the nicol required.

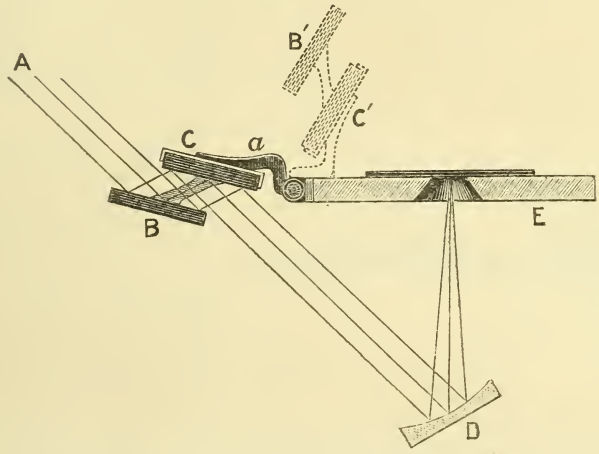
Fig. 40 represents the arrangement proposed by the firm of Zeiss, in which the polarizing nicol is replaced by two piles of glass plates.

* Zeitschr. f. Instrumentenk., x. (1890) pp. 202–7.

Light from the source A, incident at the polarizing angle on the first pile of plates B, is reflected to the second pile C, and thence to the mirror D. The two reflectors are fastened to the frame *a a*, which is firmly fixed to the foot *b* of the instrument by the binding screw *c*. A very slight turn of the mirror is sufficient to direct the unpolarized rays, represented by the punctuated lines in the figure, upon the object, so that the change from ordinary to polarized light and *vice versâ* is very easily effected.

Fig. 41 shows a similar arrangement made at the author's suggestion, by O. Behm, of Karlsruhe. The frame carrying the two reflectors B and

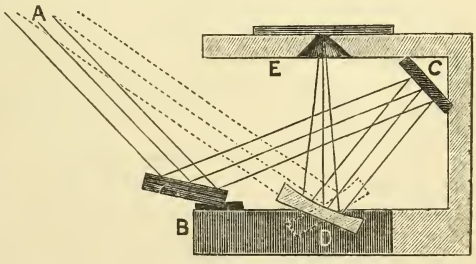
Fig. 41.



C (each consisting of five large cover-glasses) is hinged to the edge of the object-stage E, so that for observation in ordinary light it may be swung back into the position B′ C′.

The arrangement shown in fig. 42, due to Voigt and Hochgesang, of Göttingen, is considered by the author as the most efficient. Rays from the source A fall upon the polarizing reflector B, and thence almost perpendicularly on an ordinary mirror C, from which they are reflected to the concave mirror D. Observation in ordinary light is effected by turning the latter into the position punctuated in the figure.

Fig. 42.



The heating apparatus of the Microscope has been simplified. For ordinary requirements a burner with small non-luminous flame is used

to replace the inconvenient blow-pipe arrangement of the old instrument.

Fig. 43 represents the burner used when only slight changes of temperature are needed. It gives a very small blue flame, and is itself so small as only slightly to interfere with the brightness of the field of view. In the other form of burner (fig. 44), used for higher temperatures, the gas issues from a ring-shaped slit. It is closed beneath by a thin plate of glass or mica, so that the flame is driven towards the centre by the draught thus produced. The brightness of the field of view is not sensibly affected by the passage of the rays through this transparent plate and the thin layer of burning gas within the ring.

Fig. 43.

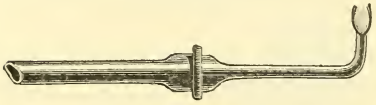
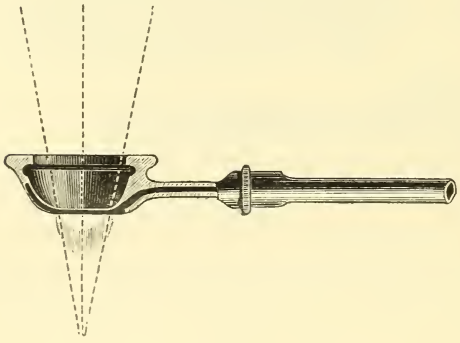


Fig. 44.



As a further improvement of the Microscope, the divided circle is enclosed in the object-stage, so that it is protected from dirt and injury from acid vapours, &c. The scale is read directly from above by means of a window in the upper side of the stage.

For experiments at very high temperatures, the larger instrument described in the Zeitschr. f. Instrumentenk., 1884, p. 369, is necessary. Into this several improvements have been introduced. In order to effect a greater concentration of heat upon the object, and to diminish the heating of the metal parts of the Microscope, the blow-pipe flame is directed through a chimney of asbestos, bound with brass, which can be fitted into the opening of the stage. A new form is given to the water screen protecting the objective. It consists of a socket 2 cm. long, with double walls and strong copper base, which fits tightly over the objective, and expands at its upper edge into a disc of about 5 cm. diameter. To prevent the condensation of water upon the objective, an arrangement is added by which a stream of air is directed upon it.

Improvements have also been made in the Projection Microscope described in this Journal, 1887, p. 291. The indiarubber tubes of the old instrument are replaced by metal ones. For cooling the alum solution, a spiral tube conveying a stream of cold water is used instead of the water screen. The mirror is not rigidly fixed as before, but can be turned about a hinge and fixed by a binding screw several degrees from its normal position of 45°. By this means a uniform brightness can be maintained when, by changes in the electric arc, the illumination of the field of view has slightly shifted. For cooling the

preparation there are three small tubes in the stage, by which a stream of air can be directed upon its under side. Instead of the black cover of the old instrument, two screens are found to be sufficient to prevent the dispersion of the light. One of these is hinged to the holder of the totally reflecting prism, while the other is fixed horizontally above it. The prism is made of a specially strongly refractive glass of the firm of Zeiss, since by the use of ordinary glass a part of the field of view is cut off by total reflection.

Remarking on this paper,* Herr R. Fuess takes exception to the remark, that the old form of instrument, whose construction was undertaken by the firm of Fuess, has the great drawback that it is not possible to observe the preparation between crossed nicols during the heating. To prevent misunderstanding he states that he did actually at one time undertake the construction of the Microscope described in Zeitschr. f. Instrumentenk., 1886, p. 325, but that pressure of business prevented him from attempting any technical improvements in the instrument, and at length compelled him to relinquish the undertaking altogether. He wishes it, therefore, to be clearly understood that no Lehmann Microscope of the form described has been made by him. He adds, that some years ago he constructed a heating apparatus for his crystallographic Microscopes, by which the preparation could be heated to a clear red glow during observation between crossed nicols.

Van Heurck's Microscope for Photography and High-power Work. —The following description of this instrument (fig. 45) is translated, with modifications, from the fourth edition of Dr. Henri Van Heurck's work on 'The Microscope,' which is now in the press:—

"In the Microscope which W. Watson and Sons have made to our specification we have attempted to combine convenience for ordinary work with the utmost possible precision, and at the same time to keep the price comparatively low.

Messrs. Watson have admirably carried out all the plans we submitted to them, and the instrument they have produced may be justly considered as realizing in various ways a degree of perfection which has never hitherto been reached.

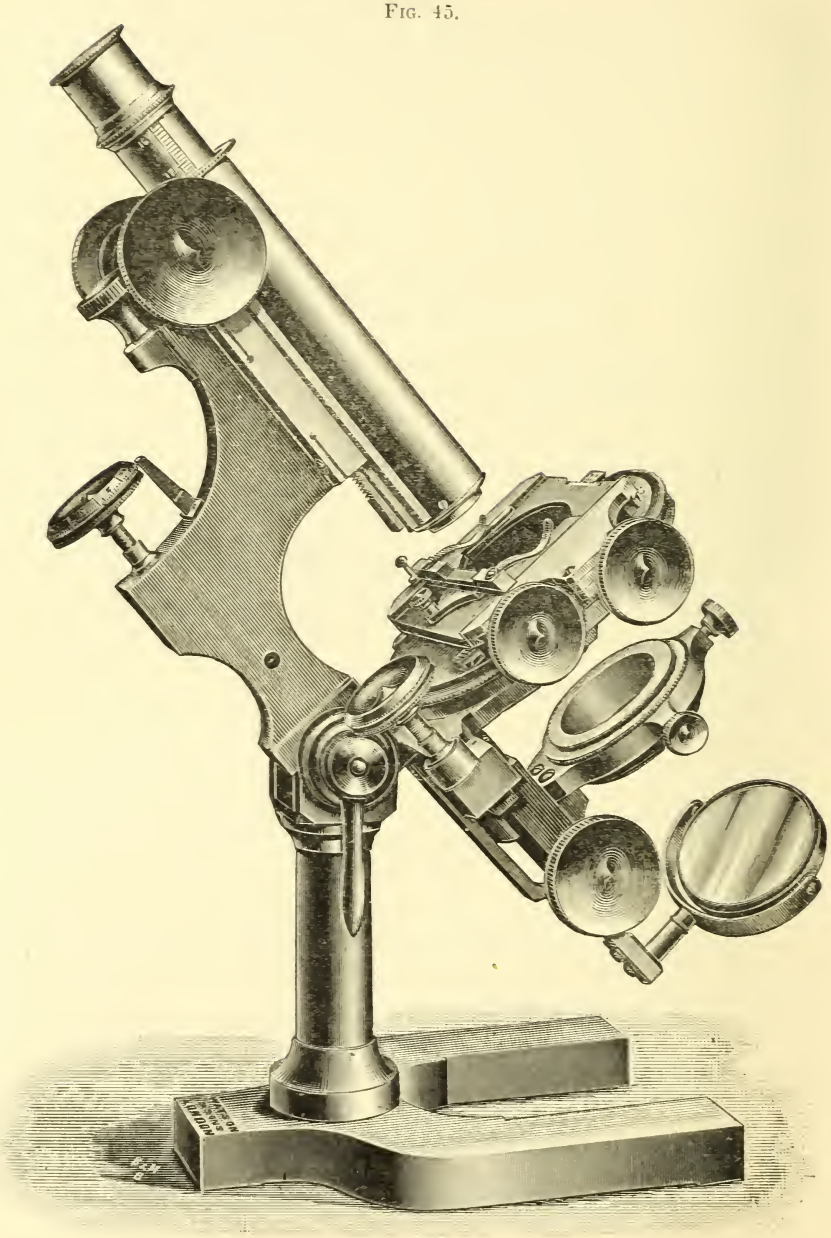
The base of the instrument is of the horseshoe form, bronzed; at the three points on which it stands slightly projecting pieces of cork are inserted, which reduce the tremor, prevent the instrument from slipping, and the table from being scratched.

A substantial brass pillar, jointed in its upper part to allow the inclination of the instrument, supports the Microscope, which can be fixed at any angle by means of a clamping screw, although the instrument is so well balanced as to render this screw almost superfluous.

To increase the general stability, all the parts of the instrument have been made as if they were cast in one piece. The stage-support is made of a single piece, and is prolonged into the articulation of the top of the pillar; the limb fits into the stage-support and is fixed by six screws, so that the whole has the same rigidity as if it formed a single

* Zeitschr. f. Instrumentenk, x. (1890) p. 261.

Fig. 45.



Van Heurck's Microscope for Photography and High-power Work.

piece. Finally, the two pieces fitted together are traversed by the clamping-screw for the inclination.

The stage rotates, and special means are provided to give a very smooth movement, and at the same time to secure perfect firmness in every position without the necessity for any toothed gearing, which seldom works smoothly for any lengthened period. The mechanical movements are effected by two superposed plates, as in the old Ross stands, actuated by lateral screws. The object rests upon a sliding bar provided with a stop-pin and clamping-screw. For ordinary work the sliding bar can be replaced by a fixed plate provided with two ledges. The horizontal and vertical movements have a range of 25 mm., and the divided scales (finders) allow a reading of the movements to 1/10 mm. by means of verniers.

The limb incloses the fine-adjustment and carries the tube in front; both the coarse- and fine-adjustments move in bearings which can be regulated as required. A screw attachment at the upper part of the limb fixes the instrument firmly in the horizontal position when it is required to photograph in that position, though we infinitely prefer photographing with the Microscope in the vertical position.

The fine-adjustment is of exquisite delicacy and of greater precision than that of any other Microscope in our collection. Each turn of the screw of the fine-adjustment corresponds to 1/13 of a millimetre. So perfect is the adjustment, that it is possible in certain cases to estimate to a hundredth of a turn, i. e. to 1/1300 of a millimetre. The mechanism of the fine-adjustment acts in an opposite direction to that of Continental Microscopes, we have therefore marked on the milled head the letters M (*monter*) and D (*descendre*), to indicate the direction in which it is necessary to turn to make the body-tube move up or down.

The body has a draw-tube; when closed up it has a length of 160 mm., which is necessary for the employment of Continental objectives; when drawn out it has a length of 260 mm. and can then be used for the apochromatics for the English tube. The draw-tube is arranged so that it can be blackened internally over part of the space covered by the eye-piece; thus all internal reflection, which is the cause of so much trouble in photomicrography, is absolutely prevented. It might be preferable to line this tube with black velvet. The lower end of the draw-tube is provided with the Society screw for use with the Abbe apertometer.

The mirror is carried by a rod having lateral movement; it can also be slid up or down within a moderate range.

Regarding the substage, which we have designedly reserved to the last, we have to point out some improvements which have not been introduced in any other Microscope. Needless to say, the condenser can be centered, and it can be raised and lowered by rack and pinion; but a fine-adjustment of great delicacy is also applied. In the few Microscopes to which a fine-adjustment of the condenser has hitherto been applied (an adjustment so necessary in certain cases and not yet sufficiently appreciated) this focusing has been simply effected by a screw which does not produce a very slow movement, and there has always been loss of time in the changes of direction. Here, however, the fine-adjustment is actuated by a lever as in the fine-adjustment of the body-tube, *and*

the milled head which actuates the movement is placed above the stage close to the fine-adjustment screw of the body-tube. By this means it is possible to obtain very great precision and to adjust the two movements simultaneously with one hand.

The arrangement of the condenser as planned by us (and employed for several months with all our Microscopes) is, we believe, an important improvement. It consists of an iris-diaphragm surmounted by the lens-holder; between these two pieces slides a plate, removable at will, provided with a central rotating ring which serves for the reception of the diaphragms. The lens-holder is adapted to receive the different Abbe condensers, the Zeiss achromatic condenser, and also adapter plates allowing the use of all the excellent condensers of Powell and Lealand, and may hence be considered of universal application.

To sum up, we have in this instrument combined all the conditions of perfection which long experience in microscopical work has taught us, and Messrs. Watson have realized all our desiderata with a care and precision which we scarcely dared hope for. If we add that this apparatus, so perfect, costs only 400 francs (16*l.*), and consequently less than the large Continental models, it will readily be admitted, we believe, that the makers have rendered a real service to serious workers by its construction."

The Graphological Microscope.* — Mr. C. M. Vorce writes:— "Among the most important of the applications of the Microscope to what are called 'business uses' is the examination of writings, books, &c. The use of the Microscope for such purposes has rapidly increased in the last ten or fifteen years, until now scarcely a case of importance whose turning-point rests on the authenticity of written or printed matter, is tried without the papers or books in question being submitted to Microscopical examination at the hands of experts, real or supposed. Among the points to which such examinations are applied may be mentioned the detection of forgery, alteration, erasure, interpolation, &c., the detection of the authorship of simulated or anonymous writing, the determination of relative age of different writings, identity or difference in inks, pencil marks, paper, &c.; detection of erased writings, the character of stains, marks, mutilations on paper and elsewhere.

Many of the questions involved require very delicate and prolonged examination for their determination, and sometimes the use of high powers, but by far the greater number of questions involve the use of but low or medium powers, and usually the examination of considerable surfaces. Probably every Microscopist who has had occasion to examine writings to any extent has felt the inconvenience of the best modern Microscopes for that purpose, owing to their limited stage room and short rack. In very many cases the examination required involves the comparison of a considerable number of papers, and often of the entire surface of a good sized sheet of paper. The examination of books, such as hotel registers, Bibles, account books, &c., is almost impossible of satisfactory accomplishment with ordinary Microscopes, the only way to proceed being usually to place the instrument on the book and focus through the stage-well. The 'Tank Microscope' of some English

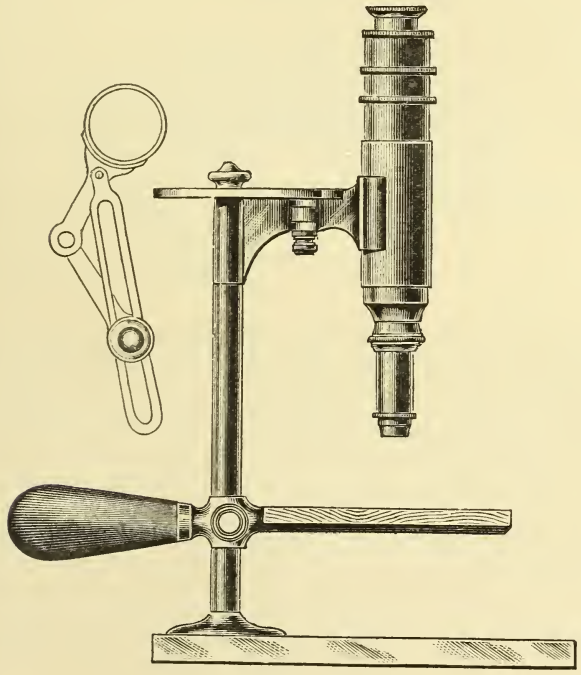
* Microscope, xi. (1891) pp. 47–50.

makers is better for this use than any other present form, but like the others, is objectionable on account of having to be moved about over the book or paper under examination. The danger of marring or obliterating some portions of the writing to be examined often prohibits the placing of the Microscope upon the writing or moving it about, and renders a satisfactory examination quite impossible.

Another serious objection to present forms of Microscope for the uses of the graphologist is the inability to use them as a class Microscope to be passed from hand to hand, with the objects to be viewed securely clamped in position and in focus.

To obviate the defects found in the present Microscopes for such uses and to produce a form adapted to the special needs of the grapho-

Fig. 46.



logist, as made apparent to me by some twenty years of my own experience in that line, and my observation of the work of others, I have devised the Microscope-stand which I have designated The Graphological Microscope, a cut of which is here given, and which is briefly described as follows:—

The pillar is a straight brass rod 5/8 in. in diameter, threaded with a long screw into a plate flush with the surface of the wooden base. The stage is of wood or hard rubber, 5 × 8 in., and rests on a forked brass plate projecting from a stout collar which slides on the pillar, and

is clamped in place by a strong thumb-screw with milled head. From the back of the collar opposite the stage a strong screw projects, upon which a handle may be screwed when the instrument is to be passed about as a class Microscope.

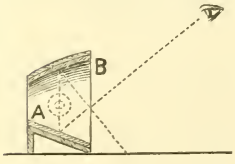
The arm is in two parts joined by a smoothly fitted joint with a nut on the pivot; the outer joint of the arm carries a slip-tube through which the body-tube is focused by sliding, and the inner joint of the arm is extended into a sleeve with a long conical bearing around the top of the pillar, insuring a smooth motion. A flat slotted plate is pivoted to the outer joint of the arm and rests on top of the sleeve of the inner joint, the top of the pillar passing through the slot, being threaded and pivoted with a strong thumb-nut to clamp the arm rigidly in place. By this construction the body-tube may be moved about over every part of a surface 6 in. square, and may be clamped in place over any part of that surface by means of the thumb-nut at the top of the pillar. The paper to be examined can be arranged on the large stage and secured in place by wire clips. In case it is desired to use the instrument as a class Microscope, the arm is clamped fast, the handle screwed on and the pillar unscrewed from the base-plate, when the instrument can be handed about as readily as a common stereoscope, and weighing but little more.

If provision is required for the use of transmitted light, which is but seldom needed, an opening in the stage is provided, and a mirror on the base like that of a dissecting Microscope. An arm for carrying a lamp may also be attached to the pillar by means of a clamping collar like that of the stage-arm, when the instrument is to be used as a class Microscope at night.

It has not been found requisite to provide for inclining the instrument in use, but if desired it can be readily accomplished by providing a slotted segment on the plate into which the pillar screws, hinging this plate to an under plate secured to the base-board, with a clamp screw to clamp the segment against a projection on the fixed plate.

The instrument, as made for me by the Bausch and Lomb Optical Company, has proved very satisfactory in use, and admirably serves the purposes for which it was designed, especially in its capability of being passed from hand to hand. An entirely unpremeditated advantage has also been discovered in the ease with which objects too bulky for examination on ordinary stands, such as large minerals, natural history specimens, &c., can be laid on the base-board (the stage being loosened and swung round out of the way), and examined with this Microscope over all their surface."

Fig. 47.



Magnifying Instrument.*—M. Th. Simon, of Paris, has devised an instrument to replace the ordinary magnifying glass. It possesses the advantage of affording a well-illuminated image. The magnification is obtained by means of a concave mirror B. This is set at such an angle to a second mirror A, that the magnified image is formed in a convenient position

* Zeitschr. f. Instrumentenk., x. (1890) p. 151.

for observation, and the illumination of the body is not interfered with by the instrument.

BERNARD, P.—Note sur un Microscope composé du 18me siècle. Lille, 1890, 8vo.

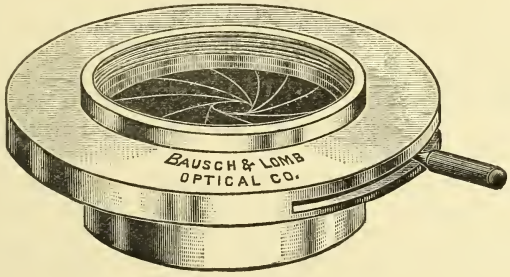
(2) Eye-pieces and Objectives.

JOHNSON, C.—The American Objective as compared with the German. *Maryland Med. Journ.*, XXI. (1889) p. 130.

(3) Illuminating and other Apparatus.

Bausch and Lomb's Condenser Mounting with Iris Diaphragm.—In addition to the form of this device which we figured in this Journal, 1890, p. 508, a simpler and less expensive form has been issued by the firm, as shown in fig. 48.

FIG. 48.



New Lens-holder with Stand.*—M. L. Malassez has constructed a new holder for use with his erecting objectives of long focus. Like the lens-holders in common use, this serves to support ordinary lenses, but will also hold a Microscope-tube provided with the new lenses. It consists of a triangular foot of cast iron and lead, very heavy, giving great stability with considerable space for manipulation. It is covered with india-rubber underneath, to avoid vibration being communicated to the arm of the lens-holder from the table. On this foot is the triangular standard with rack, on which the socket carrying the horizontal arm moves. This arm is not fixed to the socket itself, but to a ring which rotates on it. The result is that the arm can be turned round the standard, without the latter being displaced, so that when, during a dissection for example, it is necessary to dispense for a moment with lens or Microscope, there is no need to move the heavy base, but simply to turn the arm aside. Two fixed stops limit the extent of the rotation, and another stop provided with a spring enables the arm to be replaced in its original position. The friction surface of the ring of the socket is a truncated cone with the base below, an arrangement which prevents the oscillations which with another form of surface would be produced by the wear and tear of the pieces.

The arm of the lens-holder is long, so that, with the arrangement of

* Arch. de Méd. Expér., i. (1889) pp. 455-7 (1 fig.).

the foot above referred to, objects of large size can be examined. Its free extremity receives either a socket for holding the Microscope or pliers for holding the lenses. For this purpose, these pieces are provided with a pin, which fits into the hollow extremity of the arm, and can be fixed in any position required by means of a clamp-screw. By this arrangement the change of the pieces is rendered very easy, and the Microscope-tube can be placed vertically, obliquely, or horizontally. This last position is very useful when objects placed vertically, such as the side of an aquarium, are to be examined. Indices mark the vertical and horizontal positions, and also that at 45°.

The pliers for holding the lens are not, as in other forms of apparatus, in the exact axis of the arm, but at right angles to it. Owing to this arrangement, there is no risk of the nose of the observer coming in contact with the arm, and he is not obliged, in order to avoid this, to turn his head on one side. There are two pliers, the larger for ordinary lenses, the smaller for objectives. One is in front of the arm and the other behind, and they can be placed on either side by turning the pin fixed to the end of the arm.

The rotating ring of the arm has on the opposite side another arm, which is shorter and is terminated by a brass ball filled with lead. It serves to counterbalance the long arm and thus to maintain the stability of the apparatus.

Heating-Lamp with Electric Regulator for controlling the Gas-supply.*—In order to prevent the escape of gas after accidental extinction of the flame in a lamp intended for keeping up a constant temperature, Herren F. and M. Lautenschläger have patented one in which a valve is inserted in the supply pipe, and this valve is kept open by means of an electro-magnet as long as the lamp burns. If this be extinguished, the mercury in the contact thermometer falls until it sinks below a wire melted into the thermometer at a suitable place. As this wire forms part of the path of the electro-magnet, the current is thereby broken, the valve closes, and the gas supply is cut off.

Polarization without a Polarizer.—We cannot congratulate the author of the following note on the originality of the wonderful discovery he has made. Wheatstone and Brewster have unfortunately been before him. We cannot answer for American skies (which no doubt "whip creation" in polarizing as well as in other effects) but in this country at least we fancy that better results would be obtained, when no polarizing nicol was at hand, by the simple expedient of using for mirror a few glass slips inclined at the polarizing angle. Interference figures in crystal sections may often be seen with tolerable clearness when the polarization is produced by simple reflection from the work-table. Mr. H. M. Wilder says:†—"I have accidentally made a quite useful discovery, which I have not seen mentioned before. In order to *polarize*, we put a polarizer (Nicol) beneath the stage, and an analyser (Nicol) above the objective (either right next to it, at the end of the draw-tube, or above the eye-piece). The selenite comes on top of the polarizer. Now I found that the polarizer is not absolutely indis-

* Zeitschr. f. Instrumentenk., xi. (1891) pp. 73-4.
† Cf. Engl. Mech., liii. (1891) p. 113.

pensable. Given a certain polarizing condition of the sky (i. e. blue, with more or less watery vapour—as either before or after a rain, snow, or fog), you can polarize very nicely with the analyser alone, and, if you want display of colour, put the selenite on top of the slide, or anywhere convenient to you—so it comes beneath the analyser. The colours (and crosses) will, of course, be somewhat fainter than when you use the polarizer too. In order to get the best display, it will be necessary to rotate both analyser and selenite until in the proper relative positions; or, to speak more correctly, the relative position of the P.A. of the selenite to the beam of light from the mirror decides the more or less intense coloration. With any other sky, the polarization is not observed. This observation is useful in so far as to enable the possessors of Microscopes, without substage facilities, to polarize fairly well—under the circumstances—and the proper condition of the sky is often obtained in our latitude."

(4) Photomicrography.

Photomicrography.*—Mr. T. Comber writes:—"Photographing with the Microscope, or, as it is now the fashion to call it, "Photomicrography," has always had a great attraction to me. My first attempts at it were made so long ago as 1858, before the days of gelatino-bromide plates, and when the "wet-collodion" process was almost universal.

At that time one of the difficulties to be contended with was the want of coincidence of the actinic with the visual focus of the object-glass. Now most of our English makers can supply objectives specially corrected in this respect, so that when a visual image is focused on the ground glass, an image equally sharp in its actinic effects can be relied upon as thrown upon the sensitive plate. The apochromatics of Zeiss I have always found to be perfect in this respect. I should recommend any of you, who may be desirous of using your Microscope for photography, to be careful to obtain objectives corrected for the purpose; but in case you may be tempted to use an objective that is not so corrected, I may mention the method by which, in those early days, we managed to overcome the difficulty; the more so as the plan constitutes a good test to ascertain whether an objective said to be corrected for photography is in reality correctly corrected. Place a flat object on the stage, for choice a micrometer, and by putting a piece of card under one end of the slide, tilt it slightly up, so that the object no longer lies square to the axis of the Microscope, but is a little nearer on one side, a little further off on the other. Then focus carefully till the division of the micrometer scale lying in the centre of the field gives a sharp image on the ground glass, the other divisions will go gradually out of focus, those on one side being within, those on the other side beyond the focus. Next photograph the scale, and if any difference exists between the visual and actinic foci, it will be found that the centre division, which was sharp on the screen, is not sharp in the photograph, but that some other division more or less distant from the centre of the scale is. Replace the focusing screen and ascertain how much the fine-adjustment has to be moved to bring sharp on the screen the particular division that was

* Journ. Liverpool Micr. Soc., i. (1891) pp. 99–110.

sharp in the photograph. This will give the measure, for that objective, of the difference between the two foci, and whenever the same objective is used, you can always, by moving the fine-adjustment to that extent, but in the opposite direction, convert the visual into an actinic focus.

In 1859 I left England for India, and for nearly thirty years, having "other fish to fry," being, in fact, engaged in the "struggle for existence," I had no leisure for microscopical studies. On recently resuming, about two years ago, one of the first things I did was to read up what had in the meanwhile been done as regards photomicrography. What a change had taken place, whether regarded from the photographic or the microscopic point of view! I found that there were now available gelatino-bromide plates, infinitely more sensitive than the old collodion, and dry instead of wet, so that there need be no limit to the time of exposure. On the other hand, "immersion" objectives, followed by apochromatics, had greatly increased the delineating power of the Microscope. I promptly provided myself with a set of apochromatics, and proceeded to mount my old hobby, intending to apply it chiefly to the investigation of the minute structure of the diatom valve. I commenced with daylight (white cloud) illumination, which in the old days had been considered the best; next proceeded to artificial light (oxy-hydrogen); and finally adopted, for high magnifications, sunlight, with which Colonel Woodward had achieved his best results. My wish is to place before you to-night some of the results that I have so far obtained; to describe the apparatus I use in its present state of development, and explain, so far as I can without a "practical demonstration," the method of working with it.

A general idea of the apparatus you will gather from the woodcuts and description, which originally appeared in the Royal Microscopical Society's Journal, 1890, pp. 429–34.

[We omit the description and figures of the Microscope and heliostat as they were dealt with in the Journal, 1890.]

Turning now to the camera. This is fixed to a base-board, which pivots on a tripod, so that it can be slewed round out of the way when not in use. There is then room for the operator to sit at the Microscope, find and arrange his object, and adjust his illumination, also to effect the necessary corrections of the object-glass for variations in the thickness of the cover-glass, if an eye-piece is to be used; but if the photograph is to be taken without an eye-piece, this correction should be effected after the camera is attached, and when the image is on the ground glass. It is well for the table upon which the Microscope stands to be of such a height as to bring the tube of the instrument comfortably to the level of the observer's eye, and the height of the tripod must correspond, being such that the axis of the camera coincides with that of the Microscope. The light-tight connection of the camera to the Microscope can be effected in a variety of ways. The one I employ is a collar, covered with velvet, which fixes on to the upper end of the draw-tube of the Microscope, and has a deep groove, into which fits a wide brass tube attached to the camera front by a small conical bellows.

The image of the object may be projected on to the sensitive plate either (1) by means of the object-glass alone, or (2) by the use of what is termed a "projection" eye-piece. Much good work has been done by the former method, but not, so far as I can judge, the very best. I

attribute this partly to the fact that in English objectives the spherical and/or chromatic aberration is often not entirely corrected, some being intentionally left for correction by a contrary error in the eye-piece; but chiefly to the object-glass being adjusted to project the image a fixed distance, which is generally 10 in., that being the usual length of the English Microscope-tube: but when the image is projected not to 10 in., but to a distance considerably exceeding this, say to a distance of 40 or 50 in., the corrections are altogether disturbed, and the delineation in consequence deteriorated. A main cause of the disturbance can be removed in object-glasses provided with a collar adjustment for cover-correction, by altering the relative distance of the different combinations of lenses in the object-glass; and I have had even a $1\frac{1}{2}$ in. objective mounted so that the distances between the lenses could be changed; but other causes of disturbance are left, or even increased, by the change, and the image is never so clear as it is at the 10 in. A projection eye-piece, however, avoids this difficulty, for it takes up the image at the proper distance, and is furnished with means for adjusting its own action to whatever distance the sensitive plate may be placed. I have used Zeiss's, but I believe several English makers supply similar ones. You will see that there are two combinations of lenses, the distance between which can be regulated, and the adjustment thereby effected. It is correct when the edge of the field is sharp and clear.

The method of illumination may vary according to the work to be done. For moderate magnification, say up to about 300 diameters, I have found diffused daylight from cloud or blue sky to give good results. The same light, or a good lamp, can also be used for higher magnification, 500 or even 1000 diameters; but the light is then so feeble that focusing is difficult, and a very long exposure necessary. I show you one photograph of a Triceratium, × 1000, taken with diffused daylight, for which the exposure was 1 hour 40 minutes; and another of an Arachnodiscus, × 800, taken with a paraffin lamp, and an exposure of 1 hour 20 minutes. With such prolonged exposures the chances of vibration, or of changes of focus arising from the expansion or contraction of the instrument in consequence of variations of temperature, are greatly increased; so that the final result is seldom so clear and sharp as with a more intense illumination and shorter exposure. For high magnification, therefore, oxy-hydrogen light is usually employed; and better even than this I consider sunlight. It has, of course, some serious disadvantages. It cannot be obtained whenever you happen to require it by merely turning on a tap. You are dependent upon the clerk of the weather; and when you do get it, it is too apt to be intermittent. Many a time I had to wait patiently, waiting for a break in the clouds. But when you do get it, I think it is the *ne plus ultra*. When using it, exposures can be reckoned by seconds, and I have a negative of *Pleurosigma angulatum*, good so far as density is concerned, taken with an exposure of only one second to sunlight, on an ordinary Ilford plate. Whether the source of illumination be a lamp, or a lime cylinder, or the sun, care must be taken so to focus the sub-stage achromatic condenser, that an image of the source of illumination is thrown on the exact plane of the object. This is all-important.

I will now try to describe, with some minuteness, my course of procedure when taking a photograph by sunlight, premising that my objects

are generally diatoms, and the magnification 1000 diameters. The Microscope is placed in its horizontal position, and the milled head of its fine-adjustment brought into gear with the focusing-rod by means of a piece of thin whipcord. The heliostat is placed in front, on a wooden stand, which carries also the fixed mirror and the alum-cell for absorbing heat-rays. Care has to be taken that the optical axis of the whole apparatus is directed due south, which is insured by the end of the board upon which it stands being cut at such an angle that when this end is placed against the plate glass of the window, all is in right direction.

The first operation is to accurately centre the achromatic condenser, using a two-thirds objective and regulating the diaphragm so that its opening may be a little smaller than the field; next, to centre the further diaphragm at the end of the brass plate; and afterwards, removing the movable mirror from the heliostat, to ascertain that the spindle appears precisely end on and in the centre of the field, which it should do if the heliostat has been properly placed. Exactness in this last adjustment is necessary, otherwise the beam of sunlight will not be motionless. The movable mirror is then replaced on the spindle, and set to reflect the image of the sun in the centre of the field. At this stage the eye must be protected by a dark-coloured glass being placed below the condenser. The object being placed on the stage, brought into the centre of the field and focused, the condenser has next to be focused to throw the sun's image exactly in the plane of the object. Sharpness of the ultimate image upon the ground glass cannot be secured without this.

Changing the objective to a one-sixth (4 mm.) I next measure the thickness of the cover-glass, or rather the distance between that plane of the object which it is desired to photograph and the upper surface of the cover-glass, by means of the fine-adjustment screw. The purpose of this is twofold. First, to facilitate cover-correction; secondly, to ascertain whether the 2 mm. object-glass, which is now put on, can get down to it, for its front lens is rather more than a hemisphere, and the mount in which it is set is so extremely thin that it has hardly any grip on the lens, and the slightest pressure suffices to displace it. My glass, with a distance of 0·18 mm. between the object-plane and the upper surface of the cover-glass, requires no correction. For a thinner cover—and the covers of English-mounted slides generally are thinner—correction is effected by lengthening the tube-length; for a thicker cover, by shortening it. Lastly, the illuminating cone thrown by the condenser has to be regulated. You are probably aware that there is great controversy as to what this should be in order to produce a "true" image. My experience is that the width of the cone should vary according to the nature of the object and the quality of the object-glass. Too narrow a cone produces diffraction fringes, that bane of photomicrography; too wide a cone, even, I think, with the best objectives hitherto made, produces haze. With thin "test objects" I find my own glass works best when about two-thirds of its back lens is filled with light; for thick objects, I get the best results with a somewhat narrower cone. Of one thing I am convinced, that to get true images, the cone, whether it be wide or narrow, must be absolutely axial. Even a very slight obliquity renders the images unreliable.

The ordinary eye-piece is now changed for a projection eye-piece, set to the distance at which the sensitive plate is to stand; the camera is attached, and the long focusing-rod coupled on. The image of the sun will be found in the centre of the ground glass. If it is not, the centering of the condenser must be wrong, and will require alteration. The sun's image should be sharp at the edge, unless the sky is hazy. Any light fleecy clouds near the sun will be visible on the screen, almost as if an ordinary landscape lens were being used, and the effect when they drift across the sun's disc is very curious. The image of the object, as seen against that of the sun, will be somewhat out of focus, but a slight turn of the focusing-rod brings it right.

With sunlight I find it unnecessary to use anything for focusing except the ground glass. The image is so bright that the details can be sufficiently seen. With other less brilliant sources of illumination it is necessary to use other means; and that which I have found most convenient is a Microscope eye-piece. The ground glass is removed, and replaced by a wooden slide, in the centre of which is a hole fitting the eye-piece. It should be so set that the diaphragm of the eye-piece is in register with the sensitive plate. Even a very faint image, when viewed through this eye-piece, is sufficiently visible to admit of focusing.

The next step is exposure. I wish I could give you some rule by which to regulate exposure, but I find it altogether impossible to do so. Its wide range has already been indicated. From one second with bright sun, to an hour and forty minutes with diffuse daylight, is a "far cry." Exposure depends not only on the source of light, but on variations of that source. A winter sun, shining through an east wind haze, is very different from a midday sun in summer, when the sky is clear. Exposure varies, too, with the degree of magnification. A magnification of 1000 diameters requires 100 times the time that one of 100 diameters requires. It varies with the width of the illuminating cone. It varies with the opacity or transparency of the object. It varies with the colour of the medium in which the object is mounted. A diatom mounted in Prof. van Heurck's high refractive medium, which is of a deep yellow colour, requires at least six times the exposure that would be proper if it were mounted in balsam, all other conditions remaining equal. All I can tell you, therefore, is that a little experience, and a few dozen spoiled plates, of which notes have been kept, will enable you to judge, almost instinctively, what exposure is required. I always make two exposures on each object, one longer than the other, and thus have a double chance.

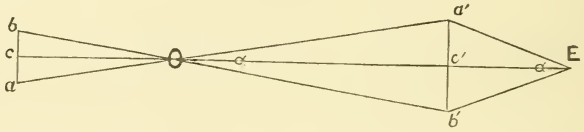
As regards plates, I recommend you to use slow ones, and to develope with hydroquinone. The usual difficulty, with most microscopical objects, is to obtain sufficient contrast, and this is most readily obtained on slow plates."

FRAZER—On Photography as an aid in Anatomical, Histological, and Embryological Work.
Report 59th Meet. Brit. Assoc. for the Advancement of Science, 1890, p. 639.
PRINGLE, A.—Practical Photomicrography by the latest methods.
New York, 1890, 8vo, 192 pp., 7 pls.

(5) Microscopical Optics and Manipulation.

Microscope Magnification.*—Mr. W. Le Conte Stevens, in spite of the distinction drawn by some authors between "magnification" and "amplification," † sees no good reason for discarding the usual acceptation of the term magnification, as denoting the ratio of the diameters of the retinal images produced with and without the magnifying instrument respectively. To obtain the magnification of a Microscope it is necessary to know the equivalent focal length of the eye-piece and objective, and also the tube-length. Unfortunately all of these data are seldom supplied by the makers. The equivalent focal length of the eye-piece is rarely given, and great diversity exists as to the points to be taken as the limits of tube-length. The tables of magnification given by certain firms are only applicable when "standard tube-length" is used, and such a standard exists only in name. Examination of such a table supplied by one maker showed that the magnification was calculated by dividing 100 by the product of the focal lengths of objective and eye-piece. This rough approximation is deduced as follows:—

Fig. 49.



Let $a'b'$ (fig. 49) denote the image of the object ab given by the objective O. O c is taken as the focal length of the objective, and O c' as the tube-length, 10 in. The magnification of the objective m is then given by

$$m = \frac{a'b'}{ab} = \frac{10}{f}.$$

The eye-piece increases the visual angle from α to α' producing a virtual image assumed to be 10 in. away. For the magnification m' of the eye-piece whose focal length is F we have

$$m' = \frac{\tan \frac{1}{2}\alpha'}{\tan \frac{1}{2}\alpha} = \frac{10}{F}.$$

The total magnification M is then

$$M = m\,m' = \frac{100}{f\,F}.$$

A more exact formula is obtained as follows:—For the objective we have

$$m = \frac{a'b'}{ab} = \frac{T}{O\,c},$$

where T is the tube-length defined as the distance from the focal plane to the point which behaves as an optical centre.

* Amer. Journ. Sci., xl. (1890) pp. 50–62. † See this Journal, 1889, p. 818.

But

$$\frac{1}{\mathrm{O}c} + \frac{1}{\mathrm{T}} = \frac{1}{f},$$

$$\therefore \quad m = \frac{\mathrm{T}}{f} - 1.$$

For the eye-piece

$$m' = \frac{\mathrm{D}}{\mathrm{F}} + 1,$$

where D is the distance of distinct vision

$$\therefore \quad \mathrm{M} = m\,m' = \frac{(\mathrm{D} + \mathrm{F})\,(\mathrm{T} - f)}{\mathrm{F}\,f}.$$

The equivalent focal length of the eye-piece can be easily calculated, if the focal length of the eye-lens is known. Thus by the formula for the combination of two lenses

$$\frac{1}{\mathrm{F}} = \frac{1}{f'} + \frac{1}{f''} - \frac{d}{f'\,f''},$$

where f' f'' are the focal length of eye-lens and field-lens respectively, and d is the distance between them.

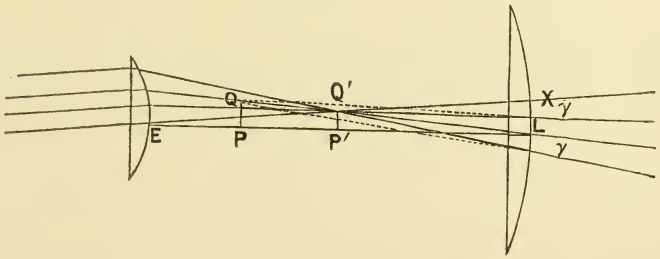
But in the case of a properly constructed negative eye-piece $f'' = 3f'$ and $d = 2f'$, so that $f' = \frac{2}{3}\,\mathrm{F}$.

To determine f' the magnification of the eye-lens can be measured by the use of the camera lucida. A micrometer is placed at the diaphragm of the eye-piece, and the Microscope is inclined until the optical centre of the eye-lens is 250 mm. (distance of distinct vision) above the paper, on which the camera lucida projects the figures of the micrometer. The magnification m' is thus directly determined and f' given by the formula

$$m' = \frac{\mathrm{D}}{f} + 1.$$

Fig. 50 explains the theory of the negative eye-piece, and shows how

Fig. 50.



the effect of the field-lens is to diminish the magnification by two-thirds. The rays $r\,r$ converging from the objective to the point Q, are made

more convergent by the field-lens L, so as to cross at Q′ in the principal focal plane of the eye-lens E.

We then have

$$\frac{1}{\mathrm{L\,P'}} - \frac{1}{\mathrm{L\,P}} = \frac{1}{f'},$$

$$\text{i.e.}\quad \frac{1}{f'} - \frac{1}{\mathrm{L\,P}} = \frac{1}{3f'},$$

since in a negative eye-piece $\mathrm{E\,L} = 2f'$ and $f' = 3f'$.

$$\therefore\quad \mathrm{L\,P} = \frac{3}{2}f' \quad \text{and} \quad \mathrm{P\,Q} = \frac{3}{2}\mathrm{P'\,Q'}.$$

The focal length of the objective is best determined by the formula of Prof. C. R. Cross.* A micrometer scale divided into tenths of a millimetre is placed on the stage, and a second, divided into millimetres, at the diaphragm in the focal plane of the eye-lens, the field-lens being removed. The magnification m of the objective is then given by focusing the image of the stage micrometer upon the eye-piece micrometer.

If p, p' denote the distances of the two micrometers from the point which behaves as optical centre of the objective, we have

$$m = \frac{p'}{p}.$$

And if l is the distance between the micrometers

$$l = p + p'$$

$$\therefore\quad p' = \frac{m\,l}{m+1}.$$

Then from the formula

$$\frac{1}{p} + \frac{1}{p'} = \frac{1}{f}$$

we have

$$f = \frac{m\,l}{(m+1)^2}.$$

Determinations, made by use of the above formulæ, of the focal lengths and magnifications of the eye-pieces and objectives of various makers showed how generally erroneous was the labelling. In the case of five eye-pieces of one of the best known of American makers, the percentage of error in the value of F varied from 2·7 to 7·4, and in no case was $f' = 3f'$ or $d = 2f'$. In ten out of eleven objectives examined, the percentage of error was greater than 4, and for two of them it reached as high as 41 and 50. Application of the formula $\mathrm{M} = \frac{100}{\mathrm{F}f}$ for various combinations was shown to give very inaccurate results as compared with determinations of the magnification made by the camera lucida.

For the application of the more exact formula $\mathrm{M} = \frac{(\mathrm{D}+\mathrm{F})\,(\mathrm{T}-f)}{\mathrm{F}f}$

* Journ. Franklin Institute, lix. p. 401.

which gives perfectly reliable results, it is necessary that makers should have accurate values of the equivalent focal length of eye-piece and objective stamped on their mountings, and also the tube-length stamped on the body-tube.

A standard tube-length should be agreed upon. The author considers that of 180 mm. of Continental makers more convenient than the 10 in. generally adopted in England and America. The upper limit of the tube-length should be the focal plane in which an image would be formed by the objective if there were no field-lens. In a negative eye-piece this plane is midway between the diaphragm and the optical centre of the eye-lens. Eye-pieces should therefore be so constructed that when slipped into position this plane should be exactly at the top of the body-tube. Such par-focal eye-pieces have been made for several years past by the firm of Zeiss. The lower limit of the tube-length should be the point within the objective which behaves as an optical centre. The distance from the top of the body-tube to the extremity where the objective is screwed on is taken a little shorter than the desired tube-length, say 160 mm. instead of 180 mm. Then in the formula $\frac{1}{p} + \frac{1}{p'} = \frac{1}{f}$, $p' = 180$ and p can be calculated, since f is known. Subtracting then from p the working distance between slide and the exposed lens, we have the distance within the objective of the point which acts as an optical centre. Allowance can then be made in the mounting of the objective to make this point just 20 mm. from the extremity of the body-tube where the objective is screwed on.

β. Technique.*

Arloing, S.—**Cours élémentaire d'anatomie générale et notions de technique histologique.** (Elementary Course of General Anatomy and Histological Technique.) Paris, 1890, 8vo, 388 figs.

Behrens, W.—**Leitfaden der botanischen Mikroskopie.** (Outlines of Botanical Microscopy.) Braunschweig (Bruhn) 1890, large 8vo, 288 pp., 150 figs.

Bonnet, R.—**Kurzgefasste Anleitung zur mikroskopischen Untersuchung thierischer Gewebe.** (Concise Introduction to the Microscopic Examination of Animal Tissues.) München (Rieger) 1890, 2 figs.

Paul, F. T.—**On the relative Permanency of Microscopical Influence of the different Staining and Mounting Agents.** *Liverpool Med.-Chirurg. Journ.*, X. (1890) p. 65.

(1) Collecting Objects, including Culture Processes.

Method for making Permanent Cultivations.†—Herr W. Praus-nitz preserves roll and puncture cultivations (and even liquid ones provided the liquefaction is not too general) by filling the tubes with a gelatin solution to which a disinfectant has been added. The tubes are placed in ice-water, the cotton wool plugs removed, and the fluid and antiseptic gelatin solution is then slowly poured in through a pipette. The tube is then plugged with a cork cut off flush with the top, and finally

* 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., ix. (1891) pp. 131-2.

sealed over in order to prevent the gelatin from drying. The disinfectants recommended are 5 per cent. acetic acid, or 1 per cent. carbolic acid. The gelatin solution is of course a simple one, and without any additions; it is clarified by means of egg albumen, and the acid added after filtration.

By this method the author preserved cultivations for two years, but he admits that sometimes, for reasons inexplicable, the gelatin liquefies.

Simplified Method for preparing Meat-Pepton-Agar.* — Mr. N. Tischutkin prepares and filters meat-pepton-agar in the short time of 2–2½ hours. The requisite quantity of agar is placed for 15 minutes in a dilute solution of acetic acid (5 ccm. acid. acet. glacial. in 100 ccm.). The swollen agar is then carefully washed free from acid and then mixed with bouillon. Boiling for 3–5 minutes suffices to make a perfect solution of the agar in bouillon. After neutralizing and cooling down the whites of two eggs are added, and the mixture placed for half to three-quarters of an hour in a Koch's steamer. It is next filtered through Schulze's paper.

