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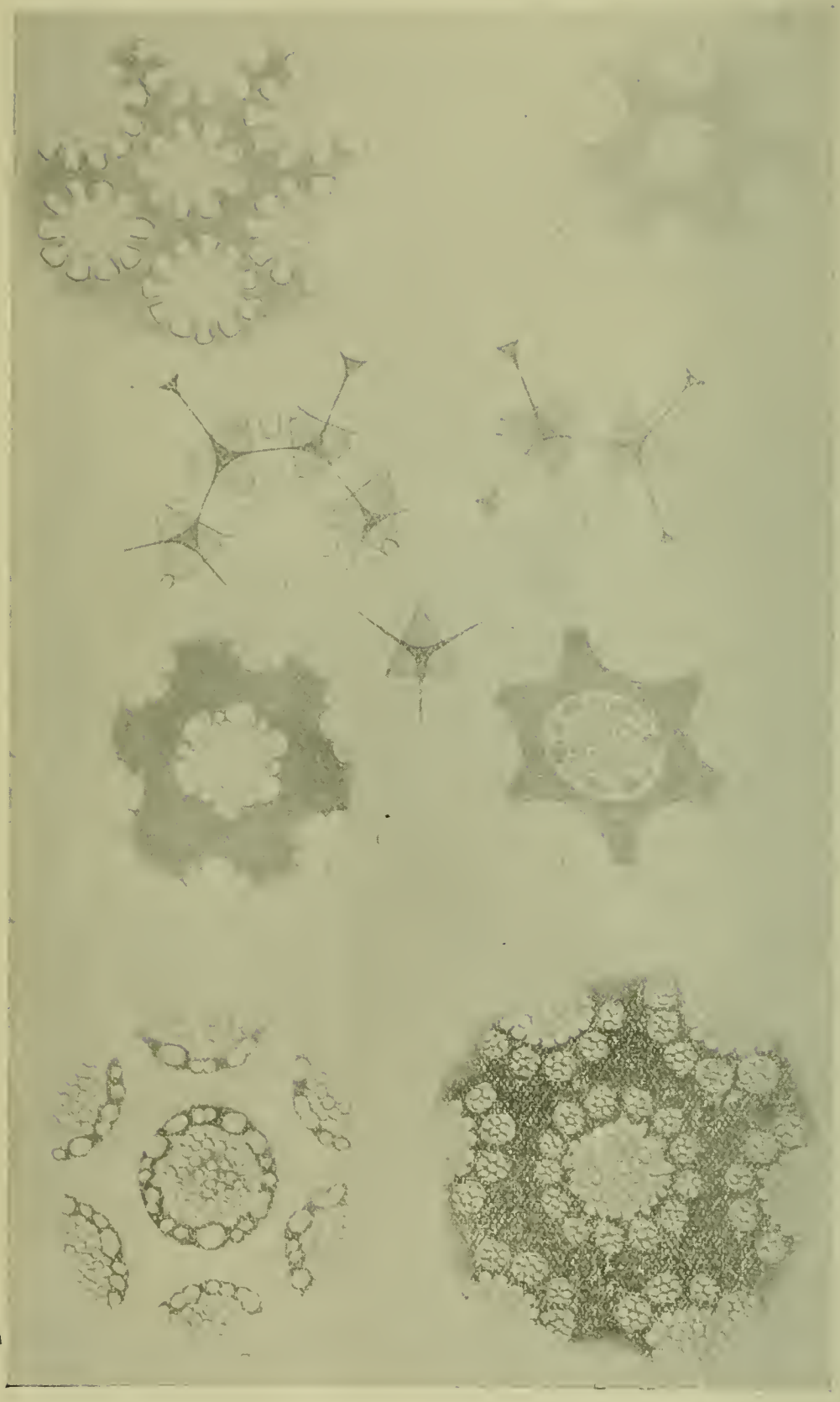

THE DIATOM, COSCINODISCUS ASTEROMPHALUS.

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CONTENTS.

On Diatom Structure.

BY EDWARD M. NELSON,

LONDON, ENGLAND.

READ BEFORE THE QUEKETT CLUB.

WITH FRONTISPIECE.

As time progresses, fresh discoveries in diatom structures become more difficult, for the simple reason that the larger or coarser structures have all been described long ago, and only the finer and more delicate ones are left. Among these, however, there is a good deal yet to be done, even with our present appliances, and when further optical improvements are effected there will be a great deal more to do. For of this we may be certain, that microscopes have by no means exhausted the details of diatom structure, any more than telescopes have exhausted the details of the solar system.

The first object to be recorded in this article is a variation of Coscinodiscus asteromphalus (fig. 1). Instead of the well-known pattern consisting of a ring of larger areolations surrounding the finely perforated membrane which covers a large polygonal cell, we have a circular ring of brackets projecting inwards to strengthen this delicate membrane or cover. The perforations in the centre of this membrane are excessively minute, and those round it are far more minute than those in an ordinary asteromphalus. These brackets are very similar in appearance to those supporting the delicate auditory membrane in the pygidium of a flea.

The second point to record is the discovery of a tertiary structute in an asteromphalus of the common form (fig. 8),

This tertiary structure must be looked for in the interior of those secondaries which encircle the finely perforated membrane. It is hardly necessary to say that these tertiaries are excessively minute. In form they are not unlike the secondaries of Asterolampra vulgaris.

This is a difficult image, not only on account of its minuteness, but because of its liability to be merely an interference image. There are certain minute microscopic images which, although difficult to see, are nevertheless undoubtedly true, for by no known method could they have been caused by interference. There are, on the other hand, some comparatively large microscopic images, which are so likely to have been caused by interference, that, apart from some special proof, they cannot be regarded as true. The Systephania diadema (Stephanopyxis corona), may be cited as an example of this latter class of objects. There, the structure, comparatively coarse, is one that is in all respects very similar to an interference image—so much so that for long I regarded it as such ; but further search revealed a broken piece with some of the supposed ghost structures sticking over the

edge, which proved the image to be unquestionably a true one. In the case now before us, however, its very minuteness prevents this test being applied, for it would be quite impossible to see at a fractured edge such a structure, even though it were several degrees coarser.

In microscopical images all fractures and edges are bounded by an umbra, coma, or undefined margin. This umbra tends to blot out all finer structures in its immediate vicinity. Thus the umbra of a primary blots out (either partially or wholly) the image of the secondary; much more, therefore, would it blot out a tertiary. For example, the postage stamp fracture in a Triceratium favus is not so easily seen as it might be on account of the umbra of the large primary structure. On the other hand there is no similar difficulty found with the postage stamp fracture of a P. angulatum or a N. rhomboides, for there is no coarser primary present.

It is obvious, therefore, that we must resort to some test of truth other than that of a fractured edge. Let us, therefore, try counting the number of spots in each aperture, for if they are ghosts they will be replicas of some pattern—e. g. six spots surrounding a central one. This, though a difficult operation, has been done, and both a five and a four have been seen, and sometimes the central one has been found wanting; further, these observations have been confirmed by an independent observer; therefore we may conclude that the image is not that of a ghost, but an indication of an actual structure.

If further evidence be required, it may be remarked that six spots with a central dot is the interference image or ghost of a quincunx pattern. Now, the images in question cannot possibly be interference images of the quincunx pattern of the primary polygonal structure of the valve, because the position of the intercostal ghosts would be in the intercostal areas; again, as a dernier resort, it may be said that it is an interference ghost of

the thirteen (more or less) spots at the periphery of the cell cap: to this it may be replied that anyone making such a statement is bound to prove that the interference ghost image of thirteen (more or less) spots in a circle is the same as that of a quincunx pattern; which of course could not be done. The crushing argument is, however, this: that there are structures precisely similar in every respect, both as to size and arrangement of spots, etc., which do not posssess this tertiary structure. The tertiaries cannot, therefore, be interference effects arising from the primary or secondary structures, otherwise they would be seen in all cases where the conditions were similar. This brings down the matter to two points: viz., that this tertiary structure is what it appears, or is an interference ghost of some as yet undiscovered quincunx membrane inside the polygonal cell. Against this latter supposition we have the dissimilarity in the patterns and number of tertiary spots. Fig. 3, is drawn from another diatom to show irregularity in pattern. There are, it will be noticed, six, five, four, and three dots round a very faint and ill-defined central spot. This central spot may or may not be an out-of-focus image of the eye spot on the lower membrane; anyhow, this is of no importance, for the argument rests on the peripheral dots. No one will contend for a moment that such an irregular pattern is merely an interference effect, and not the image of an entity; its very irregularity negatives the idea. Moreover, it should be noted that this is a coarse structure.

Now, with regard to the meaning of this tertiary structure, my theory is that it is a stage in the evolution of the central perforated membrane; the large spots at the periphery become divided up and thrown into the central portion, while other large ones are formed to be broken up in their turn until the valve has reached its adult size.

Fig. 7, shows this going on, for we have the large peripheral secondaries breaking into two dots, and the two dots thus formed becoming an integral portion of the central perforated membrane. It will be noticed that the large peripheral secondaries are in all stages; some are only just beginning to notch, others more advanced are nearly cut in two.

That something of this kind takes place, the figures in illustration of my paper on the "Formation of Diatom Structure," go to prove; further, Figs. 2, 4 and 9, illustrate the same thing, and are especially interesting because they are the first stages in the formation of the delicate perforated cap of the Asteromphalus; these may be found forming a graduated series from the elementary triangle up to the Asteromphalus pattern, as generally known.

The evolution of the peripheral dots from the triangle is interesting. I am now able to give a more complete account of this, owing to the discovery of some intermediate forms since my previous paper.

In the first instance, for a terminus ad quem, we have merely the plain polygonal (practically speaking hexagonal) structure with the usual eye-spot layer attached. Next we find an equilateral triangle at each intercostal, the apices of the triangle pointing to the centre of the hexagons, and the sides of the triangle cutting the sides of the hexagon at right angles (fig. 2). Next we find the triangle growing larger and the apices of it becoming blunted. Next a large dot is formed between the parallel sides of two adjacent triangles at a point about half way between the intercostal points. By this means six large perforations are formed in each hexagon, not at the angles of the hexagon, but at the bisection of its sides (Fig. 9). About this period the blunted apices of the triangle became notched, the notches deepen, and eventually become perforations. These perforations

which are at the intercostal angles, are not so large as those at the bisection of the sides of the hexagon. At this point, then, we have twelve perforations round each hexagon, six large ones at the bisection of its sides, and six small ones at the intercostal angles. At the next step the small intercostal perforations become elongated,then notched, and eventually divide into two. The microscopical resolution of these is precisely similar to the splitting of close double stars with a telescope (Fig. 6). Up to this point we have not had any trace of the finely perforated central membrane, but now it begins to appear round those intercostal spots which have divided. Fig. 5 shows this stage, and the suggestion I offer is that the minute perforations in the central portion are formed by the breaking up of the six peripheral intercostal dots. Thus far in no case has the large dot at the bisection of the side of the hexagon been seen to break up : the activity is confined solely to the intercostal dots; in short, it is from the intercostal point that the whole secondary structure originates. This brings us to what has been considered as the mature Asteromphalus pattern, a number of large perforations, more or less similar in size, surrounding a finely perforated sieve-like membrane, the whole forming a cap to the primary polygonal structure. The discovery of the new tertiary structure would seem to show that the ordinary Asteromphalus is not the terminus a quo of this diatom, but that after a time the whole of the peripheral dots, those at the bisections of the sides as well as those at the intercostal angles, break up and form an extended perforated membrane. By this means the intercostal silex becomes so reduced that the operation need only be repeated two or three times, when a uniformly perforated membrane will be formed over the whole of the valve. Diatoms of this form do exist : may they not be regarded as the final forms, while the common Asteromphalus is merely a stage in the evolution.

The following is an enumeration of the steps in the evolution:—

1st. A small equilateral triangle is formed at the intercostal junction of the polygonal cells (Fig. 2).

2nd. The angles of the triangle become blunted.

3rd. The blunted end becomes notched (Fig. 4).

4th. The notches deepen, and eventually becoming circular, form a perforation at each intercostal angle. At the same time the sides of the triangles form a larger perforation between them. The larger perforations are situated at the bisection of the sides of the hexagons (fig. 9).

5th. The peripheral perforations situated at the intercostal angles break into two, and by repeated subdivisions form the central finely perforated membrane (Figs. 6 and 7).

6th. The peripheral perforations, when the central membrane is complete, become more or less of a uniform size, and then break up into tertiaries (Fig. 8).

7th. Repetitions of the sixth process produce a uniform and delicate perforated membrane over the whole of the valve.

Beyond the sixth we cannot with certainty go, for we have no evidence concerning the seventh or final stage. The sixth stage may be the last, or it may go on repeating itself, after the manner of the fifth, until there is formed, not merely a cap to each polygonal cell, but a complete membrane covering the whole surface of the valve; and further, may not this be the adult form of the valve?

I have frequently seen such valves: they consist of a primary honeycomb structure, with an eye-spot layer below it precisely similar to an Asteromphalus, but having, in place of the well-known caps to the cells, a delicately perforated membrane extending over the whole surface of the valve. The suggestion (merely a suggestion, for I am

quite unable to prove it) I now throw out is that a valve consisting of polygonal primaries entirely covered with a uniform and delicately perforated membrane is the adult form of the diatom known as Coscinodiscus asteromphalus, and that this is formed by the continual repetition of the seventh stage.

All who have conscientiously worked at these images will, I believe, admit that all the stages up to the sixth are proved. With regard to the last, it may be left sub judice until further evidence is forthcoming. In conclusion, let me say that the diatoms were from the Nottingham deposit, and the slide was kindly given to me by Mr. Ingpen. All the stages up to the fifth inclusive have been observed in many different mounts, but the sixth has only been seen in that slide, and another old slide mounted by Moller : probably careful search would reveal the structure in almost any slide containing Nottingham Coscinodisci.

Let us in conclusion turn back for a moment to our first step. It would be very interesting to learn what was really the terminus ad quem of this species. Is it the primary polygonal structure, or the eye-spot layer; or is the eye-spot layer formed out of the polygonal structure, or vice versa ? As there is nothing particularly minute about these structures, it ought not to be difficult to find an answer to these questions.

In these Nottingham slides there are examples of a diatom which possesses large circular primaries with broad intervening silex. Very similar valves are also seen having a large number of primaries and consequently narrower intervening silex ; others have a still larger number, and so on until we find the circular primaries compressed into hexagons. It is important to note that this compression into hexagons commences at the periphery of the valve, the last primaries to retain the circular form being situated at the centre of the valve. This

is strictly in accordance with the law of diatomic growth viz., mature at the periphery, immature at the centre.

Although these forms have specific names allotted to them, I regard them as being early stages in the evolution of the Asteromphalus.

With regard to the eye-spot layer, it is altogether absent in the first stages, but makes its appearance when the primaries assume their hexagonal or polygonal shape. The terminus ad quem may be said to be a circular plate of silex sparsely perforated with circular primaries.

DISCUSSION.

Mr. Nelson said he was indebted to Mr. Ingpen for this beautiful diatom slide, the material of which came from the Nottingham (Maryland) deposit. It was just the sort of slide he liked to get hold of for this purpose, with the diatoms smashcd up all over the slide.

Mr. Ingpen said the slide had been sent to him as a sample of mounting in high refractive media; he believed in this case the medium was antimony. The deposit itself was a very interesting one. Professor Hamilton Smith at that time was experimenting a great deal on media of high refractive index, such as bromide of antimony, bromide of arsenic and piperine, giving an index of a little over 2, and they certainly made the objects mounted in them very distinct. There was another thing about this particular culture worth mentioning, and that was, it had never been doctored to any great extent, and therefore the structure stood well in the process of mounting; and the fact of Mr. Nelson finding these tertiary structures was no doubt due to the material not having been over-washed. When mounting in these high refractive media he always found this particular culture was much more successfully dealt with than any other. He had not mounted these slides for the sake of the beauty of the specimens, but rather to see the effects of the different media, and it was found almost impossible to mount many of these forms without fracture. Mr. Ingpen thought it highly probable

that if the diatoms were cleaned with acid and were not properly washed afterwards some of the mounting media would be affected in the way suggested, the perfection of the structure in his slide was no doubt due to the fact that the material had not been previously over-cooked.

The President thought that in considering the special forms which silica might take in the structure of a diatom a possible explanation might be found on the theory that the process of deposit might be a vital process and not a mechanical one.

Mr. Ingpen said he was much inclined to this view, and often thought that the development of a diatom might be somewhat the same as the formation of a shell, where the animal absorbed lime in a soluble form on one side and deposited it in an insoluble form on the other side. The tertiary structure might possibly be the very origin of the diatom.

On Some Micro-Cements for Fluid Cells.

BY CHARLES F. ROUSSLETT.

READ BEFORE THE QUEKETT CLUB.

Three years ago I read a note on Clarke's Spirit-proof Micro-Cements, which had proved reliable for many years in securely sealing micro cells containing methylated spirit. I then recommended this same cement for cells containing watery fluids. It appears, however, that I too hastily jumped to the conclusion that, because this cement is good for spirit mounts, it would also be equally good for watery fluids, a conclusion which seems natural enough. After three years' experience I must pronounce it a complete failure as regards watery fluid mounts, while the spirit mounts remain as good as before; and I hasten to communicate these facts, so that others may profit by my experience.

The possession of a thoroughly good and reliable cement for fluid, and especially watery fluid mounts, is so important to microscopists, that I hope all who have any

special knowledge and experience in this direction will communicate their experience. Owing to the failure of the cement to keep in the water, I have had to remount over three hundred slides of Rotifers. I have a few deep cells filled with fluid which have kept very well for four or five years, but it appears the greatest difficulty is met with in small shallow cells, which contain only a fraction of a drop of water.

I have continued my search for a cement which will absolutely prevent the evaporation of water. It seems that all cements containing solid particles become porous, or at least slightly pervious to watery vapor, when once quite dry, and therefore it appears to me that one of the objects to be aimed at is to prevent the total evaporation of the solvent.

A cement that has been specially recommended is a mixture of two third parts of gum damar dissolved in benzole, and one third coachbuilder's gold size. This has undoubtedly very good qualities ; it flows readily from the brush, is very adhesive to glass, and makes a perfect joint between the cover-glass and slide. The water or solution of formalin in the cell has no effect upon it. On the other hand I have been warned against the use of gum damar, because when its solvent is all evaporated it can be scraped off as a white powder; but the admixture of gold size modifies this property considerably. Gold size alone has long been known as a good cement, and I have been recommended by a maker to use the variety known in the trade as extra stout, which is not so liable to run in, and which never becomes absolutely hard and dry, and when scraped comes off in strings. Pure gold size, however, does not adhere so strongly to glass as when mixed with gum damar. The solvents of gum damar and gold size are benzole and turpentine, and it has been suggested to me that the evaporation of the last trace of these solvents might be prevented by covering the gold size with

a ring of another cement, having a purely alcoholic or naphtha solvent.

Such a cement is Ward's brown cement. In some respects this cement resembles Miller's caoutchouc cement, but it contains no caoutchouc and is more finely grained and better in quality; it dries with a fine gloss, never cracks, and is not attacked by benzole or turpentine; its composition is not known, and its best solvent is a mixture of wood naphtha and methylated spirit.

Taking all these considerations into account, I have of late sealed my fluid cells, which are hollows ground out in the glass slip, containing mounted Rotifers, as follows: —First I close the cell with the gum damar and gold size cement, which fixes the cover-glass firmly to the slide: when this is dry I put on a ring of the pure gold size, and when that is dry a third ring of Ward's brown cement, taking care that each succeeding coat slightly overlaps the previous ring.

In this way I hope that my slides may remain permanent, but it will take years of experience before a definite judgement can be pronounced.

Recently a friend sent me an old slide, an anatomical preparation, mounted in a deep glass cell in some watery fluid with a small bubble in it, which he said was at least forty-five years old. He could give me no further information, except that the bubble had always been there, and had most probably been left in the mount intentionally to act as a safety valve against the expansion of the fluid. Here, then, is a fluid mount which can be called permanent, and I therefore tried to find out who the mounter was and how it was closed. It has two coats of cement, first a yellow cement like gold size, and over that some black alcohol cement. Mr. W. Suffolk, gave me full imformation on this slide, and valuable suggestions regarding fluid mounts in general. He say:

"The slide you send me is by Hett, a well-known

mounter. The period you name is correct; I have a few of his slides purchased about 1854-5. All I have are still in good condition ; the included air bubble is in all of them. I consider the yellow varnish to be gold size, which was much in use by the best of the early mounters ; the black finish is probably a mixture of shellac and lamp black, added merely for ornament and harmless under the circumstances; the bare gold size would not have been sightly enough for sale purposes. The usual mounting fluid for such preparations (thick injected organs) was dilute alcohol ; sometimes a saline solution, sometimes a little creosote has been used with the alcohol, with or without glycerine. In remounts I have successfully replaced it with distilled water plus 5 per cent carbolic acid plus 5 per cent glycerine ; this mixes freeiy with any of the above fluids and gets rid of the volatile alcohol—which I think it well to do. The enclosed air bubble I consider good, and when a cell contains any quantity of fluid I always use it : it acts as a spring, the expansion of the fluid in a perfectly filled cell sooner or later forcing the weakest point of the cell when it expands from increased temperature. The included air bubble is a very different thing from an intruded one, which always means mischief.

"I have had no experience of formalin, but from its composition should consider that, like alcohol, it would attack shellac, therefore think you have done right in using gold size. I have rather a dislike to varnishes and cements of which the composition is unknown, as, however well they may be spoken of, you are quite in the dark as to their action on mounting fluids.

"My varnishes are practically two : gold size,—this is composed principally of boiled linseed oil, possibly combined with a resin dissolved in turpentine, in fact a first-rate oil varnish. It should be obtained at a good shop and be of the best quality. My other varnish is shellac, which I always make myself ; it is almost as easy to make

as gum-water. Simply place the best shellac you can obtain in a large bottle not more than half or three-quarters full with strong alchohol, shake up frequently until the lac is dissolved : it is best made at ordinary temperature. The result is a turbid mixture. If you make about half a pint, add a teaspoonful of red lead to the solution and shake it up; in a few days the red lead will have carried down all the thick matter, leaving a fine clear varnish, which decant.

"Shellac varnish resists glycerine and its compounds, but has the defect of being brittle, unlike gold size,which retains considerable toughness after the lapse of many years. For ringing balsam mounts I add twenty drops of castor oil to 1 oz. shellac varnish, but for other purposes use it pure.

"The principle of securely closing a cell containing fluid is to use first a varnish not affected by the medium, irrespective of its brittleness, and when the fluid is secure, use with a reliable varnish for security.

"My practice with glycerine and compounds is this, which will show what I mean. When I have put on the cover I clear away with a fine pipette and suction as much fluid as I can, absorbing the rest carefully with scraps of blotting paper; I then ring with a solution of damar in benzole, a weak and bad varnish, but which has the useful property of sticking to wet glass. When dry I wash under a tap so as to remove all traces of glycerine; this is important, as no sound joint can be made so long as glycerine or other fluid that persistently adheres to glass is in the way. I follow with a ring of shellac (pure), when dry wash again and then give two or three coats of shellac, washing if there is any doubt about the joint being perfectly clean. The glycerine is now secure, and the mount is then secured with gold size, which should be laid on thinly and at least six coats. I have never found a slide to leak. If gold size had been used at once the

very penetrating glycerine would soon have found its way through. This is the general principle, varying the varnishes as circumstances and the mounting fluid require. Marine glue, much in vogue for securing cells to glass, has failed to stand the test of time; india-rubber compounds are notoriously unstable. For fixing glass cells on slips I have made a firm joint with a mixture of red lead and white lead after the manner of the blacksmith's hot-water joint. I take flake white from the tube and work powdered red lead into it with a palette knife until it becomes too stiff to work comfortably; I then dilute with gold size only a small portion at a time as I want it, as it hardens very rapidly when the gold size is added. Press the cell hard down so as to have the thinnest secure film of cement, and keep in a warm place for a few days; turn off the surplus ooze before it has become too hard, and do not use the cell for at least a fortnight. Your excavated cells and solid cast ones used by Hett are of course better than any built-up ones."

It will be noticed that he advocates five or six thin coats of gold size instead of one thick one. The reason is that oil varnishes, after the evaporation of the turpentine, harden not by evaporation of the oil, but by its oxidation, forming a tough layer, and therefore the process goes on best in a thin layer. Finally, I would recommend for extreme durability for fluid mounts such as mine, in which the mounting fluid is formalin, or similar watery fluids, first a coat of pure damar in benzole, then a coat of the mixture of damar and gold size, followed by three or four thin coats of pure gold size at intervals of twenty-four hours, and lastly a finishing coat of Ward's brown cement. The "extra stout" gold size I am inclined to discard again, because it does not adhere to glass so firmly as the ordinary quality.

DISCUSSION.

In discussing the above paper, Dr. Measures said it al-

ways seemed to him that they should keep in mind the two great principles involved in the use of any cement—that it was wanted to secure the contents against evaporation, and to prevent the cover from moving. His experience was that they must prevent by all means the escape of the last trace of solvent from the cement employed, whether the object was mounted in balsam or in fluid, and to ensure his the best way was to cover the cement with another which was dissolved in a different solvent—gum damar was a good material when the solvent was benzole, and a spirit cement over that would prevent the escape of the last trace of benzole and be perfectly durable. He has some slides which were cemented with gum damar and gold size overlaid with shellac and spirit cement, and these had remained good after a number of years. He had also some which had been sealed with Brown's cement, but this had failed. The first requisite was to get something which would not be acted upon by the fluid in the cell; and the next was to give this an overlapping layer of some other cement which was not acted upon by the solvent of the first.

Mr. Karop said he had a series of slides of Tipula preserved in glycerine, which were ringed with Miller's caoutchouc cement, which seemed quite good. Mr. Morland's idea was of course very good, but it would, he thought, be found rather difficult to make one's own cement in the small quantities required, especially such materials as gold size. Hollis's glue was good, and he believed this was made of caoutchouc dissolved in *wood* naptha, *not* mineral naptha.

Mr. Earland said he had recently received three slides, dated 1851, containing desmids, and the fluid in these was intact; they were sealed with what looked like gold size.

Mr. Nelson said he had some very large insect preparations, and none of these had ever burst through the expansion of the fluid. Another thing worth mentioning in connection with this subject was that he had been told by a mounter always to make the cover-glass a little concave, and then the changes of temperature would not be so likely

to lift the cover when the fluid expanded, as the cover-glass would be able to spring sufficiently to resist the pressure.

Mr. Hinton said all those which he had seen of this kind had an air bubble in them, but he did not think they were any the worse for this ; no doubt it allowed of the expansion and contraction of the fluid in the cell.

The President thought they had had a practically useful discussion. About thirty years ago he belonged to a small microscopical society at Manchester, and the members used to practice mounting objects ; bnt they got into very considerable difficulties with regard to aqueous fluids. Some few of their members, especially one man, however, seemed to have no trouble, and specimens which he mounted then were as good as ever at the present time. He thought that personal equation came in very considerably into the matter—one man failed whilst another seemed to know exactly how to do it without knowing why. He thought Mr. Morland had hit the right nail on the head in saying that the cements sold under the same name were not always of the same composition, and therefore they might succeed at one time and yet fail at another with cement of the same name, although purchased from the very same place. He felt pretty certain that very much depended upon the ability of the person who did the mounting, as also upon the way in which the slides were mounted, and to what temperature they were afterwards exposed.

Microscopy by the General Practitioner.

BY W. N. SHERMAN, M. D.

MERCED, CAL.

No instrument yet devised by the ingenuity of man equals the microscope in its universal application to research in the broad domain of science, and its practical relation to medicine is well known. The greatest advances made in placing scientific medicine on its true foundation date from the application of the microscope to physiological investigations. The microscope alone re-

veals the true nature of many diseases—hence what a beautiful and useful thing it is to settle so many points that are a great trouble to the physician.

The younger graduates in medicine have received instruction in practical microscopy and bacteriology,but the older ones did not enjoy the same advantages when persuing their medical education. If, therefore, the senior practitioner expects to continue his calling as an up-to-date man, he must dig it out himself, with the aid of good modern text-books, and earnest personal effort. This is not a difficult matter if given intelligent study. We too often imagine a scientific subject hard to master, but after starting we are surprised that our interest and pleasure therein has made it easy, and gradually and almost unconciously we progress toward the end of a complete and practical knowledge of our subject.

The first necessity is a good modern instrument and a few good objectives—this is vital to success—for inferior instruments will only hinder and discourage the worker. With a little attention to details and the directions furnished with instruments, one is soon able to acquire sufficient skill in manipulation to begin the study of simple objects. Starting with such clinical work as the microscopical examination of urine, and a careful study of urinary crystals, deposits, tube casts, pus corpuecles, etc., he is soon ready and anxious to take up a higher class of work, such as the examination of the blood. With these studies one may readily acquire sufficient skill in the manipulation of a good instrument to do all ordinary work in practical microscopy. As the interest increases the pleasure also grows and one soon finds his enthusiasm a strong stimulant to greater effort and better work.

I shall now briefly allude to a few of the uses of the microscope that will serve to make us better physicians, to increase our interest in our calling, and to render us more worthy the confidence and esteem of our fellow

practitioners and of our patrons. The microscope will be found of great service in the diagnosis and prognosis of disease and a trusty guide to point out indications for treatment. Probably the most frequent use will be the examination of sputa for the tubercle bacilli. This is the quickest and surest method of diagnosis and the best guide as to the progress of a case under treatment. Sometimes the sputa reveal streptococcus infection which is an indication for treatment by an antistreptococcus serum. The same test and treatment has been very successful in many forms of septicemia.

The subject of hematology is of itself a vast field for study. It has become an important matter in the diagnosis and treatment of many diseases and is the only reliable method of differentiating idiopathic anemias, pernicious anemia, leucocythemia, and the various forms and degrees of blood impoverishment. The discovery of bacteria and other micro organisms in the blood has prompted more frequent examination and careful study of this vital fluid. Ehrlich's method of staining blood corpuscles has widened our knowledge of the part played by the phagocyte in its warfare against bacterial invasion. Virchow's teaching of the emigration of the white cell in pathological processes and Schultz's observation of the morphological dissimilarity of the white cells in circulating blood, are important advances in hematology, which we cannot afford to ignore. So many conditions and diseases are illuminated by a careful study of the blood, that it has become a very important and valuable procedure in medical diagnosis. The Widal test applied to the blood of typhoid fever patients has become a reliable means of diagnosis and is used by the Board of Health of the city of New York as the official test.

Before attempting to work with the microscope in clinical or other studies, it is essential to future success and good work that one should become thoroughly acquaint-

ed with the instrument and its manipulation, and that one learns how to see with it. The fact of having a fine microscope and accessories in one's possession, is not evidence of ability to use it and work with it. It is well for the medical microscopist to commence with the primitive forms of life and to observe and study them closely.

The study of the white blood corpuscles can never mean much to the man who has never studied an ameba; still, there is no reason why every medical man or student should not frequently see this primitive form of life so nearly representing true active protoplasm. Amebas are easily obtained from the horse trough, ponds, or ditches, and may be observed undergoing their characteristic changes in form, and to the student of phagocytosis, examples can often be offered of amebas devouring bacilli, some thriving on them and others dying from the poison developed by the ingested bacteria. It is not alone the white blood corpuscles whose prototype is found in our ponds and ditches. The action of the cilia of ciliated epithelium is difficult to see and understand, yet the vorticella, a comparatively large organism will nicely illustrate a cell of ciliated epithelium. This one-celled animal, when expanded, presents a cup-shaped form whose motion is capable of creating currents to or from this cup.

The fresh water algæ present a good example of procreation in their earliest development. The diatoms offer a fascinating field of study. They are valuable as test objects and teach us to observe fine, delicate outlines and structure and to test the defining and resolving power of our objectives. They are common and may be found in both fresh and salt water, in oyster juice, in polishing powder, earth, sand, and in our drinking water. These are profitable studies to the medical man who desires to master his instrument and will teach him to judge accurately the value of what he sees and to gain a clearer insight into the life of individual cells than he can ever

learn from the more practical medical microscopy of the schools.

The efforts being made in favor of pure food must necessarily bring the scientific physician into this field of research and his services will be required to assist the proper authorities in their regulation of this matter. He should be able to detect adulterations in nearly all forms of food products. The public regards him as the guardian of its health; to merit the confidence reposed in him he must of necessity know something of these questions and how to investigate them. A special microscopist or chemist cannot reside in every country village, and the simple forms of this work must be done by the physician. He should be able, by modern methods, to detect a tuberculous cow and to analyze and examine infected milk. At present an overwhelming weight of evidence points to the presence of bacilli in the milk of infected cows. It is not necessary that tuberculous ulcers should be present on the udders. Bacilli have been demonstrated in the milk from udders without tuberculous lesions discoverable to the naked eye. One should be able to carefully make the tuberculine test, and, if necessary, to verify it after death by a microscopical examination.

The physician should be able to inspect meat, and to detect impurities in lard, spices, starches, and various other food products. In the great pork packing establishments of Chicago, female microscopists are constantly employed and from each slaughtered hog, a small piece of the diaphragm is extracted and placed under the microscope in order to detect the trichina spiralis if present. This was made necessary by discrimination abroad against our meat products. It is high time we at home took some steps to protect ourselves, and demand an official inspection of all meat sold to the public, by a competent officer. The physician must prove the best qualified person for this work, if he is well informed in mi-

croscopy and bacteriology. With the constant advances of sanitary science there will be increased demands for the services of scientific physicians, in the country as well as in the city.

The physician is often called as an expert and if he is able to use the microscope, in its application to forensic medicine, his knowledge will be in demand and his evidence of greater value. The microchemistry of poisons is of itself a vast field of research. The detection of crime is often made easy by means of the microscope in honest and skilful hands. It may be of great value in detecting erasures and changes in manuscript, the character of ink, lead penciling, tracings, and the detection of forged signatures. The identification of blood stains, seminal stains, hair fiber, etc., often leads to the discovery of the guilty, and has given to the microscope its reputation as an "unerring detective."

The application of the microscope to agriculture, horticulture, viticulture, mineralogy, contagious diseases of animals, food adulterations, the analysis of water and milk, and the micro-chemical investigations of poisons, etc, might be profitably dwelt upon did time permit.

For the ambitious investigator or student there is a vast field for investigation outside the limits of medical microscopy. There is scarcely a microscopic object whose every detail cannot with the camera's aid, be reproduced on paper, hence the great value of photomicrographs for illustrations or reproductions. Glass negatives of microscopic objects have been admitted as evidence in courts of justice. From such negatives the lantern slide may be made and the same object be projected upon canvass screens by means of sunlight or electric light. This is a valuable method of teaching classes and illustrating lectures. Charcot illustrated pathological conditions in his clinical lectures in this manner and it was looked upon as a marvelous, novel, and attractive method of teaching.

What shall we say in regard to practical bacteriology for the physician? In modern clinical diagnosis and the application of pathological methods, microscopy and bacteriology are so intimately associated as to be inseparable. In the physician's laboratory the microscope and culture tube go together. If we expect to keep abreast of the times and to give our patients the benefit of modern medical science, we must be able at least to solve the ordinary clinical problems of the laboratory. Our microscope must be a modern instrument to be used alongside the clinical thermometer and the stethoscope. It is as necessary in general practice as are aseptic procedures in surgical practice.

The prevailing opinion that the necessary laboratory apparatus are intricate and expensive is an error. A laboratory can be furnished in a simple and inexpensive manner, and the essential laboratory equipment, like the essential knowledge of its manipulation, may be gradually acquired. The items of expense are not an obstacle, for any ingenious mind may devise and a skilful hand construct, most of the necessities. Aside from a good microscope stand and good objectives, a microtome, hematocrit and centrifuge are desirable and the incubator, sterilizer, and water bath are indispensable. A sharp razor, a cork, and a little paraffin, answer the purpose for a modest beginner until he is able to afford a hand or machine microtome.

For a centrifuge the tubes may be purchased for a trifle and their adjustment to a rapid rotating apparatus can be improvised. If one can command a small electric motor, it is an improvement over a hand apparatus. The Arnold steam sterilizer is a cheap and efficient device and may also be used for sterilizing surgical instruments. If this is not attainable, one may use an ordinary Hoffman's iron water bath, and upon it place a copper pail with perforated bottom, and an inside rack for holding the tubes.

When the steam escapes from the tube, in the cover, it is evidence of the proper interior temperature. The oven of a small gasoline stove answers the purpose of a dry sterilizer and incubator. The chemical reagents and glassware are simple and inexpensive. Most of the large cities now afford special instructors in bacteriology, but when one cannot afford the time and means for such a course, he must content himself with the only recourse left, viz; to work it out, by practice alone, with the aid of a few good books.

Every general practitioner should possess the ability to give to his patients the benefit of as thorough diagnosis and scientific treatment as a progressive science affords. At the present time there is no excuse for the physician who does not prepare himself to perform thoroughly the work demanded in routine daily practice. Two of the most common errors in diagnosis apply to typhoid fever and diphtheria, both of which are easily recognized by means of laboratory methods. The clinical impossibility of making an accurate diagnosis of diphtheria without a bacteriological examination should prove an inducement to a progressive physician to equip his own laboratory for accurate examinations. Boards of health in cities often supply the physician with sterilized test tubes and swabs for collecting material from suspicious cases when they are returned for laboratory examination and report. Where such advantages are not available, as in country practice, a careful bacteriologist might make a satisfactory examination with a bake oven, wash boiler and other kitchen utensils, as substitutes for the regular sterilizers and incubators.

The same truths apply to many other diseases common to every day practice. If time does not permit the making of the various necessary culture mediums, they may be obtained ready for use from the laboratory of Parke, Davis & Co., or that of some near-by bacteriologist·

Gelatin, agar, blood serum, etc., may be preserved for a long time by taking the following additional precautions, which shortens the labor of frequent preparation. Test tubes are cleaned, plugged in the ordinary way except the cotton is only one-half the usual length. They are then sterilized in a hot air sterilizer and filled immediately; after filling, the cotton plug is pushed into the tube half an inch below the top and the plug of antiseptic cotton put over it. After thorough sterilization the tubes are closed with a rubber cap and placed in an air-tight fruit jar to prevent evaporation. The antiseptic cotton used for this purpose is best soaked in a solution of water 100, alcohol 20, and copper sulphate 3, then dried slowly.—*Occident. Med. Times.*

EDITORIAL.

In 1899.—The Journal is now entering the twentieth year of issue and with the exception of last year (1898) has made some perceptible advance in every volume. We purpose this year to still push ahead and make a better journal than ever before. Many recent occurrences have encouraged us to think that we can do so. We have a list of friends who have stood by us through thick and thin, whom we prize highly and for whom we purpose to do all in our power. Our circumstances are now more favorable for making a first class periodical than heretofore and without any boasts or promises we are going ahead to do our best during 1899. We hope to send out each number on the first day of the month in which it is published. Microscope dealers have more and more declared that the business does not warrant them in advertising and certain manufacturers think it wise to enter the field of journalism themselves so as not to have to pay us for advertising. We are therefore obliged more and more to rely upon our subscribers for the money with which to pay printer's bills and shall appreciate prompt action on their part in making remittances. A word to a friend is of course sufficient.

Our list of foreign correspondents has largely increased and we now have friends all over the world in nearly every nook and corner. We trust our home lists will correspondingly increase during the new year. To all we extend a hearty greeting.

LETTERS TO THE EDITOR.

GATES' DOUBLE MICROSCOPE.

Unless he can make opposite defects in each correct each other I do not see what he can gain. I tried a somewhat similar experiment with the telescope or spyglass in my schoolboy days, but it did not take me long to find out that mere magnification without a corresponding improvement in deffinition and resolution was worthless. I think it possible that a double objective might be made so that defects of one system might be corrected by the opposite defects of the other, and in this way a much greater working distance be obtained with a greater depth of focus and equal resolution. If my health was better I should try some experiments in this line myself.—Wm. A. Terry.

Will Report own Work Later.—"I have corresponded with Mr. Gates as to his Double-Microscope. I have thought favorably of it notwithstanding Dr. Stokes' strictures, especially as I made some experiments thirty years ago on it. I got a 1-5th and a 1-5th in the eye-piece and a 1-15th and a 1-5th in the eye-piece. That was as high as I could go. I had not higher objectives to try, and only sunlight. The definition was good enough. I intended to publish a paper on the subject—and still intend to do so when I can get the time. But I must submit my paper to both gentlemen. The Journal shall hear from me on it soon."—Arthur M. Edwards, M. D.

Get Actual Results Properly Certified.—"I am too busy to write an article on Dr. Elmer Gates' tandem microscopes. Would it not be much better to get some one right on the ground who knows Dr. Gates and can see his laboratory, etc. I would suggest Dr. Frank Baker. Every

one would readily assent to any statements he might make concerning actual observation of the results obtained by him. Perhaps a suggestion can be made in passing, and that is to call attention to the saying. 'By their fruits ye shall know them'. People are a little suspicious of too tall claims. No one is suspicious of actual demonstrable results, and those are not yet forth-coming so far as I have seen."—S. H. GAGE.

Amplification is not Resolution.—"I regret to say that my impressions regarding the instrument are most unfavorable. Why? Because the result obtained is *solely* an increase in amplification (over that of a single microscope); and further, because increase in amplification beyond that easily obtained with a single microscope, *if not accompanied with a proportionate increase in resolving power*, is practically useless. What users of the microscope most urgently want today is increase in resolving power. This only increases with what Prof. Abbe has termed numerical aperture. Doubling, or trebling, a microscope in no way increases numerical aperture and, therefore, in no way increases resolving power. The writer has used methods similar to those used by Mr. Gates. Photos 14 and 18, for instance illustrating "An Experimental Study of Aperture as a Factor in Microscopic Vision," *Trans. Am. Microscopical Society*, 1896, were taken with essentially a double microscope. The idea is not new to others, although new to him.

In 1892, the writer applied the idea in astronomical photography, using a portrait lens instead of one of the microscope objectives. Great amplification (for portrait lens) was obtained (as with the Gates double microscope). Resolution, however, was wanting (as with the Gates double microscope). An account of this apparatus and of the defects in its products, illustrated with photographs of an eclipse of the sun, may be found in *The American Annual of Photography and Photographic Times Almanac* 1897, P. 155. Corresponding defects, as to resolving power, are inseparable from the products of the Gates' double microscope camera."—A. CLIFFORD MERCER.

Methods Unscientific.—"I think Prof. Gates has rushed into print too soon with his expectations based upon some very crude experiments. He discards expensive, high grade lenses and obtains better results with two microscopes in tandem, fitted with any old things for objectives. To prove his point he exhibits a confessedly 'bad' photograph. At length surmising that the ordinary objectives are not bearing the strain very well he has ordered specially corrected objectives and some new apparatus,—things which would have been done and the whole tested fully, by a wise experimenter before writing at length and drawing on his imagination. Any distinct advance on these lines will be through the aid of specially corrected objectives of the highest grade, and much very expensive apparatus, and the whole promises to be a very unwieldy laboratory instrument projecting an 'invisible image.' There is nothing better in sight, at the present outlook, than the 'expensive' but handy homogeneous immersion objective which projects a visible image."—S. G. Shanks.

MICROSCOPICAL APPARATUS.

Ohmann-Dumesnil's Camera.—It must be capable of drawing out quite a distance to get amplification. Hence a bellows is absolutely required. At a photographer's supply house, an old second hand camera can usually be got for a dollar. The box with bellows is thus provided cheaply. The box opening should be about 8 x 10 inches. Then procure a truncated four-sided pyramid of tin whose base is the size of the front frame of the camera and whose smaller end is 3 inches square. Its height, about 10 inches. Any tinner should make this for 50 cents. In a piece of wood ¼ inch thick, bore a hole in the centre, of diameter the same as that of the microscope tube and close the small end of the tin with it. Secure the other end of the tin extension to the front of the camera. Paint the tin a dull black inside and outside as well. Cover the edge of the round aperture in front with velvet so as to secure a

snug fit around the tube of the instrument. At the back part of the camera must be attached a frame grooved to admit sliding in the plate-holder easily. A similar plate-holder may be used to receive the pane of ground glass. Use sunlight, kerosene, gas or incandescent electric light at pleasure.

Jones' Excelsior.—W. Jones and Co., 5 Devonshire St., Holborn, W. C., London, offer for 67 cents post paid a microscope with nickel plated tube and extra finished solid brass stand, adjustable reflector and object glass magnifying 3,000 "volumes"—whatever that may mean. Probably it may magnify 10 diameters. That is very good for 67 cents. Not being worth one dollar, probably it can get within the U. S. Chinese wall free of duty and will make a nice present for children. International postal order for 2 shillings 9 pence can be got at all money order offices.

MICROSCOPICAL MANIPULATION.

Frozen Sections.—To prepare the tissue, fix and harden it in a mixture of one part formol to ten parts Muller's fluid, during three hours. Wash and put in dilute alcohol, afterward up to 95 per cent alcohol. Remove to solution of acacia and sugar. Then freeze, or place in 4 per cent formaldehyde for 15 minutes and then freeze. When prepared in some such manner or even if perfectly fresh, put the tissue with some formol and gum acacia fluid upon the specimen holder of the microtome. Then play down upon it a small stream of ethyl chloride, methyl chloride or anestile (a mixture of the two). Hold the tube of ethyl chloride about a foot above the specimen, move it from point to point in the specimen until the upper portion is coated with crystals of ice which are very minute and do no harm. The specimen is frozen in 40 to 60 seconds. After cutting, place the sections in 50 per cent alcohol and mount as usual. Stain when desired. Fifteen minutes will suffice for the entire operation.

Urinary Sediment.—These may be photographed with an exposure of 40 to 60 seconds according to rapidity of

the plate's action and the degree of magnification of the object. Gaudet used a wooden box for a camera. It is of the shape of a four-sided truncated pyramid. The lower opening slides over the microscope tube and the upper carries a frame of ground glass. He first removes the eye-piece and places the camera on that part of the tube which holds the objective. The slide is well fixed to the stage and focused as usual but the head of the observer is covered with an opaque cloth. Remember that the image of the object will be reversed but real and magnified. After exposure the plate is developed and the prints made as usual.

BACTERIOLOGY.

Tick Fever in Cattle.—C. J. Pound has worked out protective inoculation for Tick Fever. Up to the present time some thousands of head of cattle have been inoculated, and the results have proved highly satisfactory, for when such cattle are subjected to gross tick infection, or injected with virulent blood, they remain perfectly immune, while the "controls," or unprotected animals, subjected to the same conditions, are readily attacked with severe acute fever, which often ends fatally. So successful have our experiments been that numbers of stock-owners, whose cattle are threatened with an invasion of tick, have lost no time in systematically inoculating the whole of their herds. He has been kept busy inoculating a number of valuable stud bulls and heifers from Victoria and New South Wales, which are to be sent to North Queensland, where the ticks are very bad. He had some bottles containing several species of ticks (preserved in 3 per cent formalin,) with notes on the locality, and the animals native or other, that he had found them on. In some species he had only kept females, as the males are never seen even by observant bushmen, who are constantly meeting with ticks. Every species of tick (other than the genuine Cattle Tick, the cause of "Tick Fever" or "Red-water" in cattle) is known to bushmen, squatters, etc., as

the "Scrub Tick." Museums throughout the colonies cannot give any reliable information respecting ticks, nor have they even a representative collection of the known species which are commonly met with. Consequently there is a vast unexplored field of research in this direction.

Laboratory.—In San Francisco is a Bacteriological laboratory which makes examinations of water, milk, blood, urine, sputum, pus, diphtheretic membrane, and tumors. It gives instruction to pupils and supplies curative materials. Dr. George L. Helms has charge of microscopy and histology.

BIOLOGICAL NOTES.

Nasal Tissue.—Dr. J. Wright has studied the nasal tissue of the bull and finds it erectile and much larger than in the ox or cow. Selecting the thickest portion of the tissue in case of a bull for comparison with that of the castrated animal, he hardened, embedded in celloidin and made sections perpendicular to the surface. The sections were stained and photographed. There is very great difference shown thereby as may be seen in the New York Medical Journal for Nov. 19, 1898.

MICROSCOPICAL SOCIETIES.

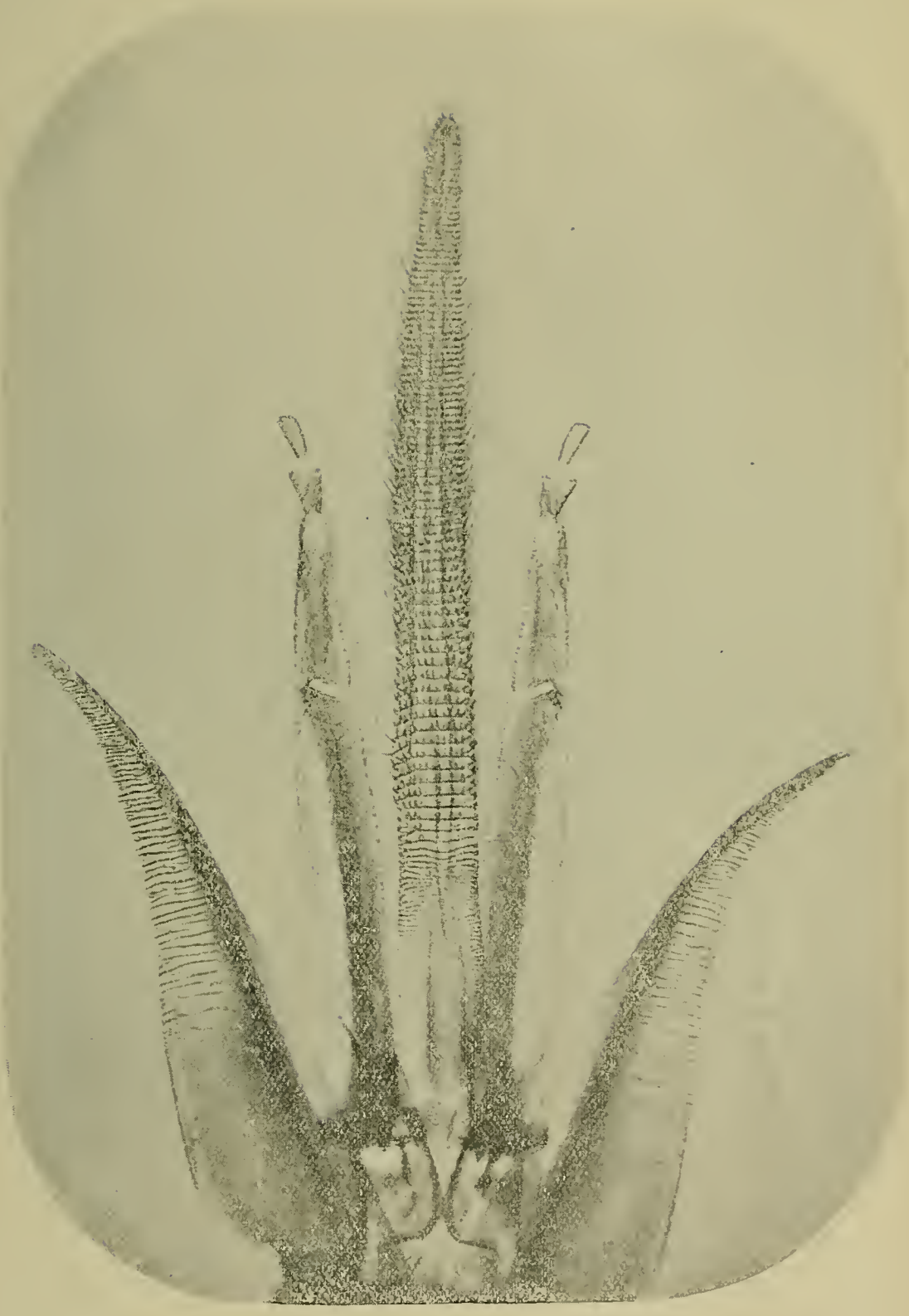
St. Louis Society.—President, Dr. H. M. Whelpley; Dr. Millard Bartlett, Secretary; Treasurer, Dr. A. E. Taussig. Meetings, second and fourth Thursdays each month at Dr. Bremer's Laboratory, 3723 West Pine Boulevard, St. Louis, Mo.