Preparing Nutrient Agar.†—Prof. van Overbeek de Meyer prepares nutrient agar in a very satisfactory manner by the aid of his disinfection oven, which insures a constant temperature of 100° to 101°. The agar is cut up into very small pieces and in the proportion of 1½ to 2 per cent. is poured into 0·5 litre of Loeffler's bouillon. To this is added 1 per cent. pepton, and 0·5 per cent. common salt.

After the lapse of about an hour, the mixture is placed in the disinfection oven, and there steamed for three-quarters of an hour at 100°. This dissolves the agar and separates out the coagulable albuminoids.

The next step is to neutralize or impart any suitable reaction to the solution, after which it is filtered through blotting-paper into flasks. The funnel is covered over with a glass, and the funnel, flask, and cover are again placed for three-quarters to one hour in the disinfection oven. In the end about 0·25 litre of perfect bouillon-agar are thus obtained. If requisite any additional substances, such as grape-sugar, glycerin, &c., may be added, after which the mass is sterilized for half an hour, and the process repeated on the two following days.

Cultivating Actinomyces.‡—Herren N. Protopopoff and H. Hammer cultivated Actinomyces on glycerin agar bouillon, potato gelatin, and in milk and eggs. The cultivations were derived from a pure cultivation prepared by Prof. Afanassiew from the pus of a person affected with actinomycosis.

By rubbing granules of the agar cultivation together with sterilized bouillon and inoculating with this emulsion, a much more rapid development was obtained than by direct transference of the granules. On glycerin agar the cultivation presented a mass of miliary granules, about the size of hemp-seeds, of a yellowish-white colour, and firmly

* Wratsch, 1890, No. 8. See Centralbl. f. Bacteriol. u. Parasitenk., ix. (1891) p. 208. † Centralbl. f. Bakteriol. u. Parasitenk, ix. (1891) pp. 163–5.

‡ Zeitschr. f. Heilk., xi. (1890). See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 63–4.

fixed to the medium. On potato the growth was particularly luxuriant and quite typical, the cultivation having a characteristically dry appearance. In bouillon the miliary nodules soon appeared, and grew up into masses the size of a hazel-nut, the bouillon remaining clear. In milk, the ray fungus throve well, the albuminoids of the milk being apparently directly peptonized without previous coagulation.

The authors found that the growth of the fungus was completely stopped at a temperature of 52° C. and that even 40° C. exerted an inhibitive action.

The authors further observed that the fungus presented in their cultivation a cyclical polymorphism, that is, the Actinomyces filaments, at first distinguished by their dichotomous ramifications, eventually assumed, by continual subdividing transversely and longitudinally, the appearance of rodlets and cocci, from which again developed the long branched filaments.

This variety of polymorphism was specially observable in potato cultivations, while in old cultivations real retrograde metamorphoses, e. g. club-shaped or spirilla forms, mucous degeneration, &c., were remarked.

The authors regard the rosette form found in men and beasts as the expression of a parasitic adaptation to the animal body.

Further experiments showed that in old cultivations the further development of the cultivation was inhibited in consequence of the accumulation of metabolic products.

The results of the experiments on animals are reserved for a further communication.

Apparatus for facilitating Inoculation from Koch's Plates.*—Herr W. Prausnitz has devised an apparatus for facilitating the inoculation of particular colonies from Koch's plates.

It consists of a metal ring which is screwed on to the Microscope-tube. From one side projects a metal piece, in which is left a linear fissure for the insertion of a platinum plate. From the lower end of the plate is excised a triangular piece. The inoculating needle is made to rest in the angle of the platinum plate, its point being about 2 mm. from the colony. The apparatus is merely intended as a device for keeping the needle steady, so that the special micro-organisms only are removed, and uncontaminated either by the medium or by adjacent colonies.

Picric and Chromic Acid for the rapid Preparation of Tissues for Classes in Histology.†—Mr. S. H. Gage remarks:—"The standard methods of hardening tissues and preparing them for sectioning require so great an expenditure of time that it is practically impossible for students in college and carrying on other university work to perform all the processes and to make any satisfactory progress in the limited time devoted to histology. Believing firmly that unless a student learns to take every individual step himself in histology, as in all other branches of sound learning, the great object is unattained, I have been experimenting for the

* Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 128–9 (1 fig.).
† Proc. Amer. Soc. Micr., 1890, pp. 120–2.

last few years in the laboratory, hoping to so shorten and modify existing methods that every step may be taken by the student himself without too great an expenditure of time. The following are the results, and they are given, not because they are the best possible methods that might be used if unlimited time were at the disposal of the student, but as methods that give excellent results in a very short time.

Picric Alcoholic Method.—The hardening and fixing solution consists of 95 per cent. ethyl alcohol, 250 ccm.; water, 250 ccm.; picric acid crystals, 1 gram.

The tissue is cut into pieces of moderate size and placed in a preserving jar containing about 25 to 50 times as much of the preservative as there is tissue. It is well also to suspend the tissue or support it on absorbent cotton, or to stir the tissue around occasionally. The tissue should be left in the picric alcohol about 24 hours. If the piece is small, 12 hours will do, and an immersion of 2 to 3 days seems to do no harm. After one day the tissue is placed for 24 hours in 67 to 70 per cent. alcohol, and then for one day or longer in alcohol of from 75 to 82 per cent. It may be left indefinitely in this. Finally, just before imbedding, the tissue is dehydrated one day only in 95 per cent. or stronger alcohol. It may then be infiltrated with paraffin or collodion in the usual manner, the whole time required being 7 days, at the longest, to harden, infiltrate, and imbed a tissue ready for sectioning.

The picric-alcohol method has given excellent results for all tissues except peripheral nerves. It is especially to be recommended for organs or parts possessing ciliated epithelium.

The double stain of hæmatoxylin and picric acid gives very sharply defined appearances, the hæmatoxylin staining the nucleus and the picric acid the cell-body and also the ground-substance somewhat.

If ammonia-carmine is used as a stain, more sharply differentiated appearances are obtained by dehydrating with the following:—95 per cent. alcohol, 100 ccm.; glacial acetic acid, 1 ccm.; picric acid crystals, 1/10 gram.

Nothing has been found more satisfactory for a clearing medium than:—Carbolic acid crystals (melted), 40 ccm.; turpentine (oleum terebinthinæ), 60 ccm.

And for a mounting medium, Canada balsam, dissolved to the consistency of thick syrup in xylol or cedar-wood oil, has given excellent results.

Flemming's Chrom-Acetic Acid Method.—This has proved satisfactory for the rapid fixing of peripheral nerves and for stratified epithelia. For the stomach and intestines it has not proved so satisfactory as the picric alcohol. Chromic acid crystals, 6 grams.; glacial acetic acid, 2·4 ccm.; water, 2400 ccm.

The tissue is cut into pieces of moderate size and placed in 50 to 75 times its volume of the fixing agent for 12 to 24 hours. It is then washed two hours or more in water and left about 12 hours in 50 per cent. alcohol, then placed indefinitely in 75 to 82 per cent. alcohol. It may be dehydrated, infiltrated, and imbedded as described for the picric-alcohol method.

Hæmatoxylin is, on the whole, the most satisfactory stain, but the staining is not so satisfactory as after the use of picric alcohol. The

staining may be hastened in this case, as in all others where it is desirable, by heating the staining agent.*

Apparatus for making Esmarch's Rolls.†—The apparatus devised by Herr N. Prausnitz for preparing Esmarch's rolls consists of a tin box 10 cm. high, 23 cm. broad, and 19 cm. deep. In the middle of the short sides two grooves are cut out for the insertion of a spindle worked by a handle. On the spindle and at a distance of 14 cm. from one another are two circular tin plates, in the periphery of which ten round holes are cut out. When required for use, the box is filled with water heated to 10°–12°, and in the holes are placed test-tubes, filled with liquid gelatin. The handle is then turned until the gelatin is set.

The best results are obtained when the tubes are one-fourth full of gelatin.

New Cultivation Vessel.‡—Dr. L. Kamen gives an account of how he devised a cultivation vessel suitable for the examination of water, &c., and how in its main features it resembles closely that invented by Petruschky.§ The main differences seem to be, from the illustrations given, that the author's vessel is 4 cm. longer and 1 cm. broader, and that the neck is indented at one side only.

A comparison of the two sets of drawings will be quite sufficient for easily understanding the trivial differences between the two forms.

Dixon, S. G.—**An Apparatus for the Collection of Dust and Fungi for microscopical and biological tests.** *Therapeut. Gaz.*, 1890, p. 308.

(2) Preparing Objects.

Demonstrating the Membrane of the Red Corpuscle of Batrachia.‖—Dr. L. Auerbach, after submitting the red corpuscles of Batrachia to a renewed investigation, comes to the conclusion that they are invested with a colourless membrane. This is demonstrable if a drop of blood, carefully protected from loss of fluid, be left alone for some hours. By this time the contents of the corpuscle have receded from the membrane, usually being massed at the poles. On the addition of physiological salt solution, the membrane swells up like a bladder. This may be still better observed after hardening in saturated picric acid solution, subsequently washed out with water. Such a preparation, stained with eosin and anilin-blue, shows the membrane blue and the adjacent layer red. Certain reagents cause the corpuscle to swell up to a thin-walled bladder which bursts, allowing the contents to escape, and leaving an empty sac behind; such are sublimate in 0·1 to 0·25 per cent. solution, 1 per cent. boracic acid, chloride of sodium, and chromate of ammonia in 2 to 10 per cent. solution. In the corpuscle can be distinguished a cortical and medullary substance, the latter inclosing the nucleus. This is well

* If the picric alcohol solution, as given above, is diluted with an equal volume of water, it makes a most excellent dissociating medium for almost all the tissues. It is especially good for epithelia and for smooth and striated muscle. The striation in the striated muscle is exceedingly clear and the longitudinal fibrillation of the smooth muscle is easy to demonstrate.

† Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 129–30 (1 fig.).

‡ T. c., pp. 165–7 (2 figs.). § See this Journal, 1891, p. 131.

‖ Anat. Anz., v. (1890) pp. 570–8 (2 figs.). See Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 511–2.

seen after the action of a 1 per cent. aqueous sublimate solution and in picric acid preparations.

After hardening in picric acid, subsequently washed out, the cortical layer may be seen as a very fine network, an unnatural condition, the result of the formation of vacuoles. In sublimate preparations the medullary substance is bestudded with dark granules, while in picric acid preparations it is quite clear, and has the appearance of a hollow space. The nucleoli in the cells of adult animals usually stain blue (cyanophilous), while in the larval condition a few are to be found which stain red (erythrophilous). In the early days of larval life there is a single large nucleolus composed of both substances.

New Characteristics of Nerve-cells.*—Sig. G. Magini, who states that the absence of chromatin in the nucleus is a special characteristic of nerve-cells, as compared with neuroglia-cells, advises for the study of this distinguishing feature methylen-blue, and also, but less effectively, vesuvin and Ehrlich's hæmatoxylin. Carmine staining is quite useless for the purpose. The objects must be hardened in Kleinenberg's fluid, or in absolute alcohol, or in sublimate. Müller's fluid is not at all suitable.

Impregnation of the Central Nervous System with Mercurial Salts.†—Mr. W. H. Cox finds that a uniform impregnation of the central nervous system is obtained when the hardening and impregnating fluids are allowed to act together for two or three months. The reaction of the hardening fluid should be as slightly acid as possible. The fluid which Mr. Cox used consisted of 20 parts of 5 per cent. bichromate of potash, 20 parts of 5 per cent. sublimate, 16 parts of 5 per cent. chromate of potash, 30–40 parts of distilled water. The preparations cannot be preserved under a cover-glass in Canada balsam or dammar, for the acidity of the medium and some other unknown cause spoil them. A freezing microtome must be used, for the alcohol involved in the paraffin or celloidin methods endangers the impregnation. The sections are placed for an hour or two in 5 per cent. solution of sodium carbonate, are washed in water, placed for a short time in absolute alcohol, then in some oil, and finally covered with some rapidly drying resin. If they must be covered with a glass, the resinous layer should be allowed to dry, and then covered with castor-oil. Then the cover-glass is put on and pressed down so as to squeeze out the superfluous oil, or by using styrax, or a mixture of gum-arabic and water, &c., the preparations may be kept intact under a cover-glass.

Preparing Nervous Tissue of Amphibia.‡—Mr. A. Smirnow adopted the methylen-blue injection method for demonstrating nerve-cells of Amphibia. 1/4 to 4 per cent. methylen-blue solutions in 1/2 per cent. salt solution were employed. In from half to three hours after injection the tissues were removed from the animal, and the stain fixed with iodopotassic iodide or picrocarmine or picrate of ammonia. The prepa-

* Atti R. Accad. Lincei Roma—Rendiconti, vi. (1890) pp. 19–23. See Zeitschr. f. Wiss. Mikr., vii. (1891) p. 519.

† Arch. f. Mikr. Anat., xxxvii. (1891) pp. 16–21 (1 pl.).

‡ Op. cit., xxxv. (1890) pp. 407–24 (2 pls.). See Zeitschr. f. Wiss. Mikr., vii. (1891) p. 511.

rations were mounted in pure glycerin, in acidulated glycerin, or in glycerin to which 1 per cent. of picrate of ammonia solution had been added.

Examining Spermatozoa of Insecta.*—Herr E. Ballowitz used male beetles, the vas deferens of which was quite full of spermatozoa. The living spermatozoa were fixed with osmic acid vapour, and usually stained with gentian-violet.

Maceration specimens showing a fibrillation of the flagellum were obtained by removing the wings and upper abdominal wall and then immersing in very dilute sodium chloride solution for some days. A piece of vas deferens was then cut out, carefully washed, and then teased out on a slide in 0·8 per cent. salt solution. A drop of this fluid was covered over with a cover-glass, and after the lapse of one to three days stained with some anilin dye. Movements of the spermatozoa were shown on Schulze's hot stage, the optimum temperature being from 30° to 35° C.

Demonstrating Structure and Termination of Muscular Nerves in Œdipoda fasciata.†—Sig. V. Mazzoni employs the following modification of the gold chloride method for staining nerve-endings. Pieces of muscle, 1 to 2 mm. in size, are placed for half an hour in a watery solution of 1/3 formic acid. When quite transparent they are transferred to gold chloride solution (1:100), wherein they remain for 7 or 8 minutes. After this they are left in the dark for 12 hours in the formic acid solution, and then mounted in glycerin.

Mounting Acarina.‡—M. E. L. Trouessart finds that dried material containing mites makes better preparations than can be obtained from fresh specimens. The material is placed in a large drop of glycerin on a slide, but is not covered. The preparation is then carefully and slowly warmed over a spirit-lamp. By this the animals are cleared up and freed from air-bubbles and any adherent impurities. For imbedding, glycerin-gelatin is recommended, but if it is desired to keep the animals this may be done in alcohol or Hantsch's fluid.

Preparing Eggs of Pycnogonids.§—Mr. T. H. Morgan found the best way of hardening the eggs of Pycnogonids was to put them into alcoholic picro-sulphuric acid for several hours, and then to gradually carry them through different grades of alcohol of increasing strength. After an hour in absolute alcohol, two to four hours in turpentine, one hour of soft and one to two hours of hard paraffin, the eggs were cut in paraffin, and fixed to the slide with albumen fixative. Again, they were passed through turpentine, absolute alcohol, 95, 80, 70 per cent. alcohols to Kleinenberg's hæmatoxylin, where they remained for from twelve to forty-eight hours. They were then washed in acid alcohol for fifteen minutes, and passed through the alcohols and turpentine into balsam. Very excellent results were obtained.

* Zeitschr. f. Wiss. Zool., l. (1890) pp. 317–407 (4 pls.). See Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 503–4.

† Memorie R. Accad. Scienze Bologna, ix. (1889) pp. 547–50 (1 pl.). See Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 504–5.

‡ CR. Séances Congrès Internat. Zoologie Paris 1889, pp. 164–75. See Zeitschr. f. Wiss. Mikr., vii. (1891) p. 502.

§ Studies Biol. Lab. John Hopkins Univ., v. (1891) p. 3.

Preserving Caprellidæ.*—P. Mayer finds that these animals may be preserved without shrivelling by placing them in a mixture of glycerin 1 part and 50 per cent. spirit 2 parts after they are taken from the alcohol in which they have been kept. The alcohol is then slowly evaporated with moderate heat. The author considers that balsam is contra-indicated since on account of its strong refraction the finer skeletal details are imperfectly seen.

Mode of studying free Nematodes.†—Mr. N. A. Cobb collects from sand by applying his knowledge of the fact that, in standing water, sand sinks at once, while small organisms sink rather slowly. "Put half a pint of sand with a pint of water into a dish of the form and size of an ordinary one quart fruit-tin. Having a second beaker or fruit-tin at hand empty, pour the water and sand rapidly back and forth until the water is well roiled, then suddenly stop; the sand at once sinks to the bottom of the dish, but the organisms remain for a few seconds partially suspended. The instant the sand reaches the bottom of the dish, pour the supernatant fluid containing the organisms into a third dish and there let it stand until clear, when the sediment of organisms may be obtained in a very satisfactory state by decanting the clear water." In collecting from mud the process must be reversed.

If the animals are to be studied in the living state they may be rendered motionless by adding a little chloral hydrate to the water. If glycerin preparations are to be made, kill with 1/100 to 1/10 osmic acid and allow the worms to remain in it till they become a trifle coloured. It is best to use warm weak osmic acid.

For the very finest histological as well as coarser anatomical work Mr. Cobb has devised a method which gives far better results than any other with which he is acquainted.

Mode of examining Calcareous Bodies of Alcyonacea.‡—Dr. G. v. Koch says that the easiest way of examining these bodies is to cut a polyp through longitudinally with the scissors, to spread out in glycerin, cover with cover-glass, and observe with crossed nicols. The spicules will appear white on a dark ground and are generally very distinct. The same method may be employed with particles of cœnosarc.

Demonstrating Structure of Siliceous Sponges.§—Herr F. C. Noll succeeded, by treating with nitrate of silver, in showing that the spicules of *Desmacidon Bosei* were covered with an organic layer, the exact origin of which would appear to be uncertain. The same reagent was used with advantage in examining *Spongilla*. Small pieces of sponge were suspended on the slide in the aquarium, and when they had properly spread themselves out, were treated for about twenty minutes with 0·25 per cent. silver nitrate, and afterwards stained with picrocarmine. The flat epithelium was by this means well preserved. For imbedding,

* Fauna u. Flora d. Golfes v. Neapel, Monogr. xvii. (1890) pp. 157 (7 pls.). See Zeitschr. f. Wiss. Mikr., vii. (1891) p. 501.

† Proc. Linn. Soc. N.S.W., v. (1890) pp. 450–2.

‡ Mittheil. Zool. Stat. Neapel, ix. (1891) p. 655.

§ Abhandl. d. Senkenbergischen Naturf. Gesellsch., xv. (1888) pp. 1–58 (3 pls.). See Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 497–8.

Canada balsam was found unsuitable, but good results were obtained with the following medium. Glycerin-gelatin is mixed with equal volumes of acetic acid and glycerin and warmed up until all the constituents have become thoroughly mixed. At a temperature of 12° R. the mass is fluid but below this it is necessary to warm it before using it. If the mass under the cover-glass be not quite firm it is advisable to ring the preparation round with some cement.

Demonstrating the Structure of Rotten-stone.*—Herr F. Dreyer adopted the usual procedure for examining the structure of rotten-stone and the distribution of the Radiolaria, viz. grinding down to one flat surface, then fixing this with balsam to a slide and then grinding down the other side, followed by balsam and cover-glass.

Isolation of the skeletons of the organisms was effected by the following ingenious device. A saturated solution of Glauber's salts was heated in a test-tube and pieces of rotten-stone, dried in the air, dropped therein. By this means they were thoroughly saturated and as they cooled down the process of crystallization effectually pulverized them. If the siliceous skeletons only be desired the following procedure is more simple. Small pieces of rotten-stone are boiled for a short time in hydrochloric acid, the carbonate of lime is dissolved and the thus separated skeletons fall to the bottom as a fine meal. The material should be washed with water in a large glass vessel, stirred up and allowed to stand for one or two hours. The supernatant fluid is pipetted off and the washing repeated several times. Finally, the material is dried in the air. By tapping the watch-glass containing some of the material, the finer may be separated from the coarser particles; some of the former can be mounted in balsam.

Collodion-method in Botany.†—Mr. M. B. Thomas advocates the use of collodion rather than of paraffin for infiltrating plant-tissues. The tissue to be treated is first dehydrated and hardened in alcohol. It is then placed in a 2 per cent. solution of collodion made by dissolving 2 grm. of gun-cotton in 100 ccm. of equal parts of sulphuric ether and 95 per cent. alcohol. In this solution it remains from 12–24 hours, and is then transferred to a 5 per cent. solution, where it again remains 12 hours. It is then laid on cork and covered, by means of a camel's hair brush, with successive layers of collodion, until it is quite inclosed in the mass, allowing each coat to dry slightly before applying the next. After a few hours the collodion will be firm enough to section.

(3) Cutting, including Imbedding and Microtomes.

Imbedding and Sectioning Mature Seeds.‡—Mr. W. W. Rowler gives some useful hints as to the best method of imbedding mature seeds in paraffin and preparing them for the microtome. The method described is that in use in the botanical laboratory of the Cornell University.

* Jenaische Zeitschr. f. Naturwiss., xxiv. (1890) pp. 471–548 (6 pls.). See Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 498–99.

† Proc. Amer. Soc. Micr., 1890, pp. 123–7 (3 figs.). ‡ T. c., pp. 113–5.

A Method of Imbedding Delicate Objects in Celloidin.*—Mr. Frank S. Aby writes:—The object, properly fixed and hardened, is placed for twenty-four hours in a mixture of equal parts of alcohol and ether. It is transferred to a thin syrupy solution of celloidin, made by dissolving celloidin in a mixture of equal parts of alcohol and ether. After remaining in this solution for about twenty-four hours, the object is covered with a thicker solution of celloidin and is allowed to remain in the same for about twenty-four hours, when it is ready to imbed on cork.

When ready to imbed the object, a small quantity of the celloidin solution is spread on clean glass (a slide will answer the purpose), and allowed to dry. Then another coat is applied and allowed to dry. This affords a firm celloidin bed upon which the object is placed and arranged, care being taken to place it in the desired position as quickly as possible, before the celloidin begins to harden. The whole is now covered with successive layers of the celloidin solution, until a firm support is built up for the object. When sufficiently dry, the celloidin is removed from the glass by means of a sharp knife, and if necessary, a pair of scissors is used to trim the bed to the proper size and form. It is now ready to imbed on cork.

The top of a cork is coated with celloidin solution and allowed to dry. This is done to prevent air from rising from the cork and forming bubbles in the celloidin. The object, in its matrix of hardened celloidin, is placed in the desired position on the cork, and fastened to it with celloidin. After drying in the air until a layer is formed on the outer surface firm enough to retain the shape, the cork is dropped into 50 per cent. alcohol. Usually the object is ready to cut after remaining in the alcohol one hour.

This method of preparing a bed of celloidin has been employed with very satisfactory results in obtaining sections of embryo chicks. Blastoderms of the earlier periods of incubation have been successfully sectioned. By arranging the embryo on the bed of hardened celloidin, it has been possible to get large symmetrical sections of the blastoderm. Celloidin contracts during the drying process, but by exercise of due care in arranging the blastoderm, distortion may be avoided.

This method of imbedding has given good results in studying *Hydra*, and the preparation of the celloidin bed may be resorted to in almost every case where delicate objects are to be sectioned.

(4) Staining and Injecting.

Vasale's Modification of Weigert's Method.†—Sig. G. Vasale says that Weigert's method for staining central nervous tissue may be rendered less cumbrous by the following procedure, for which three solutions are necessary. (1) Hæmatoxylin 1 grm. dissolved in 100 grm. water by aid of heat. (2) Neutral acetate of copper, saturated filtered solution. (3) Borax 2 grm., ferridcyanide of potash 2·5 grm., dissolved in 300 grm. water.

The sections taken from spirit are placed in solution 1 for three to five minutes, then for same length of time in solution 2, whereon they

* Microscope, xi. (1891) pp. 58–9.

† Rivista Speriment. Freniatria e Med. Legale, xv. (1889) pp. 102–5. See Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 517–9.

become black. They are then washed quickly in water and transferred to solution 3, whereby the ganglion-cells, the neuroglia, and degenerated parts are quickly decolorized, the medullated fibres remaining stained dark violet. Finally, the sections are well washed in water, then dehydrated in absolute alcohol followed by carbol-xylol (3 parts xylol to 1 carbolic acid) and balsam. If a contrast stain be desired, alum-carmine or picrocarmine or Pal's method are recommended.

Upson's Gold-staining Method for Axis-cylinders and Nerve-cells.* —Dr. A. Mercier describes two new methods, the invention of Dr. Upson, of Ohio, for staining axis-cylinders and cells of central nervous system, the results of which are stated to be wonderful. The pieces are hardened in the dark for four to six months in potassium bichromate, beginning at 1 per cent., afterwards increased up to $2\frac{1}{2}$ per cent. The hardened pieces are then washed in water, and after-hardened in spirit, beginning for the first two or three days with 50 per cent. alcohol, and ending with 95 per cent. spirit, until the pieces are of a greenish colour. The sections may be made either with or without imbedding; in any case the sections are to be thoroughly dehydrated before either method is applied.

Method 1. The section is placed for one to two hours in 1 per cent. gold chloride solution to which 2 per cent. hydrochloric acid has been added. Wash in distilled water. Transfer on platinum or paper lifter to following solution for half a minute:—Potash, 10 per cent. solution, 5 ccm.; ferricyanide of potash, a trace. Wash for half a minute in 10 per cent. potash solution. Wash well in distilled water, and transfer to following solution:—Acid. sulfurosum, 5 ccm.; tinct. iodi, 3 per cent., 10–15 drops. Mix, and add liq. ferri chlorid., 1 drop. In this fluid the section is allowed to remain until it assumes a rose colour; it is then thoroughly washed in distilled water, dehydrated in absolute alcohol, oil of cloves, and balsam.

Method 2. The section is immersed for two hours in the following solution:—Gold chloride, 1 per cent., 5 ccm.; saturated solution of ammonium vanadicum, 10 drops; acid. hydrochlor., 3 drops. Having been washed in distilled water, it is immersed for thirty to sixty seconds in the following mixture:—Caustic potash, 10 per cent., 5 ccm.; ammonium vanadicum, a trace; permanganate of potash, 10 per cent., 10 drops. It is again washed in distilled water, and thereupon placed in the following fluid:—Tin solution, 15 drops; aq. dest., 3 ccm.; iron solution, 3–5 drops; acid. sulfurosum, 3 ccm.

The tin solution is made by adding so much chloride of tin to 3 per cent. tincture of iodine until the colour is white or yellowish. The iron solution is a saturated solution of ferrum phosphoricum in aq. dest.

When the section has become red it is then treated as in method 1.

The author states that although this method may appear somewhat complicated, in reality it is not more cumbersome than most other procedures, and that the results are splendid.

Three new Methods for Staining Medullary Sheath and Axis-cylinder of Nerves with Hæmatoxylin.†—Dr. M. Wolters describes the following method for staining the medullary sheath. The nerve-fibres

* Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 474-9. † T. c., pp. 416-73.

appear of a blue-black colour, while the cells are yellow or yellowish brown. The specimens hardened in Müller's fluid and afterwards in alcohol were imbedded in celloidin. The sections were then placed for twenty-four hours in a paraffin stove at a temperature of 45° in Kultschitzky's hæmatoxylin solution (hæmatox. 2 g., alcohol abs. q. s. ad solut., acetic acid, 2 per cent., 100 ccm.).

After this the sections were immersed in Müller's fluid, and then treated with 1/4 per cent. permanganate of potash, after which they were decolorized in acidum oxalicum 1·0, kalium sulfurosum 1·0, aq. dest. 200·0. Then, having been washed in water, they were dehydrated, cleared up, and mounted.

The second method stained beautifully the protoplasmic process of Purkinje's corpuscles in the cerebellum. In this the procedure consisted in hardening and sectioning as before, and then using the following mordant:—Vanadium chloratum, 10 per cent., 2 parts; aluminium aceticum liquidum, 8 parts. Herein the sections remained for twenty-four hours, they were then washed for 5–10 minutes in water, and then having been stained with hæmatoxylin as in the first method, were decolorized with Weigert's fluid.

In the third method the pieces were hardened by Kultschitzky's method,* and after-hardened in 96 per cent. spirit. The section mass was imbedded in either celloidin or paraffin. The sections were then immersed for twenty-four hours in the following mordant:—Vanadium chloratum, 10 per cent., 2 parts; aluminum aceticum liq., 8 per cent., 8 parts. After having been washed for ten minutes in water the sections were placed in the hæmatoxylin for twenty-four hours. The staining was then differentiated with 80 per cent. alcohol to every 200 parts of which 1 part HCl was added.

When they assumed a bluish-red hue the acid was removed in weak spirit, after which the sections were dehydrated in absolute alcohol, cleared up in origanum oil, and mounted in balsam.

By this method the large cells of cerebrum and cerebellum, their protoplasmic processes, axis-cylinders, and the glia-cells were well stained.

Staining Osseous Tissue by Golgi's Method.†—Sig. V. Tirelli found that Golgi's method was suitable for studying osseous tissue, and very advantageous for flat bones; for example, the skull bones of an almost mature rabbit embryo. Against a yellow background the bone-corpuscles stand out stained more or less dark-brown, the staining in the centre of the elements being less pronounced than at the periphery or in the processes.

The reaction does not affect every individual element, but occurs usually in groups of five to thirty; and this is an advantage rather than not, since it allows the recognition of delicate details of structure.

Impregnating Brain of Amphibia by Golgi's Method.‡—Herr A. Oyarzun calls attention to the fact that in Ramón y Cajal's modification

* See this Journal, 1888, p. 510.

† Atti R. Accad. Lincei Roma—Rendiconti, vi. (1890) pp. 24–6.

‡ Arch. f. Mikr. Anat., xxxv. (1890) pp. 380–7 (2 pls.). See Zeitschr. f. Wiss Mikr., vii. (1891) p. 509.

of Golgi's silver method the observance of definite lengths of time for the different stages of the procedure is important. In frog's brain the best results were obtained by allowing the hardening and impregnating fluids to act for twenty-four hours. For the brain of the triton and salamander twenty-four hours were sufficient. If the fluids were allowed to act for thirty to forty hours, the results were very unsatisfactory.

Staining Medullary Sheath of Nerves of Spinal Cord and of Medulla.*—Dr. A. Mercier says the following simple procedures give satisfactory results for sections of spinal cord and medulla. The sections, according as they contain much or little of the chromic acid salt, are immersed in one of the two following solutions:—

Solution 1. Weak alcohol, 100; hæmatox., 2; aq. dest., 100; alum, 2; glycerin, 100.

Solution 2. Strong alcohol, 120; hæmatox., 2; aq. dest., 130; alum, 2; glycerin, 50. Dissolve the hæmatoxylin in spirit and the alum in the water, add to the latter the glycerin, and then mix with the hæmatoxylin in spirit.

Herein the section remains from twelve to twenty-four hours. It is then washed carefully in water, after which it is transferred to a modified Weigert's decolorizer:—Aq. dest., 200; ferricyanide of potash, 6; borax, 4. When sufficiently decolorized it is washed in distilled water, dehydrated, cleared up in oil of clove, and mounted in balsam.

It was found, however, that if the first decolorizing solution were followed by a second composed of potash, 10 per cent., 2 ccm.; aq. dest., 10 ccm.; æther sulphureus, 1 ccm., the differentiation was more satisfactory.

Demonstrating Nerve-end Plates in Tendons of Vertebrata.†—Sig. G. V. Ciaccio adopted the following method for demonstrating nerve-endings in tendons of Amphibia. The pieces were taken from a living animal, or from one just dead, and placed at once in 1/1000 hydrochloric acid, or better in 1/500 acetic acid until they were quite transparent.

They were then immersed for five minutes in a mixture of gold chloride and potassium chloride solutions (1/1000 each).

This imparts a pale yellow colour.

The pieces were next placed in a large quantity of 1/500 acetic acid and kept there in the dark for a whole day, and then exposed to the sun for two or three hours. When the tendon has assumed a pale violet hue, it is taken out and placed for a day in 1/1000 osmic acid solution and finally mounted in glycerin to which 0·5 per cent. of its bulk of acetic or formic acid has been added. The medullated fibres are stained dark violet, their extreme terminations being also violet, but passing into red or blue. The tendon itself is stained a pale yellow or light violet.

Preparing and Staining Testicle.‡—Sig. H. Brazzola in studying the testicle and the formation of spermatozoa, found that Podwyssozki's

* Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 480–3.

† Memorie R. Accad. Sci. Bologna, x. (1890) pp. 301–424 (6 pls.). See Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 507–8.

‡ Memorie R. Accad. Sci. Bologna, viii. (1888) pp. 681–94 (1 pl.); ix. (1888) pp. 79–85 (1 pl.). See Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 516–7.

modification of Flemming's chrom-osmium-acetic acid was very suitable for the purpose. The objects were placed in 60 per cent. and absolute alcohol for 24 hours each, after which they were imbedded in celloidin. The sections were fixed to the slide with Mayer's albumen-glycerin. For staining purposes the Pfitzner-Flemming safranin, afterwards washed out with very dilute acid (0·1 to 0·25) gave the best results. A good double stain was effected with picric acid by Podwyssozki's procedure. Instead of the Pfitzner-Flemming safranin, a saturated aqueous solution of safranin or a 1 per cent. aqueous solution of gentian violet, or better still, one of these followed by the other may be used.

Gram's method followed by eosin made excellent preparations, and these were still better if the sections were further stained with safranin. The chromatin granules, like the mitoses, were stained red, the achromatic substances a pale blue.

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

Deterioration of Mayer's Albumen-Glycerin Fixative.*—Dr. J. Vosseler draws attention to the fact that Mayer's albumen-glycerin is extremely apt to lose its adhesive property after the lapse of a few months.

The loss of this essential property, its *raison d'être* in fact, is usually accompanied by a slight browning of the colour and a decrease of the viscidity, and the change is so gradual that it is easily overlooked. At first the author was inclined to lay the blame on the corks with which the bottles were stopped, or on the salicylate of soda added as antiseptic. Both these views turned out to be untenable. Little or no effect was observed from using different antiseptics, the least unsatisfactory being camphor. After noting that the peculiar decomposition was more liable to take place in summer than in winter, probably from being hastened by the increased light, air, and temperature, the author came to the conclusion that the glycerin was at the bottom of the mischief, and confirms his view by adducing the frequency with which preparations mounted in glycerin deteriorate.

Hints for fixing Series of Sections to the Slide.†—Dr. H. Suchannek has now altogether given up the use of mica plates, and employs glass slides or cover-glasses. These must be perfectly clean and free from grease. If greasy, spirit when run over a slide shows a tendency to form in balls and not to spread itself out in an even layer. The best adhesive is Mayer's albumen-glycerin, which is rubbed on the slide with the finger. The layer should be extremely thin and perfectly even. To this the sections will firmly adhere in about half an hour at a temperature of 40°.

If the sections be thin and betray any tendency to crumpling and will not lie quite flat, then Gaule's method is undoubtedly the best to pursue. This consists in fixing the sections with 50 per cent. neutral alcohol. The slides are then placed on top of an incubator with a sheet or two of blotting-paper interposed in order that the glass may not be heated above 40°. This causes the gradual and regular evaporation of

* Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 457-9. † T. c., pp. 463-6.

the spirit and leaves the section smooth and adherent to its underlay. A higher temperature always fails to get rid of some little amount of moisture owing to the unequal rapidity of the evaporation; hence the author lays it down as an axiom that the lowest possible temperature is an indispensable condition for the production of a successful preparation. The rest of the procedure is that which is commonly adopted.

Preparation of Venetian Turpentine.*—Dr. H. Suchannek, while recording his estimation of the value of Venice turpentine for microscopical purposes,† advises that it be dissolved in neutral absolute alcohol. About equal volumes of these ingredients are mixed together in a tall glass vessel and placed in a porcelain tile oven. It is necessary to shake the mixture frequently. In from twelve to twenty-four hours the turpentine is dissolved and has deposited its impurities, and in from twelve to eighteen hours more it will have acquired the necessary consistence.

Vosseler's Cement and Wax Supports.‡—Dr. J. Vosseler recommends that paper or cardboard slips should be cemented on the slide by means of a cement made of commercial bleached shellac. Thoroughly broken up shellac is placed in a glass vessel, and alcohol of 90–96 per cent. poured over in quantity just sufficient to cover it. The vessel covered over is then placed on a paraffin stove. In a comparatively short time a clear brownish-looking mass of a syrupy consistence results. It is at once ready for use and, according to its inventor, is a very valuable cement.

The wax supports are made out of a mixture of Venetian turpentine and white wax. A quantity of wax is melted in a porcelain vessel, and thereto is added, stirring continually the while, from half to two-thirds its bulk of Venetian turpentine. Addition of turpentine softens, addition of wax hardens the mixture; the desired consistence is easily ascertained by letting fall a few drops on a glass plate or into water.

Although sufficiently plastic or impressionable it adheres very firmly to glass, hence the position of the supported cover-glass may be altered by slight pressure with a needle on one or all of the supports.

The medium may be used instead of the compressorium in the examination of fresh specimens of living Crustacea, the restless movements of which are easily restrained by fixing the cover-glass to the slide.

(6) Miscellaneous.

Use of Polarized Light in Observing Vegetable Tissues.§—M. Amann describes the results of a long series of observations made on the tissues of Mosses under polarized light, which have led to some curious results. The different cell-walls present, under these circumstances, very different appearances, depending largely on their degree of cuticularization; and it is possible in this way to define the characters of the cells belonging to the different organs in a moss, and even to a certain extent to distinguish between the characters presented by different families.

* Zeitschr. f. Wiss. Mikr., vii. (1891) p. 463. † See this Journal, 1890, p. 258.
‡ Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 459–62.
§ Arch. Sci. Phys. et Nat. xxiv. (1890) pp. 502–8.

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.

Edited by

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VIII.—*A New Illuminating Apparatus.*

By E. M. Nelson, F.R.M.S.

(*Read* 20*th May*, 1891.)

In the direction of monochromatic light very little has been done. This may be attributed to the unsatisfactory results already obtained. Who, for instance, has seen a critical image with monochromatic light? Many have witnessed attempts, and some of us have ourselves tried experiments. There has been one universal verdict to all these efforts—viz. that of dismal failure. The experiments have taken two forms: first, that of obtaining monochromatic blue light by interposing absorption media, the other by prism dispersion.

The first may be dismissed in a word: so far as is known there does not exist a medium that will only pass a blue ray. Ammonio-sulphate of copper passes any amount of red light, blue glass does the same. The examination with a spectroscope of the light which has passed through any of these absorbing media immediately dispels the idea that it is monochromatic. Before proceeding I may say that the best results in this direction were obtained by using two thicknesses of pot cobalt glass supplied by Messrs. Powell and Lealand. The spectrum through these curiously agrees with that through Rainey's light-modifier, which was composed of three different tints of glass; an account of which will be found in the Transactions.* For monochromatic light in the strict sense we are thrown back on prism dispersion. Instruments on this plan have been made, notably by Zeiss on a Hartnack model. The Zeiss monochromatic apparatus is probably very suitable for obtaining a spectrum, but in its design the requirements of the Microscope are absolutely ignored. It consists of a slit; a low-angled achromatic lens having the slit at its principal focus; two prisms; and another low-angled achromatic lens very similar to the first. The rays diverging from the slit are parallelized by the first lens, and after passing through the prisms are received by the second lens and by it brought to a focus. With this apparatus the use of a substage condenser is impossible because it yields convergent rays.

It must be borne in mind that none of Abbe's condensers will focus parallel rays; much less therefore will they focus convergent. If it is used, as intended, without any additional apparatus the cone from the low-angled condensing lens is too narrow to be of any service. As therefore this apparatus will work neither with nor without a condenser, it becomes mere lumber in the microscopist's cabinet. I never heard of one that had been used after its first trial. The apparatus I am exhibiting this evening is, as may be seen, only a makeshift; but I claim that it has for its end the requirements of the modern Microscope.

* Trans. Micr. Soc. London, N.S. ii. (1854) pp. 23-4.

It is composed of a base-board, on which slides a piece of wood holding an adjustable slit. Screw adjustment to the slit is quite unnecessary, as clear definition of the lines in a spectrum is not what

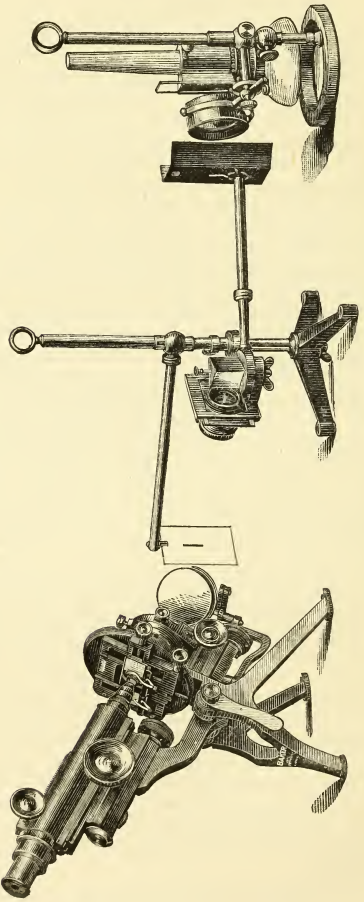


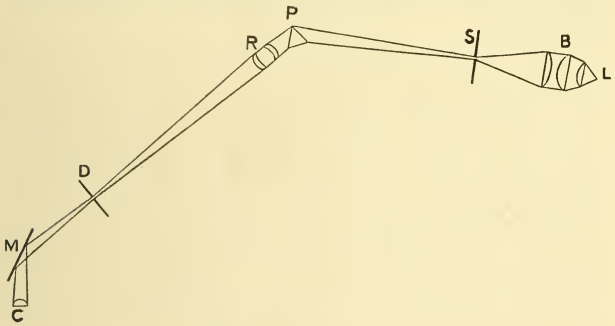
Fig. 51.

Nelson's Apparatus for obtaining Monochromatic Light.

we have in view. On the same piece of wood that carries the slit another piece of wood slides which carries the prism. This piece of wood rotates on its axis; connected to this is another piece of wood which rotates round the prism as a centre. This holds a photographic lens known as a Wray 5 × 4 R.R., working at $f/5\cdot6$. Lastly there is a card on a movable stand. The method of use is as follows. A strong beam of light is condensed on to the slit S, fig. 52, which is kept about 1/12 in. open, by means of one of my new bull's-eyes, B to which an additional lens has been added. From the slit the light passes to a dense flint prism P which by means of its rotating holder is set at minimum deviation.

The Wray photographic lens R, which is eminently suitable for this purpose, both on account of its being corrected for rays high up the spectrum and also on account of its large aperture, is rotated

Fig. 52.



round the prism until the refracted beam falls directly on it. The prism and this lens, which is attached to the same fitting, are now both moved to a distance from the slit equal to twice the focal length of the lens, the image of the slit, i. e. the spectrum, being focused on the card D, which is placed at a similar distance from the lens, and on the other side of it. The card is then moved so that the kind of light required may pass through an aperture in it to M the mirror of the Microscope. The apparatus has also been made in metal (see fig. 5), attached to a firm bull's-eye stand; another form is also made in wood, suitable for direct illumination without a mirror. The condensing system is not absolutely necessary; by placing the edge of the lamp-flame close to the slit a good light can be obtained, but the light is more intense when the condenser is used. We then proceed with the manipulation of the Microscope in the usual way, the slit

in the card being treated exactly as if it were the lamp-flame. By a slight rotation of the Wray lens any colour of the spectrum is made to fall on the aperture in the card, and by this means the required colour for the illumination of the Microscope is obtained. For resolving purposes the blue-green will probably be found the most suitable.

The next question is—What do we gain by this apparatus? In the first place, with regard to resolving power with blue-green light, it practically adds ·1 to the N.A. of the objective: thus a D.D. of ·8 becomes a lens of ·9 N.A., and that too without incurring any increase of spherical aberration. Secondly, as there can be no secondary spectrum, an ordinary achromatic lens performs as well as an apochromatic. For photomicrographic work it will be useful in taking the place of the coloured screens which are so necessary when isochromatic plates are used. For purposes of resolution, however, I do not think that it will prove of any assistance to photomicrography, Mr. Comber having pointed out that the plate itself is a monochromatic light-selecter.

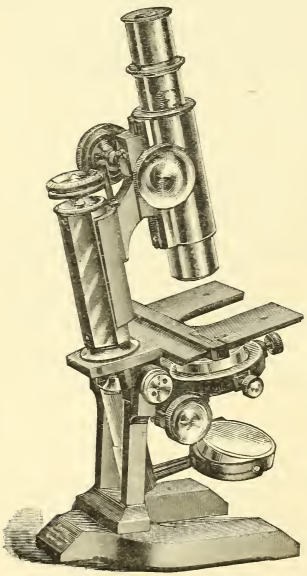
MICROSCOPY.

a. Instruments, Accessories, &c.*

(1) Stands.

Baker's Student's Microscope.—We give a figure (fig. 53) of the Student's Microscope lately made by Messrs. Baker, to which Mr. E. M. Nelson called attention at the March meeting.†

Fig. 53.



Zeiss's Crystallographic and Petrographical Microscopes.‡—Dr. S. Czapski describes the three latest forms of the Zeiss petrographical model.

The base of the large model (fig. 54) is of the usual horse-shoe form. The body-tube, &c., can be inclined and clamped in any position down to the horizontal. The illuminating apparatus, which is movable by rack and pinion, consists of the condenser and the diaphragm and polarizer holder.

The condenser has a numerical aperture of 1·4 and is movable in a socket, so that it can be easily removed and replaced by other illuminating arrangements, such as

(1) The achromatic condenser, or the special achromatic illuminating apparatus for photomicrography, by which a sharp image of the source of light is projected on the plane of the object.

(2) The Hartnack illuminating apparatus, for monochromatic light.

(3) The Engelmann microspectral objective.

(4) The spectro-polarizer of Rollet.

The polarizer holder carries the iris-diaphragm next to the condenser, and has the nicol P beneath. It is rotated by rack and pinion R, and the positions of 0°, 90°, and 180° are marked by a stop. To convert from polarized to ordinary light, the holder is simply pivoted aside (as represented in fig. 55).

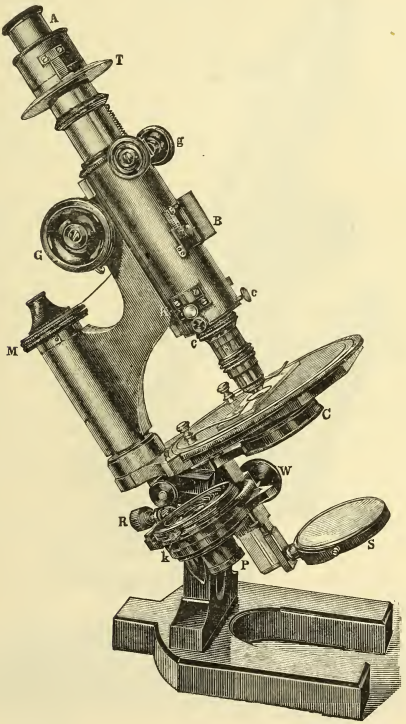
* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

† See *ante*, p. 298.

‡ Zeitschr. f. Instrumentenk., xi. (1891) pp. 94-9 (3 figs.).

The circular stage, graduated in degrees, is about 120 mm. in diameter, and is rotated by hand. For the orientation of the object, it is provided with a millimetre scale (100 mm.) along two diameters at right angles, and the diameters inclined to these at 45° are also provided with a division. The upper part of the stand is of the same form as in other

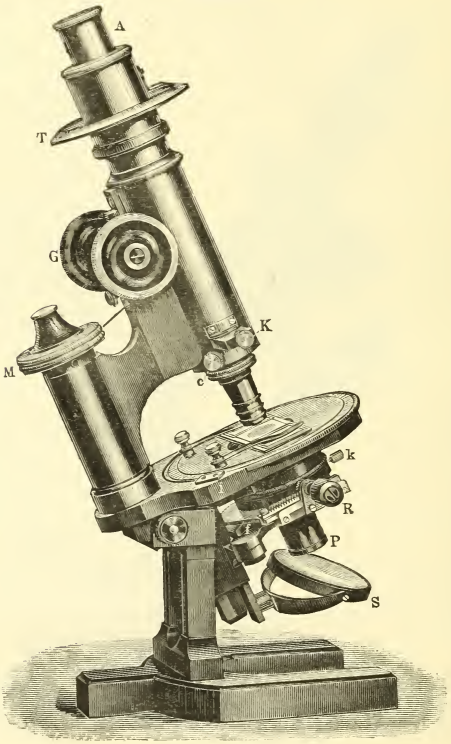
Fig. 54.



Zeiss models. The lower part of the body-tube has a centering arrangement *c c* provided with the Society screw. Near the end of the tube is a side opening in which slides a frame by means of the knob K. In the frame are two apertures side by side, one of which serves for the reception of a quartz plate, quarter-wave plate, &c., while the other usually remains empty, but may be used to receive a second plate. The draw-

tube is movable in the body-tube by rack and pinion *g*, and has a millimetre division giving the total tube-length. The eye-piece, which is provided with cross wires, is placed in the draw-tube from above. Above the eye-piece the analyser A (Hartnack-Prazmowski prism) is

Fig. 55.



applied, the mounting of which carries an index showing the orientation of the analyser on the divided circle T.