N.Y. Society.—The New York Microscopical Society has a membership of 114 (87 Active, 20 Corresponding, 7 Honorary). It was incorporated in 1877 and holds an annual meeting on the first Friday in January. Its semi-monthly meetings are held on the first and third Friday evenings of each month from October to June at the Mott Memorial Library, 64 Madison Avenue. From May 1897 to May 1898, 16 meetings were held and there was an average at-

tendance of 25 persons. The admittance fee is $5 and the annual dues are $5. Thirteen volumes of its Journal have been published. The library contains 2,000 volumes and the cabinet 5,000 specimens. The officers for 1899 are Dr. Frank D. Skeel, President, Vice president, F. W. Leggett; George E. Ashby, and Rev. J. L. Zabriskie Secretaries; Treasurer, James Walker; Ludwig Riederer, Librarian; Curator George E. Ashby. The Committee on Admission is H. C. Bennett, G. H. Blake, T. S. Nedham, Fredk Kato, G. E. Ashby; On Publications, J. L. Zabriskie, H. A. Parmentier, G. W. Kosmak, James Waker, F. D. Skeel.

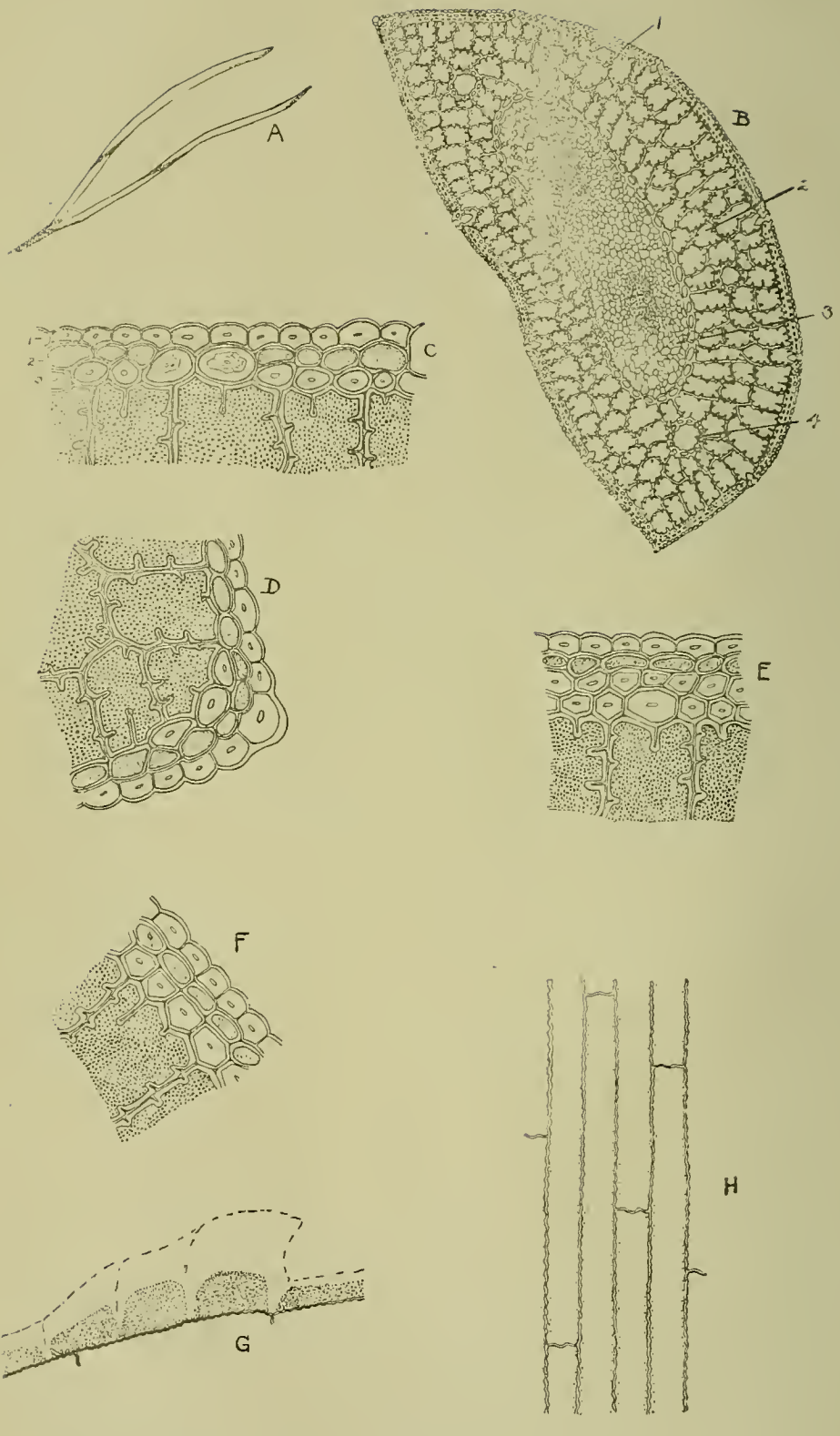
NEW PUBLICATIONS.

New Book.—The Subconscious Self and its Relation to Education and Health by Louis Waldstein, M. D., New York, Charles Scribner's Sons, 171 pp. $1.25. This is a very interesting contribution to the study of psychic phenomena such as telepathy, mind reading, hypnotism, hysteria, trance, neurasthenia, mind healing, dreams, insanity and similar phenomena. The author, like other medical men, sees material things and phenomena rationalistically and writes carefully from that stand-point. He gives no evidence of knowing the ancient and eastern teachings of the existence and power of soul and spirit as separable entities from physical bodies. If he would add this study to his other work he would greatly enrich his knowledge. The term subconscious self is paradoxical and absurd from the non-material stand point. It is undesirable from the psychic standpoint but it is useful to men like the author who do not wish to grant that man is spirit, has a soul, and sometimes lives in a physical body. When it is known that spirit (in soul) exists consciously and independently of body at times and what they do apart from the body, the theory of a subconscious self falls flat. But we welcome Dr. Waldstein's book cordially and recommend it to all our medical readers, which includes more than half of our constituency.



TONGUE OF HONEY BEE.

ENLARGED 40 DIAMETERS.



THE SCRUB PINE LEAF,

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CONTENTS.

Histology of the Leaf of Pinus Virginiana.

BY FLORENCE A. McCORMICK.

WITH FRONTISPIECE.

Pinus Virginiana, scrub pine, is a slender and usually small tree, but sometimes attains a height of 110 feet. It grows in sandy soil, from Long Island to South Carolina, west to southern Indiana and Kentucky. The tree can be identified by the straight prickles on its ovate-oblong cones. There are usually two leaves in a fascicle, rarely, however, three are found. The transverse section of a leaf from a fascicle containing three leaves is almost semi-circular in outline, but that from a fascicle containing three leaves is almost triangular, with the dorsal side curved outward.

The leaves are from four to nine centimeters in length, fig. A. For study, leaves were gathered in October at Knoxville. Portions from the middle of the leaf were

embedded in paraffine by the usual methods, and sections were cut with the microtome. The sections were double stained with eosine and gentian-violet. In examining a transverse section from the periphery towards the centre, three distinct regions can be easily identified. These regions are the cortical, fig. B, 1, the mesophyll, fig. B, 2, and the fibro-vascular, fig. B, 3. The average long diameter of a transverse section is 1.6 mm., and the average short diameter is 0.8 mm.

Cortical Region.—The cortical region includes epidermal and hypodermal or "strengthening cells" and the guard cells of the stomata. Along the angles of the leaf are serrations perceptible to the touch, fig. G. These serrations are prolongations of the epidermal cells with the cuticle greatly thickened.

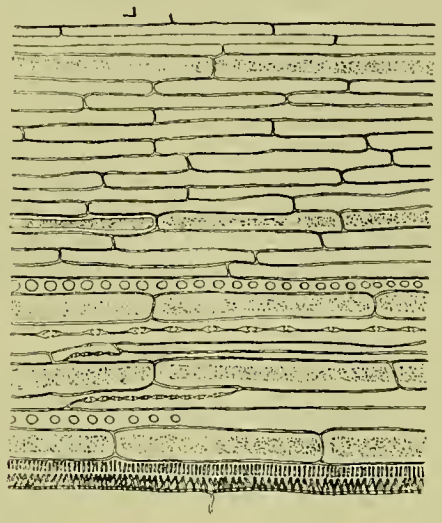
The number of rows of cells in the cortical region varies. The dorsal side of the leaf has from three to four rows of cells, fig. E, while the ventral side has from two to three rows, fig. F. There is only one row of epidermal cells proper, fig. C, 1. In some places one, and in others two rows of strengthening cells are found. The row of cells immediately underlying the epidermal cells is somewhat different in appearance from the other cells of the region, fig. C, 2. In transverse section, they appear somewhat elliptical in outline, and are filled with granular contents. The epidermal cells have a greater uniformity of shape than the other cells of the region. The outer wall of these cells is curved bow-fashion and the inner wall is curved or straight according to the character of the wall of the adjoining cells. In tangential section the cell walls of the cortical region, perpendicular to the surface of the leaf, are sinuous, but the walls parallel to the surface are straight. Accordingly in radial longitudinal section and also in transverse section the cell walls are plane, but in tangential section they are sinuous. The average length of the epidermal cells in cross

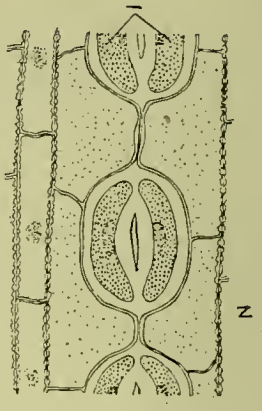
section is 14 2-3 mm. and the average width is 10 mm. their average length is 252 1-5 mm. In cross section the "thin-walled" cells above referred to are 16 2-3 mm. long and 8 mm. wide. The strengthening cells vary greatly both in size and shape. The average length in cross section is 18 2-3 mm. and the average width is 15 1-3 mm. A noticable difference between the epidermal cells and the other cells of the region is in their length. The strengthening and thin-walled cells are much shorter than the epidermal cells. The average length of the strengthening cells is 108 mm.

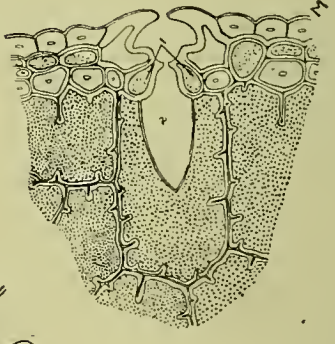
The Stomata.—These are arranged in rows along the surface of the leaf. The dorsal side of the leaf has more stomata than the ventral side. In a space of 1332 square mm. on the ventral side of the leaf there is an average of 164 stomata. In a space of similar size on the dorsal side there are 172 stomata. The guard-cells are depressed below the surface of the leaf and are almost on the same plane as the innermost row of strengthening cells. A thin surface section of the epidermis shows merely the mouth of the stoma looking like a small round opening in the epidermis. To obtain a view of the guard-cells in the tangential section, a thin section of the epidermis must be first removed. The stoma in tangential section is illiptical in shape, fig. N, 1. Measured through the centre it averages 46 2-3 mm. in width and 58 2-3 mm. in length. The guard-cells, as seen in transverse section, are almost oval in shape, with the exception of a slight extension on the end lying towards the mouth of the stoma, fig. M. These cells have very thick walls. A sharp prolongation extends from each guard-cell slightly above the epidermis, thus forming a small cavity between the surface of the leaf and the guard-cells. Below the guard-cells is the respiratory cavity, which has an average depth of 45 2-3 mm., fig. M, 2.

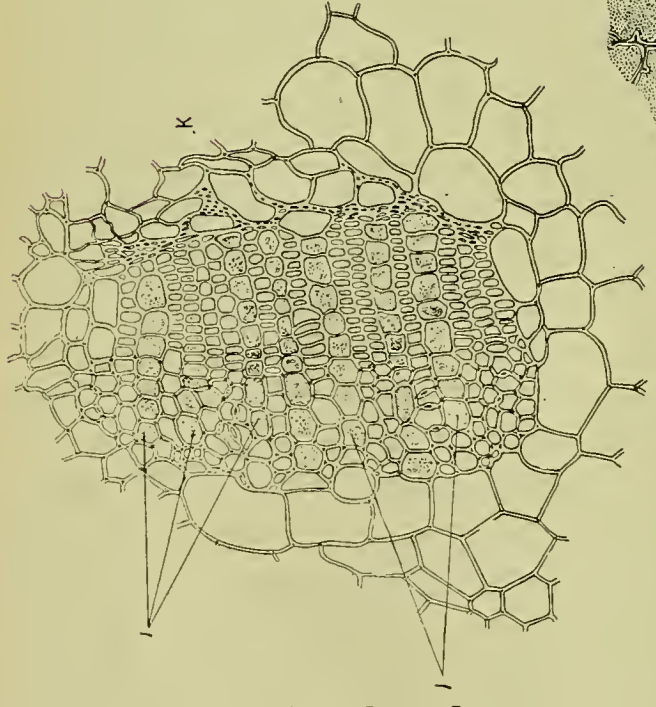
Mesophyll.—The greater part of the leaf is composed

of very thick-walled parenchymatous cells, fig. B, 2. The infoldings of the walls vary in shape. Some are narrow prolongations of the walls with a knob-like end. Others









lack the rounded end, and some are merely short, blunt thickenings of the walls. In no case are the infoldings branched. The cells are very irregular both in shape and

in size, so that it is difficult to obtain an idea of the average size. In general the cells on the ventral side of the leaf are smaller than the cells on the dorsal side. The mesophyll is broken by resin ducts, of which there are two, fig. B, 4. Three ducts are frequently found and sometimes even four. The ducts are entirely surrounded by parenchyma, and so may be classed among the "parenchymatous ducts." The number of the cells in the resin ducts varies from ten to fourteen. A fully developed duct measures, on an average, 100 mm. in diameter. The resin ducts have usually only one row of sheath cells, but in some ducts there are portions which have two rows of cells. In addition to these cells there is a row of narrow, very thin-walled secreting cells lining the resin duct. These cells are very irregular in outline, and there are about half as many of them as of the larger cells.

Fibro-Vascular Region.—The central part of the leaf is occupied by the bundle region, which is surrounded by a well defined endodermis. There are from fifty to fifty-seven cells in the endodermis. These cells measure on an average 47 mm. in width, 27 mm. in thickness, and 137 mm. in length. In tranverse section they are almost oval in shape. The leaf has two fibro-vascular bundles which are completely surrounded by a fibrous tissue. The tissue which lies next to the bundle sheath is pitted, but that which lies in the centre of the bundle region and between the two bundles is entirely free from pits and has very thin walls. Scattered in the tissue next to the sheath are some cells which are free from pits. These cells are rounded in shape. They have an average width of 40 mm., a thickness of 38 mm., and a length of 65 mm. The pitted cells are so very irregular in shape that no measurements were taken of them. The thin-walled cells between the two bundles are much more uniform in size and shape. These cells have an average length of 598 mm. The vascular bundle occupies an average space

of 216 2-3 by 125 mm. The xylem of the bundle lies next to the flat side of the leaf. The cells on the outer edge of the phloem are flattened and have very thick walls. Through both xylem and phloem are rows of parenchymatous cells which form medullary rays, fig. K, 1. These rays in the xylem contain starch, but in the phloem they contain protoplasmic substance. In the xylem next to the fibrous tissue are groups of spiral vessels. These cells have thicker walls and are more rounded than the other cells of the xylem. In studying the fibro-vascular bundles double staining with eosine and gentain violet is most profitably employed. The phloem is stained red and the xylem is stained a beautiful blue.—*University of Tennessee.*

Practical Hints on Mounting.

By R. G. MASON.

We take for granted that the worker is provided with the following apparatus and materials. Turntable, 3 × 1 glass slips, thin glass covers of various sizes (5-8in. and 3-4 in. are the most useful), tin cells, a couple of sable pencils, (writer's lining pencils are the best), a bottle of good cement, black and white finishing vanishes, two or three wire clips, and two needles fixed in handles.

Dry Mounting and Cell Making.—By mounting dry is always understood that there is no medium, used between the object and the cover glass. Objects mounted dry are always placed in a cell: of such objects, those most suitable for this method are micro-seeds, vegetable hairs attached to their leaves, skin of sole, and Foraminifera. A cell must be of sufficient depth for the cover glass to clear the object, but at the same time not be too high. It just clearing the surface of a specimen enables a higher power to be used if required.

To make cells, take a 3 × 1 slip, centre it on the turntable, spin it round at a moderate speed, charge your

brush with the black varnish, turn a disc say three-quarters of an inch in diameter. It is best to make a number of these at a time. Place aside to dry for say twenty-four hours at least, before it is fit to use for this purpose. When dry, take some dull black paper gummed on the white side, cut some discs a little smaller than the black disc on the slip, say about 5-8in. These discs are easily cut out with a gun wad punch, or may be purchased at a very cheap rate ready punched. When the black varnish disc is quite dry, stick one of these in the centre, take a tin cell of a size suitable for the object you are intending to mount, apply a little cement to one side, set aside for ten or fifteen minutes to harden the cement a little. Now with a pair of forceps, place it in position on the black paper, press down and set aside for five or six hours to dry. If a deep cell is required, several thin ones may be fastened together to the required height with the cement. The object of the black varnish disc on the glass is to give it a neat appearance on the under side. Take a piece of skin of sole as a sample object, apply a little gum to the black paper, and when it is dry moisten a little with a brush, and place specimen in position (this method will prevent the gum being absorbed by it, causing an unsightly mark), be sure all is quite dry before you proceed to put on the cover glass ; having placed the slide under a low power, to see all is clean, take a little cement and place it round the top edge of the cell, put it aside for ten minutes. Have your covers ready cleaned, take one up in the forceps, place one edge on the cement, and gently lower it down into position. Press down with the needles on the cell and not in the centre of the thin glass. If these directions have been properly carried out, the adhesion between the cement and the glass will be perfect, and if the proper quantity has been used, there will be little or no excess. The slide may now be put aside to harden the cement, for an hour or so. It is possible to

finish a slide right off when mounted as described, but it is better to let it stand for a short time. Cement will effectually seal up glycerine mounts, and may be used as described to fasten tin cells to glass, or to turn cells of the cement itself on 3 × 1 in. slips. The use of Gold size is only a nuisance from its tendency to run between the cover and the object. The only remaining operation is to apply the finishing varnish, either white zinc or some other.

Likely Failures.—1.—Specimen leaving the gummed paper in the cell.—Cause: gum not thick enough at bottom of cell.

2.—Zinc varnish running under cover.—Cause: the ring of cement on top of cell imperfect.

3.—Damp or condensation on under side of cover.—Cause: object or cell not being well dried.

Mounting in Canada Balsam or Canada Balsam and Benzole.—This covers the largest number of objects one has to deal with. If proper care is taken in preparing specimens before they are placed in contact with the balsam there is little fear of air bubbles, and in the case of balsam and benzole they will go by themselves. Time will cure them if they are not too large.

Canada balsam may be used for mounting the majority of transparent objects, whether anatomical or botanical, Foraminifera, Polycistina. Many of the Elytrons or brilliant Coleoptera are much improved by being mounted opaque in balsam, but no attempt must be made to mount the wings of Lepidoptera opaque if their brilliancy is to be retained. These are as a rule mounted dry.

We will now suppose we have some sections prepared, and in the oil of cloves. Some advise xylol for the former as a final instead of the clove oil. There are two methods of balsam mounting—the quick and the slow. The quick method is more often adopted with the balsam and benzole. Canada balsam very often is rather thick. If so, add a little spirits of turpentine, rinse the specimen

in spirits of turpentine to clear, draw it up onto a spatula with a brush, place a little balsam in the centre of a 3 × 1 in. slip. Having got rid of your excess of spirits of turpentine, place the section in the middle of the slip. See that the balsam covers it, and if not add a little more. Place it under a low power to see that there are no foreign bodies attached to it. If so, remove with one of your needles. If all is right, take a clean cover glass of suitable size, place it one edge down, lower it gently so as to keep the object in the centre. Experience will greatly help in this matter. When it is down on the specimen, gently press it with the needle points. If the right quantity of balsam has been used, very little will exude from the edges. At first and until experience shows how much to use, a good deal will come out, but this cannot be helped, and must be cleaned off afterwards. The slide may now be put aside for an unlimited time. It need not be touched for months, but if required to dry it quickly, resort must be had to a drying stove. Or it can be placed in the oven at night after the fire is out. A simple contrivance may be constructed out of a square tin, so that a number of objects may be done at once. A very small gas jet should be placed underneath which will keep up the required temperature for any length of time. About 100° Fahrenheit is a suitable temperature. Do not let the temperature get too high, or the balsam will turn brown and the slides be spoiled.

The quick or exposure method is principally used where balsam and benzole is the media. Take a clean 3 × 1 in. slip, also a cover glass. Hold the latter in the forceps, breath on it and place the side where the moisture is down on the slide. Gently press it with the needle point, place a fair amount of balsam and benzole on the cover, and immerse the section in it, care being taken to get rid of much of the spirits of turpentine or whatever it may have been in. See that it is covered with the bal

sam and free from specks of dirt. Now put it away on the slip, in a place free from dust, for from two to twelve hours. Delicate specimens will not stand exposure like the more hardy ones. In the case of rock sections, this process may be extended to 18 or 20 hours. After exposure take the cover glass in the forceps, place a very small portion of the balsam and benzole in the centre of the slip, place the cover glass in position, gently warm it over a spirit lamp with the flame turned down low. Press down keeping the specimen in the centre as nearly as possible. When cold, clean off any excess of the media. This is done with methylated spirit. Having cleaned all off, run a ring of cement round and when this is dry, finish to taste.

Other objects may be mounted in balsam, Polycistina, Foraminifera, etc. Pure balsam is the best for these. Take an ordinary retort stand and on one of the rings, place a piece of metal, iron or brass, about 1-16 in. thick, 3in. or 4 in. wide, and 4 1-2 in. long. This will hold four slips at once. It is best to fasten metal to the ring in some way. A small piece of solder will do. This is only to prevent its being upset, slides and all. Take a piece of tin and bend it into the shape of a shallow box without a lid, about 1-2 in. or 3-8 in. deep. If this is made about 1-8 or 1-4 in. smaller all round than the metal plate, it will form a sort of miniature oven when placed on the plate. It is principally to keep off dust, but it must be used, if good results are desired. Have your objects all ready, quite dry and clean. Take your 3 × 1 in. slip, place a drop of balsam in the centre, about 3-8 in. in diameter or less. Next take some up on the point of a scalpel, or in a small piece of paper. Shoot them into the centre of the balsam, gently stir with a needle point to make them sink, place your slide on the metal plate you have prepared, adjust to proper height over your lamp and turn the flame down very low so as to get gentle heat. Very little heat is re-

quired but it must last for some time. Having now lighted your lamp, proceed to prepare others in the same way, or go on with other work for at least thirty minutes, giving an occasional look to see all is going right. When ready, take a cover glass in the forceps, place a very small drop of balsam in the centre. Now put the cover in position, not edge first in this case, but as flat as possible. By a judicious use of the spirit lamp the cover will go down into its place. If any assistance is given to it the touch must be very light indeed, as the forms are very fragile. If the above operation is neatly done and the right quantity of balsam used, there will be no excess of balsam, but a nice even bevel will be formed. When this is the case, run a ring of cement round, and finish in the usual way. The object of the above process is two-fold: to get rid of the air contained in the forms and let the balsam take its place, also to harden the latter, thus saving any further drying. To mount opaque objects in balsam, take a 3 × 1 in. slip, with a cell of suitable size attached. See that all is clean, run a ring of cement round the top. Now take a clean brush, dip it in spirits of turpentine, and paint round the inside in the extreme corner of jointure with slip. This is to expel all air. Drop in your balsam, which must in this case be rather thin. Fill cell. Next put in your specimen, which must have been previously soaking, either in spirits of turpentine or benzole. Get rid of as much excess of this as possible. If the specimen has a concave side, place it in the cell with the convex side downwards. Turn it over in its cell. This will prevent any air from getting imprisoned underneath. Examine under a low power to see that all is right. Fill up your cell with more balsam, so that the top is quite convex. Have ready a cleaned cover, just a little smaller than the diameter of the cell. Place it down edge first the edge farthest from you. Gently lower it into position onto its bed of cement

and apply gentle pressure to the edge (on each side at once) by means of your needle points. If everything has been done right, it may be set on one side for twelve hours to harden the cement. Then clean it off with a brush and sprits of turpentine or benzole. When all excess of balsam is removed and the slide is dry, finish with another ring of cement. When that is dry, it may be finally finished in the usual way, a piece of black paper being fastened under the cell. Some recommend lining the inner portion of the cell with varnish, but this is likely to affect the specimen in time.

Likely Failures.—1.—Cloudiness round edges of sections.—Cause : not being thoroughly dehydrated by means of the alcohol before going into the oil of cloves.

2.—Balsam drying up and leaving a small space or spaces at edge of cover.—Cause : insufficient balsam used in mounting. It can be filled up from the outside.

3.—Section lifting cover glass.—Cause : sometimes happens with hard sections or fish scales. Use a wire clip until balsam is set.

4.—A bubble of air in the cell.—Cause : insufficient balsam in cell before putting on cover.

To Mount Insects and Insect Parts With and Without Presure.—Mounting the different parts of insects to show their structure, is very interesting. This may be done in two ways : with presure, i. e., flattened out between the glasses ; *or without,* in a cell, keeping them as near as possible in their original form. For a sample of the former method, we take the Soldier Beetle to be mounted entire. The same treatment will apply to many insects or parts of same, but must not be taken to include the more delicate specimens, as many of them require very special treatment. Our specimen being ready prepared, and in spirits and water, take it from the solution. Place it in clean water, say from ten to fifteen minutes.