For observing the optic axial figures in convergent light, an Amici auxiliary objective can be applied by the knob B through an opening in the outer tube, and fitted into a slot at the lower end of the draw-tube.

The eye-piece forms with this objective an observing Microscope,

which can be adjusted on the optic axial figure by means of the rack and pinion of the draw-tube.

Fig. 55 shows the medium size model which is generally similar to the preceding. It differs from it only in being of smaller size, and in having no Amici auxiliary objective. Instead it is arranged for the "axial image eye-piece," and consequently has no draw-tube.

The small model is of the English tripod form. The polarizer and condenser of aperture 1·0 are fastened in one socket, and can be rotated by an arm. When drawn down in the socket a few millimetres, so that the condensing lens comes beneath the stage-plate, they can be shifted to one side by a lever. The stage is movable, and is provided with a divided circle. The body-tube can only be moved by rack and pinion, but the mechanism is sufficiently rigid to allow of the use of objectives, up to a focal length of 4 mm. At the upper end of the tube is a divided circle for the analyser, and at the lower end are the centering arrangement and the slit for the Biot-Klein quartz plate.

(2) Eye-pieces and Objectives.

New Objective Changer.*—The firm of Klönne and Müller have recently brought out an apparatus which is intended for the rapid and easy substitution of objectives. The apparatus is constructed something like a pair of pincers, the upper limb of which screws on by means of an arrangement like that of the ordinary revolver nose-piece to the Microscope-tube. From the under side of this upper limb a conical piece, which is encircled by the adapter-ring screwed on to the objective, projects downwards.

The two limbs of the apparatus are kept firmly together by means of a spring. In order to insert or change an objective it is merely necessary to press the limbs together and then put the objective into the half-collar of the lower limb.

The apparatus can be used in any position of the Microscope, and can be fitted with a centering arrangement.

(3) Illuminating and other Apparatus.

On a new arrangement in Microscopes for the rapid change from parallel to convergent light.†—Herr R. Brunnée, of the firm of Voigt

FIG. 56.

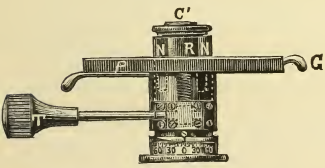
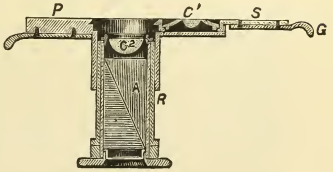


FIG. 57.



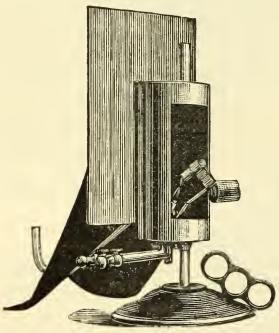
and Hochgesang, in Göttingen, has devised a method for the rapid change from parallel to convergent light, which he claims to be superior

* Central-Ztg. f. Optik u. Mech., xii. (1891) p. 46 (1 fig.).
† Zeitschr. f. Instrumentenk., xi. (1891) p. 136.

in simplicity and convenience of use to any other. In the plate P (figs. 56 and 57), by which the polarizer is connected with the instrument, is a slide S′, which, as soon as the tube R is lowered by the pinion T, has the effect of raising the lens C′ from the lens C^2. A pull on the arm G is then sufficient to move the lens C′ to one side into a depression in the plate P, and the polarizer, thus left only provided with the lens C^2, can be again adjusted in height and used for parallel light. To change again to convergent light, the tube R is lowered and the slide S pushed in, when the two lenses will again be connected together by means of the conical piece of the lens-holder C^3. To assure the correct position of the lens C′ in the ring of the slide S, the tube R is provided with four slots, in which fit four corresponding projecting pieces in the ring.

Kochs-Wolz Improved Microscope Lamp.* — The modifications introduced into the Kochs-Wolz lamp† are declared by Prof. P. Schiefferdecker, who describes the improvements, to make it an ideal lamp for microscopical purposes. The principal deviation from the original consists in a different form and method of illumination. In the present lamp a cylinder of zirconium is rendered incandescent by the combined action of an oxygen and coal-gas flame. The essential parts are fixed to a stand consisting of a heavy base supplied with a grip-handle and a vertical upright M S (fig. 59), up and down which they may be moved by means of a rack-work, the milled head of which is seen at S S. Within the metal case M C is fixed the zirconium cylinder L K, against the middle of which plays the flame from the burner B. The burner is connected with two tubes S *r* and G *r*, through which the coal and oxygen gases pass. Both these tubes can be stopped off by the cocks S *h* G *h*. The glass rod G is fixed in a tube-like prolongation on the front of the metal case M C, its inner end lying over-against the zirconium cylinder, while its outer end, bent to a convenient curve, lies underneath the diaphragm of the Microscope. In order to intercept any heat or light from the lamp, a blackened screen S*ch* is placed in front, and from the lower end of this a dark cloth T hangs down over the glass rod. The correction glasses are cemented on to the outer end of the rod. To set the apparatus going the gas-jet is turned full on, lighted, and then the oxygen-tap turned on until the flame just hisses. When the zirconium is white hot, the tap is turned down carefully till the hissing ceases.

Fig. 58.



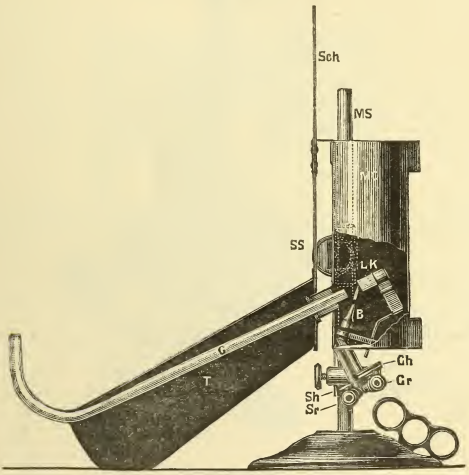
* Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 450-7 (2 figs.).

† See this Journal, 1889, p. 126.

One of the chief advantages of this lamp is the facility with which the intensity of the light is graduated, an advantage which, coupled with the fact that it preserves the natural colours of pigments, renders it even superior to daylight.

If this lamp is to be used in conjunction with an Abbe condenser, then instead of the curved glass rod a straight and very thick one is used. The outer end is adjusted about 9–10 cm. from the centre of the concave mirror, so that the light may fall on the very middle—a procedure much easier in practice than might be expected.

Fig. 59.



In a further communication * Prof. P. Schiefferdecker adds that the zircon cylinders have lately been rendered so much more durable that no cracking need be feared. It is advisable to use no more light than is absolutely necessary, otherwise the images are less well defined and the eye becomes fatigued. The glass rod should not be placed immediately beneath the diaphragm opening, but somewhat lower; and this is especially necessary when delicate colourless objects are under examination. Finally, new tubing should not be used for conveying the gas, since the dust which it usually contains will have the effect of partially stopping up the burner.

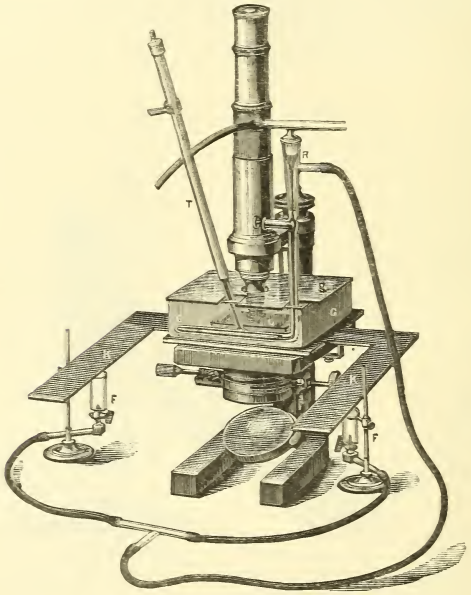
New Hot Stage and Accessories.†—Prof. W. Pfeffer describes a hot stage which keeps the temperature of the object and its environment

* Central-Ztg. f. Optik u. Mechanik, xii. (1891) p. 137.

† Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 433–42 (4 figs.)

more constant than the ordinary apparatus. The general arrangement of the whole may be gathered from figs. 60 and 61. The water receiver is a rectangular box about 110 mm. long, 70 mm. broad, and 35 mm. high, and covered over with a glass plate *g*, perforated with three apertures for the Microscope, thermometer, and regulator. In this is placed

Fig. 60.



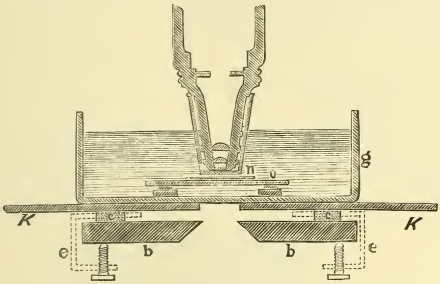
General arrangement of the apparatus when in working order.

the slide *o*, raised above the bottom from 4 to 8 mm. by strips of glass. The water is warmed by means of a copper plate *k*, heated by gas-jets *f*, the flame of which is regulated by a Stricker's regulator *r*. The pieces of vulcanite *c*, fig. 61, upon which the copper plate rests, are 3–4 mm. high, and are fixed on to the stage by the screw-clamps *e*. The thermometer *t* and the regulator *r* are kept in position by means of a stand (not shown in the illustration) to which they are clamped.

The light from the mirror is made to pass through a circular aperture in the copper plate, and then through the bottom of the glass trough on to the object. The bottom of the trough at this part is polished on both sides. For observing the object a water-immersion lens is he

most appropriate and convenient, but if a dry lens is to be used then the objective is surrounded by a conical glass or metal case *n*, fig. 61, to the end of which a cover-glass is cemented on. The case is adjusted to the objective by packing it on with cotton-wool. For

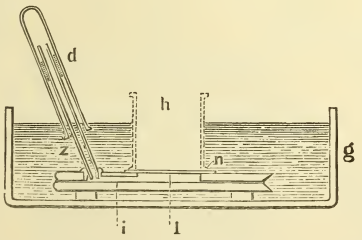
Fig. 61.



Vertical section through the objective, the water vessel *g*, the slide *o*, copper plate *k*, and Microscope-stage *b*. At *e* are shown the clamps, but these really lie in a different plane. About two-thirds natural size.

constructing an air-chamber suitable for most purposes, the author uses a couple of slides with central circular apertures. When these two slides are fixed or cemented together, and closed in above and below with a cover-glass, a fairly large air-chamber is obtained. If renewal of

Fig. 62.

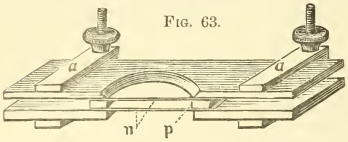


Vertical section through the water-vessel, as in fig. 61, showing the moist chamber with air-passage through *z*. The glass tube *b*, drawn in dotted line, is only used when the object is to be inspected through air. About two-thirds natural size.

the air be required, this is obtained in the manner depicted in fig. 62. A glass tube *z* communicates with the air-chamber by means of a passage *i* ground out of the uppermost slide. The tube is protected by inverting over it a test-tube.

The examination-chamber may be made of variable sizes by the method shown in fig. 63. Two cover-glasses separated by caoutchouc rings are fixed together by nickel plates with central circular apertures, and these plates are kept in position by the clamps *a*.

Fig. 63.

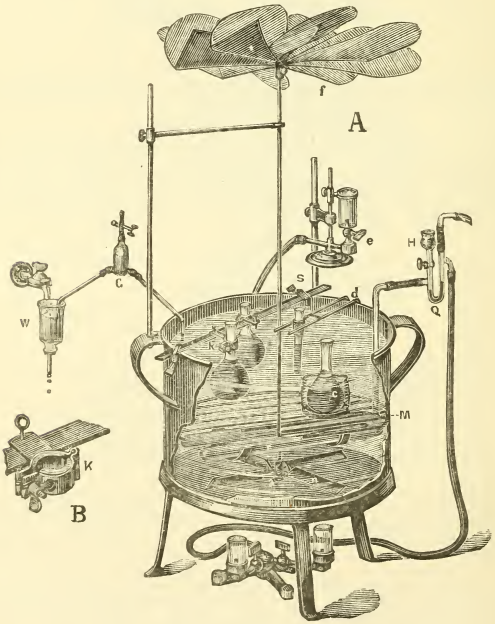


Chamber made with nickel plates. Bisected; natural size.

The temperature of the water-bath was found not to vary more than 0·1° C. in 12 hours when the water was kept throughout at 50° C.

Pfeffer's Water Thermostat.*—Prof. W. Pfeffer describes a water thermostat which, as it maintains a very constant temperature, is very

Fig. 64.



* Zeitschr. f. Wiss. Mikr., vii. (1891) pp. 442–7 (1 fig.).

useful for bacteriological and other purposes. The water-vessel, to hold 10–40 litres, is made of enamelled iron. Near the bottom is a floor made of brass bars M; beneath this is the U-tube, filled with 30 per cent. chloride of calcium solution, and the regulator *r*, which stops off access of gas to the flame in the usual manner by means of mercury.

An equable temperature of the water is effected by the working of the four scoops *n* driven by the fans *f*, which are set in motion by a gas flame *e*. The connecting-rod between the fans and the scoops is pivoted in an agate cup *a*. The water is maintained at a constant level by means of a siphon apparatus.

Flasks are fixed in position within the thermostat by means of the clamps shown in fig. B, or placed on the floor M, as at *c*. Test-tubes may be suspended by the device shown at *d*. Here they are placed in a cork which is jammed in between the two parallel bars.

Over the air thermostat this water thermostat possesses these advantages:—the cultivations are more rapidly brought up to the temperature of the surrounding medium, the water temperature is but little altered on the insertion or removal of the flask, and a greater constancy of temperature is attained.

(4) Photomicrography.

Baker's Photomicrographic Apparatus.—This apparatus, as recently supplied to Mr. Andrew Pringle, is shown in fig. 65. It consists of a substantial teak base-board 6 ft. 11 in. long, and 1½ in. thick, on which the camera with its support is placed, the other end carrying a teak-wood turntable clamping to the base. On the turntable a quadrangular metal frame is fixed, having a metal trestle to support the upper end of the limb of the Microscope when in the horizontal position, and two clamp-screws are fitted to receive the front feet of the Microscope. By this arrangement the instrument readily serves both purposes, either for ordinary observations or for photographing, the attachment in the latter case being easily and rapidly effected. The compound bull's-eye condenser, with centering adjustments, is carried by a pillar attached to the turntable; and beyond this is a support for the oxy-hydrogen lamp which is furnished with the usual mechanism for regulating the position of the lime-cylinder.

The Microscope is that known as the Nelson model, having the differential-screw fine-adjustment with actuating milled head at the lower end of the limb. It has a graduated rotating mechanical stage, and rackwork centering substage with differential-screw fine-adjustment. The body-tube is 150 mm. long, with racked draw-tube and an extra sliding draw-tube extending to 300 mm. An adapter with Society screw is fitted to the sliding draw-tube to allow the use of low-power objectives without racking the body-tube too far from its normal bearings, by which method the field of the objective is not cut off by the body-tube. The nose-piece is removable. The camera can be used at any length from 6–50 in.; it is provided with an exposure shutter and with a connecting tube sliding easily into a cap fitting on the eye-piece end of the Microscope. The camera can also be moved laterally and clamped to the base-board.

Focusing-rods run the whole length of the base-board, and con-

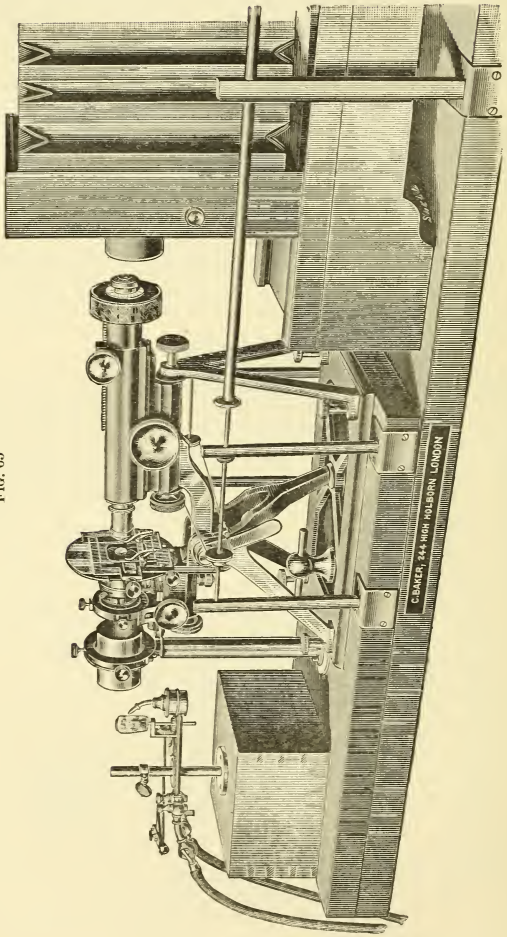


Fig. 65

Baker's Photomicrographic Apparatus.

nect readily with the milled head of the fine-adjustment by means of a silk cord.

We are requested to note that in the woodcut the apparatus appears reversed from right to left.

(6) Miscellaneous.

A Method of Drawing Microscopic Objects by the Use of Co-ordinates.*—Dr. Cooper Curtice writes:—"The method which I am about to detail is one that I found in use by Dr. George Marx, of the Division of Illustrations, in the U.S. Agricultural Department, when I first engaged studying animal parasites in 1886, but it was originated some eight years earlier, as he informs me.

It is a method that has such obvious merits that I take pleasure in placing it before students of the Microscope, but I present it as a relater of a valuable method rather than of original work. Its simplicity, its cheapness, its accuracy, the ease with which a figure of any magnification or reduction may be made, and the rapidity with which a beginner adapts himself to its use, all serve to recommend it.

A small glass slide, of the size of an eye-piece micrometer, or a disc ruled into squares, is inserted into the eye-piece, so that the lines seem to rest upon the object. Tracing-paper is placed over cardboard ruled into squares. The drawing is then made freehand, the various points located in a symmetical position with respect to the lines underlying the paper that they occupy in the apparently ruled image. The drawing made on the tracing-paper may then be either transferred to drawing-paper without reduction or be reduced by applying the same methods that produced the picture, and then be worked up.

Dr. Marx prefers using the slide. It is ruled into squares 1 mm. on each side, every third line being slightly deeper, to make it prominent. I prefer for most uses the finder made by Zeiss. It is a circular disc, upon the centre of which are ruled two sets of ten lines at right angles to each other, the lines being 5/10 mm. apart. The lines are very neatly ruled, and covered by a thin cover-glass cemented to it with balsam.

It is apparent that the system has a wide application, so far as the magnifications to be attained are concerned. The equation giving the magnification is $x = \frac{b}{a} \times c$, a being length of object, b the length of image, c the ratio of the image to the drawn figure.

Suppose that the amplification of objective is $5 \times$; that the lines on the eye-piece slide to 1/2 mm. apart, and those on the cardboard be 6 mm., then $x = 5 \times 6 \times 2$, or 60, for the unit of the card squares is twice those of the eye-piece squares.

To use a series of objectives, or of squares for the eye-piece and for the cardboard, are easy matters. A single glass ruled to half millimetres, made to fit a low-power eye-piece, is sufficient to try the plan. Card-boards, either of Bristol boards or heavy calendered Manilla paper, may be ruled into squares 3, 5, 7 mm., &c., until the student has all the combinations desirable.

By adopting this plan of drawing figures, I have found that objections

* Amer. Mon. Micr. Journ., xii. (1891) pp. 52–3.

which I find to using the camera are avoided. The lighting is not interfered with, the image moves but little, if any, with the movement of the head, and the image cannot be distorted. It is true that the accuracy of the figure depends on the skill of the artist, but a short trial of the method will satisfy most students that the actual variation of the drawing in symmetry from the image is less than that in figures made by the camera.

The objection now existing that American makers have not on hand necessary slides will be gladly removed by them as soon as they see a demand."

Carl Zeiss-Stiftung in Jena.—The following notice is of interest:—"By the present official notice the undermentioned firm has the honour to announce that their former proprietors, Dr. E. Abbe and Dr. R. Zeiss, have this day withdrawn from the firm, after having made over, according to agreement, the optical workshops in their entirety to the Carl Zeiss-Stiftung in Jena. The latter enters into all the rights, and accepts all the liabilities of the late proprietors. The firm itself remains unchanged. Dr. E. Abbe has been appointed by the Carl Zeiss-Stiftung as its authorized representative in all matters pertaining to the optical workshops, and to him, in conjunction with Dr. S. Czapski and Dr. O. Schott, the whole internal and external management has been transferred. Power of procuration has been granted to the two last-named gentlemen.—Jena, July 1st, 1891. Carl Zeiss Optische Werkstätte.

Extract from No. 153, 2. Juli, 1891 (2te Beilage) des 'Deutschen Reichs- und Preussischen Staatsanzeigers.' By a decree of His Royal Highness the Grand Duke, the undermentioned institution, inaugurated by deed of May 19, 1889, by Dr. Ernst Abbe, has been by law established, and received the right of legal personality.

The objects of the Institution are:—(1) The cultivation of the branches of scientific industry which, by the efforts of the founder, have been established in Jena by the optical workshops of Carl Zeiss, and the glassworks of the firm of Schott and Genossen; while at the same time attention is paid to the economical maintenance of those two establishments, and to the continued fulfilment of the social duties imposed upon the founders of the institution towards those who belong to it. (2) The advancement of mathematical and scientific studies by research and instruction.

The institution bears for all time the name Carl Zeiss-Stiftung, 'in honour of the man who first laid the foundation for the above undertaking, and in lasting remembrance of his own peculiar merit in having in his field of work always aimed at the co-operation of science and technical skill.' The management of the institution is by law transferred to the Kultusdepartement of the Grand Ducal Ministry of State; its judicial seat is Jena.

To the preceding public announcement must be added the fact that, after the Carl Zeiss-Stiftung had become the proprietor of the optical workshops of Carl Zeiss and co-proprietor of the glass technical laboratory of Schott and Genossen, (*a*) Dr. Ernst Abbe was appointed as authorized representative of the Carl Zeiss-Stiftung, with right of signing for the firm in all matters pertaining to these two establish-

ments, and Dr. Siegfried Czapski was allowed to act as deputy for him in his functions. (*b*) Privy Councillor Rothe, in Weimar, was appointed as commissioner of the management of the institution.—Weimar, June 24, 1891. Grossherzoglich Sächsisches Staatsministerium, V. Gross.

In our business register the following entries respecting this day's decree have been made:—in Fol. 49, Bd. 1, for the firm Carl Zeiss in Jena, and under the headings—

(*a*) Proprietor:—No. 5. The two proprietors named under No. 2 and No. 4, Dr. Med. Roderick Zeiss and Dr. Ernst Abbe, have withdrawn. No. 6. The Carl Zeiss-Stiftung in Jena is the sole proprietor of the firm.

(*b*) Representative:—No. 2. Dr. Ernst Abbe in Jena is the authorized representative of the Carl Zeiss-Stiftung in Jena, with the right of signing for the firm. No. 3. The power of procuration granted to Dr. Otto Schott in Jena, named under No. 1, has been renewed by the Carl Zeiss-Stiftung. No. 4. Dr. Siegfried Czapski in Jena is procurator.—Jena, June 30, 1891. Grossherzoglich S. Amtsgericht, Abtheilung IV. Dr. Jungherr."

Death of Mr. Mayall.—It is with the greatest regret that we have to announce the death, on July the 27th, of Mr. John Mayall, jun., one of the Secretaries of the Society. His death will be felt as a severe loss wherever the Microscope is studied scientifically. We must postpone till the next number a detailed account of the services rendered by our deceased friend to science, to the Society, and to this Journal.

The late Mr. Tuffen West, F.R.M.S.—Tuffen West, whose death at the age of sixty-eight we have recently had to lament, was one who has had few equals in devotion to natural history, and especially to its microscopic side. He was unrivalled as a draughtsman and a manipulator, and his love for his subject supplied him with never-failing energy. Severe bodily illness had for the last twenty years secluded him from contact with his fellow-workers, and robbed him of that public recognition of his services which he was about to reap. There are, however, still living many who well remember him, and can testify to the importance which was attached to securing his services in the production of any work requiring illustrations. As he was possessed of but a small income, and in the earlier part of his career of none at all, he made his dexterity with his pencil the source of his support. It was not, however, by any means solely for his artistic ability that his collaboration was eagerly sought by authors, for it was well known that he was both able and willing to give help in the most varied directions of scientific and pathological research. Work by others which had passed through his hands not only obtained a very considerable security against error, but not infrequently received important additions and elucidations. His good nature in these matters was occasionally somewhat imposed upon, and papers and books were published which really owed quite as much to the man whose name appeared only as artist, as they did to him who

assumed the rôle of author. In a general way he rendered these services with pleasure and because he delighted in his work, but there were instances of this kind of partnership which he felt to be unfair, and concerning which he would remark with a smile, "My poverty, but not my will, consents."

Tuffen West was the eldest son of a not undistinguished man. William West, of Leeds, his father, was F.R.S., and in a foremost position as a consulting chemist in the northern counties. He was one of the founders of the British Association for the Promotion of Science and of the Leeds Philosophical Society. He was much engaged as a medical jurist in cases requiring chemical knowledge, and it is said that his son's devotion to microscopic work was, when quite a youth, much developed by his being employed in the examination of blood stains in a case of murder which was tried at the York Assizes.

Those interested in tracing the hereditary descent of special faculties may hold it not superfluous to record that William West's father was cousin to Benjamin West, the distinguished President of the Royal Academy. Artistic taste had shown itself also in other members of the family.

West's parents were members of the Society of Friends, and Tuffen was educated at an excellent school at York belonging to that sect. There was a museum in the school, and much attention was given to the study of botany and zoology. The name of its master, John Ford, deserves to be recorded as one to whose kindly assistance Tuffen West, in common with many others, owed much in the development of his early tastes. As a schoolboy he was an indefatigable collector, and every moment that could be stolen from his lessons was devoted to insects, plants, and skeletons. Not far from the bottom of the school cricket-ground ran the Foss, a stream which yielded to the young naturalist uncounted treasures. In connection with this river an anecdote is told which illustrates alike West's habits and his character as a boy. The head-master having found that his boundary rules were often broken, proceeded on one occasion to make inquisition of his pupils. Calling them in succession before him, the question was put, "How many times hast thou been out of bounds during the last fortnight?" Some denied the charge altogether; some owned to once, some to more, but when it came to Tuffen's turn he replied frankly, to the astonishment alike of his comrades and the master, "Please, John Ford, every day."

After leaving school Tuffen West was apprenticed to Mr. Henry Brady, of Gateshead, a surgeon of scientific attainments himself, and who had the singular, possibly unique, good fortune to see three of his sons in succession elected into the ranks of the Royal Society. Although not much is known as to the details of his Gateshead life, it may well be supposed that in such a family a taste for natural science would certainly be fostered. Towards the end of his apprenticeship an accident occurred which put an end to his prospect of a medical career. By some inadvertency in a chemical experiment in his father's laboratory an explosion occurred, and in addition to other injuries Tuffen West incurred the irreparable loss of hearing. Through the whole of his subsequent life he was so deaf that in spite of mechanical aids it was impossible for him to listen to ordinary conversation. This was a terrible

deprivation to him, for it not only excluded him from the profession for the practice of which he had been trained, but it shut him off almost wholly from social converse. This to a man fond both of society and societies, was a most heavy blow. Thrown back on himself, Tuffen West now turned with increased zeal to his Microscope and his pencil. The result was that he rapidly developed unrivalled excellence in the use of both. Neither, however, offered much prospect of remunerative occupation, and for long Tuffen West lived in the most frugal manner. During some years he was engaged in continuous work in connection with the Queen's University at Belfast, and resided there. Subsequently he came to London, where a younger brother was in business as a lithographer, and was able to put scientific work into his hands. He now became well known, and his services were soon in great request. The Transactions of the various learned societies were year after year constantly illustrated by his hand. He was at the time of his first sudden illness in receipt of a good income, and overwhelmed with work. This was twenty years ago, and although he afterwards repeatedly resumed his pencil he was never able to undertake much. He resided during the latter part of his life in a house which he had built for himself in the beautiful neighbourhood of Frensham, near Haslemere. In personal appearance Tuffen West was thought by some to bear a resemblance to our present Prime Minister, Lord Salisbury. He was, however, of slighter build, and the regularity of his features had been somewhat marred by the accident to which reference has been made. He was a man of an affectionate disposition and of singular simplicity of character. He was twice married, but had the misfortune to lose his only son. Although an ardent Darwinian he retained an orthodox creed, and on one occasion protested with vehemence that nothing should ever make him give up his belief in the literal truth of the narrative of Jonah's escape. He took no part in politics; he read but little in poetry or fiction; he was deaf to music; he never in his life handled either rod or gun, nor did he often wear skates or mount a horse. He was, however, an invaluable companion in a country excursion, and could make himself and others happy anywhere if only a magnifying glass and a pencil were at hand. His Microscope and its accompaniments were his invariable companions. He was a diligent note-taker, and his memorandum books were crowded with pencil sketches of the objects which he described. The writer of this was on very intimate terms with him during the busiest part of his career, and often accompanied him in country excursions. On one of these they reached their destination, a lone farm-house close to the sea, a few miles from Hunstanton, near midnight and in darkness. Both were up by daybreak. They met at breakfast. "Well, Tuffen, how do you like the sea?" "To tell the truth, I haven't seen it. I got into a ditch at the back of the house, and I found it so full of interest that I did not go any further." On the same occasion, pockets crammed and arms burdened with specimens, he was stopped while trespassing by a landowner, attended by two gamekeepers. This was a not infrequent occurrence, and West on such occasions was accustomed to oppose to his enemies two deaf ears, with the result of much display of temper on their part and victory with little loss on his. He had a great contempt for the exclusiveness of proprietors,

and took a pride in going wherever he wished. His taste for natural scenery was not probably great, but his determination to secure any botanical or entomological specimen which he coveted was such as no gamekeeper could thwart.

From the nature of his occupations it almost followed as a matter of necessity that West did not do much work in his own name. He had to earn his livelihood in a very ill-paid and most engrossing occupation, and although he loved it in all its branches he yet felt somewhat keenly the fact that it took up all his time. He was accustomed to rise early and to work long into the night, yet his work was often in arrears and his employers clamorous. Nothing that he undertook was ever scamped. Thus it follows that but few original papers are to be credited to his pen. His work stands chiefly in other men's names. A paper on the mechanism of the feet of insects was of his own contributions to science the one in which he took most pride. Four years of his life were devoted to the illustrations of Blackwall's volumes on English spiders, and five to those of Smith on Diatomaceæ.

He was a fellow of the Linnean Society from the year 1861, and also of the Royal Microscopical Society. He was an honorary member of the Zoological and Botanical Society of Vienna, of the Tyneside Field Naturalists' Club, and of the Leeds Naturalists' Club.

Joseph Leidy.*—The following is part of a sympathetic notice of our late Honorary Fellow:—Dr. Joseph Leidy, the eminent comparative anatomist, zoologist, and palæontologist, died at Philadelphia on the 30th of April. He was born in the same city on the 9th of September, 1823. His father was a native of Montgomery County, Pa., but his ancestors on both sides were Germans from the Valley of the Rhine. While yet a schoolboy, minerals and plants were eagerly collected and studied, and also anatomical dissections were begun. He entered the Medical School of the University of Pennsylvania in 1840, and devoted his first year to practical anatomy. Having taken his medical degree in 1844, he became the next year, then twenty-one years of age, prosector to Dr. Horner, professor of anatomy in the university; and at the death of Dr. Horner, in 1853, he was appointed his successor.

In 1844 he made the many remarkable dissections of terrestrial molluscs, the drawings of which cover sixteen plates and illustrate thirty-eight species, in Dr. Binney's fine work on the Terrestrial Molluscs of the United States, showing in all not only remarkable power as an anatomist, entitling him to high rank, as Dr. Binney remarks, among philosophical zoologists, but also great skill as a draughtsman. Thus from the first Dr. Leidy was the thorough, minutely accurate, and untiring investigator.

After the publication of Dr. Binney's work in 1845, Leidy was elected a member of the Academy of Natural Sciences of Philadelphia, and from that time he was its most active member, hardly a volume of its publications appearing without one or more papers on the results of his researches. His contributions to zoology and comparative anatomy have a wide range. The lower invertebrates occupied a large share of his time. Besides multitudes of short papers, he published in 1853 a

* Amer. Journ. of Science, xli. (1891) pp. 523-5.

work of sixty-seven pages, illustrated by ten plates, on 'A Flora and Fauna within Living Animals,' of the botanical part of which Dr. Gray said in this Journal, "A contribution of the highest order, the plates unsurpassed if not unequalled by anything before published in the country." In 1879 appeared his large quarto volume on the freshwater rhizopods of North America, containing forty-eight coloured plates, the material of which was in part collected during two seasons in the Rocky Mountain region. As a portraiture of the doctor over the little member-less species, we quote from his concluding remarks:—"The objects of my work have appeared to me so beautiful, as represented in the illustrations, and so interesting, as indicated in their history which forms the accompanying text, that I am led to hope the work may be an incentive, especially to my young countrymen, to enter into similar pursuits. 'Going fishing?' How often the question has been asked by acquaintances as they have met me, with rod and basket, on an excursion after materials for microscopic study. 'Yes,' has been the invariable answer, for it saved much detention and explanation; and now, behold, I offer them the result of that fishing. No fish for the stomach, but as the old French microscopist, Joblet, observed, 'Some of the most remarkable fishes that have been seen, and food-fishes for the intellect.'" He delighted in his work because he knew that there was no fact in connection with the structure and functions of the simplest living things that was not profound and comprehensive, that did not reach up through all species to the highest. The vertebrates described by him were mainly fossil species. Dr. Leidy has the honour of having opened to geological science a general knowledge of the remarkable mammalian fauna of the country, and especially that of the Rocky Mountain region. Species had been before described, but through him the general range of North American species began to be known. In 1847 he published on the fossil horse; in 1850, on the extinct species of the American ox; 1852 and 1854, on the extinct Mammalia and Chelonia from Nebraska Territory, collected during the survey under Dr. D. D. Owen; in 1855 on the extinct sloth tribe of North America; in 1869, on the extinct mammalian fauna of Dakota and Nebraska, a thick quarto volume published by the Philadelphia Academy of Sciences, based on materials that had been gradually and continuously accumulating for the last years; and in 1873, contributions to the extinct fauna of the Western Territories, making the first quarto volume of the Hayden Survey. The last two works mentioned contain over eight hundred pages of text and nearly seventy of plates. Besides these large works numerous short papers from time to time appeared.

Dr. Leidy retired from this field when questions of priority began to start up, it being no part of his nature to quarrel, and having the firm belief, as he said, that the future would award credit where it was deserved. His work among the fossil vertebrates extended also to fishes, batrachians, and reptiles of different geological periods. Dr. Leidy's zeal never flagged; his labours came to an end only with his sudden death. Eight days before it he delivered his last University lecture. Beginning original work before he was twenty, his published papers and larger books continued to appear through half a century, and number over nine hundred. As is well said in one of the many tributes to him

published in the Philadelphia papers after his decease, "He possessed to the end of a long career the freshest capacity of seeing the opportunities and openings for discovery and research offered by familiar phenomena. His vast store of exact and diverse knowledge in the whole wide field of animate nature was under the command of a logical judgment and synthetic powers which saved him from vagaries. These high intellectual powers were served by an untiring capacity for work and equal skill of eye and hand. These are rare gifts; but they are none of them, nor all of them put together, as rare as his character. His simplicity, his transparent sincerity, his ingenuous anxiety to serve science and to serve science alone, his freedom from all desire for the rewards, the honours, and the recognition after which lesser men go a-wandering, were as remarkable as his scientific powers." Never were words more truthful. Honours came to him from all parts of the civilized world, and more because unsought.

β. Technique.*

(1) Collecting Objects, including Culture Processes.

Preparing Tuberculin.†—Herr O. Bujwid prepared tuberculin by cultivating the bacilli in glycerin-bouillon at a temperature of 38° C., after a period of three weeks the cultivation fluid was sterilized thrice, being kept for ten minutes each time at intervals of ten hours at 100° C. The fluid was then filtered and the filtrate inspissated in a water-bath to one-fourth its previous volume. At a pressure of 20 mm. the boiling-point was found to lie between 30°–34° C. A fine precipitate which then formed was filtered off and the fluid further inspissated to the consistence of syrup. Thus obtained, the tuberculin was thinner and lighter than Koch's lymph. Experiments were then made on healthy and tuberculous guinea-pigs: the former bore well the injection of 1/2 ccm., while the latter manifested a general and local reaction. In two lupus patients who had been already treated with Koch's lymph the characteristic reaction occurred after injection of 10 mg., but without any rise of temperature.

The author considers that his tuberculin is about half as strong as Koch's fluid, and does not believe it is a toxalbumin, but is rather inclined to hold that it is a ptomaine, or an intermediate between a ptomaine and an enzyme.

Preparing Pepton-agar for studying Pyocyanin.‡—M. Gessard gives the following ready method for making the pepton-agar so useful in studying the formation of pyocyanin. In each test-tube is placed 0·25 grm. of finely-chopped agar, and then 5 ccm. of neutral 2 per cent. pepton solution and 5 drops of glycerin are added. The tubes are then

* 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.

† Gazeta Lekarska (Polish), 1891, No. 4. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 579–80.

‡ Annales de l'Institut Pasteur, 1891, p. 65. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 541–2.

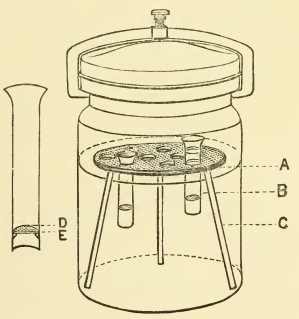
heated for some time to boiling-point in a water-bath in order to drive out the air from the agar. After this they are sterilized for five minutes at 120° C., and allowed to set in oblique position.

Simple Method for sterilizing Catgut.*—Mr. G. R. Fowler sterilizes commercial catgut by boiling it for an hour in 97 per cent. alcohol. The control experiments were made with anthrax and suppuration cocci. It was found that catgut which had been soaked in these germs was rendered perfectly sterile in an hour.

(2) Preparing Objects.

Dehydrating Apparatus.†—Mr. M. B. Thomas writes:—"A very convenient form of Schultze's dehydrating apparatus can be made as follows:—In a 9 × 9 in. Whittall-Tatum museum jar a disc of plaster of Paris is supported about 2 cm. from the top by means of legs made of glass rods (fig. 66, A and C). The disc is perforated to allow tubes of sizes varying from 2 to 4 cm. in diameter to pass through. These are the so-called dehydrating tubes (fig. 66, B). The plaster of Paris diaphragm can be made by first making a mould of the desired size with a paper bottom and a cardboard hoop for the outside. This must be placed on a level surface. The plaster of Paris is then softened with water and poured into the mould to about the depth of 1½ cm. While it is yet soft the three legs can be inserted near the edge, and holes for the dehydrating tubes cut in the disc with a knife, or pressed out with glass tubing of convenient size. When the plaster of Paris is thoroughly dry the hoop can be removed and the disc placed in position in the jar.

Fig. 66.



The jar is then filled with alcohol to about 2 cm. of the under side of the disc. The dehydrating tubes should be about 12 cm. long, and can be made by cutting off the bottom of large test-tubes. At the bottom is placed a diaphragm of chamois skin, which can be fastened in place by means of a spring made of steel wire, and forced inside of the chamois skin in the tube, thus pressing the former firmly against the latter (D, E). A rubber band around the tubes prevents them from falling through the holes in the disc, and enables them to be lowered to any desired depth in the alcohol.

The tissue to be dehydrated is packed closely in the dehydrating tube, and enough 50 per cent. alcohol poured over it to just cover it. It is then lowered through the hole in the disc until the two liquids are

* New York Med. Record, 1890, pp. 177–9.
† Amer. Mon. Micr. Journ., vii. (1891) pp. 7–8.

just at a level. After from 12 to 24 hours the two liquids will be of the same strength. The tissue can then be taken out and placed in the infiltrating bath at once.

This method for hardening has been tried in the botanical laboratory at Cornell University on nearly all kinds of plant-tissue, and in every case it was found to be successful. For the most delicate tissues, where slow hardening is desired, 5 per cent. alcohol can be placed in the dehydrating tube and thick chamois skin used for a diaphragm, and for some of the more delicate algæ it has been found advisable to use as low as 1 per cent. alcohol in the tube. The strength of the alcohol in the jar can be kept up by adding to it from time to time some calcium chloride. This will not injure the alcohol in the least.

The jar should be tall enough to allow the cover to be kept on while the tubes are in position, and thus prevent evaporation of the alcohol. An apparatus of such a form, having thirteen dehydrating tubes, has been in constant use in the botanical department for a year without changing the alcohol, and is yet in good working order.

Experiments have been made with one of smaller size, and it is found that all hardening agents, such as picric, chromic, acetic, or osmic acid, can be used in it with equal success.

The advantages claimed for the apparatus are these:—Not more than 24 hours is necessary for dehydrating and hardening nearly all kinds of plant-tissue. The apparatus does away with the transferring of the tissue from bottles containing alcohol of different strengths, and as no sudden transition from solutions of different strengths occurs, the tissue is less liable to shrink. The simplicity of the apparatus places it in the reach of all.

Many different materials may be used for a diaphragm, and almost any desired speed of dehydrating obtained. The apparatus can also be made of any size to adapt it for private or general laboratory work.

It would seem that such an apparatus would work equally well for animal tissue, but as yet I have not been able to make an extended trial of it; however, in the case of some insects hardened in it, it was found to be admirably adapted to the purpose."

Method for fixing Preparations treated by Sublimate or Silver (Golgi's Method).*—Sig. A. Obregia gives a method for rendering preparations treated by Golgi's sublimate or silver procedure so permanent that they may be afterwards stained and protected with a cover-glass.

The sublimate or silver preparations are sectioned without any imbedding or after having been imbedded in paraffin or celloidin. In the latter case care must be taken not to use alcohol weaker than 94 or 95 per cent., at any rate for the silver preparations. The sections are then transferred from absolute alcohol to the following mixture:—1 per cent. gold chloride solution, 8–10 drops; absolute alcohol, 10 ccm., which should have been made half an hour previously and exposed to diffuse light. After the sections are deposited therein the vessel containing them is placed in the dark. The silver is gradually replaced by gold, and

* Virchow's Archiv, cxxii. (1890) pp. 387 *et seq.* See Zeitschr. f. Wiss. Mikr., viii. (1890) pp. 97–8.

the mercury changed into gold amalgam. Finally, black delicate designs appear on a white field. According to the thickness of the section, the fluid is allowed to act for 15 to 30 minutes, but even longer is not harmful. Thereupon the sections are quickly washed first in 50 per cent. alcohol, then in distilled water, and finally in a 10 per cent. solution of hyposulphite of soda, in which, according to their thickness, they remain for 5 to 10 minutes. A longer immersion bleaches too much, so that the finer fibres disappear. Last of all they are thoroughly washed in distilled water twice renewed.

Sections thus fixed can afterwards be stained by any method—e. g. Weigert's, Pal's, &c.—after which they are cleared up with creosote, imbedded in dammar, and protected with a cover-glass.

Throughout the procedure the sections must be manipulated with glass instruments, and not allowed to touch any metallic substance.

Decalcification of Bone.*—In discussing various methods for decalcifying bone, and after indicating the shortcomings of the different solutions, most of which have been in vogue for a long time, Dr. R. Haug points out the advantages of phloroglucin in combination with acid. The introduction of this reagent was due to J. Andeer, who used it with a solution of hydrochloric acid.† According to the author this method was not altogether satisfactory, since the results were not invariable. By substituting nitric acid for hydrochloric a decalcifying fluid is obtained which effects its purpose very rapidly. Days and hours are only required where formerly weeks and months were necessary, and this without any damage to the tissues generally.

The solution is prepared by warming 1 grm. phloroglucin in 1 ccm. of pure non-fuming nitric acid (sp. gr. 1·4). This must be done slowly and very carefully, with slight agitation. After a very lively reaction a clear, dark ruby-red solution is obtained. To this combination of nitric acid and phloroglucin, which may be called nitrate of phloroglucin, 50 ccm. of water are to be added. In order to obtain a sufficient quantity of decalcifying fluid, to this stock solution 50 ccm. of water and 10 ccm. of acid are again added, and further additions of like percentages of water and acid may be made until the quantity reaches 300 ccm., which is the limit of the protective influence of the phloroglucin. Of course, if a further quantity of the decalcifying fluid be required, a fresh stock of solution must be made, and so on.

Fœtal or young bones of lower Vertebrata are completely softened in half an hour; older and harder bones, such as femur, temporal bone, &c., require a few hours. Of course, the pieces are small and the material previously washed. For teeth the amount of acid may be increased to 35 per cent.

When sufficiently decalcified, the preparations are to be placed in running water for about two days, in order to thoroughly remove all traces of acid. The after-treatment is as usual. If a less rapid decalcification be desired, the following formula suffices to give very good results:—Phloroglucin, 1; nitric acid, 5; alcohol, 70; distilled water, 30.

Other decalcifying methods are also discussed by the author; these

* Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 1–11.

† See this Journal, 1887, p. 504.

are those most in use, and it will not be necessary to recount them. But it may be useful to give the formulas of solutions made with hydrochloric and nitric acids:—Hydrochloric acid, 2·5; alcohol, 500; distilled water, 100; sodium chloride, 2·5. A variation of the preceding is:—Hydrochloric acid, 1–5; alcohol, 70; distilled water, 30; sodium chloride, 0·5. These solutions decalcify somewhat slowly, but the structural relations of the tissue are well preserved.

The formula for the nitric acid combination used by the author is:—Nitric acid (sp. gr. 1·5–1·2), 3–9; alcohol, 70; distilled water, 30; sodium chloride, 0·25. This solution decalcifies rapidly, but without destroying the tissues, and may be used for bone of all ages and densities. Its action may be hastened by using an incubator. Preparations stain remarkably well after this method.

Demonstrating Mucin in Tissues.*—From a very thorough examination Herr H. Hoyer shows that the mucin in mucous glands of goblet-cells of Vertebrata and Invertebrata can only be demonstrated by the basic anilin dyes, the acid salts having no effect. The various carmine solutions behave like the acid anilins, and the aluminated hæmatoxylin solutions like the basic.

Double staining with methylen-blue and triamido-benzol, known as Bismarck or Vesuvin brown, are found even in dilute solution to impart a deep stain very resistant to alcohol; other pigments named as giving satisfactory results being methylen-green, dimethylphenylen-green, metamidomalachit-green, and safranin. This last produces a metachromatic staining of the mucin, imparting thereto an orange colour, while the tissue and nuclei are red.

Another pigment giving excellent results is thionin or Lauth's violet, a derivative of indamin containing sulphur. To the tissue Lauth's violet imparts a bright blue colour, while the mucinous elements are red-violet.

For demonstrating mucin the author treated fresh pieces of tissue for two to eight hours with 5 per cent. sublimate solution, and then with 80 per cent. spirit. After imbedding in paraffin and cutting the tissue, the sections, stuck on a slide, were stained with dilute watery solutions (2 drops of a saturated watery solution of the pigment to 5 ccm. of water) for 5 to 15 minutes. Other details relative both to the pigments and to the technique are given.

Preparing and Examining Glandular Epithelium of Insects.†—Dr. V. Grandis recommends insects, and especially *Hydrophilus*, for studying glandular epithelium during secretion. After the animal's legs and wing-cases have been removed a cut is made down the whole length of the back, and then two others perpendicular to the first, one on either side. In making these incisions care must be taken not to tear the abdominal air-sacs or the tracheæ. The animal is then laid on a piece of cork, in the centre of which is a circular hole with a diameter of about 1 cm., on the under side of which is cemented a cover-glass,

* Arch. f. Mikr. Anat., xxxvi. (1890) pp. 310–74. See Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 67–70.

† Atti R. Accad. Sci. Torino, xxv. (1890) pp. 765–89 (8 pls.). See Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 86–7.

and it is so disposed that the abdomen lies in the cell. The lymph flows into the cell, and after adding to it some 0·7 per cent. salt solution, the viscera are placed therein, and the intestine, having been spread round the edge of the hole, is fastened down with needles. By this means the Malpighian vessels can be observed in the living condition.

To iodine-green they behave during life in a manner quite different from that after death. In the first case the nucleus does not stain at all, while the protoplasm assumes a purple-violet hue. After death the nuclei, which have then acquired an acid reaction, stain green, and the protoplasm bluish-green. Another differential stain is the Ehrlich-Biondi solution, which colours the nuclei green and the protoplasm orange. The other stains mentioned imparted a diffuse coloration or were otherwise imperfect.

Preparing and Staining the Ova of Chironomus.*—Herr R. Ritter obtains the ova of *Chironomus* from the water in which they have been laid during the twilight. The secretion which holds the eggs together swells up into a gelatinous mass. The egg-mass is then killed with hot 30 per cent. alcohol to which some sublimate has been added, and afterwards treated successively with 70, 90, and 100 per cent. spirit. It is then imbedded in paraffin after having been soaked in chloroform.