Gently rinse it to get rid of any foreign bodies. If the specimen has been in pure spirit, as is sometimes wrongly

done, moderately warm water may be used. *This will relax it.* Take a 3 × 1 in. slip, clean it, and place the insect in the centre. Proceed to arrange all the organs, feet, head, wings, elytra, etc., in as natural a position as possible. This will require patience and delicate handling. After all are in the desired positions, take two pieces of thin card of about the same thickness, not thicker than the specimen. Place one on each end of the slip. Take another clean slip and gently lower it down onto the one with a specimen you have arranged in position. The specimen may move when the operation must be gone through again until a successful issue is obtained. Now place two india-rubber bands round both slips to keep them together, or tie with stout soft cotton.

Have a wide-mouthed bottle ready, three-fourths full of methylated spirits. Place your slips in, see all is covered, let it remain there from twenty-four to thirty-six hours or longer. This will fix the object in position. Carefully take it out, undo the strings or take off the bands. Take specimen up. *It will now be quite stiff enough to handle.* Have some clear clove oil in a saucer. Float it on top. As soon as it sinks it is ready for mounting in balsam, by either of the methods described under balsam mounting, and finish as directed there.

We will now prepare to mount some organ *without pressure.* Take for example, the head of the Garden spider. Owing to the formation of this creature, although not really an insect, it will serve our purpose very well as an example of mounting in the above method. The head and thorax being in one part known as the Cephalo-thorax, we shall take the whole just divided at the junction with the abdomen. As it is desirous to show the falces or siezing organs, it must be mounted on its back or upside down. Wash in clean water as by other method. Prepare a cell exactly as when you mount in a cell in balsam. Now place the specimen in a saucer of methylated spirits.

Extend the organs it is desired to show, and hold them out for about ten minutes, until they have had time to fix by the spirits. Having seen the organs inclined to stay out, it may be left in the spirit for thirty minutes. Next place in clear clove oil. When thoroughly soaked in this mount, the specimen may be placed in spirits of turpentine before finally mounting. It improves the transparency, but this is a matter of fancy. Causes of failure in this case will be exactly the same as the last, and the same remedies will apply.

To get balsam and benzole in perfection, you must either make it yourself, or go to some reliable person. A very large proportion of that sold in the trade is of no use whatever, owing to the cost of production. In evaporating there is a loss of about fifty per cent. Consequently to produce it cheaper, or something that will appear the same, it is only half-dried. Then the benzole is added, so there is really a mixture of the natural turpentine of the balsam and the added benzole. To prepare balsam and benzole, the balsam is subjected to the action of a hot sand bath by being placed in a common tin dish surrounded by sand (ordinary silver sand), in another and larger tin dish over a small gas jet. Better still it can be stood on a closed stove, until the balsam is perfectly hard. It will then chip and break out clean from the dish. It is now placed in a bottle and benzole added to it until it is re-dissolved. Should it appear to have any pieces of dirt in it, it must be filtered, by placing it in front of a fire, only just to keep it warm. Cover with a larger glass vessel and filter in the usual way, through course filtering paper. If care is taken to see that everything is clean during the making, there will be no need of this. Care must be taken not to dry it too quickly, or it will darken the color. When done, it should be quite clear and very little darker than pure balsam.

The best form of bottle for containing either balsam,

glycerine, or in fact any media where small quantities are required at a time, is one invented by Dr. Needham, a gentleman of great experience in microscopical matters. It consists of a specially made bottle with a glass dipping rod of its own. This rod is provided with a small flange, fitting into a shoulder, ground out in the neck of the bottle. When not in use, the dipper or rod is placed in the bottle, and taken up as required. The surplus media, whether it be balsam or any other gets into the shoulder in the neck, preventing all evaporation, and at the same time it never holds tight enough to fix the rod. It is always ready for use, and going back into its place when not required, is the cleanest apparatus of the kind yet made. Balsam may be kept for any length of time without evaporation. One is saved the trouble of getting a cork out, and consequently no pieces can be left behind. It is superior to the bottles that are made with ground glass cap. In them the cap is always getting fastened on, then resource has to be had to heat, and the bottle is often broken.

Mounting in Glycerine.—Many delicate vegetable and other tissues are best mounted either in a cell, or in glycerine in the form of a jelly. Take as an example, the spinnaret of the Garden Spider to be mounted in the former without pressure in a cell. This is a most beautiful object with the spot lens and 2 in. objective, well repaying the trouble in mounting. Take a 3 × 1 in. slip, with cell of suitable depth fixed on it. A transparent bottom to the cell will be required. A cell of about 5-8 in. or not less than 1-2 in. is a good size for this object. Run a ring of cement round the top as with other cell mounts. With a clean brush dipped in distilled water, damp the cell in the inner edge all round, also on the bottom. *This will get rid of any air that may cling to the cell.* Get rid of any excess of water with a small piece of blotting paper. Carefully put in the glycerine until the cell is

nearly full. Have your specimen soaking for about thirty minutes in a mixture of glycerine and distilled water, about two of the former to one of the latter. Take up the specimen with the forceps. Put it convex side downwards, just the reverse way to that which you want to show it. Turn it over in the cell. This will expel any air that would get under it. This is easily done with the forceps and a needle point. Now add some glycerine until the top is quite convex, examine it under a low power. If all is right, place the cover glass on in same way as directed in former paragraphs on mounting in cells. Gently press down and set aside to dry. When dry, in say twelve hours, carefully wash away with a brush, (either under a running tap or in a small saucer) all excess of glycerine. Set on one side to dry from the water. When all is dry, run an extra ring of cement round the edge of the cover glass. When this is dry again, finish according to taste in the usual way. The directions given will be the same for any fluid mounting.

Likely Failures.—1.—Glycerine appearing full of air bubbles.—Cause: its being shaken up; glycerine should always be carefully handled, and be allowed to stand in a warm place for two or three days before using.

2.—Cell appearing to contain a number of little specks of dirt.—Cause: the media must be filtered occasionally before using.

3.—One large air bubble, or two small ones appearing in the cell.—Cause: insufficient media in the cell before putting on cover glass.

Quartz Crystals.—Collection of 35 for 70 cents. A. B. Crim, Middleville, N. Y.

Chinch Bug.—An 82-page Bulletin giving resume of present knowledge by F. M. Webster, is published by the Department of Agriculture for free distribution (1898).

For Sale.—A $45 microscope stand for $25. Address: W. A. Murrill, Ithaca, N. Y.

Diatoms Prove an Occidental Sea on the East of the Rocky Mountains.

By ARTHUR M. EDWARDS, M. D., F. L. S.

The Occidental Sea I have endeavored to describe, at first in the American Journal of Science for 1891 and elsewhere in various publications, and why I describe it as Eocene, below the Miocene of California, I have also stated. But the sea was only stated to be west of the Rocky Mountains, between that range and the Sierra Nevada. Some specimens I had from the east of the Rocky Mountains seemed to be Eocene but I was not sure that they were more ancient than the Iceberg period or Champlain. In fact, I did not get any specimens that were surely Eocene. Now, I have one that comes from the "Public Lands" on the branches of the North Canadian or Beaver Creek about thirty-five or forty miles from Englewood in the northeastern part of Kansas. This, Prof. F. W. Cragin of the Colorado College calls the Loup Fork terraine, and I have to thank him for an opportunity of examining it. It is also east of the Rocky Mountains and therefore extends, as I expected, the Occidental Sea to the eastward. I hope ere long to extend it on the coast side of the Sierra Nevada and into California. In fact, I have it from Shasta and Anacapa Island on the coast, showing that it covered a vast extent of country and was a large body of water. When a sample was shown to Prof. Cragin, in 1888, the chalky marl was thought to be of Cretaceous age and also carbonate of lime, in fact the same as chalk. But, he visited the locality in 1890 and found that it was impossible, as fossils in the same bed were land and lake animals. In 1896, he visited the locality again and confirmed the conclusion that the Loup Fork was lacustrine. He says that "the great Loup Fork Lake which extended from middle Kansas to the Rocky Mountains and from Texas to British

America must, however, have been of a more or less saline water." He thinks this is indicated by the Bacillaria found in it by Wolle. But the forms I have found in it are all fresh-water forms, and certain forms which Wolle found may also be fresh, as I will indicate. The chalk of the Cretaceous is marine or salt-water along the shore as is shown by its containing coccoliths and rhabdoliths which problematical marine organisms are not animal and not vegetables. They are seen now living in shallow marine water and the Loup Fork marl has been submitted to an Eastern zoologist who found these organisms in it. This, Prof. Cragin thinks may be a mistake, but it can hardly have been so. The sample may have come from the Loup For' and may have contained coccoliths and rhabdospheres and yet not have been marine at all. For reckoning on the salt or fresh-water origin of a sample from the microscopic organisms found in it is very unreliable. In the lacustrine time, which these marls are thought to represent of Plantanus, a land tree, and Planorbis, Sphærium and crushed specimens of an Anodonta or Unio, fresh-water mollusca which now inhabit shallow streams, and scales and spines of percoid fishes which grow in shallow brooks also. In fact, it was fresh and afterwards brackish and then dried up entirely. Prof. Cragin thinks it was a large lake then which he calls Loup Fork Lake. This was after the time of the Occidental Sea. One part of the Loup Fork Lake was brackish, very brackish, salt, and Bacillaria lived in that which held salt water forms.

The specimen I have is extremly interesting, being of a light cream color. When examined by means of the microscope, it is found to be made up of semi-crystaline particles having a few fresh-water forms of Bacillaria or Diatoms mixed with it, for I call Diatoms the dead lorica or shells of Bacillaria (Diatomaceæ). When treated with acids it effervesces and almost all dissolves, a few Diatoms

being left. This semi-crystaline mass effervesces and therefore seemed to be a carbonate of lime and hence the reason for supposing the marl to be chalky and therefore cretaceous. But when examined by means of the microscope, it is not amorphous (as chalk is) but is mineral and called Thinolite by Clarance King in 1878. The name Lahontan was given by him to a great Quaternary Lake in Northwestern Nevada, of which the present Walker, Carson, Humboldt, Winnemucca, Pyramid and Honey lakes are the relics.

The water of Lahontan Lake was fresh, but, as it evaporated, the water itself went off and the solid matter was left. This resulted in the precipitation of the matter which was called calcareous tuffa. When examined chemically, it is found to be not calcareous alone but a compound of carbonates of lime and magnesia, and oxides of iron and alumina, along with phosphoric acid. The mineral Thinolite (from the Greek for shore since it forms on the shores of the lake). When the Thinolite is acted upon by nitric or hydrochloric acids the lime and magnesia are dissolved and the Diatoms, which consist of opal, a hydrate of silica, are left, and they can be examined. They are found to be the following:

Cymbella cistula, H.; C. lanceolatum, C. G. E.
Fragilaria construens, C. G. E.
Gomphonema affine, F. T. K.
Melosira distans, F. T. K. Common.
Navicula firma, F. T. K.; N. (formosa?) var. liburnica, A. G.; N. placentula, C. G. E.; N. sphærophora, F. T. K.; N. viridis, F. T. K. Common.
Nitzschia spectabilis, (E.) J. R.
Stauroneis phœnicenteron, C. G. E.
Synedra ulna, P. A. C. N.
Tetracyclus lacustris, J. R. Common.
Sponge spicules probably from Spongia fluviatilis, L.
Shells of Hyalosphenia cuneata, F. S.—chitinous.

Those not marked common are rare. Prof. Cragin's list furnished by Wolle does not agree with the above. Wolle enumerates:

Cymbella cistula—more frequently fresh-water than brackish.

Navicula peregrina—brackish water; N. tenella and N. lanceolata which may be forms of Schizonema and hence marine.

Coscinodiscus woodwardi,E.—most decidedly marine.

Melosira granulata,(E.) R.—brackish or marine water.

Of these, Cymbella cistula is always found in fresh-water now. The Navicula, I have none of. Coscinodiscus woodwardi, Eilenstein, is figured in Schmidt's Atlas der Diatomaceen-kunde, 1878, plate 61, but no description has been published. It is seen in H. L. Smith's Species Diatomacearum Typicæ Studiis. Schmidt's figure looks like C. centralis, C. G. E. C. minor, C. G. E. is found in fresh-water. It is then supposed to be Melosira distans F. T. K. C. woodwardi is only found in the fossil condition. It looks like and may be only an end view of M. distans. In my specimen of the Loup Fork marl, I see disk-like forms that may have been classed as Coscinodiscus. M. granulata may be M. distans. In fact, they are indistinguishable the one from the other. Thus the marine habitat of the Loup Fork marl fades away and fresh-water habitat is established in it. But then, these brackish diatoms may be there still. In fact, it is extremely likely that they may be found, for brackish water merely results from fresh-water and has not been introduced from the ocean at all. It must be remembered that, in a gathering several feet away, the forms may differ very much. Such is the case now. So that the reasoning of the marine or brackish nature of the Loup Fork marl from the forms one finds which is usual may be and is very unreliable. We must find larger plants and animals, brackish or marine, to rank the layer, and they are fresh-water.

In fact, the marl is fresh-water and became brackish by evaporation. All natural water contains some solid ingredients,—chloride of sodium, and also some silica, or oxide of silicon. Now let us see what Ehrenberg the earliest observer found in the marls of the Great Plain. In the report of the geological exploration of the fortieth parallel, Vol. 1, p. 120, Clarence King says: "Specimens of the white strata were subjected to microscopic analysis by Dr. C. G. Ehrenberg of Berlin, who found forty-six distinct species of diatoms. Of these, 28 have been classed as Polygastera and 18 of Phytolitharia, the most abundant species being Gaillionella granulata, G. sculpta and Spongiolithis acicularis. In a lavender colored bed far up in the series above the acidic tuffs, further sundry beds are observed in the same sections containing more or less infusoria, in which the following were found by Mr. Charles E. Wright: Gaillionella - ——? Spongiolithis acicularis, Pinnularia inæqualis, Coscinodiscus radiatus.

G. granulata is Melosira granulata and indistinguishable from M. distans as is G. sculpta. P. inæqualis is Cymbella lanceolatum. C. radiatus is the last and evidently the round end of M. distans. S. acicularis is the sponge spicule I have found and comes from Spongia fluviatilis. In this way, Wright's and Ehrenberg's specimens agree with mine. This shows that the Occidental Sea covered also a part of Kansas east of the Rocky Mountains and makes of it a vast sea. Later I shall discuss the facts in California. I have lately received specimens from the Arizona bed of that sea. They were sent by Professor W. P. Blake, director of the School of Mines, Tucson, Arizona.

Diatomaceous Earth from Arizona Containing Cyclotella.

BY A. M. EDWARDS, M. D.

I have recieved from Prof. Wm. Blake, Director of the School of Mines, of the University of Arizona at Tucson,

a very interesting earth. It is white, almost like chalk, not calcareous but siliceous and found in Arizona. Under the microscope, it appears made up entirely of frustules of Bacillaria (an older word than Diatomacea). Whether marine or fresh-water the microscope alone can determine. It consists largely of rather large discs very beautiful in form and which look like Actinocyclus, but are Cyclotella. This genus contains both marine and fresh-water forms. When examined closely I found it to be the same as certain specimens from the N. W. Boundary Survey (1870). See Am. Jour. Science, 1891, "On the Infusorial Earths of the Pacific Coast." In my paper on the Diatomaceæ of the Geological Survey of California, I called it Cyclotella pulcherima on account of its beauty. I now consider it only a form of Cylcotella berolinensis, C. G. E. which was found in Berlin years ago. Being found at Niagara it was called Stephanodiscus niagaræ C. G. E. I have living specimens of it from Lake Erie. It shows how forms may be modified in passing from fresh-water to brackish and to salt where it becomes Actinocyclus.

Species as commonly known do not exist in Nature but forms do. Forms are changeable until they merge one into another or fade out. As forms change so genera change. One genus changes into another and one family may merge in the next. Hence forms can be placed with difficulty at times. They may be Bacillarian, Radiolarian or Sponge.

Actinocyclus is common in guano at Ichaboc, Africa, and on every coast of the sea. It is found fossil in the clay of Hatfield swamp, N. J., in the Pasaic river, and in the drinking water of Paterson, N. J. There it is called A. ralfsii. Ehrenberg first described it in 1845 (Bericht. Berlin Akademie) as Stephanodiscus brolinensis. Found in the Nile, it was called S. ægyptiacus; at Canton, S. sinensis; in the Ganges, S. bramaputræ and here as S.

niagaræ. Van Heurck (Synop. des Diat. de Belg. 1880, plate X C V, fig. 1-14) published it as S. carconinensis, Grun.; S. astrea (Ehr.) Grun. (Discoplea astrea, Ehr. Cyclotella rotula, Kutz.); St. astrea var spinulosa, Grun. (S. agyptiacus, Ehr.); St. astrea var minutula, Grun. (Cyclotella minutula, Kutz.; Discoplea oregonica, Ehr.); St. hautzschianus, Grun.; (Cyclotella operculata, Hantsch. Rab. Alg. Europ. 1104, St. balticus, Schur.); Idem var pusilla, Grun. S. C. (bellus A. Schmidt var?); Novæ Zealandiæ, Cleve; St. niagaræ, Ehr. (Cyclotella spinosa, Schumann).

The foregoing are all Cyclotella with or without spines. They are C. berolinensis, C. G. E. They include Discoplea astrea, C. G. E. Or, they are Discoplea greca, C. G. E. 1840, Ber. p. 208. I think they are sporangia (i. e. large forms) of Melosira granulata, C. G. E. Abhandeungen (Amer.) p. 187. M. granulata is very common in the deposits of the Occidental Sea. I have at least a dozen or two from the North and the South, from Washington and from Texas, from Kansas to California.

There is also present, and nearly as common as the Cyclotella, another form which it would be difficult to explain in a strictly fresh-water deposit. It is Hyalodiscus scoticus, F. T. K. and is decidedly marine. It is found everywhere on the sea coast growing in salt-water. It was first found on the coast of Scotland and named Cyclotella scotica by Kutzing in 1844 (Die kieselschaligen Bacillarien oder Diatomeen). It includes Podosira and is included in Melosira. How it was evolved is difficult to understand but as Melosira is extremely common and varies in form almost indefinitely it may have come from one of its common forms.

In Van Heurck's Synopsis, pl. 134 are 24 figures included in Hyalodiscus—Podosira, seven genera and several so-called species, and yet they are all one form.

Washed-Out Red Blood Corpuscles in the Urine as an Aid in the Diagnosis of Renal Calculus.

By G. D. HEAD, M. D.

Blood occurs in the urine in a large variety of pathological conditions. Its appearance depends upon the source in the urinary tract from which the hemorrhage originates. If the blood appears in clots the hemorrhage is usually from the lower urinary tract, the bladder or urethra being involved. If the blood appear unclotted in the urine and if the hemorrhage originates in the kidney or the pelvis of the kidney, the urine will present a dark smoky appearance.

But blood may occur in urine and give to the naked eye no evidence of its presence. Such a condition is not infrequent in the hemorrhage produced by a stone in the pelvis of the kidney, or the ureter, and failure to examine the urine microscopically will rob the clinician of a valuable aid in diagnosis. Microscopically studied, blood exhibits itself in the urine :

(a) As the normal red blood corpuscle.

(b) As the crenated red blood corpuscle.

(c) As the washed-out red blood corpuscle, or the so-called "Shadow corpuscle."

Particular interest centers about this third form of the washed out red blood corpuscle because it is present in freshly excreted urine only when the hemorrhage is high up in the urinary tract.

A washed out red blood corpuscle is one whose hæmoglobin has been nearly or quite dissolved out. It appears under the microscope as a pale yellow, or colorless ring with a very delicate but distinct border. It is somewhat smaller than a normal red blood corpuscle, and frequently exhibits little irregularities in its contour. Sometimes so completely has the coloring matter been removed that only the faintest shadow of the cell remains, and the

corpuscle is easily overlooked. To produce this appearance in red blood corpuscles artificially one needs only to run a little dilute acetic acid solution under the cover glass in a mount of normal blood, when these pale rings will make there appearance. They are usually present in small numbers in the urine. They never form a sediment visible to the naked eye, and are only seen on microscopical examination. They are a common constituent of blood casts some of which they completely fill.

If normal red blood corpuscles are allowed to stand in urine for 48 hours or more, many of them will become washed out, a fact which should not be forgotten in examining old specimens of urine.

Washed out red blood corpuscles occur in a number of pathological conditions. Von Jaksch names acute nephritis, acute congestion of the kidney, and miliary tuberculosis of the kidney, or its pelvis. Simon Purdy, Diaber, and Peyer speak of their existence, but do not specify the particular diseases. In none of these authorities is there any mention of their presence in the urine passed during or after an attack of pain caused by renal calculus. That they are many times present in this condition is certain, and their presence is of diagnostic value in excluding the causes of acute abdominal pain, such as appendicitis, gall stone, ulcer of the stomach, intestinal colic, floating kidney, and gastralgia. The urine passed during and for some days following the attack should be examined fresh. Care should be taken not to confound the spores of certain fungi with washed out red blood corpuscles; they are somewhat alike in size, but the spores have more distinct borders and usually contain yellow colored granules in their interior.

In the following cases of renal calculus washed out red blood corpuscles were found in the urine passed subsequent to or during the attack.

(1) Examination of urine: Sp. gr. 1026, high color, no

alb., no sugar, urea 4 per cent., uric acid increased. On microscopical examination there is seen in the field a moderate number of pale colorless rings (washed out of red blood corpuscles), also a few feebly colored red blood colpuscles. Subsequent examination failed to reveal any such bodies. One month later a second attack, similar to the first. Urine, Sp. gr. 1028, high color, no alb., no sugar, urea 3 per cent. Microscopical examination revealed a few delicate, ring shaped bodies (washed out red blood corpuscles). One subsequent examination failed to show any such bodies. One day later a patient brought in a small phosphatic stone passed by urethra.

(2) Urine, Sp. gr. 1028, high color, a trace of albumen, no sugar. Microscopical examination reveals a number of pale, colorless, delicate bordered rings (washed out red blood corpuscles), no casts, a few leucocytes. The woman recovered the following day. An examination of the urine one day later revealed no such bodies.

(3) Microscopical examination of urine revealed from five to ten red blood corpuscles in the field; some of them washed out. No casts. A few triple phosphate crystals. Specimen taken two days later showed no such bodies.

LETTERS TO THE EDITOR.

GATES' DOUBLE MICROSCOPE.

Lenal's Apparatus.—Seven years ago, in Zeitschr. f. Wiss. Mikr., Dr. Lenal described his microscope with a second microscope attached to it in place of an eye-piece by means of which he claimed to get greater magnification of the primal image that has been hitherto secured by any combination of eye-pieces and objectives. Dallinger, Nelson and other English microscopists pointed out that the working power of any objective could not be increased by subjecting the primal image of an approximately perfect object-glass to examination by a second microscope or other complex combination of lenses. The greater magnifica-

tions obtained are simply those of details of the image which has been brought about by diffraction in the first objective and therefore there cannot by any possibility be a single detail added, while the details that the accurate image does disclose must be blurred and tortured tenfold more than when subjected to the legitimate action of well-constructed eye-pieces. Professor Gates' apparatus is a perfected form of Lenal's apparatus which was pushed out of sight by scathing criticism. Science-Gossip for January reproduces our illustration of Gates' apparatus and makes the above statements regarding Lenal's apparatus.

Very Noncommittal.—I have been exceedingly interested in Gates Double Microscope, and feel that it is a matter of such importance that one should not give an opinion at once, for while it seems to be contrary to some accepted ideas, other things of the same character in the past have proved to be most valuable discoveries. Time and experience alone can demonstrate the value of this plan which is certainly unique.—*Henry B. Ward.*

EDITORIAL.

The Journal of 1899 will be a 32-pp illustrated magazine devoted to Microscopy in all phases and applications. The general panic of two years ago in which nearly all the periodicals devoted to this subject went down has subsided and the outlook for 1899 is such that we expect to publish the best Microscopical monthly that has been seen in the United States and to have the aid and sympathy of all microscopists. The price is $2 but those who are prompt to pay can deduct 50 cents therefor. If any of your back numbers are missing it is well to get them at once. The friends who received the Journal in 1898 and do not intend to pay for 1899 should give immediate notice, and refuse to take it from the mails unless marked sample copy.

Yeast.—Yeast may be ground up with quartz sand for the purpose of disrupting the cells and subsequently submitted to the high pressure of 500 atmospheres. This

breaks the yeast cells into fine particles but if any are left unbroken the microscopical examination shows that the liquid contents are gone. This powdered mass will nevertheless produce fermentation, which proves erroneous the hitherto held theory that perfectly organized and living cells are necessary to fermentation. The cell-life has vanished. If life is there it is in the particles. The chemists say the action is due to the chemical power of an amylolyte acting in a manner similar to digestive ferment, but they cannot yet isolate the fermentative enzyme.

New Periodical.—Messrs Lund & Company, Amen corner, E. C., London have issued an Illustrated Annual of Microscopy devoted to the progress made in research and the newest devices in apparatus and methods covering photo-micrography, bacteriology, technic of mounting, pond-life and the theory of micro-optics. Among the contributors are Van Heurck, Cole, Hartog, Beck, Karop, Soar, Spitta, each upon his speciality. The price is one dollar if ordered through us. We shall reproduce its most most valuable features from time to time.

MICROSCOPICAL APPARATUS.

Bacteriolgoical Laboratory.—In Yokohama there is a microbe-proof house built of glass blocks. There are no window-sashes. The closed doors are air tight. A pipe containing cotton-wool filter admits the air partly purged of bacteria. It is further sterilized by being driven against glycerine-coated plate-glass. The house is flooded with sunlight which still further destroys micro-organic life.

Slides Loaned.—Mr. C. Baker, 244 High Holborn, London, has opened a loan collection of microscopical slides to general circulation.

Micro-photography.—The difference between the chemical and visual foci must be found by experiment, and marked down for future reference. The ground glass ought to be replaced by a piece of plain glass, and a focusing magnifier used ; a cheap one will answer perfectly.

This must be previously focused on a scratch or on a fly's wing placed on the inside surface of a plane glass. If you prefer using ground glass, make a small pencil cross on it, and then you cement over this, by means of Canada balsam, a thin microscopical cover-glass; then focus the ærial image till it is as sharp as possible. A large number of the invisible rays can be cut off by interposing a tank made of two pieces of glass, like a magic-lantern tank, filled with a weak solution of fluorescine between the light and the microscope. Perhaps other fluorescent substances like quinine would answer. The photographic objective must be removed, and the microscopic objective used alone; but with a short camera an eyepiece is an advantage. The proper one is a projection ocular, but the ordinary one will often answer; they, however, vary in performance. As microscopical objects are seldom flat, and as the objectives have little depth of focus, the image is always indistinct at the edges; for this reason only the central part of the image is used. It is necessary to have the light and condenser quite central with the objective. It is advantageous to lengthen this quarter-plate camera by means of a cone of paper, blackened inside, fastened on to the front. Be careful to fix the camera firmly to the table by a clamp or weights, to avoid shaking it when putting in the slide, as the image is easily thrown out of focus.—Eng. Mechanic.

MICROSCOPICAL MANIPULATION.

"Method of Fixign Blood for Microscopic Study."— I note in your December issue under this heading, that dissatisfaction is expressed with the ordinary method which is employed for obtaining slides, viz., the placing of two cover glasses together and then pulling them apart. For a number of years, I have tried this method and it is on very rare occasions that I have succeeded in obtaining a satisfactory spread. Some years ago, while working with Professor Ewell in measuring blood corpuscles, we obtained easily and quickly a large number of specimens in the following manner: The blood-drop taken from the ear or

the tip of the finger, projecting as it does above the surface, admits of an ordinary mounted needle being drawn quickly across its surface. The needle is then, with a circular motion, swept over the surface of a cover-glass held between the thumb and finger of the left hand. With a little practice, this method gives very satisfactory results, quite an even spread, and the corpucles are very little distorted. Necessarily in blood measurements, this latter is of the utmost importance.—*Harold N. Moyer.*

Drilling Lenses.—Make a lubricant with 1-2 drachm aqua ammonia, 1 1-2 drachm sulphuric ether, 1-2 oz. gum camphor, 1 oz. spirits turpentine. Keep it corked and in a low glass stopped bottle. The drill must be of steel as hard as file steel 2 1-2 m. m. in dia. by 4 c.m. long. Fit it in a handle of rubber with loose cap screw. Grind an oval head entirely around the piece of steel, heat it red and plunge in cold water, grind the head on two sides flat but without drawing the temper at all. Stroke on an oil-stone to give a sharp finished point, rotating it while stroking. Hold the lens or glass with the left hand with the point to be drilled on a rounded knob of wood or cork securely anchored. Keep the pariscopic side down. Drill half through and then turn and drill opposite. Hold the screw driver between thumb and second finger. Put the index finger in the revolving cap. Dip in the lubricating solution as required. The first hole may be enlarged by a round file moistened with the same lubricant. Watch crystals, plate glass, or any other glass may be drilled in like manner.

MEDICAL MICROSCOPY.

Inflammation.—In man, it is characterized by four cardinal symptoms; redness, swelling, heat, pain. But in the frog heat is lacking. Virchow held that the essence of inflammation was action of tissue cells. Acted upon by some irritation, they began to proliferate or multiply by division, thereby producing new material.

Cohnheim observed under the microscope what took

place in the inflamed mesentery of a frog; was struck by the changes in the circulation; observed the dilatation of the blood vessels, the inital acceleration of the blood followed by its slowing up, the mural implantation of the leucocytes in the veins and capillaries, the transmigration (or diapedesis) of the leucocytes. He looked upon the vascular system as the prime factor, and thought that a molecular change in the vessel-walls was the chief cause of inflamation. The adhesion of the white corpuscles (leucocytes) to the venous and capillary walls was explained by a greater adhesion, a sort of sticky condition: the slowing up of the current made the lighter leucocytes congregate at the walls, while the specifically heavier red corpuscles continued to swim in the middle; through a molecular change in the formerly impervious walls, they became able to let not only a greater amount of plasma transude, but also to allow the formed elements to go through by a peculiar process, and without making a hole in the vessel wall. Metschnikoff began to study the lower organisms as subjects of irritation. He went down to the very lowest organisms that we recognize as animal, and began with the ameba.

This is the most simply built of all animals; it consists in fact of one single cell, a mere mass of living protoplasm, with a nucleus, and without even an enveloping membrane. If an ameba is cut in two, the part that retains the nucleus regenerates itself and the lost part grows out. If the ameba meets a bacterium, it throws out jelly-like prolongations, that embrace the enemy and gradually coalesce with the mass of the animal, whereby the bacterium soon finds itself enclosed within the ameba; a contest begins, in which the bacterium by its toxines, secreted by its metabolism, impairs the vital functions of the host. If this is strong enough to resist action of the toxines the ameba disintegrates the bacterium, in the cell substance of which we see small granular masses, that finally fall apart, and the bacterium is destroyed; its remnants are then gradually expelled, and the ameba goes on living. If, on the other hand, the bacterium prevails, the ameba dies un-

der the formation vacuoles, and the bacterium growing on its substance, lives on and multiplies. Reaction against noxious influences in the ameba consists in regeneration, digestion, expulsion. This type is followed by all animals, but the process is more complicated in higher organisms. Following up this line of research, Dr. Knut Hoegh of Hamline University finds inflammation to be nature's protection against infection, a friend and not an enemy to be fought.

Kentucky School of Medicine.—Each lecture room is furnished with a powerful electric light, magic lantern or projector, provided with stereoscopic, polariscopic, spectroscopic, and microscopic attachments, with a set of microscope objectives which yield brilliant results. Minute microscopic objects can by this means be shown to hundreds at once. A large assortment of lantern photographs, micro-photographs, and microscopical specimens are available to students. Recognizing the importance of the study of Microscopical Anatomy, they have made additional improvements in the laboratories. The course given consists of demonstrations with the microscope, of lectures and practical work in the methods of preparing, hardening staining, and section-cutting, and mounting specimens for the microscope. Students make permanent mounts, and are furnished with all the necessary material, except the slides and covers used for their individual collections. Practical laboratory work following the lectures and demonstrations constitutes the course in bacteriology. Ample room, a superb and up-to-date equipment of microscopes, dry and steam sterilizers, thermostats, plate culture apparatus, etc., enable the student to do most excellent work. Cultures of all the most important bacteria, and many of the rarest, are kept constantly on hand. The student is taught the methods of sterilization, of pure cultivation, of the examination of colonies in cultures and of the microorganisms under the microscope. The student is drilled to practical mastery of all that is needed by the modern physician. The laboratory is open the entire year, and opportunity is given advanced students and graduates to

do special and original work. John R. Wathen, is Professor of Normal and Pathological Histology and Bacteriology.

BACTERIOLOGY.

Malarial Microbe.—Surgeon Ross has proved that malaria can be acquired from a mosquito bite. Insects are the media for its propagation. Particular species of malaria microbes accompany particular species of mosquitoes. But, of course, as is known, these parasites must fall into depleted constitutions in order to live and propagate. Certain ascetics get their bodies so purified that they sit quietly and unharmed in the midst of swarms of mosquitoes not one of which ever stings them.

Cheese Rust.—By this is meant the spots and rings seen in cheeses. It is due to an organism 1-15,000 to 1-25,000 of an inch in length. Connell has isolated and mounted it after staining with aniline in cultures. It stains best with carbol-fuchsin or aniline water, gentian or violet.

Widal's Test.—The Bacteriological Laboratory of the the city of Denver, Colo., is now making Widal's test for typhoid fever. The fact that an very expert bacteriologist is required to make the test is offset by the ease of transportation of specimens of dried blood, which long retain the power of reaction; and it is greatly to be hoped that further possible improvements in technic may place this most ingenious test upon a firmer practical basis than can at present be claimed for it.

NEW PUBLICATIONS.

Bacteriological Methods.—A laboratory manual of 90 pages outlining practice for students has been published by Professor Veranus A. Moore of Cornell University presumably for the use of his students. There are 60 lessons but the work is not a text-book on bacteriology. It will be useful to teachers in other colleges who wish to lead students through carefully arranged experiments whether

longer or shorter. His classification of bacteria, following Migula is: (1) Bacilli,—motile rodshaped organisms with flagella attached to all parts of the body; (2) Pseudomonas,—such organisms with polar flagella; (3) Bacteria, —the same without any flagella at all.

Histology.—E. K. Dunham, M. D. professor in Bellevue Hospital College has just published a text book of Normal and Morbid Histology but some of the medical journals criticise it. Messrs Lea Brothers & Co have not yet sent us a copy for review.

Text Book of Histology.—Including the Microscopic Technic. By Dr. Phillip Stohr. Edited with Additions by Dr. Alfred Schaper. Royal 8vo., pp. 424, with 292 Illustrations. P. Blakiston's Son & Co. 1898. Price $3.00.

This book is written in a very systematic manner. Beginning with the purely microscopic technic of the preparation of sections, the author then enters upon microscopic anatomy proper. In this part the microscopic anatomy of the cells and the tissues is taken up. Then follows that of the organs, including the various systems and the organs of the special senses. The blood is very well and thoroughly handled, as also the lymphatic system. The nervous system requires quite a share of attention. Nerve cells are considered too; dendrites have no notice accorded to them. In fact, the author very properly deals only with those subjects which are established and capable of demonstration and not with theories and late advances not yet universally accepted and proven.

DRUG AND FOOD ADULTERATION.

How to Study Drugs.—Acquaintance with the anatomy of flowering plants obtained by practical work with the microscope is an essential to the acquisition of a sound knowledge of the histology of drugs. In order to obtain this the student should first make himself thoroughly familiar with the various kinds of cells and vessels and the tissues which they form—epidermis, cork, fundamental

parenchyma, endodermis, pericycle, phlœm, xylem, medullary rays and pith as well as the varieties of stereom. He should then make himself acquainted with the anatomical characteristics of roots, stems, leaves, flowers, fruits and seeds as exhibited in the types found in the usual biological text-books, at the same time learning to recognize with care the different tissue systems both by means of the elements and the reactions the elements give with reagents. Then he should pass on to study in detail the various peculiarities of the different cells ; the varieties of thickening of epidermal cells, and the appearance of their walls in surface view ; the forms and sculpturing of lignified elements, stone-cells, vessels, fibres ; the frequency, characters, and disposition of hairs and glands ; the nature and disposition of cell contents, crystals, starch and its size and form of granules, inulin, tannin, resins, and oils.

MISCELLANEOUS.

Objects.—Foraminiferous sands, unmounted, from Mauritius, Aden, India, Montereau, Java, Sicily, Paris basin, Cyprus, Cherbourg, Beauchamp, Malstricht, etc., 24 packages for $1.00 ; or, cleaned unmounted diatoms, from Modena, Pitruame grande in Mexico, Estuaire de la Rance in France, Newcastle in Barbados, Pornic pelagique in France, Cwm Bychan in Wales, Godfry's bog in Massachusetts, La Guadeloupe with Meridion circulare, Tabellaria flocculosa, Surirella gemma and Pleurosigma angulatum, $1.00 per series of 12 tubes. Also 5 series of microfungi at 50 cents each and 7 series of wood sections at 50 cents each series can be had from W. West, 15 Horton lane, Bradford, England. They can be brought within the chinese wall as specimens of natural history objects free of duty according to the law.

A Printer's Ink that will stand washing is made from 5 parts acetic acid with one part lunar caustic dissolved herein. After it has stood 24 hours, add 20 parts copalt varnish and a little lamp-black. The lunar caustic gives it a brown shade which may be replaced by green if the

printed matter is moistened by water containing a little potassium iodide in solution. This ink must be made only as required for use and be used as fresh as possible. The ingredients require to be kept in closed flasks that they may not deteriorate.

Paste.—Dissolve 2 drams alum in 1 quart water. Add flour to make thick cream. Stir into it 10 powdered cloves and 1 drachm rosin. Pour the above slowly, stirring continually, into 4 ounces boiling water. Let all boil till of mushy consistency, stirring to prevent burning. If kept covered and in a cool place this paste will keep through the hottest summer.

Aniline.—A mixture of one part soap, 19 parts water and one part aniline is said to remove the most obstinate grease spots which may have penetrated into the fibre deeply.

Label Varnish.—Macerate together, shaking occasionally until the solids dissolve which will take a long time in the cold: Sandarac, 60 pts; mastic, 25 pts; camphor, 1 pt; oil of lavender, 8 pts; venice turpentine, 4 pts; ether 6 pts; alcohol, 44 pts.—*Nat. Druggist.*

Marine Specimens in Formalin.—Jar of Marine Algæ of many kinds, 50 cents; helix, 10 cents; starfish, 15 cents; and a list of many other specimens for sale by W. F. Webb, Albion, N. Y.

Second-hand Microscopes.—Beck high class Binocular with 1 1-2, 2-3, 4-10, 1-5, and 1-10 oil immersion lenses with complete outfit is offered for about $80, it being but a fraction of the original cost. Also many other microscopes. Clarkson and Co., 28 Bartlett's Buildings, Holborn Circus, C. E. London, England.

Botanical Objects.—As you know, I have had Nos. 22, 27, 38, 65, 81, 94, 97, 109, 112, 121, 123, 129, 130, 131 and all the starches. I now enclose $6 for which please send me all the remaining numbers of White's objects which you now have in stock.—*C. Williams.*

Wolle's Diatomaceæ of North America with plates for sale cheap. Address the Editor.

SPURGE PLANT: EUPHORBIA.

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CONTENTS.

The Life-History of a Parasitic Fungus.

[Compiled from Quecket Club Journal.]

WITH FRONTISPIECE.

One of the strangest facts in plant life that has been worked out by the botanists during the past 100 years is the following. There are plants called fungi which not only live parasitic upon other plants but require two or more species as hosts. This fact was not settled scientifically until 1816 when a Danish schoolmaster rubbed the underside of a barberry leaf infected with æcidia onto some plants in a field of rye. The rye plants were then marked. In a few days, the fungus had spread itself widely over the plants in question while all the others were free from infection. In 1760, the farmers of Massachusetts had felt so sure of a connection between wheat mildew (Puccinia graminis), and barberry bushes that

they enacted a law requiring people to destroy their barberry bushes as a protection to wheat fields, but they did not understand why. The English farmers had the same suspicion and fortunately acted upon it.

The fungus, Uromyces pisi, as its name implies is a pest to pea-growers. It belongs to the Uredinea family, a group of 500 parasitic fungi. It lives parasitic on the spurge, Euphorbia cyparissias, and also on the leaf of the common pea, Pisum sativum, going through five different spore relations which is the maximum number known to science.

The æcidia or "Cluster-Cups" of certain fungi are highly esteemed as opaque objects to be viewed by reflected light or in sections under the microscope, being very beautiful and interesting. The spores can be germinated in cold water containing one per cent of cane sugar. They should be mounted in glycerine jelly after having been soaked in glacial acetic acid to prevent shrinkage.

The effect upon the spurge plant resulting from carrying the fungus mentioned is striking and extraordinary. Examine figure 1 in the frontispiece and you will see that the healthy spurge is large and vigorous. It has thin, linear, and somewhat deep-green thick-set leaves, seven to fourteen times as long as broad. The infected spurge is shown in figure 2, with short, thick leaves which are wide apart, dusty yellow in color, only four times as long as broad. The stems are naked, stalky and unbranched. The floral organs are aborted and sterile. These two plants differ in color, tissue, habits and appearance so that they would hardly be recognized as of the same genus. The attack of the parasite causes elongation of the stem and shortening and thickening of the leaves. Figure 3 shows an enlarged leaf dotted with æcidia. There are usually about 50 æcidia to each leaf. They are about one-third of a millimeter in diameter and each contains some 250,000 spores each capable of inocu-

lating a healthy plant under proper conditions. Fig. 4 is a section of leaf through an æcidium and figure 5 is an æcidiospore.

To Professor De Bary we are most of all indebted for knowledge of these fungi which he worked out from 1861 to 1864. The five spore stages are: the promycelial spore, the spermogonium, the æcidiospore, the uredospore, and the teleutospore. De Bary discovered heterœcism or metœcism, by which is meant that the æcidium is only a stage or special form of fructification in the life-cycle of certain groups of fungi.

The first case of this cyclic development noted was that of mildew of wheat, the fungus being called Puccinia graminis.

"The mycelium of the æcidium or 'cluster-cup' stage of Uromyces pisi (De Bary) is perennial in the tissues of the host; hence, when plants are once attacked they never recover, but produce the fungus year by year. The æcidiospores, produced in the 'cluster-cups,' are dispersed by wind at maturity, and those that happen to alight on a leaf of the common pea (Pisum sativum) germinate within a few hours if the surface of the leaf is damp; the germ-tube bores through the epidermis, enters the tissues of the leaf and there forms a mycelium. This mycelium, or vegetative portion of the fungus, consists of a net work of anastomosing and transparent tubes. These tubes, containing protoplasm, are divided by septa at rare intervals into elongated cells. The septa become more frequent at the points where sori are formed, and, in Uromyces pisi and other species producing spores of that color, orange granules appear in the protoplasm.

About fourteen days after infection, the mycelium produces dense tufts of uredospores, which burst through the epidermis at maturity, and are distributed by wind and rain. Those uredospores that alight on pea-leaves germinate quickly, enter the tissues of the leaf, and, in

turn, produce other clusters of uredospores. The clusters of uredospores are of a pale brown. Uredospores are called summer-spores, their function being to enable the fungus to extend its area of distribution. Produced in enormous quantities, and very rapidly throughout the summer, it is easy to understand the swiftness with which the disease can, and does spread; the only limit being lack of supply of pea-plants for it to attack.

"In the autumn, when the host-plant is waning, a second form of spore is produced from the same mycelium that produced the uredospores earlier in the season. This second form of spore is known as a teleutospore, or winter spore, and differs from the uredospore in requiring to remain in a latent condition before it can germinate. The function of teleutospores is to tide the fungus over that part of the season—its season of discontent—when the host-plant is not present for it to prey upon. After remaining in a resting-stage during the winter, the teleutospores germinate in the spring, and give origin to still smaller spores called sporidiola or secondary spores. These exceedingly minute secondary spores are, as usual, dispersed by wind, and those that alight on the Euphorbia leaves germinate, enter the tissues, and, in course of time, produce the æcidiospores. The secondary spores produced by the teleutospores germinate only on the Euphorbia and not on the pea, whereas the æcidiospores formed on the Euphorbia germinate only on the pea.

"To effect a cure for the disease, it is only necessary to remove from the vicinity one of the two host-plants essential to its existence."—MASSEE.

The mycelium in the Euphorbia not only goes on producing the æcidia as long as the host lives but by the use of suitable reagents, such as caustic potash, can be seen penetrating almost every part of the plant, descending mainly by its vessels. During the winter,

when the Euphorbia dies down, the mycelium hibernates in the rhizome.

The spermogonia are generally formed below the upper surface of the leaf bearing the æcidia, and are developed from the same mycelium. Sometimes, however, they occur on the lower surface, and in juxtaposition with the æcidia. These flask-like growths in the sub-cortical tissue, with brushes of hair-like filaments at their projecting apices, pour out at maturity vast numbers of spermatia—extremely minute bodies resembling spores—which have been held by some to be the male sexual element. There seems, however, to be a consensus of opinion, amongst fungologists, that there is not sufficient warrant for this idea. The spermatia are associated with a viscid fluid. The largest examined are only the 1-2500 of an inch in length and the 1-100,000 of an inch in width. Their function is not known; but it is difficult to believe that they have none, useless indeed they are merely a survival of structures which were useful under conditions that have long since passed away. This is probably true in most instances, but spermatia of some species have been caused to germinate and produce a mycelium like that of the uredo condition of the fungus. Sometimes the spermogonia occur in conjunction with the uredospores, and sometimes in conjunction with the teleutospores, though this latter is apparently not the case in the genus Uromyces.

The æcidiospores are produced in basipetal chains, the oldest spores being at the apex of the chain, the youngest at the base: they are 1-1,250 of an inch in diameter. As the terminal spores mature they become free and form an orange powder, which fills the whitish-edged and slightly fringed cluster-cup. They are, at maturity, globular or polygonal, and roughened on the outer surface. The walls are thinner in certain places, and it is through these so-called germ-pores—and as a rule from

only one—that the germ-tube issues. It is these germ-tubes which, finding their way through the stomata of the host-leaf, start densely woven mycelial growths in the intercellular spaces of its tissue. The mycelial tubes not infrequently develope tubular suckers, called haustoria, which penetrate the cells themselves, abstracting their manufactured food-stuffs for the benefit of the parasite.

The mycelium present in the leaves of the host-plant (*i. e.* the pea) resulting from inoculation by the æcidiospores, gives origin to the first crop of uredospores, which are produced singly at the tips of short branches of the mycelium. The epidermis is ruptured, and they appear on the surface as orange-colored patches or sori. The uredospores thus formed germinate on other pea leaves, and produce successive crops of uredospores throughout the summer; and finally in the autumn, the same mycelium produces a crop of teleutospores, which form dark brown sori on the surface of the leaf, and these, as said before, rest during the winter either in the soil or on the surface in connection with the decayed leaves, producing in the spring the pro-mycelial spores which inoculate the newly developed foliage of the spurge.

The uredospores are spiny, of a yellowish brown, and 1-1250 of an inch in diameter. They are said to have as many as six germ-pores, and never less than two. The germination and mode of attack on the host-plant are the same as in the case of the æcidiospores.

The teleutospores, darker in color than the other forms, are produced generally at their period of decay. In some species this is the only form of spore known. They are unicellular in U. pisi, this being the invariable rule in the genus; and there is not more than one spore developed at the end of each pedicel. They are 1-800 of an inch in their long diameter by 1-1250 of an inch in their shorter one. The teleutospores of all Puccinias are bi-cellular.

At the apex of the teleutospore there is a single perforation only, or at any rate a thinning of the outer cell-wall which looks like it, and it is through this that the germinating process protrudes; in other words, the exospore is pierced by the endospore in germination. When the teleutospores are bicellular the germ-pore of the lower cell occurs just below the median septum. The germ tube of the teleutospore does not form a mycelium, but is broken up near the apex into three or four short cells, due to the formation of transverse septa. Each cell gives origin to a very slender outgrowth or sterigma, the apex of which eventually bears a pro-mycelial spore, capable of producing a mycelium.

Practical Hints on Mounting.

R. G. MASON.

FARRANT'S MEDIUM.—This is another preparation of glycerine and gum. It is useful for some anatomical preparations, Epithelium, etc. Upon a clean slip, place a little of the media. This must be carefully done as this mixture holds air bubbles with greater tenacity than anything I know of. It is best to warm the slip first over a lamp. Now having the object in soak in a weak solution of the above media and water, place the object in position, warm a cover glass, and cover gently, press down, set aside to dry. If, on examination, air is imprisoned under the cover with the specimen, the whole process must be repeated. The bubbles will not disappear by themselves as in the case of balsam and benzole. This media will take some days to dry. So it is best to look at the slides every day and press down if required. When fairly hard at the edges, clean off with a soft brush and water, set aside to dry, run a ring of cement round as before, and finish in the usual way.

Likely Failures.—Air bubbles round object or in media.

—Cause: slide not warmed—media having been shaken up, you cannot handle this too carefully.

2.—Cover shifting during cleaning.—Cause: not dry enough, or too rough handling; this media never gets hard like balsam.

Mounting Foraminifera, Polycistina, or any Small Bodies Dry in Cells.—Cells must vary according to the specimen that is to go into them. It would look very clumsy to see small objects like the above in a very deep cell For general purposes there are nothing better than the tin cells before mentioned. Some are made in vulcanite, but these are very ugly and are never used by professional mounters. Then we have them in glass, which are very useful for some anatomical specimens. Some have recommended them to be made in card, but these are very unreliable, as they absorb moisture and cause condensation on the inner side of the covering glass. These cells being pure tin, may be used for any known preservative, and are easily put on. Their cost is very low. Our specimen, in this case, we shall have perfectly dry, and in the case of good material clean. Construct your cell and gum the bottom with ordinary gum arabic solution. Place aside to dry. When quite dry, see there is no dust in it. Breathe on the cell so as to make the inside damp, *but from the breath only.* Take up the specimens and tip a sufficient quantity in to cover the whole bottom. Take the slip in your left hand and gently knock your right hand against it, holding it in a horizontal position so as to spread the specimens evenly. Breathe again on it if required. Place it under a low power to see if all the bottom of the cell is covered, as it looks bad to see the ground of the cell in spaces between the objects. When all is satisfactory take a piece of paper, place it on the table, stand the slide on end and gently knock it. Do the same at the side. This will detach any specimens that are not secure enough. Examine again, and if there

are any bare spots, breathe on the slide again and repeat the operation. Run a ring of cement round the top of the cell. Place a cover on and finish as before directed. Selected specimens may be mounted in the same way, by picking up the single object with a hog's bristle fastened to an ordinary light handle, such as is used for inserting into the camel hair pencils.

There are not likely to be any failures in the above as the process is so simple. The only thing that may happen is the varnish running under the cover glass.

Mounting Rock Sections, Teeth, Bone, etc.—Take a rock section on its glass, place it in a saucer in clean benzole for twenty-four hours or until it leaves the glass of its own accord. Now take it up on a spatula. A square ended one is best as when the section is on it, all excess of benzole is easily got rid of by standing it square end downwards and gently tapping the end, if needs be, on a glass slip. Don't use forceps to get the section onto the spatula, but your brush. Place the spatula just under the section and draw it on while in the benzole. Wash again in clean benzole, and have your 3 × 1 in. slip ready, with some balsam and benzole on it. Place your section in position (having first got rid of all excess of fluid). Add more of the media until it is well covered. Place it aside to dry. Rocks are best mounted by the exposure method before described. An oval-shaped cover is a great improvement, but of course any shape cover may be used, provided it is of suitable size. To mount teeth sections, a little different treatment is required. First, soak them off the glass (*they are ground on in benzole*), then wash in clean benzole, and put aside to dry. Make a rather thin solution of gum arabic, adding two drops of carbolic acid to about one oz. When the teeth are quite dry, take a section in the forceps, dip it in the gum solution. Having your slip ready cleaned, place it in the centre, press down, and set aside to dry. The object of the

gum solution is to prevent the balsam running into the structure of the tooth, and so rendering it too transparent. When dry, add balsam and benzole until it is well covered. Set aside in a place free from dust and mount by the exposure method.