The author succeeded in staining the ova (a very difficult task) by placing the whole egg-mass for at least four days in picrocarmine, the transference from the absolute alcohol to the staining fluid being made very gradually. The sections may be contrast stained with hæmatoxylin.

Preserving Larvæ of Lepidoptera with their Colour.†—Sig. F. Crosa places the caterpillars in a 5 per cent. solution of chloride of zinc, and then heats the fluid almost to boiling. This hastens the process and prevents putrefaction. The objects are then placed successively in 10, 15, 20 per cent. solutions of the same salt, and remain therein until they sink to the bottom. For a caterpillar of medium size eight to ten days are necessary. After the last solution they are placed in glycerin. The zinc chloride must be perfectly neutral and contain no trace of iron salts. For this purpose commercial zinc is dissolved in pure hydrochloric acid, taking care that the zinc is always in excess, in order to prevent the formation of iron chloride; afterwards it is filtered. If commercial zinc chloride be employed, this is dissolved in water acidulated with hydrochloric acid and then boiled for some time with zinc.

It is advisable that before the treatment is commenced the caterpillars should be made to fast, and that they should be killed with chloroform. It is stated that, prepared by this method, caterpillars retain their colours (even the green and yellow hues) for quite two years, and that they are quite suitable for histological purposes.

Method of observing Pectinatella gelatinosa.‡—Mr. A. Oka states that this Polyzoon is remarkable for the ease with which it can be killed in an expanded condition. When 70 per cent. alcohol is gradually

* Zeitschr. f. Wiss. Zool., l. (1890) pp. 408-27 (1 pl.). See Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 87–8.

† Boll. Mus. Zool. ed Anat. Comp. Torino, v. (1890) No. 85. See Zeitschr. f. Wiss. Mikr., viii. (1891) p. 86.

‡ Journ. Coll. of Science, Imper. Univ. Japan, iv. (1891) pp. 91-2.

poured into a vessel containing the colonies, more than half the polypides die protruded. With such reagents as chloral hydrate or cocain chlorohydrate every polypide dies expanded. Some colonies were fixed with a saturated solution of corrosive sublimate or a weak (0·1 per cent.) solution of chromic acid. Borax-carmine and picrocarmine were chiefly used for staining. Sometimes a whole colony was imbedded.

The development of the polypide within the statoblast was thus studied: a statoblast was hardened in alcohol, and its edge was then cut between two pieces of elder-pith so as to make an opening in the chitinous shell; it was then stained and kept in alcohol until cut. In cutting the statoblast celloidin was indispensable, owing to the hardness of the shell. Fresh specimens were put on a slide after stupefying with cocain. The habits of the colonies may be studied by keeping them in vessels through which water is always flowing.

Demonstrating Tactile Papillæ of Hirudo medicinalis.*—In order to show well, says Dr. S. Apáthy, the tactile papillæ of *Hirudo medicinalis*, strong spirituous solutions of sublimate should be added to the water in which the starved animal is kept until it moves no longer. Having been stretched out with pins, 10 per cent. sublimate or 70 per cent. alcohol is poured over it. This makes the tactile papillæ stand out from the smooth ventral surface.

Examining Ova of Gordius.†—In examining the yolk-stalk of *Gordius*, Sig. L. Camerano fixed this animal in one-third alcohol or picric acid. Mayer's carmine stained germinal vesicle and spot well. For ova the author recommends as fixative 3 per cent. nitric acid or a mixture of equal parts of absolute alcohol and acetic acid, and as stain, borax-carmine or a mixture of malachite-green and vesuvin.

Study of Nematodes.‡—Mr. N. A. Cobb recommends the following method:—"On capturing a worm with the medicine-dropper, I eject it forcibly into 20 ccm. of concentrated solution of corrosive sublimate, kept at 50°–60° C. by floating it in a porcelain dish on the surface of hot water. If the sublimate solution is much hotter than 60°, the bodies of some species burst. The worms should remain in the hot sublimate solution at least an hour, better longer. When a sufficient number of worms has been captured, pour the sublimate solution, worms and all, into a flat glass dish placed on a black background, and pick out the worms with the aid of a magnifying glass and a fine-pointed medicine-dropper, and put them into the prepared object-glass of a differentiator. Stain and bring into balsam by means of the differentiator. Most of the smaller species stain readily in borax-carmine, which is one of the best of stains for this work. *Oxyuris vermicularis* (adults, not the young) and a number of other parasitic species, however, do not stain in borax-carmine. Mayer's carmine rarely fails to stain these exceptional species. Overstaining is corrected by adding hydrochloric acid to the proper differentiator fluids. I can recommend this method very highly, not

* Zool. Anzeig., viii. (1890) pp. 320–2. See Zeitschr. f. Wiss. Mikr., viii. (1891) p. 81.

† Mem. della R. Accad. di Torino, xl. (1890) pp. 1–19 (2 pls.). See Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 80–1.

‡ Proc. Linn. Soc. N.S.W., v. (1890) pp. 451–2.

only for Anguillulidæ, but also for numerous other groups of the smaller animals and plants."

Mode of Studying Phagocata.*—Mr. W. M. Woodworth found that the best reagent for killing these worms is hot corrosive sublimate; an excess of the salt is added to the saturated aqueous solution, and the whole is heated to the boiling point; by this means a very strong solution can be obtained. A modification of Kennel's process, viz. a cold saturated solution of corrosive sublimate in 50 per cent. nitric acid, was used with entire success. For the study of the intestinal tract, unstained specimens were cleared in clove oil. For staining, Grenacher's alcoholic borax-carmine, followed by differentiation with acid alcohol, proved to be the most useful method. Good sections for topographical study were obtained by staining in this carmine for twenty-four hours, and cutting, in the horizontal plane, sections 30 μ in thickness. With this light staining the nerve-tissue takes none of the colour, and in such comparatively thick sections the finer branches show as white lines against a red background. Orth's picrocarminate of lithium is a valuable reagent for all glandular tissues, as the picric acid brings them sharply out; this is also an excellent reagent for macerating. The osmic-acetic method of maceration was also successful. Isolated living pharynges were killed in hot 1 per cent. silver nitrate for the purpose of demonstrating the epithelium. Depigmenting was accomplished by the use of a 1 per cent. solution of potassic hydrate, which was allowed to act for a few minutes on sections fixed to the slide with Schällibaum's clove-oil collodion fixative.

Study of Rhizopods.†—Mr. S. H. Perry recommends the mounting of testaceous Rhizopods in glycerin-jelly rather than balsam, as the specimens do not become too transparent, and the protoplasm is preserved. Examples should be picked out singly with a fine camel's-hair brush, under powers of from 25 to 125 diameters, and transferred to a drop of glycerin, where they can be kept till required for mounting.

Demonstration of Cilia of Zoospores.‡—Prof. J. E. Humphrey recommends for this purpose, especially in the case of Fungi, a 1 per cent. solution of osmic acid, which is left for a few minutes to fix the spores thoroughly, and then drawn off by means of filter-paper. A staining-fluid is then applied, consisting of a drop of a moderately strong solution in 90 per cent. alcohol of Hanstein's rosanilin-violet composed of equal parts of fuchsin and methyl-violet. This stains both the cilia and the body of the zoospores very quickly and deeply. By this method the author was able to demonstrate that the zoospores of an *Achlya* allied to *A. polyandra* are ciliated.

(3) Cutting, including Imbedding and Microtomes.

Preparation and Imbedding of the Embryo Chick.§—Messrs. S. H. Gage and G. S. Hopkins write:—"An excellent method of preparing blastoderms of the chick, of from 1 to 96 hours incubation, both for

* Bull. Mus. Comp. Zool., xxi. (1891) pp. 6–7.
† Amer. Mon. Micr. Journ., xii. (1891) p. 80.
‡ Bot. Gazette, xvi. (1891) pp. 71–3.
§ Proc. Amer. Soc. Micr., 1890, pp. 128–131.

surface views and for sectioning, is given in Whitman's 'Methods in Microscopical Anatomy and Embryology' (p. 166).

With slight modifications, the method is as follows:—

(1) Break the shell by a sharp rap of the scissors at the broad end; then carefully break away the shell, beginning at the place of fracture and working over the upper third or half.

(2) After removing as much of the white as possible without injury to the blastoderm, carefully turn the yolk into a dish of nitric acid (10 per cent.) deep enough to float the yolk, taking care to have the blastoderm on the under side of the yolk.

(3) The coagulated white should next be removed from the blastoderm by the aid of a brush or feather, and the egg then allowed to remain in the acid from 20 to 30 minutes.

(4) Cut around the blastoderm with sharp-pointed scissors, taking care to cut quickly and steadily. After carrying the incision completely round, float the blastoderm into a watch-glass, keeping it right* side up and flat.

(5) Remove the vitelline membrane by the aid of dissecting forceps and the yolk by gently shaking the watch-glass and by occasional use of a needle.† The yolk can sometimes best be washed off by means of a pipette.

(6) Wash in water (several times changed).

(7) Colour deeply with carmine or hæmatoxylin.

(8) Remove excess of colour by soaking a few minutes in a mixture of water and glycerin in equal parts, to which a few drops (about 1 per cent.) of hydrochloric acid have been added.

(9) Wash and treat 30 minutes with a mixture of alcohol (70 per cent.) 2 parts; water, 1 part; glycerin, 1 part.

(10) Transfer to pure 70 per cent. alcohol, then to absolute alcohol (95 per cent. alcohol answers every purpose), clarify with creasote or clove oil, and mount in balsam.

For sectioning, blastoderms prepared by this method should be dehydrated, either before or after staining, as is thought best, and immediately transferred to a thin solution of collodion ‡ (2 per cent.), after which they are placed in a thick solution of collodion (5 per cent.) and then arranged for imbedding and sectioning. To accomplish this, the following procedure has been found useful:—

With a camel's-hair brush transfer the blastoderm from 95 per cent. alcohol to a paper box. It is better to fill this box partly full of alcohol (95 per cent.) before transferring the blastoderm to it, as the alcohol partially floats the blastoderm and thus facilitates its removal from the brush. As soon as the blastoderm is safely in the box, remove the alcohol with a dropper (do not try to pour it off, otherwise the blastoderm will curl up), and carefully pour in enough thin collodion to cover the

* We find it more convenient to remove the yolk from the blastoderm when it is kept ventral (or wrong) side up.

† We find that a small camel's-hair brush is the best thing with which to remove the yolk.

‡ We have found collodion more satisfactory, on the whole, than celloidin, and it is less costly. To make a 2 per cent. solution, dissolve 2 grams of gun-cotton in 100 ccm. of sulphuric ether and 95 per cent. alcohol, equal parts of each. For a 5 per cent solution use 5 grams of gun-cotton instead of 2.

blastoderm to the depth of about 1/2 cm. The box is now placed in a tightly covered jar to prevent too rapid evaporation and the consequent solidification of the collodion. After the blastoderm has remained a sufficient length of time (from one to three or more hours, depending on the size of the blastoderm) in the thin solution, the collodion is removed with a dropper, and the thick solution poured on. After infiltrating sufficiently with thick collodion, 2–10 hours, open the jar and allow a film to form on the surface of the collodion, then fill the paper box with alcohol (60–80 per cent.) and allow it to remain till the collodion becomes firm and tough; 2–4 hours is usually sufficient. Now with a sharp knife a square or rectangular piece of collodion including the blastoderm is cut out and arranged on the cork in any position desired; the block is fastened to the cork, as any ordinary tissue, by simply pouring over it thick collodion, which is hardened by immersing in alcohol (60–80 per cent.) for from 5 to 15 hours.

For holding the corks under the alcohol the following apparatus has been found more economical and convenient than the method of attaching

FIG. 67.

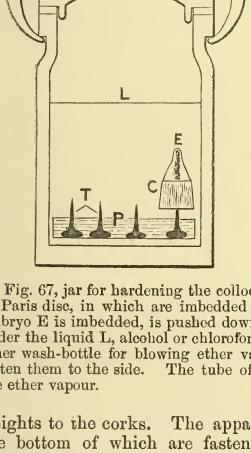


FIG. 68.

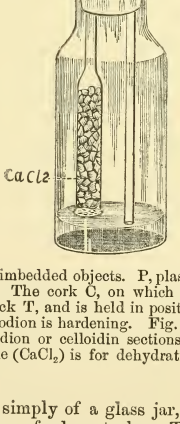


Fig. 67, jar for hardening the collodion of collodion-imbedded objects. P, plaster of Paris disc, in which are imbedded the glass tacks. The cork C, on which the embryo E is imbedded, is pushed down upon a glass tack T, and is held in position under the liquid L, alcohol or chloroform, while the collodion is hardening. Fig. 68, ether wash-bottle for blowing ether vapour upon collodion or celloidin sections to fasten them to the side. The tube of calcium chloride ($CaCl_2$) is for dehydrating the ether vapour.

weights to the corks. The apparatus consists simply of a glass jar, in the bottom of which are fastened several rows of glass tacks. The materials necessary for its construction consist of a wide-mouthed jar, a few pieces of glass rod, and a little plaster of Paris. The tacks are made by heating the glass rod and drawing it out to a rather sharp

point. It is then cut off at the right length and the cut end softened by heat and then quickly pressed upon some hard surface, so as to form a sort of head. The tacks are then arranged in rows in some shallow dish, previously oiled, and enough plaster of Paris poured around them to form a layer from 1½ to 2 cm. deep. When this hardens, the tacks are firmly held in an upright position, and all that remains to be done is to place the plaster disc in the bottom of the glass jar.

To use the apparatus, fill it partly full of alcohol (60–80 per cent.). As the specimens are imbedded on the corks, transfer them to this jar, sticking each cork upon a tack."

An improved Method of preparing large Sections of Tissues for Microscopic Examination.*—Mr. J. C. Webster writes :—" Hitherto we have employed two methods of preparing large sections for microscopic study, viz. the freezing and the celloidin. In the former the Hamilton or Bruce microtome is used, and in the latter the Schanze. Each of these processes has connected with it certain difficulties which limit the range of its employment.

The objections to the first method are the following :—

(*a*) It is impossible to prepare delicate or friable tissues in large thin sections, because, after being cut, they either break into pieces when placed in water, or during the mounting process get torn and destroyed. The placenta, for example, cannot be cut into sections suitable for the finest microscopical work, as the villi and the blood-corpuscles in the maternal sinuses are almost entirely scattered when placed in fluid.

(*b*) The relations of parts cannot be preserved. Thus, for example, one cannot mount undisturbed a section through bladder and uterus, or through brain and membranes.

(*c*) The difficulties and discomforts connected with the working of a large freezing microtome are considerable.

The objections to the second method are :—

(*a*) It is impossible to prepare sections thin enough for examination by high powers. Those which can be made are only fit for study with low powers, or for lantern demonstration. This is the case with even the most easily cut tissues.

(*b*) The microtome employed—the Schanze—is complicated and expensive; its knife is with great difficulty kept sharp, and does not always cut large sections in slices of uniform thickness.

(*c*) The materials used in preparing the tissues for cutting are expensive.

The method which I am about to describe is not only free from these important objections, but possesses several distinct advantages.

(1) *Preparation of Tissues.*—Tissues may be hardened by any of the known methods, the last stage, however, being a twelve or eighteen hours' soaking in absolute alcohol.

The following method gives splendid results :—

Place the fresh tissue in a boiled saturated solution of corrosive sublimate for one night. Then wash in water, and place for 24 hours in a mixture of one part of methylated spirit and two of water; then in a mixture of equal parts for two days. Gradually increase the propor-

* Rep. Lab. R. Coll. Physicians Edinb., iii. (1891) pp. 266–70.

tion of spirit in the mixture, and at the end of eight or ten days place the tissue in pure spirit, and leave it until it is desired to examine it. A slice is then cut 3/16 to 1/16 in. in thickness, and placed for 12–18 hours in absolute alcohol. It is then soaked in pure naphtha for 24 hours. It is then placed in a mixture of equal parts of naphtha and soft paraffin, and exposed to a temperature of about 115° to 120° F. in a water-bath for 18–20 hours. The advantage of naphtha over turpentine is that it dissolves paraffin at a much lower temperature, thereby allowing the water-bath to be kept in such a condition that there is no danger of overheating the specimen. Throughout this process the temperature is kept lower than in the ordinary methods. The advantage of naphtha over chloroform and xylol is its cheapness. It is next placed in melted soft paraffin, and kept in the bath at about the temperature mentioned above for 24 hours. Then it is changed to a mixture of one part of soft and four or five parts of hard paraffin for the same length of time at a higher temperature. Care must be taken that the thermometer does not rise above 140° F.

(2) *Imbedding.*—Paper on thin cardboard boxes, about 1 in. in depth, and slightly more than large enough to hold the tissue, may be used. Nearly fill with a warm melted mixture of soft and hard paraffin in the proportions already mentioned. This mixture is better than the hard paraffin alone. The sections do not curl up as they generally do when pure hard paraffin is used; they can be cut in a much lower temperature, and they are not so brittle. With a pair of warmed forceps place the piece of tissue in the box, the face to be cut to be laid on the bottom. The paraffin should now almost fill the box which is at once placed in a flat dish of cold water. This is an important step; rapidly cooled paraffin makes a better bed, and is less apt to retain air-bubbles than the slowly cooled material. The boxes are removed from the water after a few hours, and can be kept until it is wished to cut them.

(3) *Cutting of Sections.*—This should be done in a room only moderately warmed. The Bruce microtome is employed. Having removed the box from the block of paraffin, pare away the upper surface of the latter, keeping always parallel with the lower surface, until there is left only the thickness of 3/16 in. above the tissue. Then place this surface on the microtome plate, gently heating the latter until a thin layer of the paraffin melts. This is then allowed to cool, and the block becomes firmly attached to the plate.

The plate is then screwed to the microtome, and the sections are cut in the usual manner. As the sections are thrown off they are caught in a dry tray. They may be mounted at once or preserved in boxes or bottles in a cool place. Some of the sections will be rolled up, others being wavy or flat. When the sections are very large, I prefer to mount the former; they can be unrolled on the slide over a very gentle heat, without any wrinkling taking place, or without air-bubbles being caught beneath the tissue.

(4) *Mounting.*—A clean dry slide is covered with a thin layer of fixing-fluid by means of a glass rod. The fluid which I have found most suitable is a mixture of collodion and clove oil. The section is flattened out on the slide by a soft hair brush above a very gentle flame.

Excess of fixing-fluid and paraffin can now be wiped from the slide. Staining can be at once proceeded with, or the slides can stand for a time protected from dust.

(5) *Staining.*—Dissolve the paraffin from the section by two or three washings of naphtha, which is allowed to stand on the slide for about a minute. Then wipe the slide, and wash off the superfluous naphtha with methylated spirits.

The following stains give splendid results:—Logwood, logwood and eosin, logwood and Bismarck brown, and alum-carmine. To get the best results with logwood, the following method should be used:—Stain the section for three minutes or more in the Ehrlich's hæmatoxylin solution. Then place it in a bowl of distilled water containing a few drops of hydrochloric acid until it appears of a pale port wine colour. The acid dissolves the stain from all parts save nuclei. Then place in a very dilute alkaline solution (sodium bicarbonate) until it turns blue. The alkali deepens the stain in the nuclei.

If eosin is to be used as a contrast stain, wash the section in water and place it in 1/3 per cent. eosin solution (if Bismarck brown, in a 1/4 per cent. solution) for two minutes. Wash in water, then in methylated spirits, and finally dehydrate in absolute alcohol. Clear up in clove oil or xylol: mount in balsam dissolved in xylol, naphtha, or benzol. It is to be observed that naphtha serves for the early stage of soaking in paraffin, for dissolving the paraffin from the mounted sections, and for dissolving the balsam which covers them.

If it is desirable to stain the tissue *en masse* before cutting, the following method is valuable:—Stain the spirit-hardened tissue for 18–24 hours in a borax-carmine solution prepared as follows:—Add 4 grm. borax to 100 ccm. aq. dest., and heat to boiling point. Add 2½ grm. carmine, and boil for 12 minutes. Allow it to cool, and add an equal bulk of a 70 per cent. solution of alcohol. After allowing it to stand for three or four days, filter.

The now deeply stained tissue is partly decolorized by being placed for 12 or 15 minutes in a mixture of acid. hydrochlor. 4 drops, abs. alcohol 70 ccm., aq. dest. 30 ccm.

It is then placed in methylated spirit for three hours, and afterwards in alcohol for 18–24 hours. Clear up in clove-oil—denoted by its sinking.

It is now ready for the paraffin process, being first soaked in naphtha, &c. When the sections are cut they are fixed on the slide in the usual manner, the paraffin dissolved out with naphtha, and the mounting completed with balsam."

Sections of Staminate Cone of Scotch Pine.*—Mr. Charles E. Bessey sends the following contribution from the Botanical Laboratory of the University of Nebraska to show what can be done by the paraffin imbedding process in cutting and mounting objects which otherwise would fall to pieces. The preparation was as follows in detail:—

The cone was first put into 35 per cent. alcohol for 12 hours. Then successively 12 hours each in 50 per cent. alcohol, 75 per cent. alcohol, hæmatoxylin, 90 per cent. alcohol, absolute alcohol, alcohol and turpen-

* Amer. Mon. Micr. Journ., xii. (1891) p. 56.

tine, pure turpentine, cold paraffin and turpentine. It was then put into warm paraffin and turpentine for 6 hours, then into melted paraffin (50°–55°) for 6 hours. It was then imbedded in the paraffin and cut into ribbons upon a Reichert-Thomé microtome, the sections being 20 μ (1/250 in.) thick. The ribbons were fixed on the slide with white of egg and glycerin. The slide was warmed to melt the paraffin, which was then washed away with turpentine, washed next with absolute alcohol, then 90 per cent. alcohol, then water (distilled), then stained with fuchsin about two seconds, next washed with distilled water, 90 per cent. alcohol, absolute alcohol, and turpentine in succession. Canada balsam in chloroform was then poured over the specimen and the cover-glass laid on. I have given every step taken in the operation. The hæmatoxylin did not penetrate, hence the staining by fuchsin was necessary."

(4) Staining and Injecting.

New Method of Injecting Fluids into the peritoneal cavity of animals.*—Dr. A. F. Stevenson and Dr. D. Bruce describe a method for injecting fluids into the peritoneal cavity without danger of wounding the intestines with the point of the hypodermic needle. The needle is curved, its anterior half being solid, while the posterior part is hollow, the opening being in the middle, i. e. at the junction of the two halves. It may be fitted to any syringe. When using it, the abdominal wall of the animal is pinched up with thumb and forefinger of two hands, and then the needle plunged through until the middle (the opening) is in centre of the pinched-up tissue. Hence when the skin is relaxed the opening of the needle is freely within the peritoneal cavity.

Demonstrating the Cerebral Vessels of Mammalia.†—For studying the distribution of the cerebral vessels of Mammalia at various periods of intra- and extra-uterine life, Sigg. G. Valente and G. d'Abundo found that an aqueous solution, not stronger than 0·5 per cent., of silver nitrate, was more suitable than all other injection masses. By the injection of coloured gelatin the vessels, especially in the embryo, were dislocated from their normal position. This inconvenience is avoided by the silver solution, while at the same time, owing to its penetrating the walls of the vessels, the endothelium and the perivascular lymphatic sheaths are made clear. Brains injected in this way cannot, of course, owing to the precipitation which would ensue, be treated with the ordinary fixative media. After being exposed for twenty minutes to direct light they were at once transferred to alcohol. For staining, Meynert's method was preferred, and it is advised to stain the sections on the slide.

Three useful Staining Solutions.‡—Dr. R. Haug gives three formulæ for staining solutions which are stated to be extremely effective.

(1) Hæmatoxylin in acetic acid-alum. 1 grm. of hæmatoxylin is dissolved in 10 ccm. of absolute alcohol, and this mixed with 200 ccm. of liquor aluminis acetici (German Pharmacopœia—see also "Extra

* Brit. Med. Journ., June 6, 1891, p. 1224 (2 figs.). See also Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 689–90.

† Atti Soc. Scienze Nat. Pisa Mem., xi. (1890) 14 pp., 1 pl. See Zeitschr. f. Wiss. Mikr., viii. (1891) p. 92.

‡ Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 51–2.

Pharmacopœia"). The fluid, at first violet-black, becomes brownish-black in the course of a few weeks, and its maturation may be hastened by the addition of a few ccm. of saturated lithium carbonate solution. It is advised to overstain the preparation with this solution, and to decolorize with hydrochloric acid-alcohol. The sections are then placed in tap water until they become blue. Any contrast dye may be used afterwards.

(2) Alum-borax-carmine with acetic acid-alum. This gives similar but better results than alum-carmine. It is prepared by rubbing up 1 grm. carmine with 1 grm. borax and 2 grm. ammonia-alum, and then boiling this with 100 ccm. of liq. aluminis acetici for half an hour or longer. It is then decanted, and after 24 hours filtered.

(3) Ammonia-lithium-carmine with ammonium chloratum. This gives a fine deep strawberry red colour in 1–3 minutes. Overstained sections may be differentiated with hydrochloric acid-alcohol. Afterwards they are placed at once in absolute (picric) alcohol. It is prepared by rubbing together 1 grm. carmine with 2 grm. ammonium chlorate, and boiling in 100 ccm. water. When cold, to the solutions are added drop by drop 15–20 ccm liq. ammonii caustici and lithium carbonicum from 0·3 to 0·5. Filter. The solution is ready for use at once, and is very permanent.

Fixation of the Stain in Methylen-blue Preparations.*—Prof. A. S. Dogiel finds that the addition of osmic acid to the picrate of ammonium solution used for fixing methylen-blue is attended with several advantages, not the least of these being that it hardens the tissue just a little, and, secondly, that it stains the medullary sheath of nerves black. The solution is made by adding 1 or 2 ccm. of a 1 per cent. osmic acid solution to 100 ccm. of a saturated aqueous solution of ammonium picrate. The stain is fixed by immersing the preparation for 18–24 hours in the mixture. It is then transferred to glycerin, diluted with water, in which the colour of the nerves will keep for quite a long time. Should it be necessary to impart a consistence to the object so that it may be sectioned, the author uses a greater quantity of osmic acid (25–30 ccm. ammonium picrate solution; 1–2 ccm. 1 per cent. osmic acid). In this solution the object remains for 24 hours, after which it may be imbedded e. g. in elder-pith, liver, &c., and sectioned.

Hints on Preparation of Tumours injected during life with anilin pigments.†—In order to examine sections made from malignant tumours which have been treated by injection with aqueous solutions of chemically pure anilin dyes, it is necessary, says Dr. R. Haug, to adopt a certain procedure. The dyes usually injected *intra vitam* by the surgeon are methyl-violet and methylen-blue. If, therefore, a piece of a tumour injected with these dyes be excised before their absorption, a blue-violet mass is obtained. This mass must be hardened, and for this purpose alcohol must be altogether excluded. Hardening may be effected in Erlitzki's fluid or some other combination of chromic acid salt and copper, or picric acid; better than these is cold saturated solution of sublimate. In this pieces, the sides of which are about 0·75 cm., are left for about 24 hours. The sublimate crystals are removed by

* Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 15-9. † T. c., pp. 11-15.

immersion in the following solution, which must be renewed until all traces of sublimate have disappeared:—Tincture of iodine, 2; iodide of potash, 1; distilled water, 50; glycerin, 50. After this the piece is placed in pure glycerin, to every 100 ccm. of which 2 grm. of anhydrous sulphate of copper are added, Herein it remains for 24–48 hours. The preparation is then to be imbedded. For this it is first saturated with glycerin-jelly or transparent soap, and then inclosed in pretty hard paraffin; but before this inclosure is made, the object must be immersed merely for a moment in quite absolute alcohol. Instead of inclosing in paraffin the object may be stuck on cork with thick jelly or jammed in liver. While sectioning it is necessary to cover both knife and preparation with a mixture of equal parts of water and glycerin, otherwise the section will be torn to pieces. The sections are then removed to water on a brush. The next step is to contrast stain, and for this purpose some of the various carmine solutions are most suitable. It is advised to place the sections for a quarter of an hour before staining in a saturated solution of lithium carbonate. If a single stain be desired, then alum-carmine or cochineal are suitable; if a double stain, then neutral carmine followed by differentiation with weak acetic acid. The sections are then placed in glycerin and water (equal parts), and then saturated solution of picric acid is added.

The sections are then transferred to pure glycerin and there examined. In successful preparations a triple stain is obtained, and we are enabled to ascertain how the pigment has acted during life, that is to say, what has been its distribution and effect; whether it has penetrated within the vascular channels, among the stroma of the tissue, whether it has obtained access to the interior of the cells, and what action it has had upon the epithelial cells.

Preparing and Staining Sections of Spinal Cord.* –In a contribution to the technique required for spinal cord, Dr. A. Ciaglíński enunciates some apparently heterodox opinions, such as the uselessness of hæmatoxylin for staining, and gives with copious details his method of procedure. The spinal cord is to be cut up into pieces 1 cm. long, and these immersed in Erlitzki's fluid (3–4 weeks), or in Müller's fluid (3–4 months). As the former solution is prone to throw down a copper precipitate, the latter is preferred. When sufficiently hard, the pieces are carefully washed in water, and then placed for a day or two in 70 per cent. spirit, and finally for one day in absolute alcohol. Thus dehydrated, the object is immersed for 24 hours in anilin oil, then for 2 or 3 hours in xylol. Then for 20 hours in a mixture of equal parts of xylol and paraffin, and kept at a temperature of 37° C., after which it is imbedded in pure paraffin. The paraffin, which for winter should have a melting-point of 45° C., and for summer of 52° C., is melted over a gas flame. To the first paraffin imbedding are added some drops of cedar oil to make it more elastic, and the preparation left in the oven for 24 hours. The second imbedding is only incubated for 15–20 minutes, and then the preparation is turned out into a watch-glass smeared with glycerin. It is important to know which side is uppermost. The

* Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 19–28.

paraffin is then set in cold water. The sections are then stuck on a slide and placed in a thermostat at 37° C.

The author then stains with safranin and anilin-blue as follows:—After the preparations have been freed from paraffin by means of xylol they are washed with absolute alcohol, and then for half an hour with distilled water. They are then covered with a watery 0·2 per cent. solution of safranin and kept moist by being put inside some glass vessel. After 1–3 days the safranin solution is poured off and the preparations thoroughly washed with distilled water, whereupon they are further stained for 1–5 minutes with anilin-blue (a saturated aqueous solution diluted with an equal volume of distilled water). The deeper the safranin stain, the longer the anilin-blue solution is allowed to act, and *vice versa*. The preparation, having been washed, is then dehydrated, cleared up with oil of cloves, treated with xylol, and finally mounted in xylol-balsam.

By this method the medullary sheath of nerves is stained orange-yellow; axis-cylinders, deep blue; ganglion-cells and their processes, blue; neuroglia-cells and their connective tissue, bright blue; walls of blood-vessels blue, while the elastic membrane and nuclei of the muscular fibres are red; pia mater, blue; white substance of cord, red; grey substance, pale blue; but if any morbid changes have occurred the degenerated parts stain deep-blue.

Manipulating and staining old and over-hardened Brains.*—M. J. Honegger, who has been making researches on the brains of Mammalia, communicates the interesting fact that old over-hardened brains, always provided that decomposition has not occurred before or during hardening, can be rendered perfectly sectionable and stainable by immersing them for several days in water which has been made nearly boiling and is frequently renewed.

For staining brains preserved in bichromate of potash an ammoniacal solution of carmine is very suitable, and the author makes his solution as follows:—The carmine is rubbed up to a thick pap with only just as much ammonia as is absolutely necessary, and having been spread all round the inside of the mortar, is allowed to thoroughly dry, and then finely powdered. After 24 hours' exposure to the air the powder is dissolved in cold water, and thus a very satisfactory staining solution is obtained.

Staining with acid-fuchsin and gold impregnation may also be adopted. In the latter case the sections are kept for 3/4 hour in 1/2 per cent. gold solution in the dark. They are then transferred to water slightly acidulated with acetic acid and exposed to full sunlight, and then kept for two days more in daylight. In this way a strong reduction is attained, and after-darkening quite avoided.

Staining Bacillus of Glanders.†—Herr E. Noniewicz advises a combination of Löffler's and Unna's method for staining *B. mallei*. The procedure, which is stated to give excellent results, is as follows:—The

* Rec. Zool. Suisse, v. (1890) pp. 201–310 (5 pls.). See Zeitschr. f. Wiss. Mikr., viii. (1891) p. 99.

† Deutsch. Zeitschr. f. Thiermed. u. Vergleich. Pathol., xvii. pp. 196–208. See Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 109–10.

sections are transferred from alcohol to Löffler's methylen-blue solution (caustic potash 1 : 10,000). They are then washed in distilled water and placed in the decolorizing fluid (75 parts 1/2 per cent. acetic acid and 25 parts 1/2 per cent. watery tropæolin O O). The time for decolorizing depends on the thickness of the sections, the thick ones requiring from 2 to 5 seconds, the thin ones much less. The preparations are then thoroughly washed in distilled water; this removes the acetic acid and a good deal of the stain. The sections are then put on a slide, and the water having been removed with blotting-paper, are dried in the air or over a spirit-lamp. Xylol is then dropped on and allowed to remain till the section is quite clear. They may now be examined or mounted in balsam. Oil of cloves, origanum oil, and anilin oil are not to be used. In this way the glanders bacilli are stained almost black, while the tissue is bluish.

Staining Pathogenic Fungus of Malaria.*—Surgeon J. Fenton Evans has found it possible to stain the organisms of malaria with an anilinized alkalized solution of rosanilin hydrochloride after treatment with bichromate of potash, and after treatment with dilute sulphuric acid by an anilinized alkalized solution of Weigert's acid fuchsin. Another method is the saturation of the tissue with a copper salt, and its reduction by sulphuretted hydrogen previous to coloration with anilinized alkalized acid fuchsin.

Characteristics of some Anilin Dyes.†—Dr. C. Vinassa, in a contribution to "pharmacognostic microscopy," communicates the results of a number of experiments made with fifty-one different anilin pigments. These results are displayed in two tables. In the first are noticed the behaviour to acids and alkalies, and the stain imparted to the microscopical preparation. Some of the dyes showed a capacity for double staining, the most noticeable of these being "Solidgrün" and "Deltapurpurin." By these the vessels were stained green and the parenchyma red.

Many other useful staining qualities and characteristics may be gathered from a perusal of the table, but for these the original must be consulted. Table 2 gives the chemical derivation, the peculiar microscopical stainings of the various tissue-elements, and the behaviour as dyes to certain commercial products, such as silk, wool, &c.

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

Mounting Botanical Preparations in Venetian Turpentine.‡—Herr F. Pfeiffer highly recommends Venetian turpentine for mounting botanical preparations, and states that it possesses qualities which render it capable of superseding glycerin-gelatin. On the whole, its manipulation is extremely simple. Sections of firm vegetable tissue are merely transferred from strong spirit (92–100 per cent.) to a drop of turpentine placed on a slide. After the cover-glass has been put on, the preparation can be ringed round. But if the sections are thin, liable to wrinkle up, and are to be stained, then certain eventualities have to be

* Proc. Roy. Soc. Lond., xlix. (1891) pp. 199–200.
† Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 34–50.
‡ T. c., pp. 29–33.

borne in mind. These are, that the preparation while standing dehydration in strong spirit is distorted when transferred to the turpentine; for such preparations, though properly fixed and hardened, will not bear the transference to strong spirit. To obviate these inconveniences the author has adopted the principle of Overton's method.* The objects, already stained, are removed from alcohol to a solution of 100 parts 94 per cent. spirit and of 10 parts Venetian turpentine. The preparation is then placed in an air-tight glass capsule in the presence of chloride of calcium, by which means the turpentine is slowly concentrated by the removal of the spirit and water.

The glass capsules and other vessels employed in the manipulation should have tall sides, e. g. 2 cm. high to 1·5 cm. diameter, and 2·5 cm. high to 2 cm. diameter. The edge inside and out should be smeared with paraffin; this is easily done by just dipping the top of the capsule into molten paraffin and allowing it to set; the width of the paraffin rim should be 3–4 mm. These precautions prevent the turpentine from running up the inside and then down the outside of the capsule.

In these small capsules the object is immersed in the turpentine solution, and then these placed inside a larger closed capsule, the diameter of which is 8–10 cm., and the height 3·5–4 cm. In a few days the object will be found saturated and surrounded by thick turpentine, and suffering from no distortion.

Tissues which crumple up when placed in strong alcohol are treated by Overton's glycerin method. The object is placed in a mixture of 90 parts of water and 10 parts of glycerin; by slowly extracting the water the glycerin is inspissated, and this in its turn is removed with strong or absolute alcohol. The concentration is hastened by using the sulphuric acid exsiccator.

Preparations thus treated may, after 12–24 hours' immersion in spirit, be mounted straight away in the Venetian turpentine. If, however, they will not bear this, the procedure originally noticed must be adopted.

(6) Miscellaneous.

An Inexpensive Reagent Block.†—Prof. J. H. Pillsbury says:—"A frequently expressed need of some convenient and inexpensive block or case in which to place the reagents and apparatus used in the biological laboratory, leads me to describe the form I have used for some time (fig. 69).

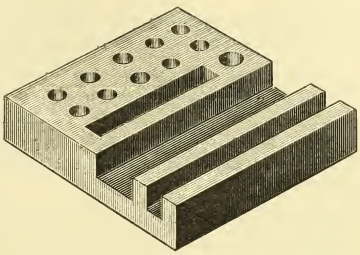
It is a plain whitewood block, 15 cm. square and 4 cm. thick. On the upper side of this three grooves are cut, each 1·5 cm. deep. The first is 1 cm. from the edge, and 1 cm. wide. The second is 1 cm. from it, and 3·5 cm. wide. The third is 1 cm. from it, and 2 cm. wide. Into one end there is glued a closely-fitting block 1 cm. long, and in the other end one 5 cm. long, leaving a trough for slides about 9 cm. long. In the place where this last block is glued is bored a hole 1·5 cm. in diam. and 1 cm. deep, into which tightly fits a paper pill-box for covers. The remainder of the block is provided with two rows of five

* See this Journal, 1890, p. 535.

† Amer. Mon. Micr. Journ., xi. (1890) p. 2.

holes, each 2 cm. in diam. and 3·5 cm. deep, for reagent phials. The first groove is used for razor, and the second for pencils, pipette, forceps, &c. The block is easily made, costs very little, is very neat in appearance, and convenient in work."

Fig. 69.



Microscopic Diagnosis of Citric Acid in Plants.* —M. E. Belzung states that, according to Schultze, citric acid may be recognized in the ripe seed of *Lupinus luteus* by treating the seeds with alcohol, evaporation, and treating the residue with water; from this solution citric acid can be separated. The author, however, endeavours to diagnose the acid by means of the formation of citrate of calcium. Young plants were grown in a weak solution of nitrate of calcium, sections of the plant were made, and examined microscopically; after a short time, numerous acicular crystals appeared, which were found to be those of citrate of calcium.

Artificial Preparation of the Sphæroliths of Uric Acid Salts.†—Herren W. Ebstein and A. Nicolaier say that if some uric acid be dissolved on a Microscope-slide in a dilute alkaline solution, and watched with the Microscope, there is, after slight concentration, a formation of round particles of urates varying in diameter from 2–100 μ. These are mixed with needles, either singly or in bundles. As solvents, sodium hydroxide, potassium hydroxide, lithium carbonate, borax, ammonia, and piperazine were used; the best results were obtained by using the uric-acid sediment from human urine.

With the polarizing Microscope between crossed nicols the sphæroliths showed a right-angled, black interference cross, the arms of which lay parallel to the polarization planes of the nicols, and, concentric with the middle point of this cross, coloured interference rings were seen.

Similar sphæroliths were obtained with sodium hydrogen carbonate, so that they may consist either of acid or normal urates.

The interest of such an observation, as bearing on the formation of urinary calculi, is pointed out.

* Journ. de Bot. (Morot) v. (1891) pp. 25–9 (3 figs.).

† Virchow's Archiv, 123, pp. 373–6; see Journ. Chem. Soc. Lond., cccxliii. (1891) pp. 760–1.

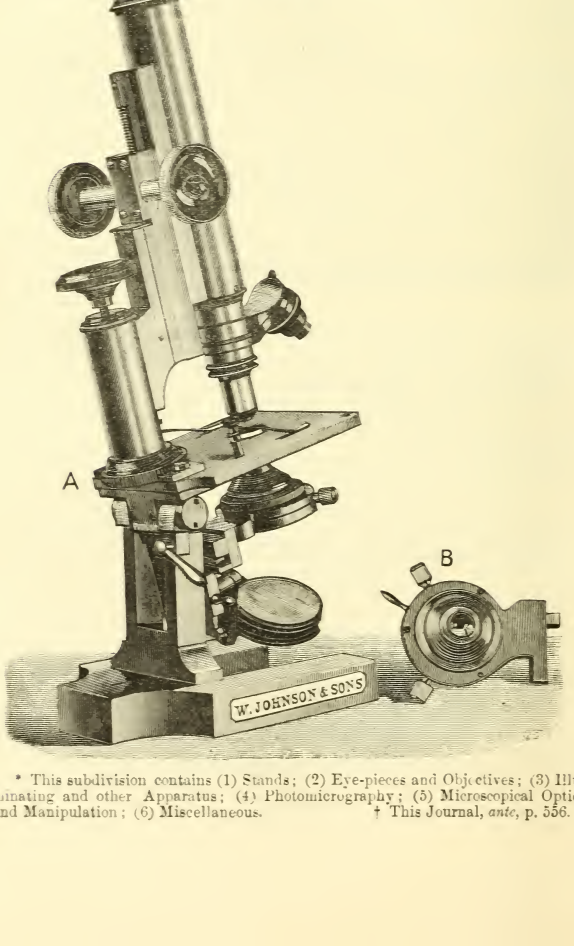
MICROSCOPY.

a. Instruments, Accessories, &c.*

(1) Stands.

Johnson & Sons' Advanced Student's Microscope.—At the meeting of the Society in June last,† Mr. T. T. Johnson, of the firm of W. Johnson

FIG. 70.



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

† This Journal, *ante*, p. 556.

& Sons, exhibited and described a new student's Microscope which he had devised.

The late Mr. John Mayall, in introducing it to the President, said the special point was in the application of a screw movement for the substage adjustment. He thought it a very economical and excellent way of applying the focusing arrangement to the substage, and it appeared to him most happily chosen for convenience, and would certainly commend itself to notice. It seemed to him that Messrs. Johnson had undoubtedly "scored 1" by bringing out this screw-focusing arrangement for the substage.

This instrument has been constructed with a view to supply the student in the higher branches of research with a suitable Microscope at a moderate cost. The foot (fig. 70, A) is of the horse-shoe form, and sufficiently weighty to insure steadiness when used in a horizontal position for photomicrography.

The patented screw substage adjustment consists of a screw placed in the axis of the substage and tail-piece, which is actuated by a milled head nut, slightly projecting at A; this being readily at command gives great facility for raising or lowering the substage, and delicately focusing the condenser, &c. The substage carrying an Abbe condenser with iris diaphragm and mechanical centering arrangement is mounted on a substantial tail-piece and slides in dovetailed fittings. The substage with its fittings (when in use) is fixed to the Microscope, and is free from lateral motion; by a simple arrangement of a clamping screw it can be readily removed or replaced (see fig. 70, B) and is, as regards durability, far superior to the pivoting system. The mirror can also be removed for direct lights if required.

The fine-adjustment is on the differential screw principle insuring delicate focusing for high powers, and the coarse-adjustment on the oblique rack-and-pinion system, giving equality and smoothness of motion, the body being supplied with a draw-tube, and marked for English or Continental objectives.

A great advantage is gained by this position of the substage adjusting knob, as in addition to its being readily at command, all liability of tilting the mirror or disarranging any of the under-stage apparatus is avoided, "accidents" which often happen where it is necessary to feel for the adjustment when placed beneath the stage.

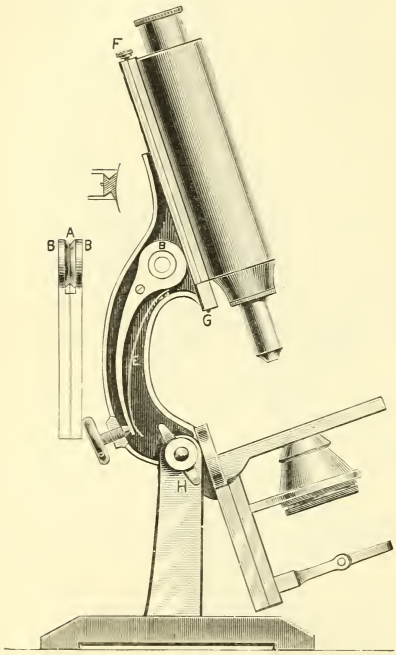
A College Microscope.*—Dr. W. H. Seaman observed:—"It may be remembered that in March 1888, *Science* published an article by me, maintaining the excellence of American Microscopes. The train of thought inspired by that article led me to make working drawings of an instrument with some novel features. These were shown to a few friends at Columbus, and were unfortunately lost from my coat-pocket at Buffalo. I did not have time to reproduce them till recently, and hoped to have the instrument itself here, but it is not quite done.

The figure (fig. 71) shows the features which are essential, in my judgment, to a good College Microscope. It will also be well adapted to the average professional man and amateur. A tripod base, rather thin,

* Proc. Amer. Soc. Micr., xii. (1891) pp. 67–8.

single foot back, wide open in front. The pillar may be single or double, but must have thumbscrew at the joint to hold it firm at any desired inclination; the mirror on swinging arm, adapted to carry a condenser if desired, and the stage just high enough to admit a short Abbe condenser; the centre of rotation of the mirror-bar just above the stage. The arm is a box-arm, Jackson model, shown with one side removed. The barrel should be of the short type and is supported on an X-shaped bar, that slides between the V's on each inside of the box-arm, as shown by detailed section. A steel tape or picture cord is fastened at each end of the X-bar, one end being the tightening screw F. This tape is wound once round the grooved wheel A, which is turned by the usual milled head and gives the coarse-adjustment to the instrument. On each side of the wheel A and on the same axis are two discs B B, that pinch the wheel A between them by a screw and act as a friction clutch. These discs are prolonged downward in the curved bar against which presses the spring E. The micrometer screw D forces the bar against this spring, and, turning the wheel A by friction, forms the fine-adjustment. Every part of the instrument is adjustable for wear. The stage is a ring, with a plate of glass dropped in it. A Zentmayer sliding holder may be used. The condenser is not shown in detail, as no special features are claimed for it. I am aware that friction fittings are not new; one was described by Mr. Wenham, vol. vii., Q. J. M.; also a chain movement was made by Pike or Grunow, of New York city, about 1850. Nevertheless, these devices do not appear to me to be as useful as that here described. The steel tape has proved successful in mechanical combinations where racks, &c., have failed, and it may succeed in the Microscope. The micrometer screw D may be replaced by a cam.

Fig. 71.

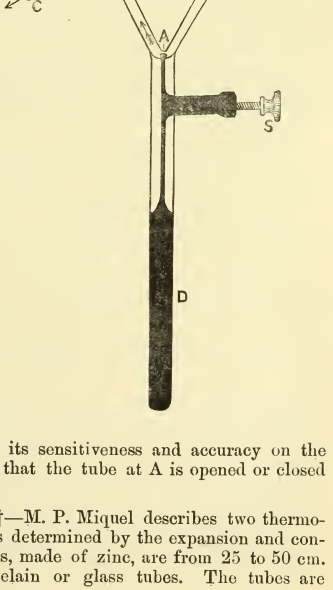


A Microscope built on the plan here outlined need not be expensive, and would be capable of all but the highest class of angular work. It may be conveniently used in its simplest form, and is at the same time adapted for the successive addition of those accessories essential to the prosecution of advanced researches with the instrument. Should it prove to answer my expectations I may refer to it again."

(3) Illuminating and other Apparatus.

Altmann's Thermoregulator.*—Herr P. Altmann has devised a very simple instrument for regulating temperatures below 100° C. The instrument works with great precision, not varying more than ± 0·05° C.

Fig. 72.



From the illustration it is seen to be made in a single piece. D is the reservoir fitted with mercury; this is narrowed above to a capillary tube, which at the side is in connection with a tube fitted with an air-tight iron screw S, which serves to regulate the apparatus for any desired temperature.

The way the instrument works is easily conceived from the illustration. When the reservoir D is heated, the mercury therein expands, and rising, cuts off, in the V tube of A, the stream of gas passing along in the direction B A C and only allows the transit of the small current along B E C. At E is a tap for regulating the supply of gas for keeping the burner just alight, and this is adjustable for any size of flame.

The instrument depends for its sensitiveness and accuracy on the large surface of the reservoir, so that the tube at A is opened or closed with great facility.

Metallic Thermoregulators.†—M. P. Miquel describes two thermoregulators, the action of which is determined by the expansion and contraction of metal bars. The bars, made of zinc, are from 25 to 50 cm. long, and are inserted in porcelain or glass tubes. The tubes are

* Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 791–2 (1 fig.).

† Annales de Microgr., iii. (1890) pp. 150–8 (2 figs.); iii. (1891) pp. 241–6 (2 figs.) and 363–74 (1 fig.).

immersed in the water-bath, and as the bar lengthens from the increased temperature, its upper end presses directly against a caoutchouc tube, through which the gas passes to the flame. Hence the flame diminishes and consequently the temperature of the thermostat. In the second model the end of the zinc bar is bevelled and its edge made to press against a lever, which is always kept opposed to the bar by means of a spring. The other arm of the lever runs between two caoutchouc tubes, one of which introduces a cooling, the other a heating medium.

The author states that having used these for some years he is able to testify that they work with great efficiency and accuracy, and gives a table recording their diurnal variations, which are certainly small. For the minute details of construction the original must be consulted.

The question of heating media is then discussed and an ingenious method for employing alcohol is described. In this case the alcohol is supplied to the flame through a tube coming from a reservoir fitted with a Marriotte's tube. The tube has an overflow pipe placed at an angle between the flame and the regulator. The regulator placed within the water-bath embraces the caoutchouc tube as it passes from the spirit reservoir to the flame, and so acts that as the bar expands it nips the tube, and thus diminishes the flow to the lamp.

Thermoregulator for large Drying-stoves and Incubators.*—M. Roux recommends the thermoregulator which has been in use at the Pasteur Institute for some years, as being very suitable for large ovens or incubators.

It is made of two metal bars welded together and bent to a U-shape. The inner bar is made of steel and the outer one of zinc. These are massive enough to prevent any springing. The length of the legs of the U are from thirty to forty cm.

The variations in temperature as recorded from the use of the large incubator at the Institute are said never to exceed 0·5°.

Capillary-siphon-dropping Bottle.†—Prof. M. W. Beyerinck says that if the V-shaped tube of a dropping-bottle be made of capillary size it will be found very useful for microscopical purposes. Thus it may be used for distributing small quantities, droplets, of any reagents from the bottle, or for capturing small animals, Infusoria, from a watch-glass, and so on.

Steam-filter.‡—The apparatus devised by Dr. P. G. Unna for filtering agar is a hollow copper sphere, the upper half of which serves as a lid.

In the bottom is a hole, through which passes the stem of an enamelled iron funnel. The top of the funnel projects above the level of the lower hemispherical segment or pan, and the distance between the edge of the funnel and the pan is about 1 cm. The pan is suspended on a tripod, from the ring of which a semicircular band passes over the pan. By means of a screw at the uppermost part of this band the lid is firmly screwed on. In the lid is also a small tap for letting off the

* Annales de l'Institut Pasteur, 1891, p. 158. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) p. 737.

† Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 589–90 (1 fig.).

‡ Tom. cit., pp. 749–52 (1 fig.).

steam. The apparatus is heated by means of a cylindrical closed pipe, projecting obliquely from the lower pan.

The agar having been cut up is boiled for half an hour on the open fire, and then having been mixed with any desired substances is placed in the funnel. In the funnel is fitted an ordinary filter-paper, filled with siliceous sinter. When the lid is screwed down, and heat applied, the pressure of the steam serves to drive the liquefied agar through the funnel into a flask placed underneath.

The chief advantages of this apparatus are its rapidity,—a litre of 2 per cent. agar can be filtered in two hours, a great economy of gas, simultaneous sterilization and clarification.

(4) Photomicrography.

The Value of using different makes of Dry Plates in Photomicrography.*—Dr. W. C. Borden remarks:—"While the variation in rapidity of different makes of plates is pretty generally understood and taken advantage of in practical work, the variations of plates in contrast and range of tones are not generally discussed in photographic literature, nor are the great benefits to be obtained by taking proper advantage of these variations understood, or generally practised. Hardly a photographic journal appears without either some new formula for a developer, or some new method of working an old one, by which it is claimed that some modification of rapidity or contrast may be produced in the plate on which they are used. Quite a large portion of photographic literature is devoted to giving these means of producing required effects in negatives, and every box of plates contains information (?) how to obtain greater or less rapidity, or contrast, as may be desired; when in fact, after a light has once struck a plate in a particular way, so changing a particular ratio, the molecular structure of the sensitizing chemicals with which it is coated, but little change in result can be produced by any developer, however much that developer may be modified. A modification, however, of the coating of the plate, giving a different chemical basis upon which the light acts, will, from the different arrangement and kind of molecules acted upon, produce a different result whatever developer may be employed. It is in this way that variations in result may be best and most surely obtained, for different makers of plates use sensitizing formulas differing in such manner that the coatings, when acted upon by light and "developed," give results differing in rapidity, contrast, and range of tones. That almost universal advice: "Get a good plate, master its peculiarities, and then use this plate exclusively," is good only so far as getting a good plate and mastering its peculiarities are concerned, for, however well the working of any one plate may be understood, results cannot be obtained from it alone, upon all kinds of objects, equal to those obtainable when different makes of plates are intelligently used, in a manner to make their peculiarities bring out, in the resulting negative, the effect sought for. For instance, if the object to be photographed has but little contrast, and a plate giving great contrast and a short range of half tones be used, a good printing negative will usually be obtained, while, if a plate having

* Amer. Mon. Micr. Journ., xii. (1891) pp. 169–72.

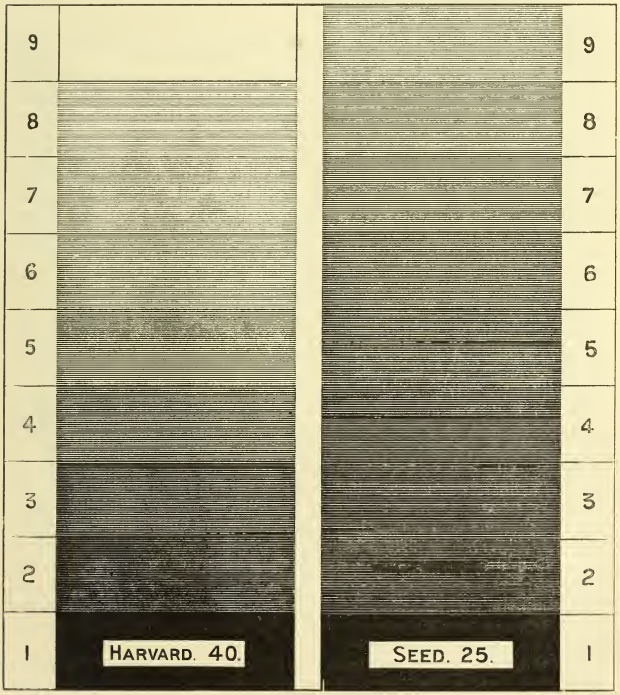
opposite qualities were used, no amount of careful exposure or development would give a negative having sufficient contrast to print properly. Similarly, with an object having great contrasts, a plate giving little contrast and long range of tones, will give a negative in which the contrasts of the object are so lessened that printable details are given in the densest parts, while were a plate having opposite qualities used, the strong contrasts of the object would be so reproduced or exaggerated that a print devoid of all detail could be obtained only. As in photomicrography, owing to the peculiar nature of the objects to be photographed, great difficulties are often encountered, the ingenuity of the operator often being taxed to the utmost, it follows that a proper selection of the plate to be used will add greatly to his resources, and will enable him to obtain results which could not be obtained were only one make of plates used, whatever legerdemain of exposure or development he might practise.

But, in order to take advantage of the different properties of different plates, it is necessary to know exactly how they differ; and this must be determined not by exposing the plates to be compared in a camera where the light may be constantly varying, and where the personal equation of the operator may enter as a disturbing factor, but in a manner by which each shall receive equal treatment. For purposes of comparison I have used a pad of thin white tissue paper (onion skin), 4 in. by 4½ in. in size, made of superimposed pieces of paper, each sheet being 4 in. long, and 1/2 in. narrower than the next sheet underneath. This pad, when placed on a piece of clear glass in a 4 in. by 5 in. printing frame, and viewed by transmitted light, gives nine gradations of density, from clear glass up. Such a pad answers for all practical purposes, though one 7½ in. long, placed in a 5 in. by 8 in. printing frame, and used with strips cut lengthways from 5 in. by 8 in. plates, will give a longer range of gradations. To test two or more plates, a strip about 1 in. wide and 5 in. long is cut from each, and placed side by side, film side down, on the pad in a 4 in. by 5 in. printing frame. They are then clamped in the frame, exposed for one instant to diffuse daylight, or for a few seconds to lamplight; and are then all developed together in the same developer.

It is best to develope for fully twenty minutes in a covered tray, with a developer containing a rather large quantity of sodium sulphite. If about thirty grains of the granular sulphite is used to each ounce of the developer, yellowing of the films, which might be produced by the prolonged development, will be prevented; and this without any ill effect on the resulting negatives. Development for fully twenty minutes is recommended in order that development be fully completed, i. e. that all the molecules of silver acted upon by light be reduced, for in this way only can the exact properties of all the strips be brought out, inasmuch as some plates develope more rapidly than others, and a stoppage of development before completion will produce erroneous results. The illustration is a reproduction of the result arrived at by comparing a "Harvard" plate, sensitometer 40, with a "Seed" plate, sensitometer 25, in the manner above described (fig. 73). It is a reproduction of the negatives themselves (not a print from them), so the lighter bands represent the thinner bands of the original negatives.

The great difference in the two negatives is seen at a glance. The greater rapidity of "Seed" plate is shown by band 9 in the plate, where the light had to act through but nine thicknesses of paper before acting upon the plate, being equally as dark as band 5 in the "Harvard" where the light had to act through but five thicknesses. The comparative rapidity of the Seed to the Harvard is therefore as nine to five; or for

FIG. 73.



practical purposes it may be considered as double. The greater contrast of the Harvard, and longer range of half tones of the Seed are shown by the same range being gone through in five bands in the Harvard, i. e. from band 1 to 5, that requires nine bands in the Seed, i. e. from band 1 to 9. In other words, a certain gradation of light in an object photo-

graphed, which will give with a Seed plate a certain contrast in the negative, will with a Harvard plate give practically double the contrast.

This comparison shows at once that the Harvard is the better plate to use when objects having little contrast are to be photographed, or when contrast is desired; and the Seed is the better plate when rapidity is desired, when an object having strong contrasts is to be photographed, or when strong contrasts are to be avoided and a "soft" negative desired. Also, that by the intelligent use of these plates, or others having similar qualities, results may be arrived at which could not be obtained by the exclusive use of either alone.

I have called attention to these particular plates, and have used them in illustration, because they have the opposite qualities, by taking advantage of which almost any Microscope object can be successfully photographed. Not but that there are on the market other plates having qualities in every way equal to the plates particularly mentioned. For instance, the "Eagle" plate, sensitometer 40, is an almost exact duplicate of the "Harvard," 40, in both rapidity and relative contrast; and Carbutt's "Keystone," sensitometer 16, is almost identical with the Seed, 25, in all properties except rapidity. All plates having the qualities of the Harvard and Eagle give great contrast and short range of half tones, and are therefore best adapted to objects having but slight contrasts. With such plates satisfactory negatives can be made from such little contrast, that were plates like the Seed, 25, and Keystone, 16, used, negatives having printing contrasts could not be made at all. Conversely, plates like the Seed, 25, and Keystone, 16, low contrast and long range of half tones, will satisfactorily reproduce the details of objects having great density or contrast, which details would be entirely obliterated if plates like the Harvard or Eagle were used. As plates similar in other qualities often vary in rapidity, as is the case with the Seed, 25, and Keystone, 16, this variation can be taken advantage of where the light is more or less strong, or where greater or less rapidity is desired, without in any way affecting the result, so far as the printing qualities of the negative are concerned.

I have, however, never found the most rapid plate too quick, even with low powers and sunlight, as I habitually use a light-filter of a colour complementary to that of the object photographed. For these filters, being generally either yellow, green, or yellowish-green, considerably lengthen the time of exposure; so much so, that while with a Zeiss 2 mm. h. i. apochromatic objective, a projection eye-piece, 4, and an amplification of 1500 diameters, a Seed, 25, plate will require about 35 seconds; a wet collodion plate, using a blue filter, would require but about two seconds.

As the Seed and Harvard plates have opposite qualities, which adapt them to almost every object to be photographed, before using other makes they should be comparatively tested, either with the plates named, or with some plate with the workings of which the operator is familiar, when their actual qualities will be demonstrated and their adaptability ascertained. Only by such testing can the operator know exactly what to expect, or be able to arrive at the best results, for this, like other work connected with microscopy, should never be of a haphazard sort.

The worker in photomicroscopy, who uses plates having opposite qualities as regards density, contrast, and range of tones, and who uses them intelligently, will obtain results which cannot be equalled by the one who uses one make of plates only, or who uses all kinds as may happen, without a knowledge of their properties arrived at by comparative testing."

Marktanner-Turneretscher's 'Die Mikrophotographie als Hilfsmittel Naturwissenschaftlicher Forschung.'*—The aim of this little work on photomicrography is to afford assistance to those who wish to make use of photomicrography in their investigations, so that they may attain their object with as little expenditure of time and trouble as possible. The theoretical considerations are supplemented by a number of very serviceable practical hints. After a brief sketch of the history of photomicrography and its uses, the author gives a description of a complete photomicrographic apparatus, and explains the various uses and modes of production of the different sources of light. He then deals with the properties of photomicrographic preparations and gives a concise but comprehensive account of the practical operations which are necessary for the production of a good photomicrogram. The usefulness of the book is increased by numerous bibliographical references, good illustrations, and well-executed photomicrograms.

(5) Microscopical Optics and Manipulation.

Diatom-Structure—The Interpretation of Microscopical Images.†—Dr. J. D. Cox in speaking on this subject made the following among other remarks:—

"In such a case the real question is one of interpretation of appearances seen under the Microscope, and what I have to say will bear chiefly on this point, with direct application to the study of diatoms.

All microscopists are acquainted with the position of Prof. Abbe in regard to images formed by diffraction. As commonly stated it amounts to a declaration that all microscopical images of structure with details smaller than ·0005 of an inch are diffraction images from which the true structure may be argued, but which cannot be taken as in themselves true representations of the structure. 'The resulting image produced by means of a broad illuminating beam,' says Prof. Abbe,‡ 'is always a mixture of a multitude of partial images, which are more or less different (and dissimilar to the object itself).'

This theory has been very vigorously assailed by Mr. E. M. Nelson, of London, from the practical and experimental side. In a paper read before the Quekett Club in May [1890], entitled "The Substage Condenser: its history, construction, and management; and its effects theoretically considered," Mr. Nelson asserts that the cone of light from a substage condenser 'should be of such a size as to fill 3/4 of the back of the objective with light; thus N.A. 1·0 is a suitable illuminating cone for an objective of 1·4 N.A.' He says that 'this opinion

* Biol. Centralbl., xi. (1891) p. 351.
† Journ. New York Micr. Soc., vii. (1891) pp. 76-87.
‡ R. M. S. Journal, December 1889.

is in direct opposition to that of Prof. Abbe,' and to maintain it he denies the truth of the diffraction theory as applied to microscopical images. He says of it: 'The diffraction theory rests on no mathematical proof—in the main it accepts the physical law of diffraction; but on experiment it utterly breaks down, all criticism is stopped, and everything connected with it has to be treated in a diplomatic kind of way.'* I state Mr. Nelson's position without any purpose of discussing it, and only to point out that it is this to which Mr. Smith refers in his paper when he says: 'This capacity of standing more light was pointed out from the first by Mr. E. M. Nelson, but has not received the attention it deserves, and the neglect of this point has stultified the efforts of many microscopists, both here (in England) and on the Continent, to get more out of the new glasses than the old objectives.'

Mr. Smith's investigation of diatom-structure is thus closely connected with Mr. Nelson's views and experiments upon the diffraction theory. Both will challenge the attention of practical microscopists as well as physicists. I have not gone far enough in my own investigations to warrant me in expressing a judgment on the questions involved; but I would urge every microscopist to make his ordinary work the occasion for accumulating evidence which may help to settle the very important debate. My suggestions are only such as are based upon the well-known history of diatom-study and my own experience. They are offered by way of clearing the field by pointing out the limits of the discussion and the known facts which ought to be kept firmly in mind in all such investigations.

It is no reproach to the Microscope as an instrument of investigation that there are limits to its powers and capabilities. Such limitations are common to all methods of investigation. If, trusting to my natural eyesight, I am trying to make out the meaning of appearances on a distant hillside, I find at once that all perception by the sense of sight is an interpretation of visual phenomena which are not in themselves decisive. They may lack clearness by reason of the mist in the air. They may be obscured by something intervening, like foliage, or may be partly hidden by inequalities of surface. A thousand things may prevent clear and easy interpretation of what I see. I may have to change my point of view before I can reach a conclusion, or even have to go to the object itself. If I cannot do this I may be left in abiding doubt as to what I have seen.

Microscopical examination is precisely analogous to this. If I am examining a mounted object I am tied to one point of view. I cannot approach nearer, and cannot do more than note the visual appearances and make theories to account for them in accordance with facts already learned. We try to vary the conditions as much as we can; we change our objectives; we try central light and oblique light; we examine one specimen dry and another in a dense medium; one by transmitted and another by reflected light; but when we approach the limit of minuteness of object or detail which our instruments will define, we are in the same situation as when using our natural eyes across a chasm, neither better nor worse: we have to account for what we see by a reasonable

* Quekett Club Journal, July 1890, pp. 124–5.

hypothesis which will make it take an intelligible place amongst natural objects.

Our skill as microscopists, apart from the technical dexterity in the use of our tools, consists largely in devising varied experiments and changes of condition, so as to enlarge the body of evidence from which we draw our inductive conclusions. To assist ourselves in this, we also catalogue such facts and methods, and such cautions and warnings, as our experience (or that of others) has taught us. Let us look for a moment at some examples.

We know very well that we are liable to illusions of sight, so natural and so powerful that even the intellectual certainty that they are illusions will not destroy them. If we are looking through the Abbe binocular eye-piece, using the caps with semicircular openings, we see a hemispherical object as if it were a hollow bowl, and, visually, it refuses to be anything else. But this is not peculiar to microscopical vision, for we do an analogous thing with the stereoscope, and by wrongly placing the pictures may make an equally startling pseudo-perspective.

We find that what we call transparent bodies are full of lines as dark as if made with opaque paint, and throw far-reaching shadows. But I see similar ones in the cubical glass paper-weight on the table before me, and know that by the laws of refraction the surface of a transparent body is always dark when its angle to the eye is such as to cause total reflection of the light in the opposite direction. By the same law we know that if the angle of total reflection in the same transparent cube were differently placed with regard to the eye, the now dark surface would become a mirror, reflecting the sky and distant objects as brilliantly as if silvered. Our diatom-shells give us constant experience in these phenomena. A prismatically fractured edge will scintillate so as to defy all efforts to define its outline. Reflected images look like actual details of structure in the object. Dealing, as we constantly are, with objects made of glass, we have constant use for our reasoning faculties to determine the meaning of all these refractions and reflections, which sometimes are almost as confusing as the broken images seen through the glass pendants of a chandelier.

In addition to these familiar effects of refraction and reflection, we have the class of phenomena which we call diffraction effects. These may be wave-like fringes of light and shadow following the outline of the transparent object, and reduplicating this outline; or they may be analogous fringes thrown off the subdivided parts of the object, as from the cup-like outline of alveoli, or from some projecting rib or groove like those along the diatom's median line.

We know by constant experience that when we throw light obliquely through a transparent reticulated object like a diatom-shell, the diffraction fringes from the separate alveoli run together across the shell in dark striæ, oblique or at right angles to the direction of the light. In the *Pleurosigma*, in which the rows of alveoli are oblique to the midrib, we very easily get the oblique striation by the use of oblique light; getting both series of lines at once, one only, or one strong and the other faint, as we please, and with very little trouble. We get, with a little more pains, a transverse striation, at right angles to the midrib,

which is fainter because it proceeds from alveoli not so closely connected in rows. It may be called a secondary striation. With still more effort we may get a much finer and fainter striation, parallel to the midrib, by throwing light at right angles to it, or nearly so. By lamplight, and with objectives not apochromatic, and not exceeding the aperture of 1·0 N. A., these lines are usually in patches, upon spots here and there, longer (in the length of the shell) than they are wide. But with sunlight this tertiary diffraction striation may be made to cover the whole surface of *Pleurosigma angulatum* by an exquisitely fine longitudinal grating over its whole surface, as was demonstrated by Dr. Woodward in one of the most striking of his photomicrographs in what is called "the Abbe experiment." * As the improvement in our lenses, both by increasing their angle, and by the apochromatic system, tends to make visible by lamplight what before could only be seen by the sun, we should expect that something like the fibrillæ shown in Mr. Smith's photographs would be visible. Finding it would not prove that it is purely the result of known laws of diffraction; but it justifies a cautious and scientific scepticism in receiving a new explanation until we have repeated the experiment often enough, and under such varying conditions as to exclude doubt.

As we increase or reduce the obliquity of the light in examining *Pleurosigma formosum*, we know that the alveoli are distorted (or may be) in varying ways and directions. Some of these are figured in 'Carpenter on the Microscope,' but they are only a few of a numerous series. Whoever will experiment a little may satisfy himself that the permutations and transmutations of the diatom markings may be made little short of kaleidoscopic. Hexagonal markings may become square, and may have short lines running off from one angle. These lines may be lengthened, and the square or hexagon reduced to a dot, so that the appearance of the surface may be that of oblique series of parallel dashes. The direction of these lines depends on the direction of the light, making a series of gratings, of which the prevalent character may be oblique in either of two directions, transverse or longitudinal. The so-called intercostal points may be enlarged and brightened until they become the most prominent marking, and the alveoli proper may be diminished to insignificance. These appearances are so like many of those in Mr. Smith's series that we, who can only see the print and cannot get our fingers upon the fine-adjustment of the Microscope and note for ourselves the effect of a change of focus, are necessarily made cautious in accepting his interpretations; but there should be caution in rejecting as well as in accepting, and he fairly challenges us to repeat his investigations under similar circumstances, and with similar objectives.

An examination of his print No. 12 with a hand-lens will illustrate what I am saying. When looked at with the naked eye, this print shows a long patch of longitudinal striation on the lower side of the valve. Immediately below the midrib we see the coarse, oblique dotting peculiar to *Pleurosigma formosum;* but if we use the lens we see at once that, in the patch referred to, the dots are twice as numerous as the

* See Roy. Micr. Soc. Journ., ii. (1879) p. 675; also Mon. Micr. Journ., xvii. p. 82.

alveoli of the shell. The interpolated ones (proceeding from above downwards) are at first very small, then larger but rectangular, and twice as long as wide, making the pattern one of alternate dots and rectangles; as we pass to the right the rectangles run into each other obliquely, making a wavy white line, the dots of the alveoli proper being in the bends of the line, very much as in the longitudinal fibrils of print No. 11. This change, distortion, and multiplication of the dots is so entirely within our common experience in diatom-study, that I have no hesitation in explaining the longitudinal striated appearance in this patch as the result of the reduplicating of the dots by the intercalation of the rectangular ones, making in fact broken lines, which on so small a scale are sufficiently even to make continuous ones to the naked eye. On the other side of the midrib in the same print (No. 12) the rectangles and round dots are of nearly equal size, but they still make a faint longitudinal striation, diverging a little from the midrib as we pass from left to right.

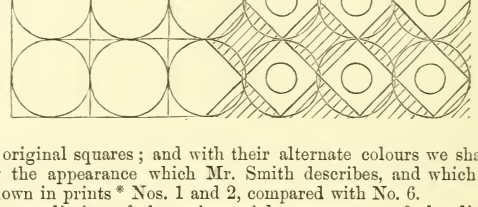
We thus have an ocular demonstration how a striated appearance may be made out of a tessellated one, when there is no question of continuous fibrils. Yet even this does not prove that the fibrils are not there. Of course all visual appearances under the Microscope have their cause in the structure of the object, considered in relation to the laws of transmitted and reflected light. The puzzle often is to tell what to attribute to each factor. I do not think it difficult to account for the tessellated appearance of dots and squares with alternate blue and red colour. To do so may require us to refer to some elementary matters in diatom-marking.

Dr. Brebisson, at a very early day, divided the regular dotted markings of diatoms into three classes: (1) *Quadrille rectangle droit*—in squares parallel to midrib, e. g. *Pleurosigma balticum;* (2) *Quadrille rectangle oblique*—in squares oblique to midrib, e. g. *P. formosum;* (3) *Quinconce*—quincunx or lozenge of 60° smaller angle, e. g. *P. angulatum.* This classification has been a good deal neglected, but has good claims to remembrance, and will assist me in explaining the phenomena before us.

In Mr. Smith's print No. 6 is well shown what I regard as the normal scheme of areolation of *P. formosum.* It will be seen to be a reticulation with meshes as nearly square as nature gives us in growing things. If the corners of these meshes be filled up, the included circles will still keep to each other the relative position of Brebisson's oblique quadrille. The diminution of the round alveoli would not need to proceed far before the approximately rectangular mass of silex between the circles would be about as large in diameter as the circles themselves. Under the laws of optics, which we have already seen illustrated in print No. 12, the tendency of approximately rectangular details is to become more strictly so in the microscopical image. In Fig. 74 I have illustrated this by a geometric diagram, of which one half shows the square reticulation, and the other the resulting tessellation of solid squares and round alveoli when the walls are thickened and the corners filled up. It will be noticed that when the corners are so filled as to make the alveoli circular, the interspaces are approximately square, and, being solid, will be red or pink by transmitted light when the alveoli are

bluish-white. On the inner side of the shell the thin circles, or "eye-spots," are usually smaller than on the outer side; the diffraction effect by transmission of light will straighten the edges of the tessellated outline; the squares will each have half the area of, and will be diagonal

Fig. 74.



to, the original squares; and with their alternate colours we shall have exactly the appearance which Mr. Smith describes, and which is very well shown in prints * Nos. 1 and 2, compared with No. 6.

The peculiarity of the quincuncial arrangement of alveoli is that when the circles crowd upon each other so as to become polygons bounded by straight lines, they form hexagons instead of squares, and even when they are circles in a continuous plate of silex the hexagonal outline is a persistent ocular illusion. We should expect, therefore, that the tessellated appearance with equal squares of red and blue would be a mark of *P. formosum* as distinguished from *P. angulatum*, under proper conditions of illumination and examination.

We are justified in concluding, therefore, that the phenomena of colour and form thus examined are not only consistent with, but strongly confirm, the generally received theory of diatom-structure, and cannot be said to indicate anything new in that direction.

Mr. Smith also expresses the opinion that only by means of a wide-angled objective, and illumination by a wide cone of light from the sub-stage condenser, can the upper and lower films of a shell like *P. angulatum* be discriminated. As he recognizes some photographs made by me, and deposited with the Royal Microscopical Society in 1884, as showing this discrimination, it is due to scientific accuracy to say that they were made with a Wales 1/15 water-immersion objective of about 1·0 N.A. aperture, and with a narrow cone of light coming from a Webster condenser under the stage having a diaphragm with a 1/4-in. opening behind it. Mr. Smith's own objects photographed could not be illuminated with a very wide cone of light, as they were mounted dry, and he tells us he used his condenser dry. There was therefore a stratum of air both above and below the slide on which the object was mounted, and the illumination could not exceed the "critical angle," 82°, in passing through the cover-glass, and must in fact have been considerably less.†

* These prints are given in an article by Mr. Smith in the journal here quoted (pp. 61–72).

† In my note-book, June 3rd, 1884, I find that I entered my observation of one of the broken shells which I photographed, as follows: "A remarkably interesting

In my own experience I have found a broad cone of illumination unsatisfactory, for the same reason that I have found oblique light in one direction unsatisfactory. It is almost impossible to centre the substage condenser so accurately that a wide cone can be trusted to be central. If you centre it by examination with a low power, it is almost certain that it will not be centered for a high power, for two objectives are rarely centered alike. The field, under a magnification of 1750 which Mr. Smith has commonly used, is so small that the least decentering will illuminate it only by the oblique rays from one side of the cone, and we then immediately get diffraction effects. I am bound in candour to say that in most of Mr. Smith's prints I recognize similar effects to those which, in my own work, I attribute to oblique light. It may be that, with improved contrivances to secure exact centering of objective and condenser, we shall find advantages in the use of the wide cone. I speak now only of my own experience under existing methods. The slightest turn of the mirror on its axis will change light from central to oblique; and I suppose we are all in the habit of doing this, so as purposely to throw light through one side or segment of the condenser for the purpose of studying the effect on an object of the changing direction of illumination. So unstable a source of light prevents our knowing very exactly when the light is strictly central, and makes it hard to return to any exact condition from which we have departed even a little. These considerations have kept me (perhaps mistakenly) in the practice of using the narrow cone of light for photography, reserving my oblique light for special resolutions of striation and for the professed study of changing effects.

Similar reasons have made me distrustful of dry mounts when high powers are to be used upon any but the thinnest objects. Refraction, and attendant diffraction, are so increased with increase of index, or rather increased difference of index, that it has grown to be a maxim with me to have the mounting medium and the object as near alike in index as is consistent with the discrimination of structure. The pale images of transparent objects are those I find most truthful, for paleness is consistent with good definition and resolution, whilst the brilliant pictures are apt to be glittering deceptions. I fully admit, however, that it may well be that with improved glasses we may add to the extent of details visible upon a surface, like that of a diatom-shell, and that it is possible that mounting in most media would obliterate the finest of these details. To a certain extent we are all familiar with this. A rather coarse dry shell like *P. balticum* will have its details instantly obliterated if water from the immersion of our objective penetrates beneath the cover-glass. Mr. Smith's print No. 50 might pass as an excellent reproduction of this effect, the fluid passing along the structural lines, obliterating part and leaving part.

But when full weight has been given to all these things, and we have put aside those of Mr. Smith's long and beautiful series of photographs

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fragment of *P. angulatum*, showing partial removal of one film, and fracture through dots over a large space." In preparing this paper I have repeated the examination with the objective named, and find the distance between upper and lower film easily appreciable in focusing.

which are liable to our criticism, there still remain several which cannot be thus disposed of.

Prints Nos. 14 and 15, taken with half the magnification of most of the others (× 875), show strips of surface marking which strongly support Mr. Smith's interpretation, viz. that the outer surface of *P. formosum* is covered by a longitudinal series of fibrils separating so as to pass round the alveoli and uniting over the solid corner interspaces. The definition in these cases is not only reasonably clear and free from the ordinary marks of diffraction effects, but, most conclusive of all, there is in No. 15 a bit of this film floated off the shell and lying detached by its side. The fibrillar structure of this bit leaves little room for scepticism, and it so exactly accords with the appearance of the similar fibrils remaining on the surface of the shell that I cannot refuse to accept it as evidence of structure. Going back from these to prints Nos. 10 and 11, we now find reason to accept these also as evidences of the same structure, though distorted by obliquity of light, so that they would not have been satisfactory taken by themselves. On No. 5 also we may recognize some of the same fibrils. The single detached fibril in No. 9 is not so directly connected with any other specimen, either in the photograph or in Mr. Smith's description, as to present the evidence on which it is shown to be part of the same structure; but the measurement of its flexures so corresponds with the areolæ of the shell that its probable connection with a similar valve may be assumed.

The interpretation of this structure which seems to me most satisfactory is to regard these fibrils as superposed upon the general surface of the shell as a protection to the thin capping of the alveoli against abrasion. It would, in that case, come under the description of those appearances which I have referred to in paragraph 4 of my general summary, viz. a "thickening on the exterior of the lines bounding the areolæ . . . . which is not in contravention of, but is in addition to," the usual formation of the shell by means of two principal plates or films. All the species of *Pleurosigma* which have the alveoli arranged in Brebisson's *quadrille* seem to have strengthened ribs between the rows of "dots"—*P. balticum*, *P. attenuatum*, &c., have them longitudinal and straight. Mr. Smith's observations seem to prove that *P. formosum* and its congeners have them longitudinal but wavy, which is a positive addition to our knowledge, since we should naturally have expected them to be oblique. The appearance of the finer square tessellation in either of the principal films of an obliquely marked *Pleurosigma* would seem to prove it to belong to the "quadrille" marked class, and I think the smaller forms which Mr. Smith has left unnamed may be identified as *P. obscurum* W. Smith, which is probably only a small form of *P. formosum* or *P. decorum*.

I do not find in the prints any conclusive evidence that the quincuncial marked species, as *P. angulatum*, have the same series of fibrils. No one doubts that all have a vegetable membrane in which the silex is deposited, and, under favourable circumstances, a fracture through a row of dots would leave the thicker connecting membrane looking approximately like a fibril. The argument from analogy is not as strong here as in the case of the "quadrille" marked kinds. The structure may be

found in all, but the evidence does not yet seem complete. There is here a good field for further investigation.

This leads me to say that the size of the fibrils shown by Mr. Smith does not seem to me so minute that any good 1/10 or 1/15 objective should not define them. We must remember that the condition of an object may count for much in the resolution of its structure. A thickly silicified shell may not show what an imperfectly silicified one will demonstrate. The former will break into small angular bits with a mineral fracture; the latter may separate into threads or membranes. The floating off of the fibrils in print No. 15 seems to show that the shell was in a peculiar condition; a sort of dissection of an uncommon kind having taken place naturally or artificially. It would be an interesting experiment to subject various species of *Pleurosigma* to the action of hydrofluoric acid for varying periods, and then mount them for examination. To extend Prof. Bailey's old experiments in this direction would be very useful, but the danger of injury to the objective is such that it would hardly be advisable to watch the action of the acid under the Microscope.

If I seem to have reduced the new matter in Mr. Smith's observations to a minimum, I should not do justice to my sense of the real value of his work unless I add that enough remains to make it, in my judgment, a very important and interesting step in the investigation of diatom-structure. It is also full of promise that still further results may be attained by pursuing the investigation on the same line. I am confident, therefore, that the Society will join with me in expressing a sincere sense of obligation to him for communicating the results of his observations, and especially for the valuable aid in understanding them which is given by his beautiful series of lantern slides and prints."

On a new Method for the Measurement of the Focal Length of Lenses or Convergent Systems.*—Sig. G. Vanni gives the following method for measuring the focal length of lenses. If F denote the focal length, p and q the distance of the object and image from the two focal points, we have $p\,q = F^2$. Small displacements of the object $\Delta\,\Delta_1$ produce corresponding small displacements of the image $\delta\,\delta_1$ in such a way that always $(p + \Delta)\,(q - \delta) = F^2$ and $(p - \Delta_1)\,(q + \delta_1) = F^2$. These three equations determine p, q, and F when the displacements are known. The plane object is movable on the optical bank and the position of the image corresponding to known displacements of the object is determined by means of a Microscope.

Proof of a simple Relation between the Resolving Power of an Aplanatic Objective of the Microscope and the Diffraction of the finest Grating which the Objective can resolve.†—M. C. J. A Leroy starts with the general theorem of Abbe that an objective in order to resolve a grating must have its aperture sufficiently large to admit at least two spectra of the grating. Abbe deduces this relation as the expression of a special function of the angular aperture of the

* See Central-Ztg. f. Optik u. Mechanik, xii. (1891) p. 152; and Atti dell' Acc. d. Nuov. Linc., 6, 90.

† Séances de la Soc. Franc. de Phys., 88. See Central-Ztg. f. Optik u. Mechanik, xii. (1891) p. 152.

objective, his "specific function of the aperture," and takes it as the starting-point of a theory of microscopic vision. To the latter the author raises objections, although he admits that the experimental data are beyond doubt, His idea is that the theorem is an immediate consequence of the diffraction on the edges of the diaphragm which limits the aperture of the objective, and that consequently the specific function of Abbe is nothing else but the diffraction produced by the edges of the aperture.

(6) Miscellaneous.

Dr. Dallinger's Address to the Quekett Club.*—The Rev. Dr. Dallinger said:—"In addressing you to-night, as President of our Club, I shall keep before me the fact that, whilst we seek as a Club to prosecute all our mutual and individual inquiries in a completely scientific spirit, many amateurs are included in and welcomed by the Club, and that we aim at promoting and aiding early efforts with our favourite instrument, quite as much as criticizing the last results of experienced research, or the latest endeavour to render more perfect the instruments with which we work.

Keeping these facts before me, I am strongly impressed with the conviction that no subject can have a more general area of interest among microscopists than the work being done regarding the nature of the animal and vegetable cell. To what extent a full and complete knowledge of animal and vegetable cellular life and history, leading to a full grasp of the comparative physiology and morphology of cells, may ultimately contribute to a completing of our knowledge of life and function in animals and man, it may not be possible to say; certain it is that what we already know has profoundly affected our insight into animal structure; but our progressive and further knowledge of the structure of the tissues that form the body, and of their physiological and even pathological action, must be concurrent with our advancement in this subject.

Upon the progressive excellence, therefore, of the Microscope as an instrument of precision on the one hand, and upon the increasing delicacy and skill with which we are enabled to prepare tissues for examination and progressive research with that instrument on the other hand, must depend our advancement.

Whatever leads to more perfect optical construction is an essentially good thing; and what is sometimes cavalierly treated as "amateur microscopy" has contributed largely to optical advancement.

It is after all "the battle of the lenses" that has led up to, and called into existence, the splendid lenses of to-day. But this was, for all practical purposes, a battle fought by amateurs and opticians. Our schools of biology in England, the Continent, and America took little, if any, part in it. Yet without question it is the students in our great biological schools who are deriving the largest benefits from the splendid improvements in quality, price, and modes of using recent Microscope objectives.

By apochromatism the study of ultimate cell-structure and cell change,

* Journ. Quek. Micr. Club, iv. (1891) pp. 304–14.

normally and pathologically, has been made a splendid possibility. The "laying" of optical "ghosts," the elision of complicated and confusing foci, by beautiful optical construction, is of incalculable value. It gives certainty and precision to all work done.

But we must be careful now not to reintroduce the ghostly element by false interpretation. I am increasingly convinced of the possible danger of employing shafts of oblique light only in one azimuth. The peril of misinterpretation is enormous.

Indeed, I have a growing conviction that all small cones of illumination may be fraught with danger, at least to the amateur.

Our German fellow-workers have only lately risen to the perception that the condenser is of value at all, but the condenser they universally employ is chromatic. Its aberration is enormous. True, their greatest microscopical optician has within the last three years seen the value of achromatism, and has made an achromatic condenser; and its value, as compared with that of the chromatic combination, is inestimable.

But surely if we are to get the purest results from apochromatized lenses, if we are to get a focal image absolutely freed from ghostly confusion, we should have an apochromatized condenser, and a condenser of the greatest possible numerical aperture.

In England those who have made microscopy a special pursuit, have long worked with fine achromatic condensers. I am glad now to know that the first apochromatic condenser yet made has been produced by the firm of Powell and Lealand, and it only needs an hour's trial in expert hands, and experienced judgment, to discover its great superiority. It has an aplanatic focus of ·9, and even if oblique beams only in one azimuth be used, their danger is reduced to its lowest. But it is by the employment of large cones of illumination, and not with small ones, that I say cautiously, but still with emphasis, the finest and truest results are to be obtained.

We may well pause before we finally pronounce on this subject, but it certainly is one that must be settled in practice, however present theory may point; and we must all feel that the remarkable paper of my friend Mr. Nelson on this subject, read to us during the past year, must be gravely considered, and made a starting point for patient research. For it is by such means that an amateur club like ours may contribute what is of permanent value to the professors and students who use the Microscope so largely in our schools of biology and medicine.

But, nevertheless, it is possible to push one phase of optical construction so far as to accomplish the object, but to leave doubtful the usefulness of the object gained.

We have all heard of the new objective produced by the firm of Zeiss, of Jena. It has a numerical aperture of 1·60. This from one point of view, is a great advantage. None would have greater reason to hail it than I, in the special work with which my life has been largely occupied.

Now, I have spent five consecutive days in the close and critical examination of one of these objectives, which, so far as I know, has been in no other hands but my own and those to whom I have shown it. I desire to take the sole responsibility of estimating its value. In my hands it is an extremely beautiful lens; it is well centered, well corrected,

and shows plainly the advantage of its enormous aperture. It is a triumph of the optical firm which produced it.

But I would hasten to say (1) That I would not trust a single result produced by its means, when oblique light in one azimuth is employed, especially with the chromatic flint condenser provided by the firm of Zeiss for its illumination. It is fatal to its truth. We can absolutely get almost any desired result with it. It is a very optical witch of Endor for calling up ghosts and ghostly visions.

(2) I did not use with it the condenser provided for its illumination. This has a dense flint front lens, and an enormous amount of aberration. It breaks the delicate balance of the beautiful objective, and is to it, in critical hands, worse than the chromatic Abbe condenser used upon fine apochromatic objectives of lower aperture; and naturally

(3) I could only successfully employ the fine achromatic condenser of Powell and Lealand with the great numerical aperture of 1·4. This, of course, could not utilize all the immense aperture of the lens, but when its full cone was employed with its relatively great aplanatic aperture of 1·1, it yielded results that to a student of delicate diatomaceous images was a vision of beauty indeed.

And it could do more than this with an apochromatic or even an achromatic condenser of its own aperture.

But now comes the pragmatic question, which we are bound to ask, "What does this objective contribute to the practical work to which, for the attainment of the highest results, the Microscope must be applied?"

I say at once for the amateur and the lover of splendid images the objective may be a delight.

But I have pointed out before that even immersion objectives, though they have a great, have nevertheless a very limited, use in strict biological inquiries of a certain kind.

This is true of water; it is doubly true of oil. If we are examining minute life under a limited cover, the fluid above, between the lens and the top of the cover-glass, will ultimately, in following the travels of the living creature, be caused to mingle with the fluid between the cover and the slip, and so destroy the work.

But in spite of this, immersion and especially homogeneous objectives have an enormous value for experiments in control and comparison.

But with the new lens of this great aperture, not only have we to use flint covers, specially and expensively ground, and flint slips, but of course we have to employ a dense mounting medium absolutely fatal to all organic tissues.

Flinty and carbonaceous animal and vegetable products, however fine, may be examined by its means; but the cell as such, to say nothing of the living cell and unicellular organisms, can never at present be subject to its optical analysis.

Now it must not be supposed that this fact was not fully known to its accomplished makers when they devised and sent it out; that would be an error. But in our inquiry as to the influence it will exert upon the special work of the Microscope in unravelling the structure and deportment of animal and human tissues it is a great factor.

In spite of the splendid result attained by it, as biologists we gain nothing. We are where we were, and studies of cells and cell life must be made with dry and immersion apochromatics of N.A. 1·4, or at most 1·5.

With this fact before us, it will be well for us to remember what we are searching for as experts, in tracking the life and behaviour of various cells, and founding, or endeavouring to found, a comparative morphology and physiology of cells and unicellular bodies.

The sphere of all research is strictly physical. Existence, not the cause of existence, succession, not the cause of succession, is our object. There can be, perhaps, but little doubt that life is a cause of phenomena, not a phenomenon in itself. As such it is impalpable to the scientific method. It cannot be the subject of experiment nor the object of a demonstration.

Certainly, in tracking the essential activities of life home to the individual cell amidst its class, or group of cells, or to the unicellular organism, we are coming into closer quarters with the mysterious cause of the phenomena of vitality. But its nature eludes us as much as before. We are, of course, no nearer to the solution of the problem of what life—the cause of all the phenomena of living things—is, than we were before.

We track its phenomena to almost their last scene of phenomenal action, but it is still only phenomena we are studying.

I do not assume that life is not a physical cause. We have no justification for doing so. But if we would go further back than the finally accessible phenomena of cell morphology and cell function it would appear that we must penetrate the mystery of atomic properties as they are found in living things, for it is, so far as our present knowledge can carry us, to the unaccountable combination of thoroughly known chemical elements that life and its properties are due. The at present unanswerable question is how not-living substances, such as C, H, O, N, with whose properties we are so familiar, should so combine, as in their combination to acquire the properties of life.

But while our inquiry will strictly confine us to phenomena, the study of the phenomena of minute cellular structure and minute unicellular organisms is essentially the highest, and, in some senses, the newest line of inquiry open to patient and enlightened study.

Its promise is enormous. But I would urge the necessity for the study of the living cell; the dead cell, dried and stained, is a poor representative of the living cell both in form and internal appearance.

The unicellular organisms, as the simplest types of cell, deserve the closest and most untiring research before broad inferences are made upon the nature and behaviour of grouped cells in tissues.

As there can be no abstract protoplasm—no protoplasm not belonging to a specific organism—and not therefore presenting itself to us as protoplasm with its own specific history and inherited qualities, so there cannot be an abstract cell. That can only exist in the imagination of the theorist. Every cell we meet with in biological realities is not only a cell, but a cell complicated with its own peculiar history and inheritance, and therefore those cells with the least complicated history should command our earliest and most thorough study; and from these we may safely advance to the more and the most complex.

Some of the cellular elements of the tissues have been noted with even simple Microscopes for over two hundred years, Dr. Hooke, in the year 1665, being among the first observers. The nucleus itself may be said to have been seen and described over one hundred years ago. It is still, however, true that the first great step leading to actual scientific advancement in this subject was made in 1831 by the distinguished botanist Robert Brown. He gave us definite knowledge of vegetable cells, and he demonstrated that the nucleus was a normal element of the cell.

What was called the nucleolus was discovered by Valentin five years later.

But even before Brown, Turpin had affirmed the physiological significance of the cell, attributing to cells distinct individualities, and affirming generally that plants were formed by their agglomeration.

But, as is now well known, the cell theory proper was founded by Schleiden, but by him it was restricted to plants. He defines the vegetable cell as "the elementary organ which constitutes the sole essential form-element of all plants, and without which a plant cannot exist; and as consisting, when fully developed, of a cell-wall composed of cellulose, lined with a semi-fluid nitrogenous coating."

To him, therefore, the cell presented itself as a vesicle with semi-fluid contents. This was in 1838. In the following year Schwann extended the cell to the animal kingdom, but to the two elements of Schleiden he added a third, that is to say, the nucleus, which he deemed essential to the existence of the cell in some period of its history. And on his authority these triple elements of the cell were universally believed to exist.

In proportion, however, as the cell theory was more and more extensively seen to characterize the animal world, it was found increasingly difficult to maintain the threefold constituents of the cell.

The conception that the cell was a "vesicle closed by a solid membrane containing a liquid in which floats a nucleus containing a nucleolus" rapidly gave way before investigation. In 1841 it was shown that cells multiplied by budding, and that the nucleus underwent fission when the cell divided; and it was contended by Goodsir that no cell could arise save from a parent cell, which was seen by Virchow to have direct application to pathology.

But it was Naegeli, a botanist, who showed first the unimportance of the cell-wall, and he was supported by Alexander Braun. But it was scarcely a universally accepted belief until Leydig, in 1857, decidedly declared it unessential, and defined a cell as "a soft substance inclosing a nucleus."

After this it was shown by Max Schultze that a cellular life might be complete even without a nucleus, as in *Amœba porrecta;* thus we come back to the cell as the ultimate morphological unit in which there is any manifestation of life.

Thus, then, by the cell theory in this form we discover that "every animal presents itself as a sum of vital unities, any one of which manifests all the characteristics of life."

I must not linger even for a recapitulation of the earlier views held regarding the living matter which constituted the cell; enough here that it was held to be a matter with an endowment of its own, possessing

properties which were *sui generis*, that is to say, that not-living matter could not, by any process we are acquainted with, take on the unique properties of matter which lived. Only from the living could the living arise. This matter was called *protoplasm*, and a quarter of a century ago was defined as "a diaphanous semi-liquid viscous mass, extensible, but not elastic, homogeneous, that is to say, without structure, without visible organization, having in it numerous granules, and endowed with irritability and contractility."

A minute particle of this, either nucleated or non-nucleated, was considered a cell.

But undifferentiated protoplasm did not long universally hold the field. It was gradually shown that a distinct structure was discoverable in some cells, and subsequently it was shown that nearly all cells and all forms of protoplasm show a microscopic network of fine fibres. In short, it became plain that the reputed structurelessness of the cell was due to the inefficiency of the lenses used, and was dissipated when competent optical aids were employed.

Since this time great progress has been made, and modern objectives, finely corrected and of great optical precision, have been very widely used, and it has been shown that the nucleus, instead of being the simple body it was at one time believed to be, proves itself to be of great complexity; and, as I believe, within it are initiated all the great changes which the cell as a whole undergoes.

This could be shown in various ways, but I have been able to demonstrate it in regard to the lowliest and least of all the organisms fairly accessible to us.

The histories of the Saprophytes of this country I have been working at for over twenty years. It is only within the last six or seven that I have been able to deal with the nucleus as an optical entity to be investigated by itself.

In my earlier work we were obliged to study the organism as a whole. Our best objectives failed us when we ventured to study the nucleus. So we were obliged to treat the nucleus as participating in or sharing the life processes of the cell. It was, in fact, to us then a mere passive instrument.

But homogeneous and apochromatic lenses have changed all that. With the objectives I can now employ I am able to deal as definitely with the nuclei of such saprophytes as possess them as I was twelve years ago with the whole organism. Yet the amplification is not greater, nor so great; but that secret of all successful microscopic investigation, a numerical aperture suited to the amplification used, is at our disposal, and this with the ghosts of injurious spectra taken away.

The result is a discovery that the apparently simple nucleus of the lowliest and the least of known organic forms is complex in a high degree; that it is the spring and fountain of vitality in the cell. All modifications to which the cell is subject in its life cycle originate in it. It is, moreover, at certain periods of development of the cell endowed with striking structure, and this structure grows more or less marked as the unicellular organism enters upon or passes certain cyclic periods of change.

In brief, the nucleus of the simplest of living cells is complex in an

astonishing degree; and, therefore, I would argue that by its careful study, and by the study and comparison of kindred unicellular organisms, we shall find nuclear complexity in its least complex condition, and, therefore, more capable of guiding us amongst the perils of the karyokinetic figures of the cells of tissues with vast biological histories and long biological inheritance.