Sections of seed-shells may be treated in just the same way as described for rocks, with this exception, that some of them are best soaked in spirits of turpentine before mounting, instead of the second benzole.

Where round cover glasses are used, it is best to run a ring of cement round before putting the finishing varnish on. This prevents all chance of its running under, and thus disfiguring or spoiling a specimen.

How to Clean Thin Glass Covers.—Always keep your glasses in a solution of nitric acid and water, about two parts water to one of acid. This will take any marks off that may be on the surface of the glass. The best way is to have them in a glass or porcelain vessel. Take them out as required, or clean a number together, immerse them in methylated spirits. Have a block of wood, about 6 in. by 4 in, tack a piece of American cloth over the top, fasten at the ends and sides, take your covers one at a time, and breathe on one surface and place on the block. A dozen may be placed in a row the damp-side down. Gently press them. All air being out from between them and the cloth on the block, they will hold. You can now clean them all together. When one side is done, take your forceps or something with a point and gently lift up one edge of each cover. Take them up in the forceps, repeating the operation of breathing. Your second surface will be clean. Should the other show any signs of contact with the cloth, this will easily clean off when the cover is used and fixed in its place, but this will be nothing to speak of if the block is cleaned occasionally. By this method, all cover glasses may be easily cleaned without breakage.

THE TURN-TABLE.—To use this instrument expertly, requires practice. The difficulty with the beginner is to set a slide central the second time, after having once had a ring put round it. Put a slide on the turntable perfectly centred. Pushing it as far as it will go up to the pillars of the springs will make it central one way. When central, make a dot on the turntable plate. A permanent one is best, just a drill tip inserted. When the slide is in position, make another dot on it with ink, directly opposite the one on the turntable. It will now be seen that no matter how many times the slide is placed on the turn-table, providing these dots are opposite and the slide pushed right against the pillars of the spring, it will be perfectly centered.

Never spin your table too quickly, or take up too much varnish in your brush at one time. Slowly turning just as the brush touches the slide will be the best. If the motion is too fast it will be easily seen that the varnish is thrown off or into the wrong place. By a little practice, the turntable becomes a most useful tool, in fact a kind of small turning lathe for forming all kinds of cells.

CLEANING OFF SURPLUS BALSAM FROM THE SLIDES.—Cleaning off the balsam in excess of what is required to preserve the specimen, is easily done if done right. If the slides have been dried by means of an oven, it may mostly be removed with a sharp knife. It may be finally cleaned with alcohol, by means of a piece of linen rag placed round the index finger of the right hand, the slide being firmly held between the thumb and finger of the left. The rag must not be tight, but fairly loose where it comes in contact with the slide. Pour out plenty of spirits in the saucer ; go over the slide gently but firmly; don't be afraid to touch it. As the balsam is dissolved by the spirits and cleared by the rag, a fresh part of the rag may be used and the slide breathed upon occasionally, when all will come clean in a short time. As with other

things, you may not be able to do this nicely the first time. I have done thousands of slides in this way.

It must be borne in mind that slides on which balsam and benzole is used never get hard all through. This is an advantage, as should a slide fall, the object is not liable to fly off as in the case of old balsam mounts, for the balsam gets brittle by age.

Caoutchouc Cement, for Making and Sealing Cells.

To Turn Cells.—Centre the glass slide on the turn-table, and with a sable-hair brush charged with sufficient cement, mark off the foundation of the cell in width and size required, the turn-table being somewhat rapidly revolved. Dip more cement and apply directly, before the first layer can set, and so on, always touching the top of the stream only, until the cell be raised to the height desired. Then lay the slide aside in a level position to dry. Slight cells dry in a few hours, deep cells, say 1-8 of an inch in two or three days. Keep the bottle tightly corked.

The brush may be cleansed with some alcohol. Before uncorking the bottle for use, always give it a sharp shake, and turn it over so as to mix the apparently thickened surface of the cement with the bottom contents. In the case of the cement becoming absolutely thickened by exposure or neglect, a small quantity of spirits of wine (not methylated) will fairly restore it; but a diluting fluid is sold in a very cheap form, a few drops only of which added will improve the working quality of it to the last drop in the bottle. It is always better to turn a few dozen cells at the time in the various sizes for circles usually preferred, and to lay them out to dry, exposed to the air until required for mounting purposes.

To Mount Objects in Glycerine, Oil, Canada Balsam, or Other Fluids.—Take a cell perfectly dry, apply or turn a sufficient layer of this cement round the top of the

cell, slightly overfill the cell with glycerine and put in the prepared object, place on the glass cover (previously tested to fit), press down the centre and edges of the cover until it is firmly in position, and with a damp brush gently remove the expelled glycerine. Test again and see by slight pressure that the cover is on the cement, and lay aside to set. The next day, the slide may be washed under the stream of an ordinary water tap, and when thoroughly freed from all traces of glycerine and quite dry, you should turn a layer of the cement over the cell, embracing the rim of the glass cover, and finish to taste (zinc cement is a strong finishing medium). This slide will then be found to be well sealed and the fluid permanently confined. All these directions apply to mounting objects in oil or fluid Canada balsam, excepting the use of water to clean them. The superfluous oil or balsam must be removed by a brush dipped in benzole, the brush being continually wiped between the folds of a cloth.

This cement is invaluable for securing glass covers on dry mounts. It does not run by capillary attraction, and absolutely prevents all finishing cements from entering the cell. It dries almost instantly and with the least possible internal evaporation, thus nearly always preventing cell dew. The result of the use of this cement for cell making, and the confining of objects in fluids generally, is beautifully perfect and reliable, and it saves more than half the labor usually required to obtain inferior results by other media.

Bacillus Tuberculosis Stained by Sudan III.

MARION DORSET, M. D.

This dye is insoluble in water, soluble, however, in alcohol with a red color, also in the various essential oils, in chloroform and xylol. Fat once stained with this material can be decolorized with difficulty. Daddi, who

first suggested the use of sudan iii in histological and pathological work, recommends that Muller's fluid and glycerin be used in hardening and fixing the tissues, and that absolute alcohol should not be used as a dehydrating agent, nor should the specimen be cleared in the essential oils or xylol nor mounted in Canada balsam.

I. *Staining of Tubercle Bacilli in Pure Cultures.*—Cover-glass preparations were made and fixed in the ordinary way and then immersed in a cold, saturated eighty-per-cent alcoholic solution of sudan iii for five minutes. The excess of stain was then removed by washing in several changes of seventy-per-cent alcohol for five minutes. The results obtained were very satisfactory, and the characteristic appearance of the tubercle bacilli could be very readily noted. The bacilli are stained somewhat better if left in the sudan iii for ten minutes and then washed in the seventy-per-cent alcohol. The germs are found stained a bright red and the beaded appearance is very distinct. Cultures of the bovine tubercle bacillus, and also of the tubercle bacillus obtained from swine, treated with this dye, were not apparently as well stained as in the case of the bacillus of human origin. The latter stained with sudan iii are not decolorized by washing for two minutes with dilute one-to-twenty-five sulphuric, hydrochloric, or nitric acid, or ammonia.

II. *Staining of Preparations from a Gland of a Tuberculous Guinea-pig, and from Sputum, which had been proved to Contain Tubercle Bacilli.*—The preparations were fixed as usual and immersed for ten minutes in a saturated eighty-per-cent alcoholic solution of sudan iii. They were then washed from five to ten minutes in seventy-per-cent alcohol. Upon examination the tubercle bacilli were found to be stained a distinct red and presented the characteristic appearance. No other bacilli present in the sputum had been stained by the sudan iii,

though they were evident in quantity when the preparation was counterstained with methylene blue. The tubercle bacilli still retained their characteristic red color and appearance.

III. *Sections of a Tuberculous Lung.*—Sections from the lung of a man who died of bronchial tuberculosis were stained from five to ten minutes in a concentrated eighty-per-cent alcoholic solution of sudan iii washed from five to ten minutes in several changes of seventy-per-cent alcohol, counterstained with methylene blue, dehydrated with absolute alcohol, cleared with clove oil, and mounted in Canada balsam. In this preparation the tubercle bacilli were stained red and could be distinctly seen lying in the tissue. The material from which the sections were made was prepared in the following way: Pieces of the lung were placed in absolute alcohol and allowed to remain for a week, then changed into a fresh lot of alcohol, and from that passed through alcohol and ether into celloidin in the usual way. The sections after staining were also dehydrated with alcohol, but in spite of this fact the tubercle bacilli were well stained by sudan iii. It is thus evident that their staining properties are not influenced by the dehydrating action of the alcohol. This differs from the method prescribed by Daddi in staining sections for fat, as he recommends that care should be taken not to dehydrate with alcohol or clear with clove oil. Possibly the fatty material in the bodies of the tubercle bacilli is not as soluble as that deposited in the tissues. It should be noted, however, that preparations stained with sudan iii and mounted in Canada balsam did not retain a bright color after a month's time.

To demonstrate further that sudan iii is apparently a selective stain for tubercle bacilli, I endeavored to stain the numerous varieties of bacteria found in decomposing sputa, pure cultures of hog cholera, glanders, typhoid, anthrax, symptomatic anthrax, diphtheria, and prodigiosus

bacilli, the spirillum of Asiatic cholera, and the *Staphylococcus pyogenes aureus*, with negative results. Preparations of the smegma bacilli were also made, but did not stain with sudan iii, although they were stained with carbol fuchsine according to the ordinary method. In a mixed preparation of tubercle and smegma bacilli, stained with sudan iii and well washed with seventy-per-cent alcohol, the tubercle bacilli appeared characteristic, while the smegma bacilli remained unstained, although this same preparation, when subsequently stained with carbol fuchsine, showed smegma bacilli present in abundance. It would appear from the results so far obtained that sudan iii may be considered as a selective stain for tubercle bacillus, and that this selective action is due to the large amount of fatty material present in the body of the germ. When stained with sudan iii the characteristic beaded appearance of the tubercle bacilli is very distinct, and, as has been suggested, this beaded appearance in the staining is probably due to the droplets of fatty substance present in the body of the germ. Although smegma bacilli stain very readily with carbol fuchsine, similarly to the tubercle bacilli, the fact that the smegma bacilli do not stain with sudan iii would indicate that their cell substance is very different from that of the tubercle bacilli.

The practical value of sudan iii as a stain for tubercle bacilli will be recognized where a rapid method is desired for staining the organism in tissues and for the purpose of differentiating without trouble between smegma and tubercle bacilli in cases, as in urinary sediments, where the smegma bacilli might be present, and throw some doubt upon a positive identification of the tubercle bacilli by the ordinary method of staining. The method takes about the same length of time as the carbol-fuchsine method for cover-glass preparations, but as the stain is a selective one for tubercle bacilli, it is well adapted to routine work.—*Bureau of Animal Industry.*

Nobert's Bands. Micrometer Rulings.

F. L. JAMES. M. D.

ST. LOUIS, MO.

Bands of rulings on glass, were first made by M. Nobert, a Frenchman, some forty or fifty years ago. Prior to the preparation of these rulings, and indeed, down to a comparatively recent period the only method of testing the amplifying and especially the defining powers of microscope objectives, was trying them on certain well-known test-objects—the scales on certain butterfly or moth-wings, certain frustules of diatoms, etc. M. Nobert devised an instrument for ruling on glass, with a spicule or splinter of diamond; lines at a definite and known distance from each other. These lines were arranged in bands, commencing with a comparatively low number to the millimeter, and gradually increasing, each band containing ten lines and consequently decreasing in width as the lines were placed more closely together. At first M. Nobert made the bands of known dimensions, 100, 200 etc. up to 500 or 1,000 or 10,000 to the millimeter, but later on he made the bands consist of lines whose spaces were an unknown quantity, thus making each possessor of one of his rulings determine the value of each band for himself. This was done on account of the very important part played by imagination or rather by the knowledge of the number, a knowledge which would cause the observer, knowing the real number, to imagine that he saw and was able to count the lines. The following is a summary of the Nobert plates as known to the writer, and he is of the opinion that it embraces all of that artist's work, though there may be other series: Ten band plates, running from 11,259 to the inch, up to 50,667, with no regularity between the series—thus No. 1 being 11,259, No. 2, 13,100; No. 3, 15,300, etc; thirteen band plates, running from 45,000 up to 112,595 to the inch;

fifteen band plates, running from 11,259 up to 56,297 ; the nineteen band plates (of which he made more than any other series, and which are most often quoted) run from 11,259 up to 112,595 to the inch. A twenty band plate was also made by M. Nobert, but the writer has never seen one. It ran from the same initial (11,259) up to 123,854. These figures are on the basis of the Paris line being equal to 0.088,813,783 inch. Up to some twenty-five or thirty years ago, M. Nobert had an absolute monopoly in this line, but about that time two or three Americans devised ruling engines almost simultaneously—Professor Rutherford, the astronomer, the late Professor Henry Rogers, of Cambridge, and the late Henry Fasoldt, chronometer maker of Albany, N. Y. The rulings, technically termed "gratings," of Mr. Rutherford are esteemed all over the world as superior to any other, and are used in making the most delicate astronomical measurements. Professor Rogers acquired a wonderful skill in making rulings, and his plates are very highly prized by those fortunate enough to possess them. They were never made for sale. Some of the most wonderful of his rulings are in the possession of Dr. W. J. Lewis. All of the gratings sent out by Nobert, Rutherford, Rodgers and those who have acquired the engines of the last-named and made rulings with them, are accompanied by "tables of corrections," which enable the observers who use them as stage micrometers, to secure absolutely correct results. The late Mr. Fasoldt, refused to issue such tables, claiming that they are unnecessary and that the rulings are absolutely corfect. The bands of rulings of all the American artists in that line, are carried far beyond the power of the microscope to separate the lines, and the distances between them are arrived at through the spectrum which they present, the calculations being based on the wave lengths of light. Professors Rodgers and Rutherford made gratings on speculum metal, and the

former made at least one grating on an alloy of platinum and gold, which, when placed in the sunlight is one of the most gorgeously beautiful objects the human eye ever rested upon. All the colors of the prismatic spectrum are reflected from the lenticular surface, and the phenomena of interference cause the little button to glow like a living coal, sending forth light rays of a myriad hues, yet so soft, withal, that the eye can rest upon it without fatigue for several minutes.—*Nat. Drug.*

Experiments in the Artificial Culture of Diatoms.

William A. Terry.

In the summer of 1895, I spent some time in exploring the marshes, ditches, and pond holes of the valley of the West River, between New Haven and West Haven, to investigate the diatoms of that locality, some account of which has been previously published in this journal. The N. Y. & N. H. railroad at that time crossed this marsh on a long trestle-work which was provided with a series of water tanks for use in case of fire. I made inquiries of the official in charge of this bridge as to the possibility of crossing the marsh in various directions. When he understood I was in search of microscopical material he called my attention to these tanks. The water they contained was saturated with salt to prevent freezing, and he said the professors of Yale College claimed they held an organism that could not be found elsewhere this side of Salt Lake, Utah. On examining these tanks, I found the one directly over West River to be swarming with Artemia gracilis, the so-called "brine shrimp." They were quite large, mature, and loaded with eggs, which looked under the microscope like bunches of grapes. The ditches on both sides of Washington street where it crosses the marsh, connect with a small brook flowing from West Haven. At low tide the mud laid bare in

these ditches and the brook is coated with a yellowish brown film composed of living diatoms. These were chiefly Pleurosigma americanum, P. hypocampus, P. brebbissonii and P. fasciola, with myriads of minute species.

I have previously published accounts of attempts at the cultivation of such finds of diatoms, which attempts all failed from the same cause, the rapid development of destructive animals which devoured them. I examined this film at different points along the brook and the ditches for more than a mile in length. It varied considerably in different places, at one point it was a yellowish green from the presence of vast numbers of Euglena. It proved to be the most extensive continuous film of living diatoms I had ever seen not even excepting the extraordinary one at Shell Beech, Leete's Island, that I described some years ago. I visited Leete's Island in the autumn of that year and found the film of diatoms had disappeared, but late in August, 1895, the film was still conspicuous at West River, and it occurred to me that as my experiments were all tried during warm weather, another trial in cold weather might have a different result. Accordingly, in September, I appeared on the ground with a large basket loaded with glass bottles, a jointed fish-pole and other apparatus. About a dozen of these were quart bottles and square, to pack closely. I filled one of these from the R. R. tank, getting an abundance of Artemia gracilis. Then fastening a tablespoon on the end of my fishpole, I scooped up a quantity of the film of diatoms from various points along the ditches and from the brook, filling up each bottle with water from the same place from which I took the diatoms. After about three hours work I had collected nearly four gallons of saltwater and diatoms which I thought abundant for my experiments. At home I poured out this material partly into glass dishes and partly into brown earthen bowls which I procured for this purpose.

As I had frequently noticed that the occurrence of these

films of living diatoms on the mud or sands was nearly always on the sunny side and never in the shade, it seemed evident that sunlight was necessary to their proper development, but direct sunlight on a small quantity of water in a warm room would produce a temperature injurious if not fatal to the diatoms, and, at the same time favor the development of destructive animals. My conclusion from observations of many years was that a temperature of about 60 F. was the most favorable to success. Accordingly all the material was placed on an open veranda, exposed to the direct warming sunlight until about ten o'clock, after which it was in partial shade. After a few hours rest a film of living diatoms had appeared on the surface of the sediment in all the receptacles.

One peculiarity of this film was, that on lifting any part of it, it cohered like a membrane, although it was composed entirely of independent diatoms, all of them rapid travellers. It seemed that in producing their motion they adhered somewhat to whatever substance they were in contact with, thus requiring some force to separate them from each other, although when a small fragment of this film was placed in a drop of water on a slip the diatoms would scatter out in all directions. A piece as large as a pinhead woud fill an inch cell so full in a few minutes that hundreds would appear at a time in the field in any part of the cell. One of the bowls was filled with material taken with a scoop-net from the bottom of a ditch twelve feet wide with water about two feet deep at low tide. This had but a small amount of sediment and contained some Oscillaria and Euglena, and was very rich in Pleurosigma fasciola. The Oscillaria soon collected all the sediment into a rounded button at the centre of the bottom leaving the sides of the bowl clean and bare.

This button of sediment in the early morning was covered with a white substance which in the bright sun-

shine soon turned pink and afterwards purple. I had seen this purple deposit covering considerable spaces in the salt marshes and had tested it enough to find that it contained a considerable amount of free sulphur, but what gave it its photographic properties I did not make out. This substance under the microscope appeared composed of very minute particles, the larger of which were of flattened ovoid form, and the smaller of irregular shapes, and all in rapid motion.

After several days exposure, the sides of this bowl became covered simultaneously and uniformly with a light brown film which the microscope showed to be composed of countless myriads of a small organism resembling miniature Nitzschia closterium but showing little trace of its characteristic markings and being apparently destitute of silex as they completely dissolved in boiling water. Their color and their motions were those of diatoms, but otherwise they resembled desmids.

I sent small vials filled with these organisms to many experts, explaining the circumstances and asking their opinions. I received various replies. Most of them were in doubt, but Prof. H. L. Smith wrote that they were certainly Nitzschia closterium, that he was very glad I had them in such quantity and such purity, and that he hoped I might succeed in keeping them long enough to observe their method of conjugation which he believed had never been seen in this species.

Now, note here, that I had made numerous examinations of this material and thought I had observed every species it contained, but had not seen a single individual of this species until the sudden appearance of this film. I had little hope of being able to protect these diatoms against their enemies for any great length of time, but to take all the chances possible, I isolated portions of the film in various small vials of different sizes, and made a large number of life slides. The life slides proved a

complete failure at once, as none of the diatoms in them lived longer than two or three weeks. In the vials they did better, some of them remaining alive two years afterward, but development was checked. They simply existed in much the same condition as when placed in the vial. In two or three, however, some individually increased in size until they were at least twice as large as the rest. After this, there was little change for some time. I tested these larger ones with acids. A very faint, shadowy and flaccid membrane of silex remained. In the original vessel the film soon showed traces of the attacks of destructive animals. Colonies of minute naviculoid diatoms, of Amphora and Nitzschia formed and grew rapidly and then the whole film would become detached and slide down to the bottom.

One of these cultures was of material from the Quinnipiac marshes. This developed abundance of Melosira borrerii. With this was Nitzchia reflexa in condition similar to the N. closterium before described, but the frustules were about twice as large, and had sufficient silex to show a faint and feeble skeleton after treatment with acid. I have found the rocks in Morris creek above the dike covered with a growth of Melosira borrerii in which the filaments averaged over six inches in length. Detached patches formed rounded masses sometimes a foot in diameter, resembling in appearance a commercial sponge.

Taken from the water they collapsed into a rope of harsh feeling filaments without any elasticity. The harsh feel was caused by independent diatoms and other organisms adherent to and entangled among the filaments. The adherent diatoms were often Pleurosigma, the specimens of the small "Pleurosigma paradoxum, H. P." described and figured by Peragallo in his monograph, were taken from a clump of Melosira from Morris creek. It may seem an extreme view to class these microscopic filaments of the culture, with the other comparatively gigantic growths, but the change from

one to the other is easily understood when their life history is known. As these papers are intended to be a mere recital of the facts observed I say little about theories. The culture filaments were about 1-4 inch in length and small in proportion.

The second morning after these gatherings were placed in the open air and sunlight, the surface of the sediment in one of the glass dishes was completely covered by a display of hydroids, the sea anemone in minature circles, of pure white pearly tentacles from 1-2 inch in diameter down to those barely visible. A slight jar would cause them all instantly to retract and disappear. The tubularian worms also began to show their presence. Most of them were of single proboscis kind but I found some with eight tentacles. Six of them were somewhat club-shaped and two considerably longer and tapering, resembling closely the arms of a squid. Among the Pleurosigma fasciola, was found the pointed cylindrical form previously found at Leete's Island. This was a little longer than P. fasciola, tapering at both ends with a slight knob at the point. It was very active, traveling faster than the Pleurosigma. It was destitute of silex. They could be bent up double, in form of an ox-bow, without injury. Then when we removed the pressure they straightened out and moved on as fast as before. My previous experience with Nitzchia closterium forced me to think that these were immature Nitzschia tænia, chiefly because quite a number of mature and normal forms were found with them. The mature forms were more than three times as long as the others and large in proportion. They were rigidly siliceous and could not be bent without breaking.

The weather becoming cold the cultures were moved into an upper room to set before the south window where they had the full sunlight. But the room was cool enough to prevent too much rise of temperature. Here new colonies developed quickly: Navicula, Amphora, Nitzschia, Melosira and others. Some were enveloped in gelatinous mucus, but

the others were without. Very many were aggregated into dense masses. The water abounded with independent individuals traveling in every direction, and also with minute spore-like forms of the same color as the diatoms and which were also actively motile. It was impossible for me to decide upon the species of these minute forms. In some cases even the genus is doubtful. In my article in the December number of this Journal, the printer makes me say as to similar forms that they were of "well-known" species. I wrote "unknown." These forms are mostly rounded even when elongated, with no sharp angles and with slight markings, without silex, and can be known as diatoms only by their color and motions. Some of the Navicula and the Nitzschia resemble each other so closely at this stage as to be easily mistaken. Although the original diatoms of this material were nearly all Pleurosigma, yet nothing developed that could be recognized as that genus. I had one bottle filled at Morgan Point rocks projecting into the Sound outside the Lighthouse Point. This I poured into a large tumbler which it just filled. It had little sediment but diatoms,—Synedra, Melosira, Navicula maculata and other large varieties. In a few days, a number of delicate and beautiful bright green algæ started and grew rapidly. These were possibly Cladophera refracta, but they did not attain sufficient maturity for me to decide with certainty. Soon a red Schizonema appeared upon the branches and finally attained more than an inch in height. It was of beautiful rosy pink in color and had a flattened, much-branched frond filleded with diatoms. In this stage, it made a splendid object for the microscope. I have always keenly regretted that I did not sacrifice it for slides, but I wanted to study its development. Other branches showed a light-golden brown fringe. This was a very delicate Acnauthes with long stipe, as long in proportion as that of Acnauthes longipes, but the diatom was scarcely one-tenth as large.

One morning, when the algæ had grown nearly to the top

of the water, I found them covered with flesh-colored worms or larva about 1-16 inch long, and soon small sand fleas appeared. These soon grew large enough to attack the larva which they soon devoured and then commenced upon the diatoms and algæ. In a few weeks nothing remained except the crustaceans and an increased sediment, a careful examination of which showed not a trace of diatoms. Not even a fragment of the shells of the large species forming the original sediment remained.

As many of the cultures had altogether too many diatoms for healthy growth, I drew up portions of the film with a dropping tube and made other cultures that thus consisted chiefly of salt water and diatoms with very little sediment. Some of the original cultures that had developed too many animals and had lost their diatoms were boiled, and after ærating sufficiently a new stock of diatoms added. From others I carefully poured off the salt water and used it as a culture without addition, boiling the sediment in a large quantity of fresh water which was reserved as a nutriment for the cultures. The boiled sediment was cleaned and slides made from the diatoms obtained from it, to be used for comparison with those obtained from the cultures later.

In this way, I soon had a stock of over 50 cultures, large and small, under observation. The most ubiquitous and also troublesome of the animals were the anguilla, or "eels." They appeared everywhere in spite of every care, but the most obviously destructive to the Pleurosigma were the ciliata. An oyster-shaped species, large enough to contain 15 or 20 Pleurosigma americanum at once, devoted themselves to the destruction of this kind entirely, while the little ones devoured the corresponding Pleurosigma, but the amebæ took both diatoms and spores. The proper habitat of Pleurosigma americanum is the estuary or tidal inlet at the mouth of fresh water creeks where the tide ebbs and flows, and I supposed from this that they would not be very sensitive to changes, but to my surprise I found

them the most sensitive of all. Any increase in the saltness of the water would produce a change in the arrangement and color of the endochrome in a short time.