Nor is this all. They may be studied in their living condition, and, I will add, only with efficiency in that condition. Stains may be to some extent used without destroying the organism, and by patience and a thorough knowledge of the nature and use of objectives and condensers, facts of immense value can be made out.

What we want to discover is, what determines the changes in so lowly and minute a nucleus, and what are the correlations between the changes in the nucleus, and the powerful changes brought about in the minute unicellular organism.

The entire organic cell, with a complete life-history definitely known, if relatively large, may be, say, the one-thousandth of an inch in breadth and thickness respectively. Cubically it occupies the four-thousand-millionth of a cubic inch.

The nucleus may be the one-tenth part of this cubically. Yet within this area all the determinate causes of vital phenomena of the whole unicellular organism are at work; and what is more, they are accessible to our perception through modern instruments—and those, when properly used, are instruments of precision.

So far as my present ability and instruments carry me, when the organism is in a fixed or static condition, whether for a shorter or a longer time, the nucleus is a glossy hyaline body with considerable refractive power and no discoverable structure.

But directly a change is about to ensue the nucleus puts on the first evidence of it. The cyclic change of these unicellular organisms is, that after growth from the germ or egg emitted from a maternal sac, and when maturity is attained, the cells go through rapid and successive fission. Their division into two or more in every case is complex, insomuch that, however complicated the flagella of the organism may be, the division is so effected as to produce for each divided part the flagella possessed by the original undivided form, and so with the nucleus.

Following upon this, after a long series of fissions extending over many hours, the final links unite with other ordinary forms, the protoplasm of each melting into the other and producing a sac in which the genetic seed arises, from which a new generation grows.

Now the added point of great moment is that I can now—previous to the first fission in a new generation—discover the initiation of this act in the nucleus. In fact a powerful change takes place. The hyaline particle becomes turbid, as I now know, with structure; this structure divides, and this initiates the division of the nucleus. Upon this follows the division of the whole organism.

This takes place in every fission.

But quite another change comes over the nucleus of the last link in a chain of fissions. Instead of becoming semi-opaque with structure, it becomes opaque by what, to our present resources, is a homogeneous milkiness, and greatly enlarges.

Once observed, there is no mistaking the nucleus in the two conditions, and always when in this last condition it seeks and effects union with another, and genetic products ensue.

I cannot but believe that we have here the act of fertilization in its simplest condition, and the act of cell-budding in its most initial state. By their study the complexities of karyokinesis may be, I believe, approached and understood. It is worthy of our best effort; and certainly is worthy of the finest endeavour of the optician and the chemist to provide us with the best possible objectives—not objectives that, though triumphs of science and art, are not adapted to our wants—but objectives that may be applied to this most difficult and most promising labour by meeting our specific and inevitable wants.

This may not be possible without the chemist's aid. It seems almost certain that mounting media of great refractive indices are indispensable; but to serve the purpose of the student of living cells they must be media applied without heat, and at least tolerant, or for some moments at least not destructive of organic tissues.

Of this I do not despair, and when I see what great mathematical and optical insight and ability have done in the past, combined with perfect lens grinding and mounting, I anticipate a nobler future for microscopic biology and microscopists of the true type."

The late Mr. John Mayall, Jr., Sec. R.M.S.—Our deceased friend, who to so many of us was the type of manly vigour no less than of great mental activity, died on the 27th of July last, from an attack of acute pneumonia; his illness was so short that many learnt of our loss only when the August number of the Journal came into their hands.

Mr. Mayall was not fifty years of age, having been born at Lingard, in Yorkshire, on January the 7th, 1842; he received his early education at the Lycée Bonaparte, where, as we may suppose, he acquired his accurate knowledge of French language and literature: on his return to England he was for a time a student at King's College, London. But, as we all recognize, a man's education depends as much, if not more, on his associates than his schoolmasters; Mayall was a friend of the great French painter Meissonier and the distinguished English mathematician Augustus de Morgan.

His acquaintance with and mastery of the theories of mathematical optics was of great service in the introduction and explanation of the views of Prof. Abbe; he translated Naegeli and Schwendener's treatise on the Microscope, and he delivered two valuable series of Cantor Lectures on his favourite instrument before the Society of Arts. He first became associated with this Society in 1867, and was a member of its Council from 1881 to the time of his death; in 1890 he was elected to succeed Mr. Crisp as one of the Secretaries of the Society. In this last office he was most energetic, undertaking the greater part of the direction of the affairs of the Society, and being a constant visitor to our rooms. He carried through the business of our removal at great trouble to himself, but none to the Society, and, even on his death-bed, he sent communications to his colleague regarding some difficult questions in which the Society's interests were involved.

The Fellows had ample opportunity of observing Mayall's acquaintance with all the details of the manufacture and manipulation of the

Microscope; he made himself personally acquainted with what was being done at Jena, and he may well be said to have been the link between English and foreign microscopists of all nations. The large collection made by Mr. Crisp was thoroughly well known to him, and he took a warm interest in everything that concerned it.

If his great knowledge of his subject had any drawback, it was one that affected him alone adversely; an inventor of a new instrument never likes to be told that much or all is old; the constructor of a faulty one objects to having his errors swiftly exposed. As Mayall was no respecter of persons, and perfectly lucid in his criticisms, he was, perhaps, a more unpopular man than he really deserved to be. To a rare knowledge he added a rare courage.

The activity of his mind showed itself in his proficiency at games of skill, and particularly of chess, but he was hardly less active of body; not only was he a good fencer, but in these days of cycling it must not be forgotten that he was the first to ride a bicycle from London to Brighton.

The thoroughness with which he put his hand to do his duty or his pleasure was equally evident when he was called upon to serve a friend or do a kindness; others beside the present writer must have been astonished at the time and trouble he would ungrudgingly devote t serve them.

The anonymous manner in which this Journal is conducted makes it impossible for any not "behind the scenes" to know how much its success has been due to his assistance; one who does know may sum it up by saying that the death of Mayall has deprived him of one of the shrewdest counsellors a man may ever hope to meet with in his earthly pilgrimage. The student of microscopy will regret that a work just commenced on the history of the Microscope will now never see the light.

We append a list of Mr. Mayall's papers and inventions:—

C. Naegeli and S. Schwendener, The Microscope in Theory and Practice. Translated from the German. 8vo, London, 1887.

Immersion Objectives and Test Objects. Monthly Micr. Journ., 1869, pp. 90–3.

The Controversy on the Aperture Question. Letters in the Monthly Micr. Journ., 1875, pp. 93–7, 150–1, 214–5, 299–301; 1876, pp. 50–1, 97–100.

Aperture Measurement of Immersion Objectives. Journ. R. Micr. Soc., 1879, pp. 842–3.

Immersion Illuminators. Journ. R. Micr. Soc., 1879, pp. 27–31.

Description of Nobert's Ruling Machine. Journ. Soc. Arts, xxxiii. (1885) pp. 707–15.

Cantor Lectures before the Society of Arts, 1886, 1888, 1889. Published in Journ. Soc. Arts, xxxiv., xxxvi., and xxxvii.

An account of his visit to Jena. Journ. R. Micr. Soc., 1887, pp. 322–5.

Various Papers on Microscopy and Microscopical subjects published in the 'English Mechanic' under his *nom de plume* of F.R.M.S.

He also devised and improved the following, of which notices were published:—

Immersion Stage Illuminator. Journ. R. Micr. Soc., 1879, pp. 837–8.

Spiral Diaphragm for Oblique Illumination. Journ. R. Micr. Soc., 1881, pp. 126–7.

Modified form of Nelson's Lamp. Journ. R. Micr. Soc., 1884, pp. 286–7.

Amplifiers for the Microscope. Journ. R. Micr. Soc., 1884, p. 607.

Stepped Diagonal Rackwork. Journ. R. Micr. Soc., 1885, pp. 958–9.

Mechanical Stage. Journ. R. Micr. Soc., 1885, p. 122.

Jewelled Fine-Adjustment. Journ. R. Micr. Soc., 1890, pp. 508–9.

Carl Wilhelm von Naegeli.*—As the son of a country physician at Kilchberg near Zurich, Naegeli was originally intended for the medical profession, and for this purpose studied at the University of Zurich. His interest in medical matters, however, soon waned, and it was not long before he turned his attention to botany, in the study of which his progress was so rapid, that in 1840 he obtained his doctor's degree at Zurich by a work on the Swiss *Cirsiæ*. After a brief sojourn in Berlin, spent in the study of Hegel's philosophy, Naegeli turned to Jena, where he became associated wtth Schleiden in editing the 'Zeitschrift für Wissenschaftliche Botanik.' In that journal he published his important discovery of the spermatozoids of Ferns as well as of the Rhizocarpeæ, first explained the importance of the apical cell, and showed by examples the astonishing regularity in the growth of the cells of plants. Journeys to Italy and England gave Naegeli opportunities for the study of marine Algæ, which resulted in the appearance in 1847 of his work 'Die neueren Algensysteme und Versuch zur Begründung eines eigenen Systemes der Algen und Florideen,' followed in 1849 by his 'Gattungen einzelliger Algen.'

Naegeli entered upon his academic career, first as Privatdocent and then as Professor at Zurich. From Zurich he soon received a "call" to Giessen, and in 1852 to Freiburg. The three years which he spent in the latter place were devoted to the work which was contained in the physiological researches published later in conjunction with Prof. Cramer: it included the exhaustive work on the starch-granules, and on the theory of intussusception. In 1855 Naegeli returned to Zurich as professor in the then recently opened Swiss Polytechnic School. In the summer of 1857 he received a call to the University of Munich, where his first work was to prepare plans for the Botanical Museum, for which purpose he made journeys to St. Petersburg and Paris.

Amongst those who received instruction from Naegeli at Munich were Schwendener, Leitgeb, Engler, Brefeld, Prantl, Peter, and Dingler. The scientific work which he next produced, including the important researches on the course of the vascular bundles, on the examination of microscopic objects in polarized light, and the classic treatment of the question of the formation of varieties and the laws of hybridization, soon led to his being regarded as the first of living botanists. It was in the winter of 1876–7 that he brought before the Aerzlicher Verein in Munich a series of papers on the lower Fungi and their connection with infectious diseases; in 1879 appeared the 'Theorie der Gärung,' and in 1882 the 'Untersuchungen über niedere Pilze.' Naegeli's contributions to bacteriology met with great opposi-

* Chiefly from a notice in the Münchener Med. Wochenschrift, by H. B.

tion, and it is true that later researches have shown that many of his theories are untenable; but the correct ideas which he was the first to enunciate have since borne fruit. For instance, he was the first to clearly explain the grounds for supposing that infectious matter could not be gaseous.

In 1884, in spite of failing health, he succeeded in completing the great work of his life on the doctrine of descent, the 'Mechanisch-physiologische Theorie der Abstammungslehre,' which will ever remain as a monument to his powers as a scientific thinker. Naegeli's principles differed widely from those of Darwin. Natural selection he was only able to recognize as a means for the removal of unsuitable forms. The production of new forms he ascribed to the principle of progression existing in the organism. To the microscopist our deceased Honorary Fellow was best known by the work on the Microscope which he published in conjunction with Prof. Schwendener, and which has passed through three editions in Germany and was translated into English. In the winter of 1889–90 Naegeli was prostrated by an attack of influenza, but recovered so far as to be able to go to the Riviera in the following winter. He died somewhat suddenly in May last.

List of all Patents for Improving the Microscope issued in the United States from 1853 to 1890.*—The following list is of interest:—

1853. H. De Riomonde: Otoscope. No. 9581.
1861. R. P. Dagron: Photo charm. No. 33,031.
1862. H. Craig: Charm. No. 34,409.
1864. J. Ellis: Seed Microscope. No. 42,843.
1865. Wales: Plain movable front to lens. No. 46,511.
1865. J. J. Bausch: No. 47,382.
1865. C. B. Richards: Friction wheels on rack motion. No. 47,860.
1866. H. L. Smith: Side reflector above objective. No. 52,901.
1866. Heath: Combined Microscope, telescope, and eye-glass. No. 54,542.
1866. R. B. Tolles: Binocular eye-piece. No. 56,125.
1866. O. N. Chase: Seed glass. No. 56,178.
1869. J. H. Logan: Dissecting Microscope. No. 93,895.
1874. J. J. Bausch: Botanical Microscope. No. 151,746.
1876. Wales' pillar fine-adjustment. No. 178,391.
1876. J. Zentmayer: Fine-adjustment carrying rack, swinging substage. No. 181,120.
1876. Gundlach: Fine-adjustment. No. 182,919.
1877. Gundlach: Glass stage, sliding carrier. No. 198,607.
1878. R. B. Tolles: Sector illuminator. No. 198,782.
1878. R. B. Tolles: Swinging illumination tube. No. 198,783.
1878. J. J. Bausch: Convex base to stand. No. 199,015.
1879. Gundlach: Pillar tube. No. 211,507.
1879. Gundlach: Eye-piece of field lens and triplet. No. 212,132.
1879. H. G. Deal: Cloth-counter for bolting cloth. No. 214,283.
1879. W. H. Bulloch: Swinging substage loose from mirror. No. 215,878.

* Amer. Mon. Micr. Journ., xi. (1890) pp. 280–1.

1879. Gundlach: Triplets as one element of lens combination. No. 222,132.
1880. W. H. Bulloch: Scroll turntable. No. 226,648.
1880. Molera and Cobrian: Binocular. No. 230,320.
1880. E. Bausch: Folding Microscope. No. 230,688.
1880. J. W. Sidlo: Cog-wheel turntable. No. 235,030.
1882. Lomb and Bausch: Trichinoscope. No. 251,721.
1882. P. H. Yawman: Differential screw fine-adjustment. No. 262,634.
1883. Foster: Socket. No. 270,296.
1883. W. J. M'Causland: Magnifier for telegraph. No. 270,907.
1883. F. B. Gould: Microphotographs. No. 271,838.
1883. L. M'Intosh: Pin arm. No. 273,752.
1883. E. Bausch: Electric light and Microscope. No. 277,869.
1883. W. H. Bulloch: Bayonet-catch nose-piece. No. 287,904.
1883. D. Tetlow: Bottle seed Microscope. No. 287,978.
1884. E. Bausch: Swinging Wenham prism. No. 293,217.
1884. W. K. Kidder: Electric spark device for Microscope. No. 295,770.
1885. E. Bausch: Microtome. No. 325,722.
1885. E. Bausch: Sheet-metal flanges to tubes. No. 328,277.
1886. G. Fasoldt: Spring nose-piece. No. 334,009.
1886. G. Klippert: Turntable. No. 334,530.
1886. G. W. Palmer: Bevelled slides. No. 336,257.
1886. B. F. Allen: Stand. No. 352,639.
1886. E. H. Griffith: Turntable. No. 354,130.
1889. S. Frost: Botanical Microscope. No. 407,192.

Newspaper Science.*—"One of the latest specimens is furnished by the *Globe-Democrat*, of this city, which a few Sundays ago printed the following:—

'Charles X. Dalton, instrument-maker, says R. B. Tolles, of Boston, now dead, was the greatest maker of Microscope lenses the world has ever seen. He once made an object-glass that magnified 7500 times. It was the first and only one ever constructed, and was made as the result of a long controversy with other microscopists in regard to the possibility of resolving what was known as Nobert's nineteenth band. Nobert was a Frenchman, who, by mechanical appliances, ruled on glass parallel lines at the rate of about 100,000 to the inch. No Microscope lens then made was sufficiently powerful to count these lines. Mr. Tolles, as a result of statements made during the controversy, started to make an objective that should magnify 7500 times. This he succeeded in doing somewhere about 1874. This objective was 1/75 in. in diameter, and is about as large as the hole made in a sheet of paper by the point of a very fine needle. This lens was afterwards sold to Major Woodward, in the Government employ at Washington, but his bill was not allowed by the auditor, and the lens was taken off his hands by one Dr. Harriman. In turn he sold it to Dr. Ephraim Cutter, in whose possession it now is. Objectives that magnify 5000 times are rare, and it is a powerful Microscope that magnifies even 2500 times. These

* National Druggist (St. Louis), xix. (1891) p. 25.

are necessary in bacteriological research, and in testing blood-corpuscles to determine, for instance, whether they are of human blood or not. A local paper recently told of a Boston physician who examined the tubercle bacillus with a powerful glass that magnified 900 times. Ridiculous! You can't see the consumption bacillus with an objective that magnifies less than 1200 times. England is the great rival of this country in Microscope-making. France and Germany are behind. I suppose that sometime an objective will be made that will magnify 10,000 times, but it will be a much more difficult task than the making of a telescope glass five feet in diameter.'

"While no one will deny that Robert B. Tolles was one of the greatest lens-makers that the world ever saw, there are a great many who would hesitate to place him above his great contemporary and teacher, Charles A. Spencer, of Canastota, N.Y. Neither of them, however, ever 'made an object-glass that magnified 7500 times.' To do this would require the manufacture of an objective with a focal length of 1/750 in., which, it is needless to say, has never yet been attempted. Nobert's 'nineteenth band' contains 112,595 lines to the inch (estimating the Paris line at 0·088·813·783 in.). The Tolles 'seventy-fifth' was not '1/75 in. in diameter,' but the combination had a theoretical focal length of 1/75 in. When used with a 1-in. ocular and with a 10-in. tube-length the combination would give an amplifying power of about 7500.

'Objectives that magnify 5000 times are rare.' We should say so —and likely to remain so, since to make one would require the construction of a combination with a theoretical focal length of 1/500 in. The balance of this sentence shows that Mr. Dalton (or the reporter) confuses the Microscope (here the combination of eye-piece and objective) with the objective alone.

The statement regarding the visibility of bacillus tuberculi is not less misleading than the balance of the farrago. Bacillus tuberculi can easily be seen and recognized with a 1/5-in. objective and a 2-in. ocular, or, roughly, with an amplification of 250. With twice this amplification (i. e. 500) it becomes a very conspicuous object. In fact, the writer rarely uses amplification over 500 in making examinations for tubercle bacilli, his favourite combination being a 2-in. ocular and 1/10-in. objective."

β. Technique.*

(1) Collecting Objects, including Culture Processes.

Preparation of Nutrient Media.†—Dr. N. K. Schultz finds that really good bouillon agar and gelatin can be obtained by attending to several details which in practice are highly important. He recommends that the precipitates formed during the preparation of the medium should be removed separately, because each precipitate has its own special properties. The reaction of the medium should be determined by titration since neutralization cannot be accurately ascertained by

* 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., x. (1891) pp. 52–64.

means of litmus paper. This is quite a simple process, and merely consists in adding a drop of phenolphthalein to 1 ccm. of bouillon, and then dropping in 0·4 per cent. caustic soda solution until a pale rose colour appears. Phenolphthalein is a greyish-yellow powder, and dissolved in alcohol (1 to 300) is almost colourless, but on the addition of an alkali turns dark red. This sensitiveness to alkalies renders it a convenient reagent for measuring the amount of alkalinity of nutrient media.

Bouillon should be neutralized before either agar or gelatin is added. Agar requires to be boiled for quite a long time before it is completely dissolved, while gelatin should only be boiled for a very short time.

Preserving Malaria-Plasmodia alive in Leeches.*—Dr. N. Sacharow finds that leeches (*Hirudo medicinalis*) may be used for keeping alive the plasmodia of malaria. The leeches were frozen in a piece of ice and kept in an ice-cellar for a week, the plasmodia being found at the expiration of this time quite unchanged. Their mobility was even greater than when taken directly from the blood of a patient suffering from malaria, though their form was somewhat altered and their size diminished.

Cultivating Spirillum Obermeieri in Leeches.†—Dr. Th. Pasternacki gives the result of fourteen observations made by means of leeches on *Spirillum Obermeieri*, from which it seems that this micro-organism is very resistant to low temperatures. The leeches were filled with blood from cases of relapsing fever, and a drop of blood was obtained for microscopical examination by placing some salt crystals on their tails: this caused the leech to evacuate a drop of blood on a cover-glass placed ready for the purpose.

Directly after sucking the relapsing fever blood the leeches were exposed for various lengths of time to temperatures varying from 0°–40°, and then if alive, a specimen of the blood was obtained in the manner described.

New Cultivation Medium for Bacteria.‡—Dr. P. Kaufmann states that he has obtained very favourable results from the use of jequirity as a cultivation medium. The solution is prepared in the following manner:—10 grms. of jequirity seeds are pounded in a mortar to remove the husks, and this reduces the weight to about 8 grms. The 8 grms. are then boiled in a steam sterilizer with 100 ccm. water for two hours, and when cold filtered. The fluid thus obtained is of a yellow colour, with a neutral or very slightly alkaline reaction, and after sterilizing in the usual manner, can be used without further addition or treatment as a medium for cultivating bacteria.

From their behaviour to the jequirity solution the bacteria were divisible into three classes:—(1) in which the colour remained unchanged; (2) in which it was discharged; (3) in which a green colour was produced. A further examination showed that the green cultures had an alkaline

* Wracz, 1890, pp. 644–5. See Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) p. 199.

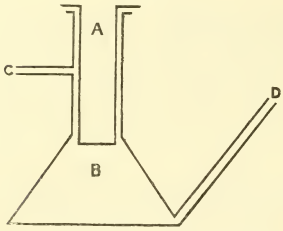
† Wracz, 1890, p. 297. See Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 198–9.

‡ Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 65–9.

reaction, and those in which the colour was discharged an acid one. This was confirmed by chemical experiment, for by adding an alkali the solution became green, while the addition of acids removed this colour. In this jequirity solution, therefore, there exists a means of distinguishing between bacteria which form acids and those which form alkalies.

The results of the addition of various substances, agar, gelatin, pepton, glycerin, alone or in combination and with neutral or alkaline reaction are exhibited in two tables. The most favourable results seem to have been obtained from the simple solution of jequirity with neutral reaction, and from an alkaline solution to which 2 per cent. of pepton had been added.

Fig. 75.



Reichel's Apparatus for Filtering Fluids containing Bacteria.*—Herr Reichel describes an apparatus which he has devised for filtering fluids, and which is expressly intended for bacteriological work. It consists of a glass vessel somewhat resembling an inverted funnel. The body B is intended for the receiver, while from the bottom projects upwards the tube D, and from the neck the exhaust-tube C. Into the neck fits the porcelain filter A. The tube D is intended for the evacuation of the filtrate or removal of small portions for test purposes. When in use, the air is exhausted by means of an air-pump attached at C, the orifices at A being carefully plugged with cotton-wool.

Organisms of Nitrification and their Cultivation.†—M. Winogradsky, who at one time ascribed the nitrifying faculty to a single species of bacteria called *Nitromonas*, has by later investigations satisfied himself that morphological differences exist in these organisms, and they are now classed together in a group of "Nitrobacteria," the common characteristic of which is the oxidation of the ammoniacal nitrogen. The bacteria were cultivated on the following medium, devised by Kühne,‡ and modified by the author:—Commercial silicate of soda is diluted with thrice its volume of water, and then 100 ccm. is thoroughly mixed with 50 ccm. of dilute hydrochloric acid. The mixture is dialysed for 24 hours in running water, and then for two days in distilled water frequently renewed. The dialysis is completed when the fluid remains quite clear on addition of silver nitrate. The solution may now be sterilized by boiling, and preserved in flasks closed with cotton-wool.

The second solution is composed as follows:—Ammonia sulphate, 0·4; magnesium sulphate, 0·05; potassium phosphate, 0·1; calcium chloride, trace; sodium carbonate, 0·6–0·9; distilled water, 100. The sulphates and chloride are dissolved and sterilized together, as also are

* SB. Phys.-Med. Gesellsch. zu Würzburg, 1891, pp. 44–7 (1 fig.).

† Ann. de l'Inst. Pasteur, 1891, p. 92. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 603–5.

‡ See this Journal, *ante*, p. 130.

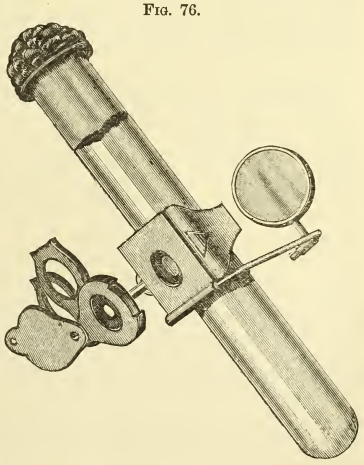
the phosphate and carbonate, and the two solutions mixed after cooling.

The next thing is to evaporate down to about one-half the silica solution in a flask until 2–3 drops set within five minutes when a drop of the salt solution is added. Ten to fifteen minutes suffice to render it firm enough to stand being scratched across. When this degree of concentration is reached the evaporation is suspended and the silica solution is pipetted into glass capsules. It is then set by adding to it one-half or one-third its volume of the salt solution, according to the degree of consistence required. The two constituents must be well mixed, and in a few minutes a slight opalescence will denote that coagulation has set in.

The material to be tested may be inoculated by mixing it with the salt solution or scratching it over the medium when solidified. For sodium carbonate magnesium carbonate may be substituted; although this impairs the transparency, it renders the colonies more evident, since this carbonate is dissolved from round about the colonies. The deep-lying colonies of the nitrobacteria are very small, while the superficial ones form a pretty thick crust along the course of the inoculation track.

Nitrobacteria may be obtained by direct inoculation of the earth, but it is better to set up nitrification in a watery saline solution by means of a bit of earth and then to transfer some of this to the solid medium. In this way are developed colonies consisting almost exclusively of nitro-bacteria, and that they do form nitrate is easily ascertainable by the nitric acid reaction with diphenylamin.

FIG. 76.



A Colony-counter. * — Mr. J. E. Line writes:— "In the study of the comparative biology of water-supplies, sewage, infusions, secretions, &c., it is necessary to fix the organisms in a nutrient medium, cultivate them to a given limit, and make a count. To do this neatly and effectively two pieces of apparatus are requisite—an Esmarch tube and a colony-counter. Glass plates and a linen-prover have been made use of, but for the more accurate results other and better means are called for. The Esmarch tube is simply a test-tube evenly coated internally with a solid sterilized nutrient medium—agar-agar, gelatin, combinations of

* The Microscope, xi. (1891) pp. 179–80.

the two, &c.—and stopped with cotton. The coating is done by pouring into the tube a quantity of medium, tipping and turning the same until no part of the surface remains untouched, except, of course, that in the immediate vicinity of the cotton stopper. When the medium has thus been evenly spread, the tube is immersed to the neck in ice-water, and then stored for future use. Some roll the tubes on ice, but the medium sets and hardens unevenly, in lumps, ridges, &c.—a condition of things likely to vitiate the count. In making a comparative determination a series of tubes are taken, a given quantity of the material under examination put into each one, "swashed" about and the surplus thrown out, or by means of gentle heat (not, however, always advisable) incorporated with the medium. At the end of a given number of hours or days a count is made, the count repeated at intervals, the results recorded, and, if it is desired to experiment further, a cultivation begun.

At this stage of the examination the counter (fig. 76) comes into play. It is simply a small Microscope adapted to tube examinations, and consists of a modification of a brass knife-clamp that grasps the tube, holding it firmly to the under side of the stage, the opening in which contains a cover-glass divided into square millimetres, or, in a more recent and better form, an opening in the stage 1×4 mm., and the greater diameter running lengthwise with the tube. The optical part is an "Excelsior" triplet, the lenses of which can be used separately or in combination; the adjustment is frictional. The substage has universal movements, and may be readily detached if window- or lamp-light is preferred direct. The Bausch and Lomb Optical Company make the instrument."

Filtration and Sterilization of Organic Fluids by means of liquid carbonic acid.*—M. A. d'Arsonval describes a quite simple instrument for the cold-filtering and sterilizing of liquids containing colloid or albuminoid substances. A wrought-iron bottle filled with liquid carbonic acid is connected by means of a narrow tube with a steel or copper cylinder which is to receive the fluid to be filtered. The receiver of course contains a porcelain filter, and this is easily removable for the purpose of cleaning or sterilizing.

In practice the pressure used is about 45 atmospheres, and this is found to be quite as efficacious in many cases as sterilizing by heat.

The effect of this method may be increased by combining a temperature of 40° with the pressure, and, by certain modifications, cultivations may be attenuated or their development retarded.

The author noticed that the richness of the filtrate in colloid substances was in close relation to the pressure, and that in mixtures containing various ferments, for example, pancreatic fluid, the action of the fluids obtained by filtration varied with the different pressures.

D'Arsonval's Apparatus for maintaining a Fixed Temperature.†—M. A. d'Arsonval has invented a new thermostat, the temperature of which is regulated by quite a new device. The apparatus, intended chiefly for embryological and cultivation purposes, consists of a double-walled case, the interior of which is filled with water. At the middle

* Comptes Rendus, cxii. (1891) pp. 667–9 (1 fig.).

† Arch. de Physiol. Norm. et Pathol., ii. (1890) pp. 83–8. See Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 102–4.

of its bottom is a folded metal plate similar to those in aneroid barometers, and under this a sort of box, into which the gas passes through a tube. From this the gas passes by two tubes, one on either side, to a couple of burners. The heat from these burners passes up through the water through two metal tubes or chimneys. When heated, the excess of water passes out through an opening in the middle of the top, and when the desired temperature is attained the aperture is closed with a caoutchouc plug, into which fits a long glass tube open at both ends. Further expansion of the water causes it to ascend in the tube, and also to press on the metal plate, and as the latter descends it presses on the central gas-pipe, and thus stops off the superfluous access of gas. In this way the temperature of the thermostat remains quite constant. An additional power of regulating the supply of gas is obtained by means of a screw fitted to the pipe, by which it is brought nearer to the flexible metal plate.

The author describes other apparatus constructed on a similar principle.

(2) Preparing Objects.

Examination of Embryonic Liver.*—In his study of the liver of the embryos of mammals Dr. O. Van der Stricht examined fresh tissue in serum and in different fluids; teasing was effected in an aqueous solution of 1 per cent. sublimate, 1 per cent. osmic acid, or Flemming's liquid, and the elements stained with a dilute solution of safranin, methyl-green, or gentian-violet. Fixing was effected with an aqueous solution of 2 per cent. sublimate, either pure or with the addition of a little chloride of sodium, with Flemming's liquid, either pure or with an equal part of water, or with Hermann's liquid; of these Flemming's was found to be the best. The best colouring agents were safranin, gentian-violet, or Ehrlich's violet. The finest preparations were obtained by using safranin and gentian-violet simultaneously. Imbedding in celloidin was found to be preferable to the use of paraffin.

Preparation of Wing-muscles of Insects.†—Prof. E. A. Schäfer cuts open a suitable insect and places it in alcohol of about 90 per cent. for twenty-four hours or more; it is afterwards transferred into glycerin, when the sarcostyles of the wing-muscles can be isolated and examined without difficulty. When stained, as with hæmatoxylin, the dark bands take the staining most intensely, but the various parts of the sarcostyle differ in their behaviour to staining reagents. A very valuable method is to apply the gold-formic method to the tissue when taken from the glycerin. If fresh muscle be so treated the sarcoplasm alone is stained, but if the alcohol-glycerin muscle be taken, the reduction of the metal takes place in the sarcostyles and almost exclusively in their dark bands. By these means there may be brought out, with a clearness which renders the application of the photographic method comparatively easy, points of structure which, with our present usual methods of investigation, have remained obscure.

* Arch. de Biol., xi. (1891) pp. 41–2.
† Proc. Roy. Soc. Lond., xlix. (1891) pp. 280–1.

Preparation of Nervous System of Hirudinea.*—Dr. E. Rohde investigated the nervous system of *Aulostomum* by means of teased preparations as well as of sections. The latter were generally prepared after hardening in sublimate; the living animals were forcibly extended and fixed in a small vessel containing wax; an opening was made along the dorsal middle line; the worms were covered with a one to two per cent. solution of sublimate and left for several hours. After this was removed they were gradually put into strong alcohol. Only after they had been for a day in 80 per cent. of alcohol was the nervous system taken out, stained, and imbedded in paraffin. The author strongly recommends this method. Of *Pontobdella* serial sections only were made. Mayer's alcoholic carmine solution is highly praised as a staining reagent, but Golgi's method is found to be useless for Invertebrata. The sections were generally 1/200 mm. thick, but for the recognition of the finest structural relations much more delicate sections were necessary. The sections were always put in glycerin; resinous media are to be avoided as they make the preparations too transparent for very fine work.

Mayer's picric glycerin mixture was found to be of no use, but salt solution was useful.

Mode of Investigating Sipunculus nudus.†—Mr. H. B. Ward attempted to kill his specimens in such a way as to prevent distortion and to preserve well the tissues; the thick impermeable cuticle and the wealth of muscular tissue made the operation one of some difficulty.

Specimens were allowed to remain for some time in clear sea-water so as to get rid of adhering sand; they were then brought into a shallow dish of sea-water, and 5 per cent. alcohol was allowed to flow gently over the surface; the spirit must be allowed to disseminate gradually. Narcosis varies with individuals, but supervenes in from four to eight hours. When the animals make no contractions on being gently probed with a dull instrument they may be regarded as sufficiently stupefied, and be transferred to 50 per cent. alcohol. After a short stay in this the introvert was cut off, and alone subjected to stronger alcohol. Material thus preserved may be well stained by all methods.

Development of Hydra.‡—Dr. A. Brauer, in his study of the development of *Hydra*, preserved the shell-less eggs chiefly in Flemming's solution, and those that retained their shells by treatment with hot corrosive sublimate. The yolk-granules were distinguished from the nuclei by double-staining with borax-carmine and malachite-green; and, later, as the nuclear stain was found to be too faint, shell-bearing eggs were alone so treated, and the others were put for twelve hours in Grenacher's hæmatoxylin and washed with acid alcohol. Paraffin was used as the imbedding material; for sections the older shelled ova alone gave difficulty; for these Heider's mastic-solution was used.

Study of Karyokinesis in Paramœcium.§—In the study of *Paramœcium*, Prof. R. Hertwig made use of picro-acetic acid, chromic

* Zool. Beiträge, iii. (1891) pp. 1–3 and 49–51.
† Bull. Mus. Comp. Zool., xxi. (1891) pp. 141–5.
‡ Zeitschr. f. Wiss. Zool., lii. (1891) p. 170.
§ Abhl. d. K. Bayer. Akad. d. Wiss., ii. Cl., xvii. Bd., i. Abth. (1889) pp. 4–5. See Amer. Nat., xxx. (1891) p. 87.

acid, and chrom-osmic acid, as hardening reagents. Picro-acetic acid followed by borax-carmine was the principal method. The staining process was aided by the heat of an incubator, and decoloration was effected by alcohol acidulated with hydrochloric acid. The preparation was mounted in glycerin or in clove-oil. Clove-oil is preferable to balsam, as it reveals more clearly the fibrous structure of the spindle, and allows of turning and pressing of the object at any time.

Clove-oil causes the cytoplasm to become brittle, so that the body of the infusorian may be broken up by pressure or blows on the cover-glass, and thus the nuclear spindles be set completely free. In this isolated condition they can be studied to the best advantage, as they are not obscured by overlying cytoplasm. For the study of the chromatic figures clove-oil is too strong a clarifying medium. Glycerin or water will serve better. Hertwig examined the preparation first in clove-oil, then isolated the nuclear figures, washed in alcohol, and mounted in glycerin. He was thus able to study all parts and figures under most favourable conditions.

Method of Narcotizing Hydroids, Actiniæ, &c.*—Mr. H. B. Ward writes:—"In order to kill Hydroids, Actiniæ, and similar forms in an expanded condition, a little expedient may be recommended which the writer has tried in many places and on many forms, and has uniformly found of value. The animals to be killed are left in a small quantity of the salt water in which they were brought in, until this becomes rather warm and stale, or until, in fact, they are weakened by the narcotizing effect of impure water. This manifests itself in one or two ways; some forms draw themselves completely together, while others hang half expanded and limp in the water. They are then transferred in colonies or in large groups into [a] fresh [quantity of] salt water, which is at the same time cool. The effect of a mass of cool, pure water is such as to cause the animals to expand fully and promptly. Immediately as the expansion is seen to reach its maximum, in the course usually of a few seconds, they are transferred by a quick motion into some rapid-killing reagent. After the long narcosis in poor water the polyps appear to lack energy to contract forcibly, as is usually the case. As killing reagents, alcoholic corrosive sublimate and picro-nitric acid have given the most uniformly good results. In this way the most susceptible Actiniæ may be easily preserved expanded and intact, and hydroids of all genera yield good specimens. The transfer to fresh sea-water is the only point requiring care. No time limit can be given, as the factors are too variable, but a little practice is sure to show the character and advantages of the method."

Method for Demonstrating the Formation of Acids by Micro-organisms.†—Herr M. W. Beyerinck describes a method for showing the acidity or alkalinity of the products of micro-organisms.

It consists in mixing a suitable medium, and one which will set well with very fine whiting, and then pouring the mixture into a glass capsule. The nutrient layer thus made is opaque and milky white. As coagulation media, gelatin, agar, or silicate may be employed. To

* Amer. Nat., xxv. (1891) pp. 398–9.

† Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 781–6 (1 fig.).

exemplify his method, the author gives in detail the procedure for demonstrating the presence of lactic acid bacteria and for isolating them from fermenting maize. 20 grms. gelatin (or 3/4 grm. agar) are dissolved in yeast-water made by boiling 8 grms. yeast in 100 ccm. tap water. 5–10 grms. glucose are then added, and the mixture having been boiled again, it is filtered, and a few drops of whiting and water added. It is then poured into glass capsules, so that the layer at the bottom is about 1 mm. thick.

The micro-organisms are obtained by shaking a drop of fermenting maize up in a flask of boiled water and then pouring the infected water over the chalked medium. The water is then poured off, but sufficient adheres to inoculate the medium.

As the colonies develope their immediate vicinity clears up, owing to the acid produced by the micro-organisms, and these transparent areas are visible even to the naked eye.

In addition to whiting, the medium may be mixed with carbonates of magnesium, barium, strontium, manganese, zinc. The mixture of zinc carbonate appears to be very suitable for lactic acid bacteria.

Besides indicating the production of acid, this method may be used for demonstrating the formation of alkalies. In the illustration given by the author of his apparatus, this production of alkali by an organism is shown by its power of neutralizing the acidity resulting from an acid-forming bacterium in an adjacent colony.

Demonstration of Suppuration-Cocci in the Blood as an aid to Diagnosis.*—Baron A. von Eiselsberg describes four cases in which the original diagnosis was confirmed by a bacteriological examination of the blood. In all four cases suppuration cocci were cultivated from the blood (*Streptococcus pyogenes*, *Staphylococcus pyogenes albus*, and twice *Staphylococcus pyogenes aureus*).

Examination of the blood in five cases of laparotomy where the symptoms soon after the operation were unsatisfactory, failed to show micro-organisms—a result confirmed by the subsequent satisfactory issue of all the cases. In three cases of phlegmon, one of acute osteomyelitis, and four of septic peritonitis, the cocci could only be demonstrated in three instances—a result which is explained by supposing that in certain cases of sepsis the phenomena are due to the absorption of certain chemical matters from the original inflammatory focus. Moreover, it must be remembered that as the cocci are only sparsely present in circulating blood, catching a visible germ in any given drop of blood is not a matter of certainty.

At any rate the author's recommendation that the bacteriological examination of blood should be undertaken as a supplementary aid to diagnosis is a good one, for while negative results only leave the matter in the *status quo ante*, a positive result is extremely valuable.

On a Method of Preparing Vegetable and Animal Tissues for Paraffin Imbedding, with a few Remarks as to Mounting Sections.†—Mr. Gustav Mann writes:—"*Requisites*—I. Picro-corrosive alcohol.

* Wiener Klin. Wochenschr., 1890, p. 731. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) p. 834.

† Trans. and Proc. Bot. Soc. Edinb., xviii. (1890) pp. 432–5.

Heat absolute alcohol to 50° C., saturate with picric acid, and then add bichloride of mercury to saturation. When cool decant. This solution may be made in quantity and kept. II. Absolute alcohol. III. Chloroform-alcohol—chloroform and absolute alcohol mixed in equal parts. IV. Chloroform. V. Solid paraffin, melting-point 46°–50° C. VI. Short wide-mouthed bottles. VII. Best cork stoppers, two for each bottle; the one fitted with a piece of glass tubing 1 cm. in diameter and 3 cm. long. VIII. Number of glass rods drawn out into fine points, as one must avoid bringing metal instruments in contact with the picro-corrosive fluid.

Method—*A.* The fixing and hardening of tissues.—Place tissue in at least fifty times its bulk of the picro-corrosive alcohol. Leave small objects (up to 1 cubic cm.) for twenty-four hours, larger objects for forty-eight hours and upwards in the fluid. Keep the bottle well corked.

B. The replacement of the picro-corrosive alcohol by pure absolute alcohol.—1. Pour off the hardening fluid till the tissue is just covered. Add absolute alcohol according to the size of the tissue in 1–10 drops every ten minutes, till the tissue is again in fifty times its bulk of fluid. After each addition move the bottle very gently to allow the added alcohol to mix with the hardening fluid. Leave tissue in this diluted mixture for twenty-four hours. In no case should this process be hurried, or strong diffusion currents will be set up, and the protoplasmic contents of the cell separate from the cell-wall. 2. Pour off the fluid till the tissue is just covered, and add absolute alcohol up to the original bulk. Move about the bottle gently every three or four hours. Most of the picro-corrosive material will thus be extracted after twenty-four hours. 3. Draw the fluid rapidly off by means of a pipette, and add absolute alcohol up to half of the original bulk. Any drying of the tissue must be carefully guarded against. Leave for twenty-four hours, and repeat the process.

C. The replacement of the alcohol by chloroform.—1. Pass, by means of a pipette, the chloroform-alcohol mixture to the bottom of the vessel, when the tissue will float on the mixture. Remove then the superfluous alcohol by a pipette, leaving only enough to cover the tissue. 2. When the tissue has sunk in the chloroform-alcohol mixture, introduce by a pipette pure chloroform, on which the tissue will float; the fluid above the tissue is removed by a pipette. After twenty-four hours the tissue may or may not have sunk in the chloroform; if not, it may be induced to do so by heating the chloroform to 20° C. (not higher); if this fail, a little sulphuric ether may be added. After the tissue has sunk, leave for twenty-four hours. 3. Place a fresh supply of chloroform at the bottom of the vessel (50 times the bulk of the tissue), and if there is a distinct line of demarcation between the newly-added and the old chloroform, the upper layer should be removed by a pipette.

D. The replacement of chloroform by paraffin.—1. Place the tissue in a warm chamber heated to 25° C.; add solid paraffin in pieces up to the size of a small pea. After each piece has dissolved, the bottle has to be moved about very gently to hasten the mixing of the paraffin, which will be in the upper layers, with the chloroform. Continue till no more paraffin dissolves. Tissue which did not sink in pure chloro-

form will always sink as soon as paraffin is added. 2. Place the tissue in a warm chamber heated to 30° C. for twenty-four hours. 3. Place the tissue in a warm chamber heated to the melting-point of the paraffin (46° C.), and after six hours replace the ordinary cork stopper (which up to this stage has always to be employed) by a perforated one. This method is adopted to ensure a gradual giving off of the chloroform, for I find that, if the latter be driven off rapidly, a good deal of shrinkage always results. When all the chloroform has evaporated, i. e. if after shaking the bottle gently one is unable to detect by smelling the faintest trace of chloroform, then the tissue is ready for sectioning. If the bottle be not shaken gently before smelling the solution, it is often impossible to detect chloroform, although a large quantity of the latter is still in the lower layers of the paraffin, as the upper layers part more readily with the chloroform. 4. The tissues should not be exposed longer than just necessary to the temperature of melted paraffin, but should be imbedded by means of Leuckart's type-metal box, or by two L-shaped pieces of metal running in an oblong box, the breadth of which corresponds to the short limb of the L. The metal boxes should be warmed and filled with melted paraffin. After five to twenty seconds, when the paraffin at the bottom of the box has solidified, the tissue is removed from the bottle by a copper lifter, and, without being allowed to cool, it is dropped into the imbedding box, put into any desired position by means of hot needles, and the paraffin cooled very gradually. It is best not to touch the tissue with any instrument till it is ready to be placed in the imbedding box, and also to avoid heating the copper lifter or the needles too much. Tissues thus imbedded may be kept unchanged for any length of time.

To get perfectly satisfactory results, the tissue we are treating must be living; smaller vegetable objects, as flower-buds, ovaries, growing apices, &c., must be dropped into the fluid as soon as separated from the plant, and animals like tadpoles, worms, and larvæ are placed directly into the fluid, where they are killed rapidly and in an extended position. Tissues of plants and animals must be placed in the fluid as soon as separated by dissection. Tissues of warm-blooded animals should be placed in the picro-corrosive alcohol of corresponding warmth. Treating tissues like brain, it is best to place into the bottom of the vessel a pad of cotton-wool or felt to allow the hardening fluid to penetrate readily; the pad must be removed before the chloroform-alcohol is placed below the tissue. My method was found to give very satisfactory results with plasmodia of myxomycetes, growing apices, developing endosperm, stem and leaf structures, human fœtal brain, frog's cartilage, muscle, myxomatous tissue, retina, tadpoles, wasp larvæ, caterpillars, &c. Karyokinetic figures are specially well fixed, and show the minutest details.

Now a few words as to mounting sections. Sections cut in ribbons (I use the Cambridge rocking microtome) are fixed to a slide by Schällibaum's method, thus:—An even layer of the fixing material is spread on the slide, the slide heated to 30° C. (melting-point of paraffin = 46° C.), and a piece of the ribbon gripped by a pair of forceps at one end and quickly laid down on the warm slide. In this way I get the sections to lie perfectly flat, and it is even possible to make a closely coiled-up

ribbon expand with the greatest ease, without causing any further trouble. The slide is next heated above a Bunsen, just enough to melt the paraffin; it is then placed in a vessel containing resinified turpentine, which latter removes the paraffin in a few minutes; the turpentine is removed by absolute alcohol, and the sections stained by any of the current methods, then dehydrated in absolute alcohol, cleared in resinified turpentine, and, lastly, mounted in Canada balsam dissolved in turpentine, as turpentine-balsam has a low refractive index."

(3) Cutting, including Imbedding and Microtomes.

Sharpening Ribbon-Microtome Knives.*—M. J. W. Moll says that the ribbon-microtome is far superior to the sliding microtome, provided that the knife be properly sharpened.

After alluding to the shape of the knife, the form and dimensions of which are figured in his illustrations, the author says the knife is honed on a glass plate, 19 cm. long, 4·5 cm. broad, and the manner of holding the knife is depicted. The first stage consists in sharpening the knife on the dull side of the glass plate with emery and water, and then having washed it, to hone it on the smooth side of the glass, using a little "chaux de Vienne." In this way an edge quite straight and without any serrations is obtained, and a 5 μ thick section perfectly smooth, without a tear and showing no knife-marks, may be cut with certainty.

To preserve Edges of Microtome Knives.†—A writer in the 'Dental Review' says:—"To render instruments perfectly aseptic, and to preserve the cutting edges from oxidation, they should be boiled for five minutes in one per cent. solution of carbonate of sodium. They can remain in this solution indefinitely without rusting or dulling the cutting edge. When required for operation they are taken out, dried with a sterilized piece of gauze, and handed to the operator. Whenever, in course of operation, they come in contact with anything not aseptic, all that is required to resterilize them is to dip them for a few seconds into the boiling solution of sodium bicarbonate."

(4) Staining and Injecting.

Staining of Chlorophyll.‡—For staining the chlorophyll-bands of *Spirogyra*, Mr. G. Mann recommends the following process:—A glass vessel is filled with two litres of water, to which six drops are added of a 10 per cent. solution of cyanin in absolute alcohol. Then a small quantity of either *Spirogyra jugalis* or *S. nitida* is placed in the vessel, which is exposed to bright daylight. After some time, varying with the temperature of the room and the activity of the threads, from 3–24 hours, the whole of the cyanin will have been taken up by the threads. The ground-substance of the chlorophyll-bands will have changed from a green to a bluish-green colour, while the oil-globules and many of the microsomes between the bands will have turned blue, showing their fatty nature. Concentrated solution of alcanna-root, or a

* Botanisch Jaarboek, Gent, 1891, pp. 541–56 (1 pl.).
† Amer. Mon. Micr. Journ., xii. (1891) p. 124.
‡ Trans. and Proc. Bot. Soc. Edinb., xviii. (1889-90) pp. 394–6.

1 per cent. solution of osmic acid, may be used instead of the cyanin, but the results are not so good.

New Application of Safranin.*—Dr. P. Kaufmann says that he has obtained surprising results with the following solution, which stains both the tissue and the micro-organisms, though of different colours, the nuclei being red and the bacteria and fibrin blue. After the preparations have been stained for two to eighteen minutes, they are treated as in Gram's method with the iodo-potassic iodide. The solution, which does not keep very long, and should therefore be freshly made, is composed of the following:—Alcohol 98–100 per cent., 2 grms.; anilin oil, 0·5; aq. destil., 30·0; gentian-violet, 0·25; safranin, 1·25. Or the last three ingredients may be formulated thus:—25 ccm. of aqueous 5 per cent. solution of safranin, 5 ccm. of aqueous 5 per cent. solution of gentian-violet, the anilin-oil and alcohol being afterwards added.

New Syringe for Hypodermic Injection.†—M. Strauss has, by a simple modification of the plug of an ordinary Pravaz syringe rendered its cavity sterilizable by steam, dry air, or boiling. The plug is made of compressed elder-pith, and in case it should become too slack, the metal discs are screwed on to the piston rod, so that the intervening pith may be tightened up.

Colourability of Tubercle Bacilli.‡—M. G. Roux thinks that the reason why tubercle bacilli frequently fail to stain or exhibit such differences in appearance when they are stained is to be sought for in the degeneration of the anilin-oil used as mordant or in the method adopted. After obtaining a perfectly pure and recently made anilin-oil, the preparations of sputum showed numerous deeply-stained bacilli, while those stained with a solution made of old dark-coloured anilin-oil showed scarcely any at all. The author also notes that with Hermann's method the bacilli appear thicker and more numerous than when stained by the anilin-oil or carbolic acid solutions.

Phospho-Molybdic Acid Hæmatoxylin.§—Dr. F. B. Mallory recommends as a useful stain in the study of nerve-tissue a mixture of 1 part 10 per cent. solution of phospho-molybdic acid, 1 part of hæmatoxylin crystals, 6–10 parts of chloral hydrate, and water to 100. Expose to sunlight for a week and filter before using. Discharge excess of stain, which acts in from ten minutes to an hour, with 40–50 per cent. alcohol, changing twice or thrice. Dehydrate and mount as usual. If the solution does not stain deeply, add more hæmatoxylin.

Methods of Differential Nucleolar Staining.‖—Mr. Gustav Mann says:—"As far as I am able to ascertain, Guignard ¶ was the first to describe a differential nucleolar stain by a certain mixture of methyl-green and fuchsin, but he does not specify any proportion of admixture,

* Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 717–8.

† Le Bulletin Méd., 1891, p. 89. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) p. 737.

‡ La Province Méd., 1891, No. 4, p. 37. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) pp. 678–9.

§ Anat. Anzeig., vi. (1891) pp. 375–6.

‖ Trans. and Proc. Bot. Soc. Edinb., xix. (1891) pp. 46–8.

¶ Ann. Sci. Nat., sér. 6, xx. p. 318.

though he repeatedly mentions the fact of the differentiation. I am unable to follow him in his method, and, notwithstanding many trials, have failed to get his differential stain, namely, the chromatin-elements of the nucleus green and the nucleolus red by means of methyl-green and fuchsin.

While endeavouring to stain the nucleolus and endo-nucleolus differentially, my attention was drawn by Dr. Macfarlane to heliocin as a good nuclear stain for *Spirogyra*. By extending its action in combination with anilin-blue to other tissues, I have succeeded in obtaining an excellent differentiation.

Method.—Tissues, both vegetable and animal, preferably fixed by my picro-corrosive method,* are treated for ten minutes in a saturated solution of heliocin in 50 per cent. alcohol; the sections are then transferred for from five to fifteen minutes to a saturated watery solution of anilin-blue. The superfluous stain is rapidly washed off by distilled water, and the sections placed again for one to two minutes in the heliocin-solution, dehydrated, cleared by resinified turpentine, and mounted in turpentine-balsam.

Effect.—The whole of the cell and the nucleus blue, the nucleolus red. In karyokinetic figures the cell and nuclear barrel are stained blue, the nuclear plate, monaster and diasters stained red.†

The chemical constitution of the heliocin I used I am unable to find out; when dry it is a brick-red powder, readily soluble in water, slightly so in absolute alcohol, and in each case showing no fluorescence. A watery solution is of an orange brick-red colour. My friend Mr. Terras was kind enough to test this heliocin chemically, and found it to act thus: The dye dissolves in concentrated sulphuric acid with a red orange colour, which on boiling becomes dark brown. Water added to the dark brown fluid does not produce any precipitate. Hydrochloric acid added to the solution in water gives no precipitate, and does not change the colour. Zinc-dust added to the acid solution decolorizes it in the cold easily, and the colour does not return on exposure to the air. Strong caustic potash added to the watery solution of the dye produces no change either in the cold or when boiled. Zinc-dust added to the alkaline solution decolorizes it in the cold.

Besides the heliocin just described, another one is in the market, a dark brownish-red powder soluble in water, with a distinct fluorescence, readily soluble in alcohol, and giving the reactions of true eosins.

One should endeavour to get the heliocin first described, for it makes a beautiful contrast with the blue, and allows one to study the finer structure of nucleoli.

Should either of the two heliocins not be obtainable, any of the eosins, or erythrosins, may be substituted, when treating vegetable tissues, while for animal tissues safranin makes a tolerably good substitute.

Another differential stain is got by placing living tissues for at least a week in a saturated picric acid solution of absolute alcohol, to which

* See Trans. Bot. Soc. Edinb., xviii. (1890) p. 432, *et supra*, pp. 686 *et seq.*

† "I may state that in dividing cells of the root of *Nymphæa alba*, we may stain the whole of the cell pink, and the nuclear plate, monasters and diasters blue, by treating sections first with alcoholic eosin and then with alcoholic methylene-blue."

that variety of nigrosin known as alcohol-soluble nigrosin has been added. After-staining the sections with eosin or Kleinenberg's hæmatoxylin causes the nigrosin to be replaced by either dye, leaving only the nucleolus of a greenish-blue colour."

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

Reference Tables for Microscopical Work. III. Cements and Varnishes.*—Prof. A. B. Aubert gives the following list:—

Asphalt varnish:—Asphalt, 450 grm.; linseed oil, 225 grm.; turpentine, 1000 ccm.; or dissolve asphalt in benzol and to the solution add gold size. In the first method, dissolve by the aid of heat; dilute when necessary, with turpentine. Not very reliable as a cement.

Bell's cement:—Probably a solution of shellac, but the exact composition is not known. This in the opinion of many is an excellent cement.

Gold size:—Linseed oil, 25 oz.; red lead, 1 oz.; powdered white lead and yellow ochre, of each a sufficient quantity. Boil the oil and red lead together carefully for three hours; pour off the clear liquid, and boil with a mixture of equal parts of the white lead and yellow ochre added in small successive portions. Let it stand, and pour off the clear liquid for use.

Gram-Rutzon's cement:—Hard Canada balsam, 50 grm.; shellac, 50 grm.; absolute alcohol, 50 grm.; anhydrous ether, 100 grm. The ingredients are mixed, and when the gums are dissolved, filter if necessary, and evaporate, away from the flame, over a water-bath until of a syrupy thickness.

Gutta-percha cement (Harting):—Gutta-percha cut in pieces, 1 part; turpentine, 15 parts; shellac, 1 part. Heat the gutta-percha and turpentine together, filter, add the shellac pulverized, and heat until a drop hardens on a cold glass plate. Used to attach cells; the slide must be warm when using the cement.

Brown cement:—Pure gum rubber, 20 grains; carbon disulphide, a sufficient quantity; shellac, 2 oz.; alcohol, 8 oz. Dissolve the rubber in the smallest possible amount of carbon disulphide, add this slowly to alcohol, avoiding clots; add powdered shellac and place the bottle in boiling water until the shellac is dissolved and no more smell of carbon disulphide is given off.

Guiacum varnish:—Gum guiacum, 2 oz.; shellac, 2 oz.; alcohol, 10 oz. The powdered gum guiacum is dissolved in the alcohol and the powdered shellac added; keep the bottle in hot water until all is dissolved.

Shellac varnish:—1, shellac, 60 grm.; 2, alcohol, 60 grm.; 3, castor oil, 25 grm.; 4, alcoholic solution of anilin dye, a few drops. 1 and 2 are dissolved and heated until quite thick, then a little of 4 is added, and for every 60 grm. of the mixture add 25 grm. of castor oil, and heat for a short time.

Electrical cement:—5 parts of resin; 2 parts of hard balsam; 1 part of yellow beeswax; 1 part of red ochre. The components are melted together.

* Microscope, xi. (1891) pp. 150–2.

This is not usually employed for mounting purposes, but may be used in cementing glass and metal parts of instruments.

Zinc-white cement, German formula:—1, mastic, 10 pts.; 2, dammar, 4 pts.; 3, sandarac, 4 pts.; 4, Venetian turpentine, 1 pt.; 5, turpentine, 20 pts.; 6, benzol, 10 pts.; 7, zinc-white. 1, 2, and 3, powdered are mixed in a well-corked bottle with 4, 5, and 6; shake well occasionally; after several days filter, and triturate in a mortar with zinc-white in quantity sufficient. Dilute if necessary with benzol.

Zinc-white, English formula:—1, gum dammar, 3 pts.; 2, gum mastic, 1 pt.; 3, benzol, 6 pts. Dissolve powdered 1, 2, and 3 in a well-corked bottle; when dissolved filter, and mix carefully in water with zinc-white.

Marine glue:—India-rubber shreds, 2 oz.; shellac, 2 oz. Dissolve the rubber in mineral naphtha, add the powdered shellac, heat until liquefied, and mix well together. This gives solid marine glue, and requires heat in its application. Great care should be observed in having all fire and flame removed while there still remains naphtha in the mixture.

Lovett's cements:—Powdered white lead, 2 parts; powdered red lead, 2 parts; powdered litharge, 3 parts; gold size. The white and red lead and the litharge must be very finely powdered; for use this powder is mixed with gold size to the consistency of cream, and the cells immediately fastened to the slide. They are secure in two weeks. This stands considerable heat, and is excellent for fluids containing some alcohol. Make a little only of the mixture with gold size at a time, as it hardens quite rapidly and becomes useless.

King's cement and lacquer.—Satisfactory, and highly recommended by some.

Brown's rubber cement.—Very good for finishing slides.

Miller's caoutchouc cement.—Sold in England by opticians. It is a most excellent and quickly drying cement.

Hollis's glue.—Somewhat similar to Bell's cement.

Nearly, if not all the foregoing can be most advantageously bought of the opticians and dealers in microscopical material.

(6) Miscellaneous.

Coco-nut-water as a Culture Fluid.*—Mr. G. M. Sternberg points out that the fluid contained in unripe coco-nuts is quite transparent, with a specific gravity of 1·02285. Chemical analysis showed that it was composed of water 95 per cent., ash 0·618 per cent., glucose 3·97 per cent., fat 0·119 per cent., albumen 0·133 per cent. This fluid forms an excellent medium for numerous kinds of micro-organisms. There is no need to sterilize it, if it be removed with the necessary precautions. As its reaction is slightly acid, it must be neutralized before being used for cultivating certain kinds of pathogenic micro-organisms.

* Philad. Med. News, 1890, p. 262. See Centralbl. f. Bakteriol. u. Parasitenk., ix. (1891) p. 834.

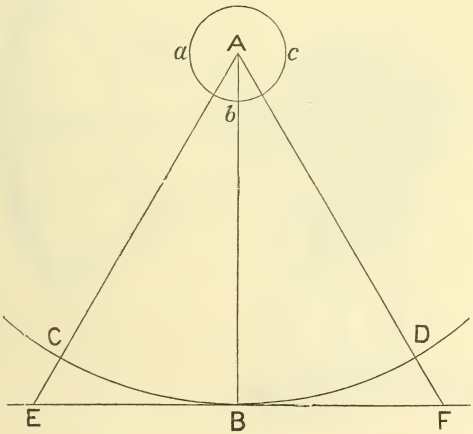
XI.—*On an Improved Method of making Microscopical Measurements with the Camera Lucida.*

By Sir Walter Sendall, K.C.M.G., M.A., F.R.M.S.

(*Read* 21*st October*, 1891.)

In fig. 77, *a b c* is a section, through the plane of the paper, of the draw-tube of the Microscope, in a horizontal position; A is the extremity of the axis of the tube, from which the line A B is drawn perpendicular to the axis, and meeting the plane of the table in the point B. If A B, the height of the Microscope from the table, be

Fig. 77.



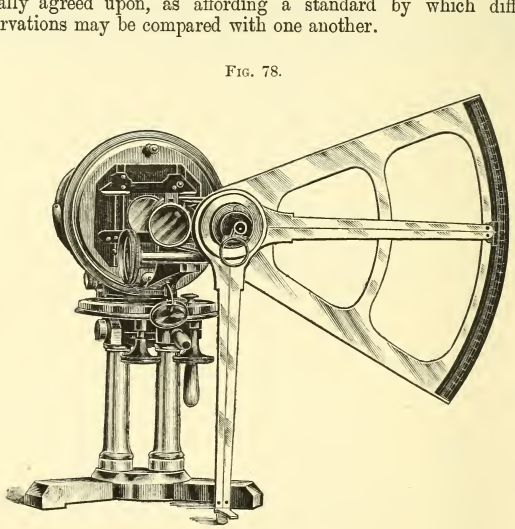
equal to ten inches, and a camera be placed at A, we shall have the arrangement commonly recommended for making drawings and measurements of the virtual image of an object, projected upon the plane surface of the table.

With centre A, and radius A B, describe C B D; take any points E, F, in the line E B F, and join A E, A F. Then, since the amount of magnification of the object, afforded by its virtual image, is dependent, at every point, upon the distance of the image from the eye placed at A, an inspection of the figure will show that it is only at the point B that we obtain a degree of magnification due to a distance of ten inches. At every other point in the surface of the

table, such as E, F, we get an amplification due to a distance greater than ten inches.

A little consideration will in fact show, that if we desire to investigate the true dimensions of an object by examining its virtual image projected *upon an area of uniform magnification*, we must employ for this purpose, not the plane surface upon which such measurements are usually made, but a concave spherical surface, having the eye at A for its centre, and for its radius a length of ten inches, or whatever other distance may be convenient to the observer, or may be conventionally agreed upon, as affording a standard by which different observations may be compared with one another.

Fig. 78.



Reverting to the figure, if E F be a measurement taken across the image of an object projected upon the plane of the table, the amount of magnification deduced from such measurement will be in excess of the magnification due to a distance of ten inches, by as much as the length of the straight line E B F exceeds that of the circular arc C B D; and any conclusions drawn therefrom, either as to the magnifying power of the glasses employed, or as to the dimensions of the object under examination, will be affected with a corresponding error.

The error here involved may be corrected by a simple calculation, provided care be taken that the line to be measured is so placed (like the line E B F in the figure) as to be bisected by the central point of

the field; and this can generally be effected with a fair amount of approximation to accuracy. The calculation is as follows.

The line A B, in the figure, being given equal to ten inches, and B E being known by observation, the tangent $\frac{B\,E}{A\,B}$ of the angle E A B, and hence the angle itself, becomes known; whence also the angle E A F, which is double of E A B, is known; and the linear value of the arc C B D can then be taken at once from the table of circular values, to be found in every collection of mathematical tables.

Fig. 79.

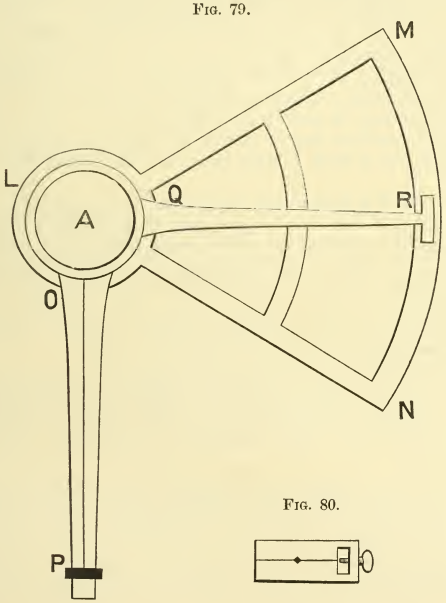


Fig. 80.

Substituting this value for the measurement E F, we obtain a quantity which accurately expresses the dimensions of the magnified image, due to a uniform distance from the eye of ten inches.

By applying a similar calculation in every case, all measurements taken with the camera upon a plane surface can approximately be reduced to their proper values; it would, however, be much simpler and more satisfactory, where accurate results are of importance, to

take our measurements directly along the circular arc C B D of the figure; and this is the object of the instrument about to be described.

A mere inspection of figs. 78 and 79 will indicate the nature of the instrument, and its use.

A, being, as in fig. 77, the extremity of the axis of the Microscope, L M N is a graduated arc of 60°, fitted accurately upon the open end of the draw-tube, and secured in the position shown in the figure, by means of pins or studs which enter into notches cut upon the shoulder of the tube. It is essential that the draw-tube should not be able to turn; in a binocular body this will of course be the case, and in all others it must be specially arranged.

O P is a radial arm, which, together with A R, attached to it at right angles, swings freely about the axis of the Microscope. At P is placed a projecting piece, shown separately in fig. 80, which may be termed the speculum; this piece is slipped over the end of the radial arm O P, and kept in position at right angles to it by a binding screw. The speculum may be placed with either face uppermost; one being white with a black central line, the other black with a white line.

The arc M N is divided into degrees and parts (not shown in the figure); and at R there is a vernier, reading to the tenth of a part.

To use this instrument, a camera being placed at A, any part of the image of an object in the Microscope can readily be brought upon the face of the speculum; one edge of the image being then brought into contact with the line on the speculum, the arm O P is swung round until the same line coincides with the opposite edge, and the angle passed through is read off upon the arc M N.

This will give the dimension of the image, with perfect accuracy, measured along the circular arc C B D in fig. 77, and therefore upon an area of uniform magnification; the linear value of the measurement, to any radius, being ascertained at once from the table of circular arcs.

It is to be noted that the instrument will give accurate measurements only in one plane; that, namely, which cuts the axis of the Microscope vertically, at its point of intersection with the reflecting surface of the camera; the image should therefore be so adjusted that the required measurements may lie along the diameter in which the field is intersected by this plane; this will coincide with the horizontal diameter of the field, as presented to the observer looking through the camera.

It is also obvious that the action of the instrument is independent of the inclination of the Microscope body, or of its distance from the table; all that is requisite is that the radial arm shall have room to swing. The graduated arc may be of any dimensions, and the speculum may be adjusted to any length of radius to suit the observer's sight. In the instrument which Mr. Holtzapfel has made for the writer, the arc has a radius of ten inches, and is graduated in

degrees, halves, and quarters. The least division therefore contains fifteen minutes; and by help of the vernier, readings may be taken to a minute and a half of arc; the linear value of which, to a radius of ten inches, is less than the 1/200 of an inch.

When constructed upon this scale the instrument requires a somewhat massive stand to support it; but it could easily be made smaller and lighter, though with some loss of range in the graduation.

The black face of the speculum will be found useful, in cases where the field of the Microscope is feebly illuminated; it being often easier in such cases to catch the outlines of the image upon a dark surface, than upon a light one.

MICROSCOPY.

a. Instruments, Accessories, &c.*

(1) Stands.

A Universal Stand.†—Dr. A. G. Field describes this stand thus:—"Fig. 81 below represents a stand adapted to the wants of the professional or amateur who uses the Microscope and camera. It consists of base A, 14 × 14 × 15 in., to which are secured by dovetail, glue, and screws, two uprights, B B, 5 × 1 in., one 3 and the other 7 ft. in height. These are precisely perpendicular to base, to bring instruments and objects in line when centered. They are grooved on edges to receive tongues or arms, C C C C, of the secondary base D, and also on the camera-carrier H. The uprights are made firmer by additional pieces extending up 30 in. from the base. The secondary base, 14 × 14 in., is corner-braced as shown, and is adjustable as to height, being secured in desired position by set-

Fig. 81.

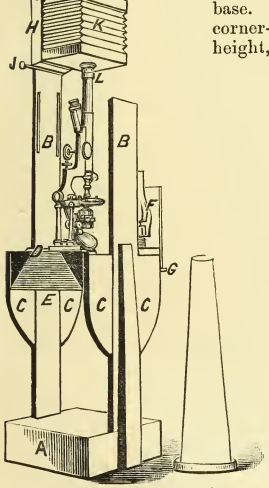
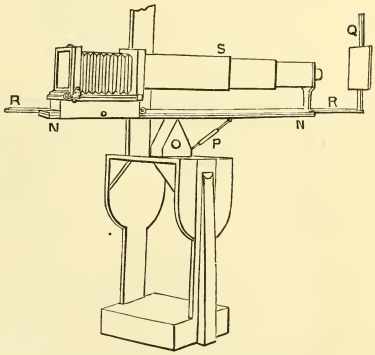


Fig. 82.



screw E. In the centre is a hole, 1½ in. in diameter, which receives the tube of the Microscope when it is placed on the base for high amplification in photomicrography, and also the gudgeon of the support of the base-board O, when used in copying or photography. G is a

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

† Amer. Mon. Micr. Journ., xii. (1891) pp. 151–2.

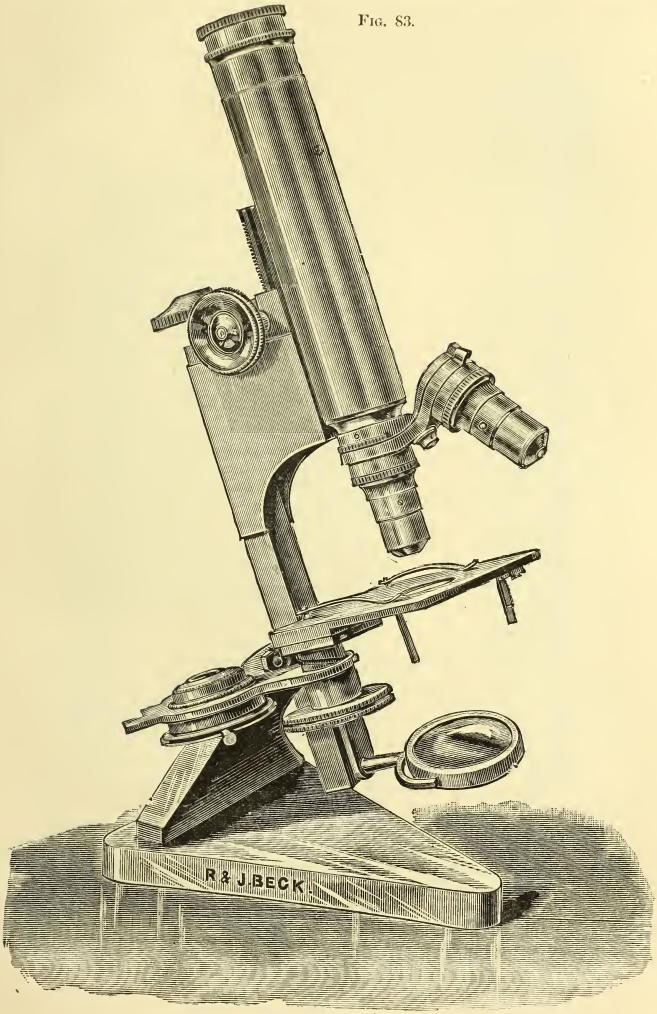
lamp-rest which slides on cleats attached to the corner braces, and has an upright for concave reflector when desired. H, sliding carrier for camera, with tongued arms of sufficient width to bring the photographic lens collar precisely over the microscopic tube when centered on either base. I, set-screw to retain it in position, and J, milled head of pinion by which it is racked down to attach camera K to eye-piece of Microscope. This light-tight connection is made with one-half of a child's rubber ball, perforated in centre to fit neck of eye-piece, and of sufficient size to fill the collar of the photographic lens. Fig. 82 illustrates use of the stand in copying enlarging, and reducing, and requires but little explanation. N, N, base-board, 5 × 1 in., 4 ft. long, grooved on edges to receive tongues on arms of camera-carrier. It is hinged to apex of wedge-shaped block O, the gudgeon of which fits snugly into the hole in centre of supplement base; S, telescopic boxes; R R, slot passing beneath the camera-carrier, with upright for carrying the picture to be copied, the distance respectively between the lens and the picture, and the lens and ground-glass, being regulated by the operator without leaving his position at the focusing screen, so that all copies may be brought to a uniform size, as for lantern slides, without regard to the size of the original. Removing the telescopic boxes and slot, we have a convenient camera stand for inside use, the lateral movements being secured by the gudgeon attachment, and the vertical by the screw brace P. If used ordinarily as a Microscope stand the instruments are always in line and position for photomicrography."

Beck's Bacteriological "Star" Microscope. — This Microscope, which was exhibited at the October meeting, is made in two forms, one with a sliding and the other with a rackwork coarse-adjustment. The fine-adjustment to both forms is that known as the micrometer screw. It is also provided with an inclining joint, a draw-tube, and a swinging double mirror. The special feature of the instrument is the movement of the substage; this is done by a milled head at the right-hand side of the instrument, by the revolution of which the substage is raised or lowered. When it has been moved to its lowest position a further turn of the milled head turns the substage out of position to the right-hand side of the instrument. The substage is fitted with an Abbe condenser and iris diaphragm.

Giant Projection Microscope.*—In the Optical Institute of Franz Poeller, in Munich, an enormous projection Microscope is now being constructed for the "World's Fair" at Chicago. Electricity plays a great rôle in this instrument. In the first place it supplies and regulates the source of light which is mounted in the focus of a parabolic aluminium reflector, and has an intensity of 11,000 candles. By means of an ingenious piece of mechanism, it also maintains the centering of the quadruple condenser and the illuminating system. It also serves to control exactly the distance of the carbon points. For this purpose the arc forms part of a shunt whose intensity is measured by a galvanometer, by the movement of the needle of which the distance of the carbon points can be read to the tenth of a millimetre. The most important innovation, however, is the arrangement for cooling the instru-

* Central-Ztg. f. Optik u. Mechanik, xii. (1891) p. 178.

FIG. 83.



BACTERIOLOGICAL "STAR" MICROSCOPE.

ment. This is absolutely indispensable owing to the intense heat of the source of light (1·43 calories per second). It consists in pouring over the whole Microscope and polariscope a fine spray of liquid carbonic acid. So great is the cooling effect produced that an expenditure of only 0·00078 grm. per second is required. The linear magnification of the instrument is, with ordinary objectives, 11,000, and with oil-immersion lenses, as high as 16,000.

Eustachio Divini's Compound Microscope.*—Sig. P. A. Saccardo describes an ancient Microscope, bearing the inscription "Eustachio Divini in Roma, 1672," which is preserved in the Museo di Fisica, Padua, where, however, nothing further is known as to its history. A Microscope of Divini is fully described in the 'Giornale dei Letterati' i. (1668).† The present instrument is in many respects similar to the one there described. It consists of four tubes of cardboard covered with parchment coloured green and gilded. These slide with friction one within the other, and each has marked upon it in gold the points of different extension (I., II., III., IV.). The largest tube has a diameter of 8 cm. When all the tubes are closed up as much as possible, the total length from eye-piece to objective is 36·5 cm. When all are drawn out as far as the marks I., II., III., and IV., the total length is 41, 49, 54, and 56·5 cm. respectively. The lowest tube carries on its lower half a broad projecting spiral band of cardboard covered with parchment, which gears into a corresponding spiral cut into the cardboard cylinder round which is the brass band bearing the inscription. This band is supported by three divergent feet of brass 15 cm. long. The objective, consisting of a biconvex lens 8 mm. in diameter and 2 mm. thick at the centre, is fitted by means of a screw cap into a brass tube 5·5 cm. long and 2·5 cm. in external diameter. On a screw-thread round this tube moves another tube, in the lower part of which, through two side slits, passes the object-holder, which is kept firm by a spring. The object is focused by raising or lowering this tube on the screw-thread.

The eye-piece is formed of a large somewhat yellow biconvex lens 6 cm. in diameter and 5 mm. thick. It is inclosed in two wooden rings into which the first tube of the Microscope enters. Thus the special eye-piece system of Divini, which consisted of two plano-convex lenses, is wanting. In all probability these have been lost, in which case the lens just described should be regarded as the field lens.

Invention of the Compound Microscope.‡—Sig. P. A. Saccardo publishes several of the documents bearing on the claims of Janssen, Galileo, and Drebbel. Criticizing these he comes to the following conclusions:—The testimony of P. Borel in favour of Janssen has no documentary value. The documents published by Govi show that the first inventor of the compound Microscope (with concave ocular and direct vision) was Galileo in 1610. The documents published by Rezzi, which are in harmony with the testimony of Gassendi and Huygens, show that Cornelius Drebbel was the reformer of the Galilean Microscope, or was in 1620 or 1621 the inventor of the Keplerian compound

* Atti R. Istit. Veneto Sci., II. vii. (1891) pp. 817–27.
† See Dallinger's Carpenter, p. 131. ‡ Malpighia, v. (1891) pp. 40–61.

Fig. 84.

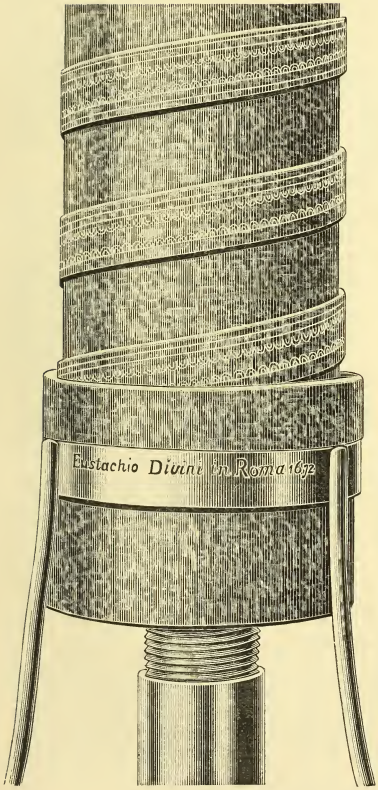
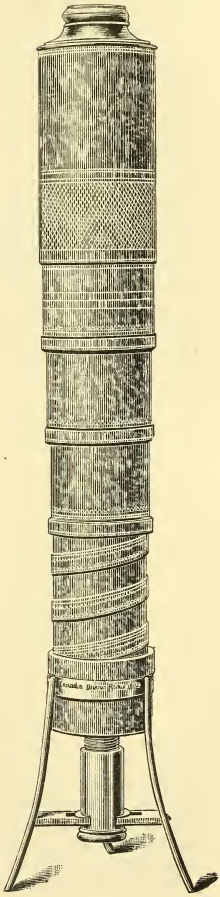


Fig. 85.



Divini's Compound Microscope.

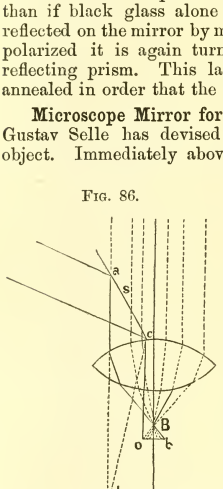
Microscope with all the lenses convex and with reversed vision. The name *microscopio* was invented in Rome in 1625 by Giovanni Faber, a physician of S. Santità.

(3) Illuminating and other Apparatus.

New Polarizer.*—Prof. S. P. Thompson read, at the British Association, a paper on "A new Form of Polarizer." He explained that owing to the great dearth of Iceland spar large Nicol prisms could not be obtained, and he therefore thought it expedient to devise some means of producing polarized light without its aid. The method proposed consists in reflecting the light from a black glass mirror, whose surface is covered with a plate of clear glass. In this way less light is lost than if black glass alone were used. The light from the lantern is reflected on the mirror by means of a total reflecting prism. After being polarized it is again turned back into its original axis by a second reflecting prism. This latter prism, however, must be very carefully annealed in order that the light may remain plane polarized.

Microscope Mirror for Illumination by Reflected Light.† — Herr Gustav Selle has devised an ingenious method of illuminating the object. Immediately above the objective system is a concave mirror, which reflects the rays incident upon it through an aperture in the side of the case of the objective in such a way that the external rays of the reflected cone *a c d* (fig. 86), by passage through the objective, are refracted through the focus B to the further edge of the object *b*, while the inner rays are refracted parallel to the axis of the Microscope to the near side *o*.

Fig. 86.



Electro-Microscope Slide for Testing the Antiseptic Power of Electricity.‡— Dr. R. L. Watkins writes:—"Fig. 87 represents an instrument that I have devised for the purpose of ascertaining whether or not electricity will destroy the life of germs. It is the result of a number of experiments to confirm a belief I have long held, that electricity is an antiseptic and disinfectant. I also learned, while experimenting, that Apostrali had made the same claim.

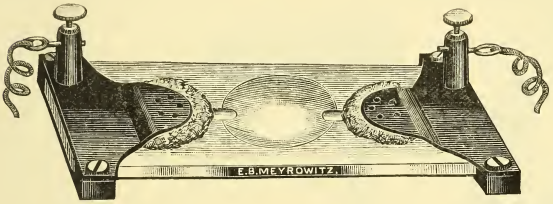
The instrument consists of a glass slide, in the centre of which is a sunk cell. Two grooves, each 3/4 in. long, run from this cell outward. Two brass pieces are fitted over the extremities of the slide in such a manner that the rounded points, the under surfaces of which are lined with platinum, will cover a portion of the grooves. These rounded points do not touch the glass, but are raised above the grooves about

* English Mechanic, liv. (1891) p. 36.
† Central-Ztg. f. Optik u. Mechanik, xii. (1891) p. 239.
‡ Amer. Mon. Micr. Journ., xii. (1891) p. 204.

1/8 in. Binding posts are riveted to the brass for connection with a battery.

In order to apply this instrument, a sufficient quantity of the fluid containing the bacteria should be used to fill the cell and grooves. A cover-glass is placed over the cell and its contents. Two small clean sponges, saturated with either the fluid or distilled water, are then placed

Fig. 87.



underneath the platinum points and in contact with the fluid in the grooves. The bacteria are now ready for observation, the electricity is turned on, and the quantity noted by the milli-ampere meter to stop all sign of germ life. They can now be cultivated on gelatin in the ordinary way should it be desired to determine whether or not their vitality has been entirely destroyed.

Other uses for the slide will readily occur to one working in this field: for example, the effect of electricity on the blood and different tissues.

I have found this instrument very satisfactory, not only as an easy, but as a quick way of finding out the amount of electricity required to destroy micro-organisms."

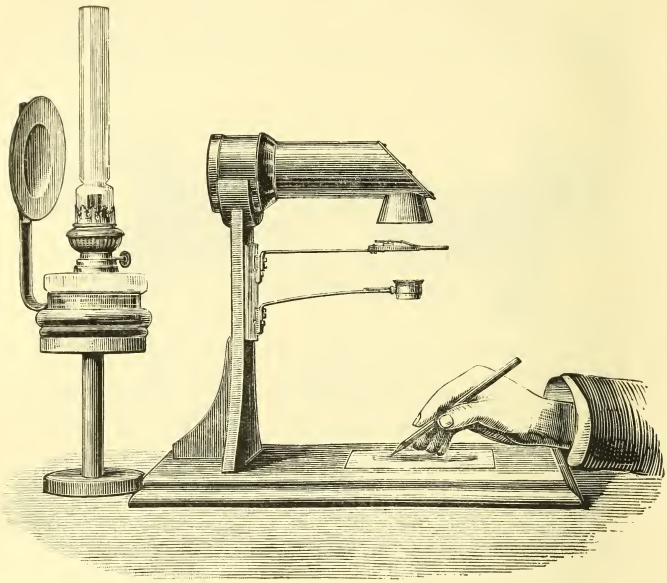
New Apparatus for drawing Low Magnifications.* — Dr. L. Edinger has devised a simple form of apparatus for drawing low magnifications (fig. 88), in which the image is projected directly upon the paper and a perfectly free movement is given to the object which lies horizontally on a stage.

The apparatus has the following construction. On a polished wooden base, which serves as drawing board, rises a wooden upright which supports a horizontal tube, closed in front by a condensing lens and behind by a mirror set at 45°. The rays of a lamp are concentrated by the lens upon the mirror. Through an opening beneath the mirror the light falls downwards upon an object-stage which is adjustable in height. Beneath the object-stage is a lens, supported in an adjustable holder, which produces on the base-plate an objective image of preparations which are placed on the object-stage. According to the adjustment of lens and drawing-board it is possible to take magnifications from 2 to 20. The apparatus, however, is supplied with three lenses,

* Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 179–81.

since it is not advisable to produce all gradations of magnification by displacement alone.

Fig. 88.



Glasses for keeping Immersion Oil.*—Dr. W. Behrens describes a convenient bottle for keeping immersion oil, which has been made by the firm of Zeiss. It is of cylindrical form, 60 mm. in height and 30 mm. in diameter. It has a wide neck with a clear diameter of 15 mm., and holds 20 ccm. of liquid. Above the ground neck fits a cap, to the centre of which is attached a cylindrical solid glass rod reaching nearly to the bottom of the bottle. This rod has at its upper end a glass hemisphere which is cemented by shellac into a corresponding hole in the glass cap. It is 60 mm. long and 1·5 mm. in diameter. At its lower end it is not simply swollen, but is terminated by a small glass ball of 2 mm. diameter, which prevents the oil from dropping off.

(4) Photomicrography.

Magnesium Flash-Light in Photomicrography.†—Dr. R. Neuhauss gives an account of different flash-lights which have been made use of for photomicrographical purposes. By mixing different powders it is

* Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 184–5. † Tom. cit., pp. 181–4.

possible to insure the presence of rays near the red end of the spectrum which are serviceable in taking coloured preparations. Newcomb was amongst the first to undertake experiments of this kind. He mixed 1 part of magnesium powder with 5–7 parts of pure nitrate of soda, and obtained thus an intensely yellow light. Röhmann and Galewsky made many experiments with a number of different mixtures and obtained good results with the following receipt:—Mixture A. Magnesium, finely powdered, 9·6 grm.; potassium perchlorate, free from water, 13·8 grm. Mixture B. Barium tartrate, free from water, 5·7 grm.; potassium perchlorate, free from water, 2·7 grm. 10 parts of A mixed with 1 part of B and 0·5 grm. of salt, free from water, added. From 1 to 3 grm. of this powder are used. Röhmann and Galewsky also recommended other mixtures, in one of which acetate of copper was employed.

As the result of a number of spectrographic investigations, the author comes to the conclusion that all complicated mixtures of salts of barium, copper, &c., such as these, must give place to the so-called smokeless flash-powder of Gaedicke. This powder consists of a mixture of magnesium and permanganate of potash which burns quickly, giving an intense light with little smoke. If the flash-light is taken with the spectrograph on an ordinary plate, not the slightest effect is shown in the red, yellow, and green, but some bright lines are produced on the border between the green and blue, joining on to the bright zone in the blue and violet. The effect is quite different on the erythrosin plate. In this case the bright zone begins already in the yellow by the Fraunhöfer line D. In the centre between the lines D and E the silver deposit on the negative is very thick, and gives the impression that here there was more light effective than in the whole of the blue and violet together. Between the lines E and F the light effect gradually diminishes. In the blue and violet the effect is the same as on the ordinary plate. By using the erythrosin plate and interposing the yellow-green Zettnow filter, the blue and violet light is completely absorbed, and there remains only the strong maximum in the yellow-green between the lines D and E. These are exactly the relations which are wanted in photomicrography, and as they are found in sunlight. The maxima and minima of the light effect of this flash-light are distributed on the erythrosin plate exactly as with sunlight, only the maximum in the yellow-green is much more intense.

Coloured Photomicrograms * — MM. Lumière, of Lyon, are the authors of a process for mechanically colouring photomicrograms. The best results have been obtained by the following methods. A carbon paper poor in colouring matter is chosen and sensitized in a solution of bichromate of potassium containing—water, 650 grm.; bichromate of potassium, 25 grm.; alcohol, 350 grm. After five minutes' immersion the paper is dried and then exposed in the press. The duration of impression is determined by means of a photometer. The image is then developed on a thin ground glass by the usual methods. The positive is washed in cold water, immersed in alcohol for ten minutes, and finally dried. If properly done the proof is faint, sometimes scarcely visible. In order to colour it, solutions of the colours used in micrography, such

* Bull. Soc. Belg. Micr., xvii. (1891) pp. 121-6.

as methyl-violet and blue, &c., are prepared. The concentration which appears to be most suitable varies between 1/100 and 1/500 according to the solubility and the colouring power of the substance. When insoluble in water the colour is dissolved in as small a quantity of alcohol as possible, and the solution is then diluted with water.

The colouring solution is poured over the positive. After a few seconds the liquid penetrates the gelatin, which retains the colour. If the coloration is too intense, the proof is washed with water. The decoloration is in this way generally effected slowly and regularly, and the washing is continued until the right tint is obtained.

When the decoloration by water is not sufficient, alcohol is used. It is then much more rapid, so that the operation must be conducted with more care. The final washing is in all cases with ordinary water.

To obtain a double coloration, as for example in the case of a microbe coloured red on a blue ground, the positive is first treated with a very intense colour. In the case of the microbe a 1 per cent. solution of magenta-red would be used. The proof is thus coloured in all its parts, the microbe deep red and the ground light red. A partial decoloration, first with water and afterwards if necessary with alcohol, is then effected. When the ground begins to lose its tint the proof is treated with the colour required for the ground. A weak solution, such as the aqueous 0·2 per cent. solution of cotton-blue, is used. For projection, it is necessary to varnish in order to get rid of the grained appearance of the surface. The projected images are then much more brilliant.

(5) Microscopical Optics and Manipulation.

Probable Limits to the Capacity of the Microscope.*—Dr. S. Czapski discusses the question of the limits to the resolving power of the Microscope. So long ago as the beginning of the century it was recognized that increased magnification was not the only thing necessary to render the details of a microscopic object clearly visible. With the same magnifying power, the same perfection in the correction for aberration, &c., and with the same method of illumination, systems having the larger angular aperture always showed superiority in definition and resolving power. The explanation of this "specific function of the angular aperture" came almost simultaneously from Abbe and Helmholtz. The theory of Helmholtz supposes the object to be self-luminous, so that it has not so direct a bearing as that of Abbe upon the ordinary Microscopic practice, in which the preparation is illuminated by reflected or transmitted light. However, the two theories, although thus divergent in their points of departure and in most of their consequences, lead in one point almost to the same result. With central illumination—i. e., according to Helmholtz, when the pencils of rays from the luminous points of the object occupy the whole aperture of the Microscope; or, according to Abbe, when the object is met directly only by one small axial pencil—the resolving power according to both theories is determined by the same formula. This formula shows upon what factors and

* Zeitschr. f. Wiss Mikr., viii. (1891) pp. 145-55.

in what way upon these, the resolving power of the Microscope depends. The deduction, thus made, that the resolving power does depend upon certain factors, leads at once to the consideration of a limiting value for it. Naturally, inquiries of this kind, as to how far we can hope to advance, have only a relative value, and can necessarily be only considered from the point of view of our present resources.

The fundamental formula for the capacity of the Microscope given by both the theory of Abbe and that of Helmholtz for central illumination is

$$\delta = \frac{\lambda}{a}$$

where δ denotes the smallest distance of the elements of a regular structure which can be distinguished by an optically perfect objective, λ the wave-length of the effective light (in vacuo), and a the aperture of the system. This equation shows that δ, the smallness of which is a measure of the capacity of the Microscope, can be diminished in two, and in only two, ways. We can either (1) increase a, or (2) diminish λ.

Since the work of Abbe and Helmholtz, increase in the magnitude of a, i. e. of the aperture, has been the great aim of all opticians who have attempted the improvement of the Microscope. Now $a = n \sin u$ where n denotes the refractive index of the medium in front of the first lens of the system, and u the angle made with the axis by the extreme ray from a central point of the object which can traverse the system. On purely geometrical grounds this angle u cannot exceed 65°, in order that a certain, even though very small, space may intervene between object and system (for the cover-glass and room for adjustment). Thus the value of $\sin u$ can scarcely exceed $0 \cdot 95$. When, as is generally the case, this geometrical limit has been reached, the aperture can only be increased by raising the value of n the refractive index of the medium in front of the objective. We are thus led to the principle of immersion systems. With respect to these it must be borne in mind that it is not sufficient simply to interpose between object and objective an "immersion liquid" of high refractive index: it is also essential that no medium shall be present between object and immersion liquid, even in the microscopically thinnest layer, whose refractive index is less than that of the immersion liquid. Otherwise, however high may be the refractive index of the latter, the aperture of the system will be reduced by total reflection to the magnitude $a' = n'$, if n' is the lowest refractive index of any layer occurring between object and immersion liquid.* Now for most preparations we are compelled to use cover-glasses. Those usually employed, which can be easily made and are consequently moderate in price, have refractive indices of $1 \cdot 52$ to $1 \cdot 53$. The limit of aperture to be attained by the use of such glasses is therefore only about $1 \cdot 44$ to $1 \cdot 45$. To obtain higher apertures, cover-glasses of high refractive indices must be used, and here many difficulties are met with.

The firm of Schott and Genossen have prepared glasses having refractive indices as high as $2 \cdot 0$. But cover-glasses made of such glass are very costly owing to the loss of material involved in their construction, since they have to be ground down to the required thickness of

* See this Journal, 1890, p. 11.

0·15 to 0·2 mm. from thicker blocks. The use of these cover-glasses also raises another difficulty, for, as above stated, no medium must intervene between object and objective with a lower refractive index than the number of the aperture, so that the object must be mounted in a medium whose refractive index has the required height. We do possess mounting media with refractive indices above 2·0; but the use of such media and the preparation of objects with them have their inconvenient side.

They consist chiefly of arsenic and phosphorus compounds which are liable to give off poisonous vapours or to explode during the preparation of the object. Experiments with the system of aperture 1·60 made with such mounting media have also shown that they are apt to attack the cover-glass so that the surface becomes rough and loses its transparency. Better results, no doubt, would have been obtained by the use of a different kind of glass, but in any case it is certain that the cover-glass of high refractive index will be more sensitive than the ordinary cover-glass, so that the choice of suitable mounting media will be considerably more limited than formerly. Altogether, then, the preparation of objects for these high apertures will be a much more difficult and costly process than with the apertures at present in use.

Another difficulty arises when the object is of organic nature and is attacked by these highly refractive mounting media. A large class also of organic bodies requires to be placed in special media as like their natural surroundings as possible. Such media have refractive indices from 1·33 to 1·6 at the highest. This circumstance therefore sets a limit for such substances to any extension of the aperture, and in this case recourse must be had to the second method for increasing the capacity of the Microscope, which consists in diminishing λ, the wave-length of the effective light.

Now the absolute energy of the sun's rays is different in different parts of the spectrum, and the sensitiveness of the eye varies for the different colours. The strength of impression of white daylight on the eye is therefore represented by a curve. The maximum point of this curve lies at $\lambda = 0{\cdot}55\,\mu$, so that from waves of this wave-length and those near to it the eye will receive by far the strongest impression, so much so that the partial images corresponding to the smaller and larger wave-lengths will be to a great extent rendered ineffective. But if these more energetic rays of wave-length $0{\cdot}55\,\mu$ and those of greater wave-length be in any way excluded, and only rays of shorter wave-length admitted to the eye, then, under favourable circumstances—i. e. with a sufficiently intense source of light—the light of these short waves can be made to a certain extent effective. Thus it is well known what an astonishing increase there is in the resolving power of an objective when, either by the use of monochromatic light, or by the interposition of absorption glasses, a preparation is observed under pure blue illumination. Often a preparation which with ordinary illumination is beyond the limits of resolution, with monochromatic blue light, with the same objective, and under otherwise exactly the same conditions, is clearly resolved. In fact, the eye is sufficiently sensitive for the wave-length $0{\cdot}44\,\mu$ to receive quite an intense impression when other light is excluded. A diminution of the effective wave-length from $0{\cdot}55$ to $0{\cdot}44\,\mu$, however,

is equivalent to an increase of the aperture, e. g. from 1·40 to 1·75, so that here we have a very considerable advance by very simple means.

Photography, as was first shown by Helmholtz, affords a means by which the capacity of the Microscope may be increased. The result, however, has not always corresponded to the theory. An important point indispensable for practical success has been often overlooked. It is in all theoretical deductions tacitly or even expressly assumed that the objective used for the photograph will give with the rays of shorter wave-length equally good images as with ordinary white light. This is, however, by no means the case. In fact, with the objectives of the ordinary type, such as alone existed a few years ago, such a result could not be attained. If the objective gave good images, i. e. was corrected for light of the wave-length 0·55 μ, the images from light of wave-length 0·44 μ were so bad as to annul the theoretical advantage of the increased resolving power. The method employed to obviate this difficulty was not very successful. It consisted in spherically correcting for rays of that wave-length, e. g. $\lambda = 0{\cdot}44$, which was most effective in the photographic process, and in effecting the chromatic correction so that the image corresponding to the wave-length 0·55 should coincide with the photographically effective image. Thus the latter could be correctly adjusted by the naked eye, but the defects remained that (1) the optically effective image was in itself bad (spherically under- and chromatically over-corrected, and (2) in the photo-chemically effective parts of the spectrum the concentration of the light was very incomplete, so that owing to the under-correction of this part of the spectrum there was danger of one part obscuring the image produced by the other.