I had a quantity of these Pleurosigma in a small tumbler on my worktable. They were left here in a warm room over night. The next day the water had dried down to less than half its former bulk and the diatoms, showed a remarkable change. The endochrome had darkened in color and separated into detached masses generally six or eight in number but several were filled with very small globular forms resembling spores. Their activity had diminished, but most of them still retained their characteristic motions. I applied a small drop of fresh water to one edge of the cover-glass. All motion was instantly arrested, and those diatoms directly in the line of flow had about half their endochrome forced out into the water, apparently through the raphe, while in those out of the direct current, the endochrome soon swelled and became apparently homogeneous, filling the frustule. Afterwards their motions gradually recommenced and they were soon traveling again nearly as fast as ever.

I repeated this experiment many times, and always with the same result. All these actively traveling Pleurosigma had invariably a small bubble of air inside, in contact with the valve at the raphe. The position of this air bubble varied definitely for each species. In P. americanum it was near the centre of the flexure, in P. hypocampus it was nearer the end, and in N. sigma it was at the point of the frustule. After it had once attracted my attention, I did not fail to find it in every case.

LETTERS TO THE EDITOR.

GATES' DOUBLE MICROSCOPE.

Not New.—This microscope does not differ in principle from that described by Dr. Royston Piggott before the Royal Society in April, 1870. He stated that the general interpretation of the Podura scale was incorrect, and he gave an illustration of what he had proved it to be by this means, but which no one has been able to see, and which other microscopists have declared (consequently ?) to be spurious, and still later that his theory was wrong. His remarks, however, created a great reduction in residuary aberration in micro-objectives. He exhibited to Messrs. Powell and Lealand an image of Pleurosigma angulatum with a power of 4,000 diameters, from an ⅛in. object-glass, with clear and distinct beading. I have used it and found it very interesting, but very difficult to get good results, and never succeeded in getting differentiation which I had not obtained before.—*R. O'H.*

Important.—The invention of Professor Gates I look upon as most important, and is on the same lines as some experiments I made about 15 years ago.—R. J. NUNN.

Slides.—Where can I get mineralogical and microscopical specimens mounted opaque without glass cover but capped to keep out the dust. Cap to be removed when specimen is put under the microscope.—C. VB.

EDITORIAL.

To those public libraries not already subscribers to this journal, (though most of the large ones are already on our list), who send subscription for 1899 ($1.50), we will make a *present* of THREE BOUND VOLUMES, 1895, 1896, 1897.

A Delicate Experiment.—Delage divided an egg of a sea-urchin under the microscope into two parts, one containing the nucleus and the centrosome the other simply cytoplasmic. Beside them he placed an intact ovum and then let in spermatozoa. All three objects were fertilized,

all segmentated, the latter most rapidly. After three days, it had become a typical gastrula, the nucleated fragment a smaller gastrula, and the non-nucleated also a gastrula. All the cells showed nuclei.

Indian Corundum.—In India, it is found in connection with basic and acid rocks. Particularly a felspar rock in the Coimbatore district of Madras yields unaltered corundum which shows no evidence of secondary origin. Corundum may be crystallized out from certain slags which contain an excess of alumina.

White's Objects.—We have received a supply of the following new preparations from Walter White. Those who have all the others will want these also.

Vine	Stem.	T. S.	Serial	Number	182
Oleander	"	"	"	"	183
Maple	"	"	"	"	184
Hop	"	"	"	"	185
Oat	"	"	"	"	186
Larch	"	"	"	"	187
Plane	"	"	"	"	188
Andromeda floribunda		"	"	"	189
Iris, Leaf	"	"	"	"	190
Mexican Agave		"	"	"	191
Cucumber, Ova		"	"	"	182
Dendrobium, Aerial Root			"	"	193
Fern, Stipe		T. S.	"	"	194
Lady Fern	"	"	"	"	195
Sea Buckthorn		Cuticle	"	"	196
Arancaria imbricata		"	"	"	197
Barbary		"	"	"	198
Horse-chestnut,	Starch		"	"	199

MICROSCOPICAL APPARATUS.

A Lantern Microscope.—Hugo Schroeder gave a lecture and demonstration of his Electric Microscope in London Jan. 11, 1899. It is capable of a magnification of 10,000 dia. Its efficacy is due to a new lamp of semi-incandescent type.

Greenough's Instrument.—The microscope, as constructed heretofore, only allowed the inspection of even surfaces, and its focus was so limited that objects of any thickness had to be first prepared between glass plates or upon a flat surface to become visible at all. This quality makes it a most difficult matter to observe objects through a microscope of great enlarging power. In order to obviate this difficulty and to enable the enlarging of small bodies as well as of flat surfaces, an American living in Paris, Mr. Greenough, constructed a microscope with a greater depth of focus, which has since been further improved by the optician Czapski, of Jena, Germany. Its lenses are so arranged that they will permit the inspection of uneven surfaces, and in order to make the vision more perfect two systems of lenses are provided, giving the image a strongly stereoscopic effect. As built by Greenough, the image was inverted, but the present form of instrument shows the correct image, the inverted picture being turned around by prisms placed between the lenses, as in the Zeiss field glasses.

Objective.—Dr. M. C. White of Yale has adopted an objective of 20 mm. focal length, and a numerical aperture of 0.95. It is a magnified copy of a 5 mm. apochromatic, the diameters and radii of curvature of all the lenses being increased fourfold. His theory is that if a certain angular aperture is necessary to secure proper definition with a magnifiying power of, say, 1,000 diameters, then a similar aperture will be necessary to secure good definition in an image projected on the screen, even if it is obtained with a 3-4ths inch objective and a projection eyepiece.

Pointer.—A convenient pointer for class demonstrations and other work may be made by cementing a human hair to a circular ring of blackened paper or card board which can be placed on the diaphragm or removed at will.

Color Effects.—By looking at objects through a screen of two glass plates, one laid upon the other, and one being of a blue tint obtained from oxide of cobalt, while the

other was of a yellow tint got from oxide of manganese and iron, M. Henri Cross finds that objects which to the naked eye appear the same in color, look different through the screen. Thus a green emerald looks a rosy violet through the glass, but a false green emerald looks green. True sapphire keeps its natural blue through the screen, and false blue sapphire appears a rosy red. An Egyptian cup of Sevres blue paste appeared blue, save a part restored, which was red. He was able to conclude that the Egyptian paste had a base of copper blue and the restored part one of cobalt.—*Optical Journal.*

MICROSCOPICAL MANIPULATION.

Spirit-Proof Micro-Cement.—Take Nelson's amber gelatine, soften with water, pour off the water, melt the gelatine with gentle heat. Add sufficient white lead in powder to render it opaque.

Focal Lengths.—It is not an easy matter to measure accurately the focal lengths of microscopic objects ; but a sufficiently close approximation can be obtained by comparing them with others, whose focal lengths are known, taking care to use the same eyepiece and the same tube-length. The best way of measuring the power of any combination of objective and eyepiece is to place a stage-micrometer on the stage and compare the graduations as seen through the instrument with one eye with an ordinary foot-rule, as seen at the same time with the other, both eyes being open and the rule being at a distance of 10 in.—A. Woolsey Blacklock, M. D.

New Stain.—Ziemann has described a new stain for demonstrating the nucleus of the malarial parasite. He uses various proportions of a one-per-cent watery solution of rectified methylene blue and a one-per-cent watery solution of eosin. He demonstrated throughout the body of the parasite certain reddish stained areas.

To Kill Invertebrates.—Take four parts of a saturated aqueous solution of corrosive sublimate and add to it one

part of glacial acetic acid. Pour it upon the organism while its organs are extended and, before it has had time to retract them, death will ensue. Then take up as much as possible of the killing fluid and replace it with thirty per cent alcohol. Repeat and add alcohol till sure the sublimate and acetic acid are gone. This will be accomplished in half an hour. Objects too minute to be seen readily or found easily may require some other reagent.

To Mount Ameba.—Dr. Overton of Zurich University gives the following: Having killed by usual methods bear in mind the brittleness of such objects. Transfer the object with a particle of alcohol upon a clean cover glass. Upon a thin piece of cork place the cover glass containing the ameba having the cork smaller than the glass so that the latter may project over on all sides and prevent the alcohol from running off from the slip. The cork is placed on a little bridge of lead and the latter in a small dish. Pour into the dish enough absolute alcohol to reach half way up to the slip. Cover and set away over night. The next day, it will be discovered that absolute alcohol has replaced the thirty per cent alcohol and the ameba has been dehydrated—freed from all trace of water. This is necessary because a preservative must saturate the object in lieu of the water.

Now apply a ten per cent solution of collodion to the object till the glass is fairly coated with it when it may be dropped bottom side up into eighty per cent alcohol to be hardened. This seals up the object and protects it from injury, and it can stay here as long as desired.

To stain, hæmalum or any other aqueous or alcoholic stain is used. Pass the object (upon the glass) through fifty and thirty per cent alcohol to pure water. Soak in the coloring agent 5 or 6 hours, in water an hour, then back through 30, 50, 75 per cent alcohol. Do not go to absolute alcohol unless you wish to dissolve the collodion and remove the ameba. If the collodion gets stained too deeply it must be taken out with alcohol or water. Amylic alcohol does not dissolve collodion and is useful to follow the seventy-five per cent alcohol. After quarter of an hour xylol

may be used to render the object transparent. Discontinue when sufficiently transparent.

To mount. Still wet with xylol, collodion side up, place on a glass slide 3x1 some very thin Canada balsam in xylol. Lower with forceps the glass slip, object-side down into its final position. When the balsam is dry, clean, polish and label the slide.

BIOLOGICAL NOTES.

New Alga.—An ellipsoidal coenobium, about 1-200 inch long, containing 32 biflagellate cells arranged in five rings around the periphery of the hyaline gelatinous matrix is called Pleodorina illinoisenses by Kofoid. Like other Volvocineae, one pole of the colony is always directed forward during locomotion. They differ from those at the posterior pole being provided with larger red pigment spots. Four of them, called vegetative cells, are much smaller than the other 28 gonidial cells. This find is in eresting because of its well-developed structural and physiological polarity. Pleodorina is intermediate between Volvox and Eudorina.

Jerusalem Mud.—Forty years ago, some samples of dry mud were taken from the ancient pool of Gihon, outside the Jaffa gate and sent to England. Being moistoned, six new species of Entomostraca were found. Although dried and moistened for eight years in succession, the species could still be found alive.

Ameba.—A new Rhizopod parasite of man has been found in abundance in the serous fluid-accumulation of the peritonal and pleural cavities of man in a case of peritonitis. It is called Amœba miuria.

BACTERIOLOGY.

Are Bacteria Fungi ?—Dr. Johan-Olsen says they are one stage in the development of fungi. He cites the species of Oospora. When their tenuous hyphæ break up into conidia, the latter closely resemble rod-shaped bacteria in

size and form which grow into genuine branched mycelia. Branched tubercle and diphtheria bacilli may be involution forms. They are usually found only in old cultures, sparingly, and under conditions unfavorable to the organism. The mycelium of Dematium casei changes into bacteria bearing endospores, the germination of which he witnessed.

MICROSCOPICAL SOCIETIES.

Royal Microscopical Society.—On Dec. 21, Mr. E. M. Nelson, exhibited a new objective by Carl Zeiss, called a "Plankton-Searcher," a low-power water-immersion objective, designed for use in examining living objects in water the definition of which was exceedingly sharp. He also exhibited an erecting eyepiece fitted with Porro's prisms, another new appliance produced by the same firm, which would be found useful for dissecting and other purposes. Mr. Keith Lucas exhibited and described a new model microscope, the design of which was to effect the coarse and fine adjustments by means of a single slide, thereby reducing the expensive work of planeing. The President directed attention to some of the various types of binocular microscopes that were exhibited. Among those referred to were Ahren's binocular eyepiece, in which both tubes were equally inclined ; and a microscope by Murray and Heath, one tube only being inclined, the other lying in the optical axis of the instrument, the construction being similar to that of Nachet. These two instruments were exhibited by the Society. There was a new binocular dissecting microscope by Leitz, exhibited by Messrs. Watson and Sons, consisting of two Brucke lenses, fitted on a bar by jointed attachments, so that the distance between the tubes could be adjusted to suit the eyes. This was likely to prove valuable for examination of objects, or for dissection under low power. Attention was directed also to a form exhibited by Carl Zeiss, made with Porro's prisms, giving an erect image ; this microscope is provided with two objectives of equal power, one for each tube, the stere-

oscopic effect being greater than that obtained by a divided image frdm one objective. Messrs. Powell and Lealand exihibted their high-power binocular prism in conjunction with a 1-20 in. apochromatic objective. A Nelson model, a Mojinie's portable binocular, Wenham's binocular with a high-power objective, a binocular micro-spectroscope were exhibited, besides various patterns of Stephenson and Wenham binocular microscopes by Messrs. Chas. Baker, R. and J. Beck, J. Pillischer, Swift and Son, and Watson and Sons. Among the other objects exhibited may be mentioned typical species of foraminifera, selected from various localities, by Mr. A. Earland, which were rare and beautiful ; and mounted specimens of hydrozoa, by Mr. G. E. Harris.

NEW PUBLICATIONS.

Die Mikroscopischen untersuchungs-Methoden des Auges. Dr. S. Seligmann, Augenartz in Hamburg. S. Karger, Karlstrasse 15, Berlin. Price, 6 marks. 240 pp.

For microscopical technique the laboratories of the Anatomical Biological Institute of Berlin are second to none in Germany. A student there, Dr. Seligmann, has complied the different methods, which he has met with, and which he has proven to be of value by personal experience in the laboratory. The histological examination of the eye differs materially in technique from that of general histology, and the worker in microscopic histology and pathology of the eye will save much time and valuable material by adhering closely to the instructions of the author. The subject is fully and well covered, the first half of the book being devoted to what may be called general remarks with regard to collecting of material, preparation of bulbus, ways of producing microscopical preparations, etc. The second half is especially given to the specific treatment in technique of various parts of the eye.

Moore's Bacteriology.—"It would be difficult to find anywhere in the world, in the same number of pages, as many important and useful suggestions."—A CRITIC.

Swiss Rotifers.—Dr. Weber has published in French a superbly illustrated monograph on the rotifers of Lake Leman. Each species is described and figured in colors. The males and eggs are illustrated in some instances.

Photo-Micrography.—A book by Edmund J. Spitta, late Demonstrator of Anatomy in St. George's Hospital Medical School, London, has been issued by the Scientific Press, Ltd., Southampton Street, Strand. It contains 40 halftones and 63 other illustrations. Price $3.00. While suited to those commencing the subject, it will assist those desirious to achieve the highest results in what is now a scientific and commercial necessity.

Fossil Plants.—Seward has issued a text book in which will be found a certain amount of matter relative to algæ, diatoms, etc.

MISCELLANEOUS.

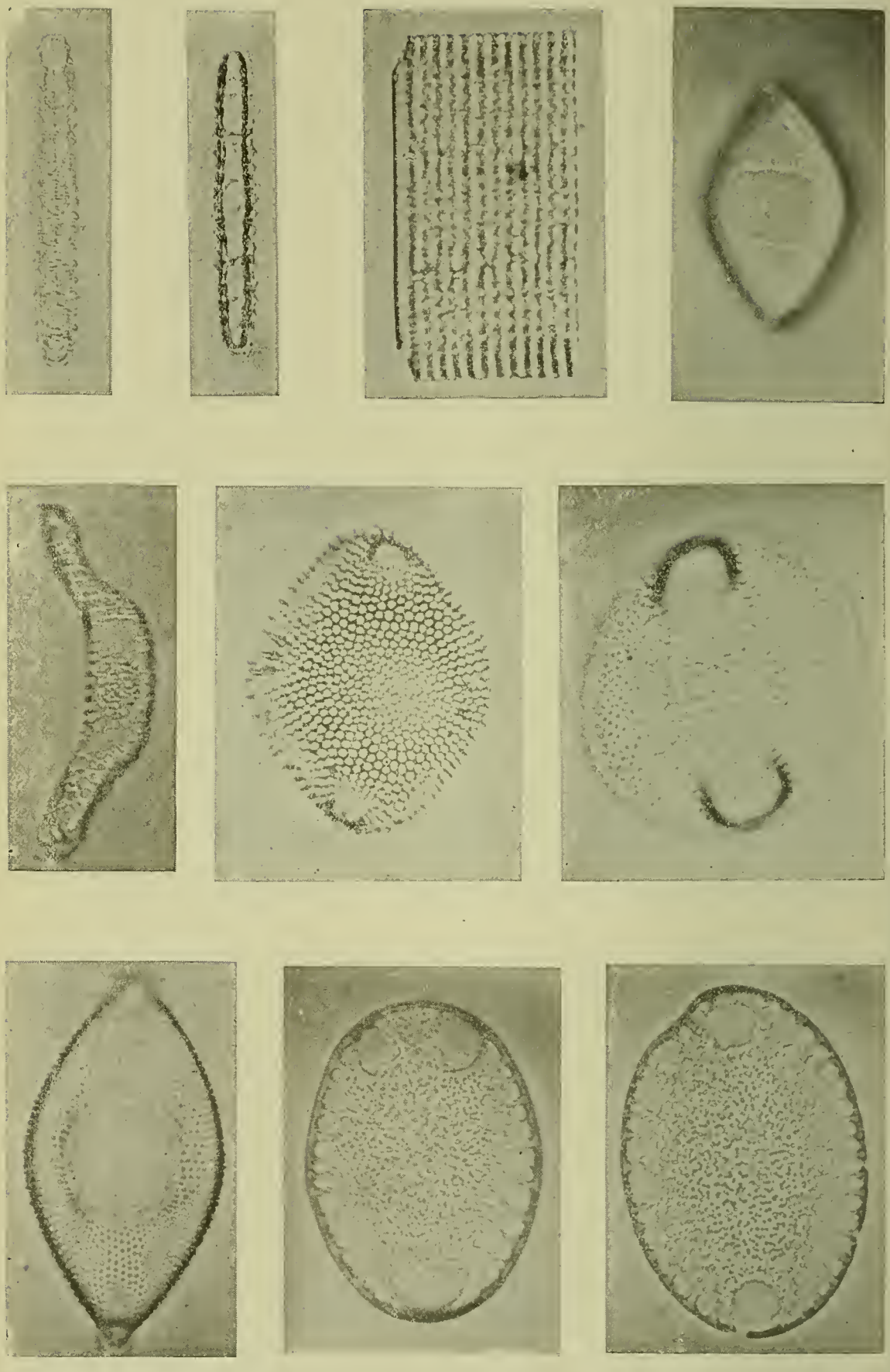
Personal.—M. S. Wiard is Secretary of the New Britain Scientific Association, Conn. The annual meeting was held Jan. 17, 1899.

Necrology.—Max Hauer, microscopist and mineralogist died August 10, 1898, at Oberhausen, Germany, aged 51.

Objects.—Foraminiferous sands, unmounted, 5 series of micro fungi at 50 cents each and 7 series of wood sections at 50 cents each series can be had from W. West, 15 Horton lane, Bradford, England.

Marine Specimens in Formalin.—Jar of Marine Algæ of many kinds, 50 cents; helix, 10 cents; starfish, 15 cents; and a list of many other specimens for sale by W. F. Webb, Albion, N. Y.

Second-hand Microscopes.—Beck high class Binocular with 1 1-2, 2-3, 4-10, 1-5, and 1-10 oil immersion lenses with complete outfit is offered for about $80, it being, but a fraction of the original cost. Also many other microscopes. Clarkson and Co., 28 Bartlett's Buildings, Holborn Circus, E. C. London, England.



NEW DIATOMS.

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CONTENTS.

Seven New Species of Diatoms.

CHARLES S. BOYER.

WITH FRONTISPIECE.

RHABDONEMA WOOLMANIANUM.— Valve oblong, with sharply crenulate margin, punctate, the puncta in transverse rows, about 6 in 01 mm. Pseudoraphe indistinct. Septa perforate, connected with each other usually near alternate ends by straight transverse diaphragms. Length of valve, .155 mm. to .231 mm. Fossil in the Miocene deposit from an artesian well at Asbury Park, N. J., at a depth of 40 ft. Not uncommon. Approaches *R. hamuliferum* Kitton and *R. musica* Brun., but it does not show either hooked septa as in the former nor curves resembling musical notes as in the latter. It also differs in the character of the margin and in size, being four or five

times larger than either. See frontispiece, fig. 1 for valve view × 284; fig. 1 a, for septum × 284; and fig. 1 b, for zonal view × 284.

BIDDULPHIA INTERRUPTA.—Valve elliptical, with small rounded processes. Surface convex, finely punctate, the puncta about 10 in .01 mm., radiating in scattered lines from the centre at which are three minute spines. About one-third the distance from centre to processes, at each end, a hyaline band produced by the interruption of puncta, crosses the valve transversely extending nearly to the sides. Valve .112 mm. Campeachy Bay. Rare. Frontispiece, fig. 2, × 310.

BIDDULPHIA VERRUCOSA.—Valve suborbicular, convex Processes very large, cylindrical, truncate. Surface coarsely reticulate, the reticulations unequal, irregular, about 2 in .01 mm. Within the reticulations are coarse puncta about 3 in .01 mm. Valve .138 mm. Fosil at Redondo Beach, Cal. Very rare. Frontispiece, fig. 5, × 302. This form, which approaches the *Cerataulus* group is distinguished by the incrusted or warty appearance of surface.

BIDDULPHIA KEELEYI.—Valve broadly rhombic-elliptical, rounded at the ends. Surface slightly convex, without depression, reticulated, the reticulations unequal, hexagonal, about 2 in .01 mm., with puncta within the reticulations about 8 in .01 mm. Three stout spines are placed on each side near the margin. Processes inflated at the base, small at the apex and placed not at the ends of the valve but obliquely opposite, near the ends. Valve .148 mm. U. S. S. "Tuscarora" Soundings, Lat. 36 ° 12′ N., Long. 133° 11′ W., 1,605 faths. Also coast of California. Rare. Only two specimens have been noticed, one of which was found by Mr. F. J. Keeley on seaweed from California. Frontispiece fig. 4, × 300.

BIDDULPHIA ARGUS.—Valve broadly elliptical, convex, with an elliptical depression at centre. Surface finely reticulate, the reticulations, more or less hexagonal, about

3 in .01 mm. at the border, and 5 in .01 mm. at the centre from which they radiate in curved lines. The central depression is encircled by from ten to twelve short spines. Processes short and obtuse. Valve .165 mm. Port Antonio, Jamaica. Not common. Distinguished chiefly by the central spines and by the size of the reticulations, whence the name. It approaches *B. Roperiana* Grev. Frontispiece, fig. 6, × 330.

BIDDULPHIA SEMICIRCULARIS ASBURYANA.—Valve arcuate with the ends produced and elevated into rounded processes. Surface not divided by costate lines, convex, punctate, the puncta rounded about 6 in .01 mm. near the hyaline excentric space from which they radiate irregularly, increasing in size to about 1½ in .01 mm. at the margin where they are irregular and occasionally confluent. Valve .181mm Fossil from artesian well in Miocene deposit at Asbury Park, N. J. at a depth of 40 ft. Not uncommon. Quite distinct from *Euodia producta* Grun. and from *B. semicircularis* (Br.) in the irregularity of the produced ends, in their elevation into processes, in the size, shape and distribution of the puncta and in the absence of costæ. Frontispiece, fig. 3, × 300.

BIDDULPHIA SHULZEI.—Valve elliptical, slightly raised toward the centre, with a large rounded process-like elevation at each end. Surface punctate, the puncta rounded, oblong, averaging 5 in .01 mm., but for the most part scattered, leaving numerous hyaline spaces, one of which appears as an indefinite, indistinct transverse band at the base of each process. Owing to the irregularity in the distribution of the puncta the circumference of the valve appears to show a scalloped border. Valve .115 mm. In the character of its markings it approaches *Tabulina Testudo* Brun. from which it is distinguished by having but two processes and in being without the hyaline lines which cross the valve in the latter. Fossil in the artesian well deposit at Weymouth, N. J. I have seen but one

specimen which was found by Mr. John A. Shulze who also discovered a variety having two processes at one end and one at the other. There appears to be no special reason why either this form or that known as *Tabulina Testudo* Brun. should be separated from the genus *Biddulphia.* The hyaline lines are not always definite even in *Tabulina testudo,* while the variety in which but three processes appear clearly indicates an approach toward the type of *Biddulphia.* Frontispiece, figs, 7, 8, × 475.

All the photographs were made by F. J. Keeley, of Philadelphia, and the plate has been loaned to us by the Philadelphia Academy of Sciences.

Malaria Parasites Observed at Camp Wikoff.

DR. JAMES EWING.

The author made four slides from cases of malaria studied at Camp Wikoff. On the first slide was a mixed infection—the æstivo-autumnal and tertian malarial parasites. In the one field were two specimens of tertian ring-shaped organisms and three of the æstivo-autumnal ring-shaped organisms. These specimens were interesting as illustrating the differences in the morphology of these two ring-shaped parasites. The tertian rings are coarser than the æstivo-autumnal, although they may be of about the same size. They show a quite distinct and large achromatic spot, two or three times the thickness of the ring. It was probably the nucleus, as it stained with hæmatoxylin. The æstivo-autumnal ring did not exhibit any demonstrable achromatic spot of the size of the other. Both the tertian rings showed one or two very fine pigment grains, whereas the early æstivo-autumnal rings were entirely without pigment—as was usual in the cases of Cuban malaria examined. In the same field of the microscope was a young crescent, thus placing beyond doubt the question of a mixed infection.

The second slide furnishes an illustration of "twinning" of the tertian parasite—a process not at all uncommon in tertian malaria, but much more common in the æstivo-autumnal form. The blood was taken about two hours before the expected chill, from a man suffering from quotidian paroxysms, and the specimen showed a segmenting body and a half-grown parasite in the same red cell.

The third slide shows another form of twinning of the tertian parasite, which has been seen only in the Cuban cases. In this specimen there were a great many cells containing parasites in which, evidently, the amœboid motion had been very active, and the parasites, instead of being in a more or less spheroidal form, were strung out into a number of fine threads with nodal thickenings. In each cell presenting this peculiar appearance there were two achromatic nuclear spots, indicating the presence of two parasites.