The apochromatics have rendered the greatest service in this direction. In fact the advantage of their use in photomicrography is even more pronounced than their recognized superiority in ordinary microscopic work. This is due to the fact that with these objectives the images corresponding to the different wave-lengths right up to the violet are practically coincident in position and magnitude. Since their introduction cases have continually multiplied in which structures have been made visible by photography which could not be resolved by other means. But even with the apochromatic the conditions have not always been kept upon which an advance in the capacity of the objective depends. The author considers that such an advance by means of photography depends upon the following conditions:—

The system employed should be suitably corrected, so that the images resulting from the short wave-lengths may be sharply defined and coincident in position with that which affects the eye. The second condition is that the light of the required short wave-length should be photographically effective. This requires that (1) the source of light must emit waves of the required shortness, and these with sufficient intensity; (2) the rays corresponding to the larger wave-lengths must be excluded in such a way that the intensity of the short-wave rays shall not be too much reduced; (3) the photographic plate must be sufficiently sensitive for the light of the required wave-length; (4) all media between source of light and photographic plate must transmit the rays of the required short wave-length. This last requirement draws the limits to the

possible advance narrowest. The ordinary glasses, it is well known, only transmit a very small pencil of light of wave-length $0\cdot3\ \mu$. It appears, therefore, that the use of light of wave-length $0\cdot35\ \mu$ is almost the extreme point which we can hope to reach without increasing the difficulties of the work beyond measure. The use of the wave-length $0\cdot35\ \mu$ instead of the mean wave-length of ordinary daylight, $\lambda = 0\cdot55$, would be equivalent to a raising of the aperture from e. g. $1\cdot40$ to $2\cdot20$, while the use of the wave-length $0\cdot30\ \mu$ would raise it to $2\cdot57$. Under these circumstances, by central illumination, structures would be resolved which contained in the length of a millimetre, in the first case 4000 elements and in the second 4667 (distance apart of elements $0\cdot25\ \mu$ and $0\cdot21\ \mu$ respectively), while the corresponding numbers now with aperture $1\cdot40$ and white illumination are 2545 and $0\cdot39\ \mu$.

Measurement of Lenses.*—Prof. S. P. Thompson, F.R.S., read, at the British Association, a paper on "Some points connected with the Measurement of Lenses." He said that although lenses were used in so many departments of practical optical work—as, for example, in the making of telescopes, Microscopes, spectacles, and cameras—yet there is no uniform system of describing the properties of a lens. Moreover, all the text-books of the subject refer only to the particular case of thin lenses. He showed how all the properties of a lens could be indicated by specifying the position of four points, the two focal points and the two so-called "Gauss points," where the principal planes of the lens intersect the axis of it. No method has previously been given for the accurate determination of the Gauss points, and Prof. Thompson described an apparatus by means of which he can do this in the case of any lens or combination of lenses. The theory of the apparatus was also explained in detail. The testing of lenses having become a matter of importance in photography, the Kew Observatory has recently instituted a special department for the purpose; but it was not proposed to guarantee any great accuracy (say, within a quarter of an inch or so) in the measured focal lengths. Prof. Thompson hopes that the committee of the British Association, which he has been instrumental in establishing, will communicate with the authorities of the Kew Observatory, and induce them to carry their measurements to a greater degree of accuracy than they have previously contemplated.

Photographic Optics.†—Mr. A. Caplatzi writes, "There has just appeared under this title a work by Dr. Hugo Schroeder, which will be welcomed by practical opticians and amateurs alike. The latter will find in it an ample reply to the many requests for information addressed to these columns, and the former a practical treatise forming a reliable guide in their lucrative business of photo lens-making. In this royal octavo of some 200 pages a hard blow has been dealt to rule-of-thumb work. Those who will carefully peruse it need no longer work in darkness and uncertainty, but can do it in broad daylight and full conviction that every step forward will bring them one degree nearer to a successful result. And those students who have hitherto derived their optical knowledge from the meagre contents of text-books only, will be surprised at the number of further considerations requiring attention before a

* English Mechanic, liv. (1891) p. 36. † Tom. cit., p. 18.

practical plan for the construction of photo lenses can be laid down, and they cannot fail to admire the skill and patience that has given us the good lenses we possess, without clearly understanding the numerous conditions they must satisfy. Though the work deals mainly with the construction of photo lenses, it will prove itself as useful for the combination of any other kind of lenses, as the formation of images and the correction of chromatic and spherical aberrations, astigmatism, and diaphragms have been masterly treated. Actinism, of course, need not be taken into account in telescopic and microscopic lenses.

The work is preceded by a valuable list of the principal optical works that have appeared since Newton in English, French, German, and Italian, including fragmentary dissertations contributed to the learned societies, with annotations by the author. Whilst it numbers some 200 works on general optics, only six or seven refer specially to photography. First among these are Petzval's, published in 1843, 1857, and 1858. Dr. H. Zinken, Voightlaender's son-in-law; Dr. Lorenzo Billotti, Schiaparelli's assistant at Milan; and Prof. Seidel, Steinheil's friend, at Munich, also contributed considerably to the perfection of photographic optics. Still, nothing complete and easily understood appeared until the work under notice was called forth by Prof. W. Vogel in Berlin to form a supplement to his new 'Handbook of Photography.'

It is unfortunate that most of this valuable information is in German. The present complete treatise, however, will no doubt soon also appear in an English dress. Meanwhile I shall be pleased to help those who may desire to know something more of the practical rules and formulæ developed by the author, if the editor will afford me space. Dr. Hugo Schroeder possesses the rare advantage of being a linguist and practical optician, as well as a mathematician, and this advantage enabled him to simplify much that was hitherto obscure, and to bring together information that was scattered about in many inaccessible writings. He dissects all the lenses in actual use, and shows on what principles they have been constructed, and how they can be still further improved."

(6) Miscellaneous.

New Edition of Carpenter on the Microscope.*—We are glad to be able to call attention to the new (seventh) edition of the late Dr. Carpenter's well-known work on the Microscope. Dr. Dallinger has been engaged on this work for a considerable time, and has devoted much attention to it. When the last edition of this work was published the new era in microscopical optics had just opened; now, ten years later, it is necessary to give a full account of the work of Prof. Abbe. The consequence is that Dr. Dallinger has had to completely rewrite the first seven chapters. These, he tells us, "represent the experience of a lifetime, confirmed and aided by the advice and practical help of some of the most experienced men in the world, and they may be read by any one familiar with the use of algebraic symbols and the

* 'The Microscope and its Revelations,' by the late W. B. Carpenter. 7th ed., in which the first seven chapters have been entirely rewritten and the text throughout reconstructed, enlarged and revised by the Rev. W. H. Dallinger, LL.D., F.R.S., &c. xviii. and 1099 pp., 21 pls., and 800 wood engravings. London, 1891.

practice of the rule of three. They are not in any sense abstruse, and they are everywhere practical."

The second chapter deals with the Principles and Theory of Vision with the Compound Microscope, and of it Prof. Abbe, who saw the proofs, says, "I find the whole . . . much more adequate to the purposes of the book than I should have been able to write it. . . . I feel the greatest satisfaction in seeing my views represented in this book so intensively and extensively."

Dr. Dallinger has not shrunk from calling to his aid a number of specialists, among whom we may mention Mr. Crisp, the late Mr. Mayall, Mr. E. M. Nelson, Mr. W. T. Suffolk, and Dr. Sorby. Many sections of the book have been rewritten, nineteen new plates have been prepared, as well as 300 additional woodcuts, for many of which the editor returns his thanks to the officers of the Society.

Death of Mr. Walter H. Bulloch.—We regret to hear of the death, on Friday, November 6th, of Mr. Walter Hutchison Bulloch, the well-known optician of Chicago. The deceased was a prominent member of the Chicago Academy of Sciences and the local Microscopical Society. He joined the Royal Microscopical Society in 1882.

Universal Microscopic Exhibition at Antwerp.* — The following particulars are obtained from the 'Chemiker Zeitung':—

The "Exposition de Microscopie Générale, de Produits Végétaux et d'Horticulture" has just come to an end. It was projected by Dr. Henri van Heurck, Director of the Antwerp Botanical Garden, a microscopist of reputation. The plan of the promoters allowed of a strange mixture of products. Thus, along with brewed drinks, "schnaps" of all kinds (i. e. inferior liquors), were to be found pianos, mineral oils, guano, and other manures.

J. D. Möller, of Wedel, in Holstein, exhibited a collection of diatoms, including not fewer than 4026 distinct forms. Not only photographs of these species were on view, but the original specimens could be examined under a number of Microscopes.

The firm of Lumière & Collar, of Lyon, exhibited coloured transparent figures of microbes, just as they appear to the eye under the Microscope.

Along with Microscopes there were exhibited ovens for the cultivation of bacteria, apparatus for sterilizing, &c.

Among the exhibitors of instruments, a prominent place belongs to the establishment of Carl Zeiss of Jena. Their display included a selection of Microscopes, from the simplest to the most complex, combined with appliances for photographic projection, a set showing all the single parts of which a perfect Microscope is composed, and a collection illustrating the production of lenses from the crude glass through every stage of grinding.

Watson & Sons, of Holborn, exhibited a large selection of Microscopes for various purposes, especially an instrument made according to the indications of Dr. van Heurck, adapted for delicate researches and for photomicrography.

M. Nachet, of Paris, displayed instruments for research, general, scientific, and technical.

* Chemical News, lxiv. (1891) p. 169.

Powell & Lealand, of London, exhibited a large Microscope, said to be the most perfect as regards its stand. Hartnack, of Potsdam, had Microscopes and object-glasses, with photomicrographic fittings. J. Deby, London, displayed a collection of instruments by various modern makers with manifold appliances for illumination, arrangement for obtaining monochromatic light, as also a rich and interesting collection of preparations.

Adnet, and also Wainsegg, of Paris, and Seibert, of Vienna, exhibited a variety of bacteriological apparatus.

It strikes us as remarkable that no spectroscopic apparatus seems to have been exhibited.

The 'Chemiker Zeitung' remarks, with perfect justice, that it is impossible for an expert to pronounce on the value of any instrument, so long as it can only be seen in a glass case.

Meeting of American Microscopists.*—Dr. J. S. Billings, of the Army Medical Staff, in welcoming the visitors to Washington, said :—

"The President, Ladies and Gentlemen : It is my pleasant duty this morning to bid you welcome to Washington and to say to you that you are to make yourselves very much at home here.

Washington, as the capital of the country, is, in fact, the natural and proper home of all national associations, and they are beginning to discover this, for the number of such gatherings here increases every year. Within the last twenty years this city has become not only one of the most beautiful cities in the world, but has become one of the great scientific and literary centres of this country. The needs of different departments of the Government for accurate and precise information upon many subjects connected with their work have brought together here in the different bureaus many men specially trained in modern methods of investigation and research, each working some particular line, and more or less of an expert upon some one particular subject, yet also interested in the general progress of knowledge and the results obtained by his fellow-workers. Hence it is that our local scientific societies are numerous, well attended, and have an abundant supply of material to interest their members ; more so, probably, than the majority of local societies in other larger cities. Among these associations, we number an active and flourishing Microscopical Society, for although the Government has no department or bureau exclusively devoted to this subject, yet in almost every department and in many of the bureaus there are, and must be men who are familiar with the use of the Microscope, or they could not answer the questions which are liable to come before them at any moment. You may be sure, therefore, that the American Microscopical Society will always find an appreciative and interested audience for its papers and discussions here.

Of the numerous bureaus of the Government which make use of and are interested in the Microscope and microscopic technique, there is none which makes more constant use of this method of investigation, and none which in times past has done more to stimulate improvements in microscopy, than the medical department of the army, including the Army Medical Museum. The improvements in microscopic objectives

* Amer. Mon. Micr. Journ., xii. (1891) pp. 193-5.

which have been made during the last thirty years, have been, to a considerable extent, stimulated, suggested, and given definite direction by the application of photomicrography to the testing of such objectives as to resolving power and flatness of field under different conditions of illumination. Photomicrography with high powers became a practical and useful process when the use of direct sunlight as a means of illumination was introduced. This was first done in this country by Prof. O. N. Rood, of Columbia College, New York, in 1860–1. It was first suggested and applied in this country to histological preparations in the spring of 1864 in a military hospital here, in Washington, by two assistant-surgeons in the army, James William Thomas and William R. Norris, both now well-known ophthalmologists in Philadelphia. These gentlemen brought the results obtained by them to the attention of Dr. J. J. Woodward, of the army, who was engaged in the collection of materials for the preparations of the medical history of the war and the formation of an army medical museum, and by his direction the process was taken up, extended, and improved by Dr. Edward Curtis, now of New York, who was then engaged in making microscopic preparations to illustrate the pathological histology of certain camp diseases. Subsequently Dr. Woodward himself took the matter up, studying especially the optical combinations and technique of illumination adapted to secure the best results, and applying these methods as a means of minutely and accurately comparing the powers and performances of different objectives, and of making of such performances records whose accuracy could not be questioned, and which could readily be compared with each other.

When Dr. Woodward was doing the greater part of his testing work homogeneous immersion objectives were unknown, and with high powers the proper adjustment of the cover correction was a matter of the greatest importance to secure the best results, and was also often a matter of considerable difficulty. Dr. Woodward's skill and patience in making these adjustments and in regulation of the illumination were unrivalled. He often spent half an hour and more in securing a single cover correction, and the makers of microscopic objectives, both in this country and abroad, came to recognize the fact that he was not only absolutely impartial to his tests, but would get from each lens the very best work of which it was capable. The result was that they were glad to send him lenses for trial and to obtain his suggestions as to the possible means of improvement, which in this way was strongly stimulated. Since his death, microscopic and photomicrographic work have been carried on steadily in the museum, but on somewhat different lines, consisting mainly in the practical application of these methods to pathological research and to bacteriology. We shall be very glad to have you spend as much time at the Museum as you can spare, and to show you what we are doing there. In connection with this I wish to invite your attention to two cases at the south end of the main Museum hall which contain a number of Microscopes illustrating the development of and changes in this instrument and its accessories, from the time of the first known compound Microscope of Janssen, in 1685, down to the present time. In bringing together this collection during the last ten years, I have been greatly aided by [the late] Mr. John Mayall [Jun.] of London, who

has had so much to do with the formation of the magnificent collection of Mr. Crisp. Permit me to remind you, that as citizens and sovereigns of the Republic, the Medical Museum belongs to you, and that as American microscopists its collection of Microscopes and of microscopic slides and material should be a matter for your special interest and care. The collection is very far from being complete, it is only the beginning of what I hope will one day be gathered and carefully preserved in it, namely a specimen of every different form of Microscope, and especially of the earlier forms of American makers, of which we have none, and also specimens of the best work of American microscopists which can be shown by permanent preparations, and to secure this I ask your assistance.

The library of the Surgeon-General's office, connected with the Museum, is rich in books and journals relating to the Microscope and its uses, especially in its applications to biology and the medical sciences, and it is available to all who wish to use it. If you are not familiar with its resources and its index, I hope you will become so while you are here."

Recreative Microscopy.*—Mr. Henry Ebbage communicates the following note:—"A pretty object for entertaining friends is the arborescent growth of silver crystals. To show this, dissolve a small crystal of silver nitrate (or a piece of lunar caustic) in a few drops of rain-water. Place a drop of this solution in the centre of a slip of glass, and arrange it under a low power of the Microscope, concentrating the light from above by means of a stand condensing lens. Now take a piece of copper bell-wire 1½ in. long, and bend it like a capital L, then bend the longer limb to form a hook, which will rest anchor-fashion when laid down. Place this at the side of the drop of solution, allowing the hook to dip into it at the edge. Chemical exchange results, copper going into solution, and silver crystallizing out.

N.B.—Do not spill the solution as it stains black."

β. Technique.†

(1) Collecting Objects, including Culture Processes.

Methods of Bacteriological Research.‡—In an article of twelve pages Dr. Kirchner gives a compressed but clear account of all the methods of bacteriological research, and this is prefaced by a review of the general morphological and biological characteristics of bacteria.

The most important of the microscopical and cultivation methods are described with an accuracy of detail so that they are available for practical work.

At the end of the article are considered the examination of water, air, and soil, and also that of infectious diseases.

* English Mechanic, liv. (1891) p. 19.

† 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.

‡ 'Handwörterbuch der Gesundheitspflege,' pp. 69–80. See Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) p. 234.

Silicate-jelly as a Nutrient Substratum.*—Herr P. Sleskin, who has used the substratum of silicic acid, relates his experience in following out the preparation of the silicic acid as directed by Kühne, and its further modification according to Winogradsky, for cultivating nitrifying organisms. Three volumes of silicate of soda diluted to a specific gravity of 1·08 are mixed with 1 volume of hydrochloric acid (equal volumes of HCl sp. gr. 1·17 and H_2O). The two ingredients having been thoroughly mixed by stirring, the solution is dialysed in running water for about eleven days. The dialyser used was 19 cm. in diameter, and the layer of silicic acid 4–5 cm. thick. The fluid thus obtained has a specific gravity of 100·1 (about), is slightly opalescent, but transparent and liquid. In this condition it may be kept, for some time at least, in sterilized flasks. The next step is to evaporate the silicic acid down to 3/5 to 1/2 its volume in flasks plugged with cotton-wool.

The nutrient salts to be added are—Ammonium sulphate, 0·4; magnesium sulphate, 0·5; potassium phosphate, 0·1; calcium chlorate, a trace; sodium carbonate, 0·6–0·9. All the sulphates are mixed together and dissolved in as little water as possible; so too are the soda and potash salts, the extremely dilute calcium chlorate forming a third solution. All three are sterilized apart and thus preserved.

The two first saline solutions are mixed with the thickened silicic acid and the calcium chlorate afterwards added. A few flakes from precipitated salts are usually visible, but these do not interfere with the transparency of the medium, which is a fluid with the consistence of oil, and which, after having been poured into capsules, slowly and of its own accord thickens in a few hours to a jelly.

Substitutes for Agar and Gelatin.†—Herr Marpmann says that a perfectly bright and clear nutrient medium having all the properties of agar may be prepared in the following manner, by using an alga, *Sphærococcus confervoides*, found in the Mediterranean:—30 parts of alga are macerated in 2 parts hydrochloric acid and 1 litre water for two hours. The mixture is then washed thoroughly with water until blue litmus-paper no longer turns red. After decanting, there are added 700 parts water, 40 parts glycerin, 20 parts Koch's liquid pepton, 2 parts beaten-up albumen. The mixture is next boiled in a steamer for 20 minutes, then neutralized and filtered through a syrup-filter.

As a substitute for gelatin the author uses chondrin, which he extracts from rib and ear cartilage by boiling in a Papin's digester under a pressure of two atmospheres. The chondrin is filtered while hot through an ordinary paper filter, and when cold it is found to have set more firmly than gelatin. Besides this greater firmness, chondrin possesses the additional advantage of being more slowly liquefied by peptonizing microbes and of not losing its consistence after prolonged boiling, at least not till 140° C. are reached.

Miniature Tank for Microscopical Purposes.‡—Dr. Thomas S. Stevens remarks:—"Any collector from ponds and ditches, who has reached over the contents of a round bottle with a lens, knows how difficult it is to see and capture the interesting objects it may contain,

* Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 209–13.
† Tom. cit., pp. 122–4. ‡ Microscope, xi. (1891) p. 156.

on account of the distortion produced by the convex sides of the bottle. At a trifling cost a small flat aquarium, or large zoophyte trough, may be made that will obviate this difficulty.

Take two pieces of plate glass about 6 in. square, and from a dealer in rubber goods obtain a strip of pure rubber packing about 3/4 in. square, and so long that when bent into a horse-shoe or U shape the ends will just come to the top edge of the glass sides, while the curve shall not quite reach the bottom. If the rubber is flush with the lower edge, or a trifle below, the tank will not stand firm when finished. This rubber strip, bent into proper form, is to be cemented between the two glass sides. This may be easiest done by marking on a soft pine board a square exactly the size of the glass, and on this square bending the rubber strip into a U shape; keep it in position by placing pins or tacks, not through, but at the sides of the packing, at various points, so as to hold it in shape. Smear the upper side of the packing thoroughly with cement, lay on one of the glass sides, being careful to have it in position, press it firmly on the cement and place a weight above it to hold it down, and leave it overnight for the cement to harden. Smear the other side of the rubber strip with cement and place the other glass upon it, being careful to have the edges of both sides parallel. Weight it down, leave to harden as before, and the tank is done. The cement that I have used is Van Stain's Strateria. No doubt there are others that would answer the purpose as well. Marine glue would probably be better. The rubber packing comes in different sizes, from 1/4 to 1¼ in. in thickness. The aquarium may therefore be varied, both in size and transverse depth, to suit the needs and taste of the maker."

Apparatus for Gathering and Examining Microscopic Objects.*—Mr. G. M. Hopkins writes:—"One of the difficulties experienced by the beginner in microscopy is the finding and gathering of objects for examination. As a rule, cumbersome apparatus has been used. The conventional apparatus consists of a staff, to which are fitted a knife, a spoon, a hook, and a net; but a great deal can be accomplished with far less apparatus than this.

The engraving (fig. 89) illustrates a simple device by means of which the amateur microscopist can supply himself with as much material as may be required. It consists of an ordinary tea or dessert-spoon, and a wire loop of suitable size to extend around the bowl of the spoon, having the ends of the wires bent at right angles and hooked in opposite directions. To the loop is fitted a conical cheese-cloth bag, and to the bottom of the bag, upon the outside, is attached a strong string, which extends over the top and down to the bottom of the bag, where it is again fastened. The spoon is inserted between the bent ends of the loop and turned, and the point of the bowl is slipped through the loop.

The instrument is used in the manner shown in fig. 89, that is to say, it is scraped along the surface of objects submerged in the water, the water passing through the cloth, and the objects being retained by the conical bag. When a quantity of material has accumulated, the bag is turned inside out by pulling the string, and the pointed end of the bag

* English Mechanic, liii. (1891) p. 426.

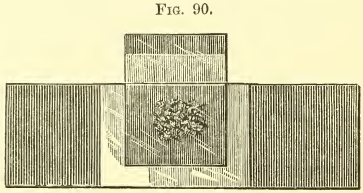
is dipped a number of times in water contained in a wide-mouthed bottle. The operation is then repeated. The objects thus washed from the bag are retained in the bottle for examination.

FIG. 89.

Gathering microscopic objects.

The common method of examining small objects of this kind is to place a drop of water containing some of the objects upon a glass slide by means of a drop-tube, then to apply a cover-glass, and remove the surplus water by the application of a piece of blotting-paper. This answers very well for the smaller objects, but the larger ones must be examined in a tank like that shown in fig. 90. This tank consists

FIG. 90.



Tank for microscopic objects.

of a glass slide, to which are attached three glass slips, by means of cement (bicycle-tire cement answers well for this purpose), the strips forming the bottom and ends of the tank. The front of the tank is formed of a piece of a glass slip attached to the strips by means of cement. To vary the thickness of the body of water contained in the tank when necessary, one or more glass slips are inserted behind the object."

(2) Preparing Objects.

Preserving Fluid.*—Prof. Strobel strongly recommends "il liquido Caggiati" as a preserving medium for entire animals and for anatomical preparations. Though it cannot be used in extremes of heat or cold, it is otherwise most advantageous; it is economical and simple, is not inflammable, and does not remove the colour of the objects preserved. Its composition in cubic centimetres is distilled water 1000, creosote 20, alcohol (at 75) 100 parts.

Investigation of Fowl's Ovum.†—Prof. M. Holl removed the ovary from a just killed hen, and fixed it either with chrom-osmium-acetic acid or 1/3 platinum chloride or Kleinenberg's fluid. After gradual hardening in alcohol, staining was effected with borax-carmine or hæmatoxylin, and after treatment with toluol, imbedding in paraffin followed.

Preparation of Embryos of Amphibia.‡—Mr. H. H. Field, in his investigations into the development of the pronephros and segmental duct of Amphibians, made use of the ordinary histological methods; many, however, of the hardening reagents and stains gave thoroughly unsatisfactory results. Embryos of *Rana* and *Bufo* can be satisfactorily killed in Kleinenberg's picrosulphuric mixture, and can be then successfully stained in Orth's lithium-carmine. The object should be exposed to the action of the stain as long as possible, but care must be taken to guard against maceration; with this object it was often found advantageous to stain the object twice, removing it after the first staining to strong alcohol. In passing the stained objects through grades of alcohol it is important to keep a little picric acid dissolved in the several fluids, in order to prevent the alcohol from extracting the yellow stain from the specimen. Embryos thus treated showed a very effective double stain; the nuclei are bright carmine, and contrast with the yellow colour imparted by the picric acid to the yolk-spherules among which they are found. Merkel's fluid is a good killing reagent, and should be followed by hæmatoxylin, and the decolorizing watched with care.

For *Amblystoma* the best treatment was Fol's chromic-osmic-acetic mixture, followed by Czokor's cochineal.

Investigation of Brain and Olfactory Organ of Triton and Ichthyophis.§—Dr. R. Burchhardt recommends for young Amphibian larvæ which still contain a considerable quantity of yolk, preservation in Rabl's fluid, and coloration with borax-carmine or alum-cochineal. For older larvæ Rabl's fluid, Altmann's process for chrom-acetic acid (1 per cent. chromic acid 10 hours, 5 per cent. acetic acid 24 hours, alcohol in slowly increasing quantities, and then 1/2 per cent. osmic acid for 5 hours). The preparations should be washed in water and stained with borax-carmine or Delafield's hæmatoxylin. Specially exact results were obtained by fixing with osmic acid and staining with hæmatoxylin. Excellent results are also to be obtained by the combination of borax-carmine with nigrosin or Lyon's blue in a weak

* Neptunia, i. (1891) pp. 301–2.
† SB. K. Akad. Wiss. Wien, xcix. (1890) p. 369.
‡ Bull. Mus. Comp. Zool., xxi. (1891) p. 203.
§ Zeitschr. f. Wiss. Zool., lii. (1891) p. 370.

alcoholic solution; fixing by picric acid will improve the results. Adult Amphibia should be decalcified and fixed with chromic and nitric acids; they should be stained with borax-carmine.

Preparing Epithelium of Mid-gut of Arthropods.*—Sig. O. Visart opens the living animal, keeping it immersed in running water, and injects by the anus a concentrated solution of methyl-blue in alcohol at 80. The gut is then ligatured, and left for a quarter of an hour. On opening the gut, the epithelium is found completely separate from the tunica propria, and furnishes most satisfactory preparations.

Mode of Preparing Crustacean Eyes.†—Mr. G. H. Parker states that most of his specimens were stained in Czokor's alum-cochineal and mounted in benzol-balsam. The agent used in depigmenting sections was an aqueous solution (1/4 per cent.) of potassic hydrate.

Preparing Segmental Organs of Hirudinea.‡—Prof. H. Bolsius found the following combination useful; after staining with hæmatoxylin the leech was washed for half an hour in a nearly concentrated solution of pure picric acid. By this double coloration the nucleus was stained by the hæmatoxylin, while the protoplasm of the segmental cells was yellow. This method introduces much variety into the coloration of the other tissues of the body. The muciparous cells are blue, the spermatozoa have cherry-red nuclei, the ova are rosy, the epithelial cells of the intestine violet-red with very deep nuclei, the ganglia are deep lilac, the nerve-chain almost black, the lymphatic and blood-cavities yellow to brown, the muscles are straw-coloured with red nuclei, and the connective tissue is of a clear yellow colour.

Eismond's Method of Studying living Infusoria.§—M. A. Certes reports that this method ‖ gives excellent results. He has attempted to improve on it by the addition of colouring matters, and he has fully succeeded with methyl-blue and violet dahlia No. 170; with the latter the species studied did not live long; with the other, survival is very much longer, unless the solution is too concentrated.

Demonstration of Presence of Iron in Chromatin by Microchemical Methods.¶—Dr. A. B. Macallum states that he has discovered a method of employing ammonium sulphide as a reagent for iron, by which he is able to show the presence of the latter in the chromatin of the nuclei of a very large number of species of cells hardened in alcohol. The iron does not here occur combined as an albuminate, but rather in a condition comparable to the combination seen in potassium ferrocyanide or hæmatin. Experiments with vegetable cells and such animal cells as those of the corneal epithelium of Amphibia show that the iron found is not due to the presence of hæmatin. Moreover, when chromatin is very abundant the iron reaction is very marked, while it is feeble in cells poor in chromatin. In the chromatin loops and filaments of karyokinetic figures the iron reaction is intense and sharply confined

* Atti Soc. Tosc. Sci. Nat., vii. (1891) pp. 277-85.
† Bull. Mus. Comp. Zool., xxi. (1891) p. 141.
‡ La Cellule, vii. (1891) pp. 5-6.
§ Bull. Soc. Zool. France, xvi. (1891) pp. 93-4.
‖ See this Journal, *ante*, p. 141.
¶ Proc. Roy. Soc. Lond., xlix. (1891) pp. 488-9.

to these structures. So far as the author's studies have gone he has not met with an instance of the chromatin of a cell not containing iron.

Culture of Terrestrial Algæ.*—Prof. A. Borzì gives the results of his long experience in the cultivation of terrestrial Chlorophyceæ, whether mixed or pure. It is essential in either case to have a contrivance for the constant and regular supply of fresh water. A porous substratum furnishes the best results, and he finds the most convenient to be a white calcareous tufa known in Sicily as "Syracuse stone" (pietra di Siracusa). The light must be allowed to reach the glass vessel in which the algæ are grown from one side only; the side where the fresh water is received and the surplus water drawn off must be the least illuminated; the zoospores will then collect on the wall of the vessel and form a green layer visible to the naked eye. It is impracticable to obtain as absolute purity in the culture of unicellular algæ as in that of bacteria. The plan recommended by the author to obtain comparative purity is a purely mechanical one,—removing the organism to be examined by means of a capillary glass tube, placing it in a drop of pure water, and repeating this process many times. He strongly approves Beyerinck's gelatin method † for the culture of algæ.

Re-softening dried Algæ.‡—Herr J. Reinke recommends eau de Javelle as an excellent medium for restoring dried algæ to an almost fresh condition. Even if they are quite black, the blackening will disappear with prolonged maceration.

Demonstrating Fungi in Cells.§—For demonstrating fungi within cells filled with plasma, Herr H. Möller advises that the fresh material should be treated with chloral hydrate either after the method of A. Meyer (5 : 2), or still better, in cold saturated solution. In this strength not only the starch but the cytoplasm are soon dissolved, and the process may be hastened by heating in a water-bath. It is necessary to constantly change the chloral hydrate, and at each interval wash the sections in water. By this procedure almost all the contents of the cell are dispersed, while the plasma of the fungi is unaffected, so that when stained a good picture is obtained.

Modes of Investigating Chemical Bacteriology of Sewage.‖—Sir H. E. Roscoe and Mr. J. Lunt have carefully recorded by means of photographs the microscopic and macroscopic appearances of the organisms found in sewage; they consider this to be of much importance, as bacteriological descriptions of organisms are frequently of little value from the want of accurate representations of the microscopic preparations and pure cultures.

For the isolation of micro-organisms the methods of gelatin plate-culture and of dilution were used, as well as two in which spore-forming organisms were isolated, or anaerobic organisms were isolated and cultivated. The anaerobic organisms were isolated by carrying crude sewage through three cultivations in pure hydrogen; spore-forming organisms were isolated by heating sterile broth in which a sowing had been made

* Neptunia, i. (1891) pp. 198–208. † Cf. this Journal, *ante*, p. 130.
‡ Ber. Deutsch. Bot. Gesell., vii. (1890) p. 211. § Tom. cit., p. 215.
‖ Proc. Roy. Soc. Lond., xlix. (1891) pp. 455-7.

from crude sewage to 80° for ten minutes; the still living spores were then further isolated by plate cultivation, either with or without previous incubation of the broth tube.

When the micro-organisms were to be photographed, they were stained with methyl-violet, and as this stain transmits chemically active rays, actinic contrast was obtained by using a coloured screen and iso-chromatic plates; the apparatus employed was of the simplest kind, and the source of illumination was a common duplex paraffin lamp.

Simple Method for obtaining Leprosy Bacilli from living Lepers.* —Dr. A. Favrat and Dr. F. Christmann state that by the following method, which also possesses the merit of improving the patient's appearance, leprosy bacilli can be easily obtained in quantity. The skin is first purified with soap, 1 per cent. sublimate solution, alcohol, and ether. One or more nodules are then burnt with a Paquelin's cautery. The cauterized place is then coated over with collodion, and lastly is protected by aseptic bandages. After 3–4 days (not later), the bandage having been removed and the sore washed with spirit, the scab is raised with a red-hot spoon and the subjacent layer of matter scraped off or inoculated directly on the cultivation medium. The sore rapidly heals, and no trace of the leprosy nodule remains.

Microscopical examination reveals an enormous quantity of bacilli, together with pus corpuscles and broken-down matter. The bacilli lie scattered about without any definite arrangement, occasionally being observed in little heaps, but never inside cells.

Cultivations made from the bacilli were unsuccessful, while the inoculation experiments are as yet unconcluded.

(4) Staining and Injecting.

Method for fixing Preparations treated by Sublimate or Silver (Golgi's Method.).†—Sig. A. Obregia gives a method for rendering preparations treated by Golgi's sublimate or silver procedure so permanent that they may be afterwards stained and protected with a cover-glass.

The sublimate or silver preparations are sectioned without any imbedding or after having been imbedded in paraffin or celloidin. In the latter case care must be taken not to use alcohol weaker than 94 or 95 per cent., at any rate for the silver preparation. The sections are then transferred from absolute alcohol to the following mixture:—1 per cent. gold chloride solution, 8–10 drops, and absolute alcohol, 10 ccm., which should have been made half an hour previously, and exposed to diffuse light. After sections are deposited therein, the vessel containing them is placed in the dark. The silver is gradually replaced by gold, and the mercury changed into gold amalgam. Finally, black delicate designs appear on a white field. According to the thickness of the section, the fluid is allowed to act from fifteen to thirty minutes, but even longer is not harmful. Thereupon the sections are quickly washed first in 50 per cent. alcohol, then in distilled water, and finally in a 10 per cent. solution of hyposulphite of soda, in which, according to

* Centralbl. f. Bakteriol. u Parasitenk., x. (1891) pp. 119–22.

† Amer. Mon. Micr. Journ., xii. (1891) p. 210. See Virchow's Archiv, cxxii. (1890).

their thickness, they remain from five to ten minutes. A longer immersion bleaches too much, so that the finer fibres disappear. Last of all they are thoroughly washed in distilled water twice renewed.

Sections thus fixed can afterwards be stained by any method, e. g. Weigert's, Pal's, &c., after which they are cleared up with creosote, imbedded in dammar, and protected with a cover-glass.

Throughout the procedure the sections must be manipulated with glass instruments, and not allowed to touch any metallic substance.

Rapid Staining of Elastic Fibres.*—Sig. E. Burci fixes the objects in alcohol, Müller's fluid, or corrosive sublimate; stains the sections with carmine or hæmatoxylin; washes them in water; dips them for a minute or two in saturated alcoholic solution of aurantia (ditrinitrophenylamine). The sections are then passed rapidly through absolute alcohol, cleared, and mounted as usual.

New Method of Spore-staining.†—Dr. H. Moeller describes the following method for staining spores. The cover-glass preparation, having been dried in the air, is passed thrice through the flame or immersed for two minutes in absolute alcohol. It is then placed in chloroform for two minutes, and afterwards washed with water; then for 1/2–2 minutes in 5 per cent. chromic acid, and again thoroughly washed with water. The preparation is then stained with carbol fuchsin, being boiled in the flame for 60 seconds; the carbol fuchsin having been poured off, the stain is decolorized in 5 per cent. sulphuric acid, after which the cover-glass is thoroughly washed with water. It is then contrast-stained by immersion for 30 seconds in aqueous solution of methylen-blue or malachite-green. The spores should be dark red and the rest of the bacterium green or blue.

Hæmalum and Hæmacalcium, Staining Solution made from Hæmatoxylin Crystals.‡—Dr. Paul Mayer highly recommends the use of two staining solutions made from hæmatein, the essential staining constituent of logwood. When pure, hæmatein is a brown-red powder and crystallizes with one or three equivalents of water. It is most frequently found in commerce as hæmateinum crystallizatum, a compound of hæmatein and about 9 per cent. of ammonia, and is more properly designated ammonia-hæmatein. When pure, hæmatein and its ammonia compounds should not only be perfectly soluble in distilled water and alcohol, but should remain so on addition of acetic acid. From hæmatein is prepared a solution called, for short, hæmalum.

1 grm. of the pigment is dissolved by the aid of heat in 50 ccm. of 90 per cent. alcohol, and then added to a solution of 50 grm. of alum in 1 litre of distilled water. When cold it may be necessary to filter, but if the constituents have been pure this is quite superfluous. The solution is ready for use at once. It may be necessary to add a thymol crystal in order to prevent the formation of fungi.

For staining sections, hæmatein is used like Boehmer's hæmatoxylin, and if required the preparations may afterwards be washed with ordinary water, distilled water, or 1 per cent. alum solution.

* Atti Soc. Tosc. Sci. Nat., vii. (1891) pp. 251–3.
† Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 273–7.
‡ Mittheil. Zool. Stat. zu Neapel, x. (1891) pp. 170–86.

Hæmacalcium, which is proposed as a substitute for Kleinenberg's hæmatoxylin, is made with the following ingredients:—hæmatein or ammonia-hæmatein, 1 grm.; aluminium chloride, 1 grm.; calcium chloride, 50 grm.; acetic acid, 10 ccm.; 70 per cent. alcohol, 600 ccm. The first two substances are to be pounded together very intimately; the acetic acid and the alcohol are then to be added, with or without the aid of heat. Last of all, the calcium chloride is added. The fluid is of a red-violet hue. After having been washed in neutral 70 per cent. alcohol the preparations are violet or blue, and rarely require to be treated with acidulated alcohol. If too red they may be treated with 2 per cent. aluminium chloride dissolved in alcohol.

Fraenkel on Gabbet's Stain for Tubercle Bacilli.*—Dr. B. Fraenkel seems to think that the method known as Gabbet's, the original communication of which was in the 'Lancet,' 1887, p. 757, is really the same in principle as one published by him in 1884. Gabbet's method consists in decolorizing with a mixture of H_2SO_4 and methylen-blue. Fraenkel's decolorizer, as given in No. 13 of Berlin. Klin. Wochenschr. for 1884, is nitric acid, besides which the formula includes alcohol. What should be the criterion for determining what is or what is not a new principle in bacteriology must remain open. At any rate, the formula given by Dr. Glorieux, published in Bull. Soc. Belge de Microscopie, 1886, pp. 44–8, is much nearer in principle than Fraenkel's, and differs from Gabbet's merely in that the latter contains no alcohol.

Syringes and their Sterilization.†—Dr. Tavel describes a syringe which is easily sterilized. Though chiefly intended for surgical purposes, it is useful in the bacteriological laboratory. The principle of the apparatus consists in avoiding the trouble of having to sterilize the piston part, which is quite disconnected from the syringe-needle portion. The piston, half the rod of which is notched, is furnished at the end with a screw and tap. To this screw is screwed on a metal cap, and into this latter fits the graduated glass holder or syringe, terminating at its other end in a steel needle. For laboratory work the author discards the piston portion, using the syringe-needle and adapting this for injection purposes by means of a Y-shaped glass tube. To the arms of the Y are fitted the syringe-needle and the bellows by means of rubber tubes.

The apparatus used for sterilizing these syringe-needles is then described. It is an ordinary rectangular vessel heated by gas, the jet of which is regulated by Reichert's thermo-regulator, but instead of water the reservoir contains paraffin. In this the syringe-needles, inclosed in test-tubes plugged with cotton-wool, are suspended, and thus are sterilized with hot air. The regulator is adjusted for 155°, so that the inside of the syringes may be kept at 150°, a temperature which is maintained for two hours. Higher temperatures are injurious to the steel of the needles. The sterilizing over, the test-tubes are taken out and wiped. The needles, kept inside till required, remain perfectly aseptic.

* Deutsch. Med. Wochenschr., No. 15, 1891. See Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 234.

† Annales de Micrographie, iii. (1891) pp. 564-73 (3 figs.).

(6) Miscellaneous.

Microchemical Reactions of Tannin.*—Mr. S. Le M. Moore distinguishes three kinds of tannin in plants, known by their different reactions with Nessler's fluid, viz.:—(1) tannin giving an immediate brown precipitate, occasionally with brown-pink tendency; (2) tannin giving a yellow colour, quickly becoming red-brown, and, finally, a cold-brown precipitate; (3) tannin giving a yellow colour, the yellow substance readily diffusing through the cell-walls into the surrounding fluid, thus leaving the cells colourless after a varying lapse of time. In addition to the functions hitherto ascribed to tannin, the author believes that it may have a more general relation to the turgescence of the cell; and that tannin is also most likely used up in the lignification of the cell-wall.

Cleansing Used Slides and Cover-glasses.†—Dr. F. Knauer says that the slides and cover-glasses of old preparations may be made as good as new by the following method, which he has adopted for some time past. 60–80 (say) slides are placed in a vessel holding about half a litre of 10 per cent. lysol solution and boiled for twenty to thirty minutes. The still seething vessel is then placed straight away under a strong current of running water until it streams back quite clear, after which the glasses are taken out and dried on a clean cloth. If the preparations be of comparatively recent date a 5 per cent. solution is quite sufficient.

Dr. J. B. Nias ‡ says:—"Bacteriologists and others who find themselves with accumulations of Microscope slides may be glad of the following hint for cleaning them. It is not given in any text-book that I can discover. Instead of warming the slides one by one over a flame, pushing off the cover, and then scraping away the balsam and cleaning with alcohol, I put all my slides together into a saucepan with a lump of washing soda, and boil them. The heat of boiling is enough to soften most cements and all ordinary resins used for mounting, and I then fish out the slides one by one, push off the cover-glasses, and put them back. The action of the soda is to convert the balsam or other resin into a grumous mass, which is easily wiped off with a little rinsing. Cover-glasses can also be recovered for future use in the same way, if desired. I think this method may be of service to laboratory attendants. Neither do I find anything on the surface of new covers and slides which will resist the action of hot water and soda; and so I prefer this way to the use of strong sulphuric acid and alcohol, or the other methods given in the text-books. The exact quantity of soda to be used is immaterial; a piece about the size of a mandarin orange to half a pint of water will do."

Method for the Estimation of the actual number of Tubercle Bacilli in Phthisical Sputum.§—Dr. G. H. F. Nuttall describes with great lucidity a method which he has devised for estimating the actual number of tubercle bacilli in sputum. Naturally enough the procedure

* Journ. Linn. Soc., xxvii. (1891) pp. 527–38.
† Centralbl. f. Bakteriol. u. Parasitenk, x. (1891) pp. 8–9.
‡ Lancet, 1891, p. 1414.
§ Johns Hopkins Hospital Bulletin, No. 13, 1891 (5 figs.).

is complicated, but as the separate stages or details are quite simple, and as the method is applicable not only to sputum but to any fluid containing micro-organisms, it seems probable that it may succeed where several other methods having a similar object have failed. Owing to its length we can only give the coarser details of the process, and for the finer ones must refer to the original, wherein the minutest particulars will be found.

The sputum is mixed with 5 per cent. caustic potash solution until it becomes perfectly fluid. The mixture is then shaken in a "milk-punch shake," in order that the bacilli may be evenly distributed throughout the fluid. The sputum is then transferred to a burette and dropped out on cover-glasses. The flow of sputum from the burette is regulated by means of a groove filed on one side of the aperture of the stop-cock. By this device the equable flow of a series of equal-sized drops was insured. The equal size of drops containing an equal number of organisms is, of course, the great desideratum. The best size for the drops was found to be 100–150 to the cubic centimetre of sputum.

The next step is to spread the sputum on the cover-glass so as to form a thin film. This is done on a turntable, the sputum being spread by means of a fine platinum needle, the point of which is bent at an angle of about 45°. The cover-glasses, kept in a perfectly horizontal position, are to be dried at 35–40°, and then surrounded by a ring of paint composed of lampblack and serum. The layer of sputum is next to be covered with a thin film of sterilized serum, which is coagulated at a temperature of 80°–90° C., and then the caustic potash must be extracted from the sputum by means of alcohol, the solvent action of the latter being aided by heating in the thermostat. The main object of the serum film is to prevent any of the bacilli being removed during manipulation. The sputum is then stained with phenol-fuchsin and decolorized by alternate immersion in alcohol and weak sulphuric acid. After having been washed with water, the preparations are merely dried on blotting-paper and then mounted in balsam.

The next part of the method deals with the actual counting and the apparatus necessary thereto. In a No. 12 eye-piece is inserted a diaphragm made of black paper in which a small hole has been cut. The aperture of the diaphragm is traversed by a hair-line. In order to be quite accurate about the fields, the latter are indicated by fixing a cork to one of the screws of the mechanical stage. The cork is armed with a thin wooden indicator terminating in a needle. The needle is made to point to the radial divisions on a cardboard scale placed by the side of the Microscope. The scale is affixed to a wooden circle, the centre of which is cut out in order to allow the stage-screw to be easily manipulated through the aperture. By this simple apparatus the size of the drop in fields is measured. The number of fields varies from 180–220, and the method of calculation is from a given case as follows:— A drop 200 field-widths in diameter is found to contain (average of 500 fields) 5 bacilli to the field; then $200^2 \times 0{\cdot}7854 = 31{,}416$ (area of drop in fields) $\times$ 5 = 157,080 bacilli to the drop.

The bacilli are counted as they pass under the hair-line of the diaphragm, and their number is registered by a machine known as the

"adding and counting register," which is fixed to the tightening-bar of the Microscope.

After giving illustrative cases, the author makes some remarks on the multiplication of tubercle bacilli in sputum outside the body, and then gives a short demonstration of the accuracy of the method.

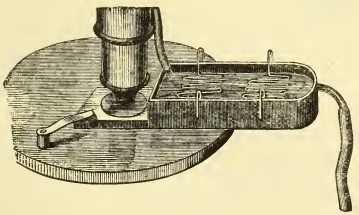
Colloidal Clay for Filtering Fluids containing Bacteria.*—Dr. H. Aronson uses argillaceous earth, from which he prepares the hydroxide of aluminium for filtering purposes in the bacteriological laboratory. The aluminium hydroxide is precipitated as a gelatin-like snowy mass from a 12 per cent. solution of sulphate of alumina or alum by means of excess of ammonia. After settling, the supernatant fluid is partly decanted and partly siphoned off, and the residue washed with distilled water until its reaction is completely neutral. The colloidal mass is then spread on the plate of Hirsch's porcelain filter and distributed so as to form an even layer, and the whole then sterilized in an incubator at 140°; previously to this any excess of fluid may be removed by a suction-pump in the usual way.

In this way is obtained a filter-mass which is at once uniform and homogeneous. Occasionally, after removal from the incubator, cracks and fissures may develope in the mass; these may be avoided by adding a little boiling sterile water before the mass have had time to cool.

The filtrates obtained by means of this medium seem to have been successful in most cases. The apparatus, however, will tolerate only very low suction-pressures, as anything like a high pressure produces cracks and clefts in the filter-mass.

Some Suggestions in Microscopy.†—Mr. G. M. Hopkins, writing in the 'Scientific American,' says:—"An object which always interests the microscopist, and excites the wonder and admiration of those who regard things microscopic from the point of popular interest, is the circulating

Fig. 91.



blood in living creatures. Nothing in this line has proved more satisfactory than the microscopic view of the circulation of blood in the tail of a goldfish. Thanks to Mr. Kent's invention of the fish-trough, the arrangement of the fish for this purpose has been rendered comparatively simple and easy.

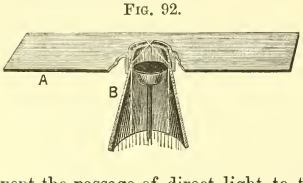
* Archiv f. Kinderheilkunde, xiv. (1891) pp. 54–8.
† English Mechanic, liii. (1891) p. 494.

The trough consists of a metallic vessel provided with a thin extension at one end near the bottom furnished with glass-covered apertures, above and below. The body of the fish between the gills and tail is wrapped with a strip of soft cloth, and the trough being filled with water, the fish is placed therein, with its tail projecting into the extension between the glass covers. The tank is arranged on the microscopic stage with the tail of the fish in position for examination. So long as the fish remains quiescent all goes well, and the beautiful phenomenon may be witnessed with great satisfaction; but the subject soon becomes impatient, and at the most inopportune moment either withdraws its tail from the field or jumps out of the tank, thus causing a delay which is sometimes embarrassing.

The uneasiness of the fish is caused partly by its unnatural position, and partly by the vitiation of the water. The latter trouble has been remedied by the writer by inserting a discharge-spout in one end of the trough, and providing a tube for continually supplying fresh water. The other difficulty has been surmounted by providing two wire grids, each having spring clips at their ends for clamping the wall of the tank. These grids are pushed downward near the body and head of the fish, so as to closely confine the little prisoner without doing it the least injury. With these two improvements the examination may be carried on comfortably for an hour or more.

In fig. 92 is shown a simple device for dark-ground illumination. Although it does not take the place of the parabolic illuminator or the spot-lens for objectives of low angle, it answers an excellent purpose.

Fig. 92.



To a metallic slide A, having a central aperture surrounded by a collar, is fitted a funnel B, of bright tin or nickel-plated metal, which is provided with a downwardly projecting axially arranged wire, upon which is placed a wooden button capable of sliding up or down the wire, the button being of sufficient size to prevent the passage of direct light to the objective. The light by which the illumination is effected passes the button, and, striking the walls of the conical reflector, is thrown on the object."

Artificial Sea-water.*—Dr. D. Levi-Morenos has some notes on the composition of artificially prepared salt water as used with success in aquaria, and in keeping oysters, for instance, in good health. Gosse's recipe suggested the following proportions:—Sodium chloride 100, magnesium sulphate 8·8, magnesium chloride 14·3, potassium chloride 3. These salts, dissolved and filtered, were added to fresh water till the average density of natural salt water was reached. In Perrier's aquarium the water contained the following salts in the proportions stated:—Sodium chloride 78, magnesium sulphate 5, magnesium chloride 11, potassium chloride 3, calcium sulphate 3.

* Neptunia, i. (1891) pp. 162–4.