On the fourth slide was a very richly pigmented leucocyte of enormous size—one of very many seen in a fatal case of mixed æstivo-autumnal and tertian infection. The importance of these leucocytes was very great in diagnosis, and their appearance, when typical, seemed to him to be quite as characteristic as that of the parasite itself. —*N. Y. Med. Jour.*

Experiments in the Artificial Culture of Diatoms.

William A. Terry.

It was now the middle of winter, and wishing to hurry matters, I filled a small teacup with water from one of the cultures and drew up carefully a quantity of diatoms with as little sediment as possible and placed them in a west window in my room where they were exposed to afternoon sunlight and a day temperature of about 60 ° F. At night they were cold, in fact sometimes the water was partially frozen. I hoped that this would discourage the animals, but it was a failure in this respect, as eels, ameba and ciliata appeared in increasing abundance. Still, I had an opportunity to note some peculiarities, There were in this lot a number of Surirella striatula of the open water type, more like Van Heurck's drawing than that of Wm. Smith. I shall have occasion to note these differing types further on. I soon began to find abnormal forms among these. Sometimes one end would be imperfect and sometimes the other, but more had one edge of the frustule bent in. Whether these abnormal forms were produced by division in a fluid deficient in silex, or whether they were older valves in process of solution I felt uncertain, but as the diatoms were rapidly diminishing in numbers, I determined to attempt to destroy the animals without killing the spores which might be present. Accordingly, I allowed the water to freeze solid, freezing and thawing a number of times in succession, then let the water evaporate until the salt crystalized, filling up again suddenly with fresh-water. This treatment destroyed the animals and also the diatoms, and the amount of sediment was increased. In this sediment soon appeared minute globular bodies which I called spores. These were of many species and soon similar kinds congregated together in separate masses and grew rapidly in size and numbers. Some were of a bright

green, these being the largest; others, a brownish red, these being the most numerous; and others were hyaline.

Soon the water became filled with very minute red spores, actively motile; the larger of these appearing slightly elongated and flattened. After the larger green spores had increased many times in size they began to show ameboid motions. These were evidently developing euglena. The larger brown spores at the same time changed their globular form and showed slight angles and a bellows shape. Was it Surirella? A smaller kind with a deeper color became rectangular in one position remaining circular in the other. These I thought Melosira. Many of the hyaline spores threw out a pointed elongation which made them comet-like in shape. This tail vibrated like the tail of a tadpole and drove them through the water with considerable activity for a time, but they finally became fixed, their tails retracted, and their changing shapes showed them to be infant ameba which soon grew large enough to attack the red spores. They were so numerous that a drop of sediment placed on a slide would show dozens of them in the field at a time, in any part of the slide. They grew rapidly and committed havoc upon the red spores to which they confined their attention. At this time the larger hyaline spores germinated and threw out threads of mycelium which soon permeated the entire mass of sediment, contracting and binding it together and destroying all other life.

I now cleaned up the whole by the acid treatment. The large abnormal Surirella were completely dissolved, showing a total lack of silex. The Pleurosigma had nearly disappeared, but I obtained a considerable number of the newly formed Surirella and Melosira, and made quite a number of slides. About one-third of the Surirella showed a curious notch or depression on one side of the valve and the melosira all showed a dot which looked like an opening on one side the centre.

It will be useless for me to attempt to report subsequent observations in detail. I must content myself with giving the general results. In August, 1896, I was away from home for two weeks, and when I returned, one of the larger cultures had a chestnut brown film on the surface of the sediment that was not there when I went away. These were magnificent specimens of Surirella striatula, larger than any I had ever seen before, and more than double the size of any in the original gatherings or any that I had ever found along the Connecticut shore. To guard against accidents I cleaned up a quantity of these diatoms and made a number of slides. I cannot account for their production in such quantities in so short a time. If it be thought that they might have grown by multiplication from specimens overlooked in previous examinations, let it be remembered that under the most favorable circumstances they could not have increased in that way more than fourfold in that time. If multiplication had been carried on much longer they would most certainly have been discovered sooner. The same argument has nearly equal force against the idea that they were the product of conjugation. They might easily have been produced from spores, but I can scarcely think that such large diatoms could have grown to maturity in so short a time. It is true that the spores might more easily have been overlooked. I am the more diffident about forming an opinion because of my failure to discover the preliminary stages when precisely the same thing happened again in the following spring.

In April, 1897, another culture suddenly showed the same brown film composed of precisely similar Surirella, but this time in company with an equal number of the very large Amphiprora pulchra. Small Amphiprora had been abundant in the culture, but of this large kind I had not previously found a single frustule. These large Surirellas were like those found in the open salt water along

our shore. The type illustrated by Wm. Smith is also abundant in this state, but is found in pools or pondholes in the salt marshes which are fed by freshwater springs. I find them in company with the remarkable colonies of Pleurosigma terryanum and Navicula maculata at Leete's Island. But my most remarkable find of them was at Branford, in a pool so nearly fresh that it was filled with growing fresh water plants, from among which I scooped up the Surirella. These were much heavier than the salt-water type, and more circular in outline. I returned afterward to get a larger supply and found the pool covered with an embankment, and a large building standing nearly over the buried diatoms. The gathering of Artemia gracilis was placed in a bowl and exposed with the diatoms, but they did not do well. In a short time, all were dead and I returned them to the bottle to preserve the eggs.

In October, 1896, more than a year after they were gathered, many of the eggs hatched, and the water was filled with young Artemia. They did not flourish but all died before reaching maturity. In many of the cultures, the slightly sigmoid Nitzschia appeared proof against the animals, and they finally rivaled in numbers the original Pleurosigma. I hoped that the Surirella being too large to be swallowed by any microscopic animal, and having developed in captivity, might live long enough to show their entire life history; but I soon saw that this was hopeless, as in every study under the microscope I saw "eels" thrusting their heads through between the side hoops, and absorbing the entire contents of the diatom in a few minutes.

I separated many of these Surirella and made separate cultures with a peculiar result. The endochrome would shrink and be surrounded by a hyaline envelope stretched out into points of attachment to the edges of the valve, precisely similar in this respect to the sarcode of a rhizo-

pod, except that the envelope of the diatom endochrome was much more visible, being highly refractive. I have kept some of these Surirellas in this state for three years without any great change.

After about 2½ years observation, fearing to loose these remarkable diatoms, as the Pleurosigma were disappearing, I cleaned up the two original cultures, obtaining quite a quantity of the Surirella and Amphiprora, but very few Pleurosigma. The surface of the glass in the dishes that held them was removed, as if it had been etched with acid. In many of the smaller cultures, the sediment resembled coagulum; was composed of minute spore-like bodies held together by hyaline threads, somewhat like lichens, except that there was no such regular arrangement. The spore bodies were much smaller and the hyaline threads much finer and were not fungus. They were of a nearly uniform length and when separated from the mass were motile, doubling up and matting together One of these masses is a dark purple, another a pale pink, another olive green, and two or three are golden brown, like diatoms. I have kept some of these, three years without further development, and am unable to tell what they are.

To Ascertain Focal Length.

E. M. NELSON.

[In English Mechanic.]

The focal length of a microscope objective can be easily and accurately determined in the following manner:—Project the image of a stage micrometer by means of the objective itself (no eyepiece or amplifier of any kind being used) on to a white paper or cardboard screen, placed about six feet with low-power lenses up to a ½ in. and three feet, with powers higher than a ½ inch, from the lens. Determine the magnifying power by dividing the

size of the image by that of the stage micrometer. Measure accurately the distance from the screen to the front lens of the objective. Divide the screen distance by the magnifying power plus one.

Example: A reputed quarter projects the image of .01 in. of a stage micrometer on a white screen distant 38 in. from the front lens. The magnified image measures 1.8 in. The magnifying power is therefore 180. Add one to this and divide 38 in. by 181 which gives .21. This shows that the reputed quarter is nearly 1-5 in. in focus. Instead of taking the trouble to divide the 38 by 181, it is shorter to divide both the numerator and the denominator of the fraction by the numerator, 38, which gives numerator 1, denominator $4\frac{3}{4}$. Having found the focal length of the reputed quarter, the magnifying power of a Huyghenian eyepiece can be determined in the following manner:— Place the microscope in a horizontal position, a micrometer on the stage, a camera—Wollaston's, Beal's, or one of similar form—on the eyepiece, and a scale divided into inches and tenths on the table, directly underneath the camera and at a distance of 10 in. from the camera. Next measure accurately the distance from the front lens of the objective to the diaphragm in the eyepiece. Now suppose that the magnified image of .01 in. of the stage micrometer, as projected on the scale lying on the table, covers 2.4 inches it is obvious that the combined magnifying power of the objective and eyepiece, with the same tube length as above measured, is 240 diameters. Now, as we know the focal length of the objective is as above, its initial magnifying power will be 47.5.

Dividing, then the combined magnifying power by this quantity, we find the magnifying power of the eyepiece, thus 240 divided by 47.5 equals 5 05.

If a camera is not at hand, the combined magnifying power may be found by projection on to the screen used above; but the screen must be placed at a distance of

10 in. from the hole in the eye-cap, and if the eyepiece has no eye-cap, then the screen should be $10\frac{1}{4}$ inches from the eye-lens.

When the power of the eyepiece is once known, the focal length of any other objective may be found, without taking the trouble to project the image on the screen, by first determining its combined magnifying power by means of the camera and the scale, as above. For example, the combined magnifying power of Zeiss AA, with the same eye-piece and tube-length, was found to be 80 diameters. Next find the initial magnifying power of the AA by dividing the combined power by the power of the eyepiece, thus 80 divided by 5.05 equals 15·8. Now divide 10 by this initial magnifying power, 15.8 and we have 2-3 (nearly), the focal length of the AA. All microscopists should measure in the above manner the focal length of at least one of their object-glasses, preferably a 1-4 in. and determine for themselves the magnifying power of their various eyepieces, instead of relying on the values assigned to them in the makers' catalogues.

Another Way to Get Focal Length.

By J. G. P. V.

Put a stage micrometer on the stage, and focus the glass on it; then turn the microscope horizontally, and pin a piece of paper vertically in line with the axis and parallel to the stage. It is most convenient to fasten this paper on the wall. Remove the eyepiece, and focus again till the image of the micrometer is sharp on the paper. This will only be the case with the centre lines. Measure their size carefully, and measure the distance between the micrometer and the image. Let D be this distance, F the required focus, and M the magnification; then F is equal to D multiplied by M, and the product divided by the square of M plus one. If the distance is a fair length—about 8 ft.—the distance divided by the

magnification is close enough for ordinary purposes. If this plan is tried with high powers, the illumination is feeble, and the light must be shaded from the image—either by putting the microscope and lamp in a box, with a hole for the tube, or by using a camera, with its lens removed, and receiving the image on the ground glass, the head being under a focussing-cloth. For these powers, however, a close enough approximation can be made by comparison. Say, for instance, you have found the focus of one lens, and it is 1-2 in. If this lens magnifies with an eyepiece 100, this will give 5 as the magnification of the eyepiece. Say another lens magnifies with the same eyepiece 500, then this gives 100 as the magnification of the objective, and the lens is 1-10 in. focus. As for measuring the magnification of a microscope, Beale's camera is the simplest, and easily made. Get a pill-box, or make a cardboard tube to fit over the eyepiece of the microscope. Cut one end of the tube to an angle of 45°, and fasten by means of cement a thin cover-glass over it, and over this make a hole about 1-4 in. diameter, and blacken the inside. To use it, place the microscope horizontally and at 10 inches from the table, and then you can easily draw anything on the stage. If a stage micrometer is placed there, you can draw the magnified image, and thus know the magnification. If 1-100 in. measures one-inch, the magnifying power is 100 diameters.

C. R. Cross on Focal Length.

CROSS'S FORMULA.—Dr. J. J. Woodward, of the U. S. Army Medical Museum, published in the *American Journal of Science and Arts*, for June, 1872: "Remarks on the Nomenclature of Achromatic Objectives for the Compound Microscope" in which he said: For some years past, while most of the Continental opticians have contrived to give arbitrary designations to their achro-

matic object-glasses, such as No. 1, No. 2, &c., or system A, etc., the English and American manufacturers, affecting a higher degree of accuracy, have undertaken to name the objectives they construct by their real or supposed agreement in magnifying power with single lenses of specified focal lengths. At first sight nothing could appear simpler or more exact than such a nomenclature; nevertheless, recent articles in the journals would seem to indicate that the general plan is capable of considerable modification in its practical application, and that grave misunderstandings have hence arisen. Under these circumstances, it appears desirable to give some account of the principles involved and of positive difficulties to be considered in their application, particularly as the microscopical text-books contain little or no information on the subject. In fact, the only scientific description of the matter with which I am acquainted is the paper of Mr. Charles R. Cross 'On the Focal Length of Microscopical Objectives' (*Journal* of the Franklin Institute, June, 1870, p. 401). This paper gives a reasonable formula for the *approximate computation* of equivalent focal lengths, and furnishes some other valuable information, but does not discuss all the points at issue.

We learn from the elementary treatise on optics that when an object is placed in front of a single convex lens, at a distance somewhat greater than its focus for parallel rays, a real image is formed on the other side of the lens, which may be received on a screen. This image will be larger, and formed at a point more distant from the lens, the nearer the object approaches to the focus for parallel rays; and two equations are given which express the relationship of the distances to each other, and to the magnifying power, viz.: $-\frac{1}{f} = -\frac{1}{p} + \frac{1}{p'}$, and $-\frac{p'}{p} = m$, in which f is the length of the focus for parallel

rays; p the distance of the lens from the object, p' its distance from the image, and m the true magnifying power, that is, the size of the image divided by the size of the object: p and p' are termed the conjugate foci, and are variable quantities; f is termed the principal focus, and has an unchangeable value for each single lens.

"If now we combine the above equations, representing $p + p'$ or the sum of the conjugate foci by l, we may deduce the formula $f = \frac{m\,l}{(m+1)^2}$, which represents in the case of any single convex lens the relationship existing between the length of the principal focus, the magnifying power. and the distance from the object to the screen. This formula, which I think rather more convenient than that of Mr. Cross, differs from it only in using m = the magnifying power, instead of n = the reciprocal of the magnifying power. It may be deduced from his by substituting for n its value $\frac{1}{m}$, and reducing; or it may be derived directly from the primitive equations. In either shape the formula yields the same numerical results, and if for any single convex lens m and l are given, the accuracy of the value of f resulting will be the degree of precision with which m and l have been measured.

"If, now, there were any such actual equivalence between achromatic objectives and single lenses as the nomenclature assumes, it would only be necessary to set up the objective to be rated in such a manner that the image of a micrometer should be focussed upon a white screen, using, of course, no eyepiece, to measure the distance from the micrometer to the screen, to determine the magnifying power by measuring the image of the micrometer, and substituting these values of l and m in the working formula to calculate the value of f. Unfortu-

nately, however, if with any compound objective we repeat this operation several times, merely varying the distances, *we obtain as many different values of f as there are distances used*, instead of obtaining but one value for all distances as we do with a single lens. Mr. Cross has already pointed out this circumstance, which results from the fact that the modern achromatic objective has considerable thickness from its anterior to its posterior surfaces, and that it has, properly speaking, *no true optical centre.*"

Dr. Woodward, in his paper, gives several examples of ascertained discrepancies while using the formula in achromatic object-glasses, when trials were made with them at varying distances to ascertain their magnifying power.

Making and Staining Cover-Glass Preparations, and Formulae for Staining Solutions.

V. A. MOORE.

[*From his New Book*].

Make two cover-glass preparations from each of the cultures made in agar, bouillon,and gelatin, and stain one with alkaline methylene-blue and the other with carbol fuchsin. Describe the appearance of the bacteria and make a drawing of a few individual bacteria from the preparations made from the agar culture. Preserve a cover-glass preparation mounted in balsam and label it. Prepare the staining fluids.

Making Cover-glass Preparations.—(1) From bouillon cultures. Place 2 clean cover-glasses on the tray. With the loop remove a drop of the bouillon culture and spread it in a thin layer over about 2-3 of the surface of the cover-glasses. One loop-full will ordinarily make from 2 to 4 preparations. Allow the liquid to dry on the cover-glasses in the air. When dry, fix the bacteria to the cover-

glasses by passing them, film upward, 3 times through the middle of the upper half of the gas flame. Each passage (complete circle) should not occupy more than one second. After fixing, they are ready for staining. (2) From cultures on solid media (agar, gelatin, potato, serum, etc). Place the cover-glasses on the tray and on the centre of each a drop of sterile water or bouillon. With the needle, touch the surface growth of the culture and then gently rinse the end of the needle in the liquid on the covers. Spread the liquid on the covers as before. From this point the procedure is the same as that for the preparations made from the bouillon culture.

Staining Bacteria in Cover-glass Preparations.—(1) With alkaline methylene-blue. With the pipette place a few drops of staining solution on the film side of the preparation which is either held horizontally with the fine forceps or left resting on the tray. Allow the stain to act for 2 or 3 minutes. Then carefully rinse off the stain in water, holding the cover firmly by one edge with the forceps. After thoroughly rinsing place the preparation, film downward, on a clean slide and dry the upper surface with a piece of filter paper. It is now ready for the microscopic examination. Use first the dry lens (1-8in. obj.) and then the oil immersion objective. If the specimen is a good one and it is desirable to preserve it wipe off the drop of oil with a piece of lens paper, run a drop of distilled water under the cover-glass which floats it, when it can be easily removed with the forceps. Place it on the tray, film upward, and when dry mount it in alkaline Canada balsam. (2) With carbol-fuchsin. Cover the film on the cover-glass with the stain and allow it to act for about one minute. Then rinse it thoroughly in water after which cover it with 1-10 p. c. solution of acetic acid or strong (95 p. c.) alcohol. Allow this to act from 5 to 10 seconds and again thoroughly rinse in water and examine as above. (For other decolorizers see textbooks).

Upon examination the preparation should be free from deposits or stained back ground. The bacteria should, as a rule, be isolated and distinct, unless they are, the preparations are not satisfactory.

Cover-glass preparations of bacteria are permanently mounted in the same manner as similar preparations made from the blood or other tissues in histology, the process being to place a drop of the balsam on the center of the slide and place the preparation, film downward, over it and apply slight pressure. Label the preparation, giving the name of the organism, its source, (kind of culture, tissue, etc., from which the preparation was made), stain used, and date. If the specimen is not preserved, the slide and cover-glass should be cleaned for future use.

Suggestions Concerning the Microscopic Examination of Stained Preparations of Bacteria.—In the examination of the bacteria in the stained condition the following points, and perhaps others, should be observed and noted. (1) Concerning their morphology. Are they spherical, rod-shaped, or spiral? Are they separated or united in clumps or chains? If rod-shaped, are the ends pointed, round, or square? Are the bacteria all of the same form and size? Note the presence or absence of spores and capsules. (2) Concerning their reaction to staining fluids. Do they stain uniformly or irregularly? Do they stain deeply or faintly? Is the center lighter than the periphery? Is there an unstained central band and deeply stained ends (polar stain)? Do all the bacteria take the stain alike?

Staining Solutions.—The basic aniline dyes are used in staining bacteria. There is a large number of these, and there are several formulae for preparing staining solutions from each. Further, as will be seen from the chapters on staining bacteria in the text-books, there are several methods of applying these stains. In an introduc-

tory course, however, it is impossible to try them all and consequently those are described which seem to be the best adapted for general use.

In addition to the ordinary staining solutions and methods there are special processes for certain species such, for example, as the tubercle bacillus, and still others for, staining certain parts of many bacteria, such as the flagella on motile forms, the spores in spore bearing organisms, and the capsule on certain other species. There is a large number of these special methods which are to be taken up in connection with the study of the bacteria requiring them.

Formulæ for Staining Solutions.—Methylene-blue, gentian-violet, methyl-violet, and fuchsin are the dyes used.

LŒFFLER'S ALKALINE METHYLENE-BLUE.

Concentrate alcoholic solution, methylene-blue...30 cc.
Caustic potash 1 per cent solution.....1 cc.
Distilled water.....100cc.

CARBOL FUCHSIN (ZIEHL'S SOLUTION).

Fuchsin (dry).....1 gram.
Alcohol (absolute).....10 c c.
Carbolic acid, 5 per cent solution.....100 c c.

Dissolve the fuchsin in the alcohol, after which add the carbolic acid solution. Instead of using the dry fuchsin and alcohol, 10 c. c. of a saturated alcoholic solution of fuchsin may be used.

Aqueous Solutions.—The aqueous solutions of methyl-violet, gentian-violet, fuchsin, and the other aniline dyes are prepared by adding 1 c. c. of the saturated alcoholic solution of the desired dye to 20 c. c. of distilled water This will impart a decided color to the liquid so that a pipette full will be barely transparent.

The true aqueous solutions are made by dissolving the dyes in water, but these are weak and not so effective as those prepared from the alcoholic solutions. These solutions deteriorate in a short time. The carbol fuchsin and

alkaline methylene-blue will keep a little longer, but they require to be filtered occasionlly.

ANILINE METHYL-VIOLET (EHRLICH-WEIGERT).

Saturated alcoholic solution of methyl-violet....11 c c.
Absolute alcohol.....10 c c.
Aniline water.....100c c.

Making Aniline Water.—Aniline water is a saturated aqueous solution of aniline oil. It is prepared by adding 1 c. c. of aniline oil to 30 c. c. of distilled water and shaking frequently for 30 minutes. It is convenient to use a stoppered vial or large test tube for mixing it. Filter through a moistened filter paper. The filtrate should be perfectly clear. If it is cloudy it should be refiltered before using.

Loffler's Method of Staining the Flagella of Bacteria.

The staining methods employed to show bacteria in sputum, etc., and of revealing spores in the bacteria do not reveal the little infinitessimally small and delicate wavey lines called flagella which fringe the organism in many species and constitute its fins or wings and are used to produce motion. The specimens easily loose these appendages and hence when it is proposed to demonstrate them fresh and vigorous material must be found and be handled with great care. Taking as small a quantity of this material as is possible by soaking it apart in water and lifting a droplet on a platinum wire loop, dry it on a cover-glass using the heat gently for it could easily burn off these little protrüberances or whips from the bacterial body. This drying may be accomplished by holding the cover-glass specimen-side away from the heat, and quickly passing it through the flame. If you do not burn your fingers and thumb in which you hold the glass y ou will not cremate the flagella. In 20 cc. water,dissolve 2 grams dry tannin, add 4 cc. ferrous sulphate so-

lution (1 : 2), and 1 cc. concentrated alcoholic solution of fuchsin. Stir the mixture, filter, reject the filtered precipitate and keep the fluid which improves as time elapses. This constitutes a mordant which has affinity both for the organism and for the dye which is to follow. Wet the fixed specimen in it after having first moistened it with water, gently heat over a flame till vapor comes off but without boiling. After 60 or 90 seconds wash it thoroughly in water to take off all the mixture from the glass which will occur if the heat has not been too intense. If it has been, scrape off the debris from the glass to make it perfectly clean for by the dye which is to follow the glass would be badly disfigured if a mordant base remained on it. The dye now follows. It consists of a one-to-fifty-part hot aqueous saturated solution of fuchsin, or of a solution made by heating 3 grams fuchsin in 100 cc. anilin water. Moisten the surface of the mordanted specimen with it, heat over the flame and wash as above directed. This dye gives colored flagella or a colorless back ground visible under a 1-12 inch homogeneous oil imm. objective if the manipulations have been skillfully made.

LETTERS TO THE EDITOR.

GATES' DOUBLE MICROSCOPE.

Defining Power.—According to Abbe's theory, generally accepted by microscopists, defining power is purely a function of numerical aperture, and if, as Dr. Gates claims, he has really succeeded in seeing detail with a combination of dry objectives, which oil-immersion objectives of high aperture employed in the usual manner fail to show, all Abbe's theories must fall to the ground. It is rather significant, though, that in spite of the enormous advances he claims to have made, no detail of any new discovery, such as the minute structure of diatoms, for example, are indicated. Even if some new structure were apparently ex-

hibited by such an arrangement as Dr. Gates proposes, it is a mooted point whether such appearances would indicate real structure or whether (as is most probable) they would be caused by diffraction spectra.—A. G., PUNJAB, INDIA, JAN. 22.

EDITORIAL.

Stains.—To see more plainly what might otherwise almost elude detection we vary its setting or back ground. This is the theory of all micro-staining. If, given a liquid containing tubercle bacillus both of about the same color, we can stain the liquid or the germ, we produce a visible contrast. Ingenuity has now enabled us to invent stains which will affect germ and containing fluid differently and even produce several different colors in the same infinitesimal speck of matter each color portraying some particular part of the organism.

Diagnosis.—It will be seen from Dr. Ewing's article that the microscope was of decided practical value in determining some of the diseases from which the soldiers suffered at Camp Wikoff after having been subjected to Cuban climate.

Terms.—An amusing misuse of terms occurred recently. Sir W. Roberts-Austen in an article on steel rails reported taking photographs of enlarged images which constitutes photomicrography but he entitled his paper Microphotography. A photograph of a large object reduced is a microphotograph. A photograph of a small object which gives an enlarged image is a photomicrograph. Thus all of our illustrations of bacteria etc. being much larger than the objects themselves are photomicrographs. But those curiosities such as the Lord's prayer visible only under an objective are microphotographs. The writer has a photograph of the "White House" brought down so small that it cannot be seen other than as a pin-point speck until placed under a lens. This is a very small or microphotograph.

MICROSCOPICAL APPARATUS.

Quekett Club.—The 364th meeting held Friday, Dec. 16, Dr. John Tatham, president. After the usual formal business, Messrs. Swift exhibited a microscope on their four-legged tripod model, which, except wearing surfaces and screws, was entirely constructed of aluminium. Also an objective, said to be the first with the whole mount in aluminium. It was very light and most beautifully made. The aluminium objectives are likely to spare the strain on the fine adjustment, in some forms at least. An advantage of the lighter metal is for the stands of traveling microscopes. It is more costly to make than the usual brass and gunmetal. Mr. H. Schroeder gave a detailed description of a new projection microscope, which could be used for exhibiting on the screen all kinds of objects, prepared or recent, including crystalization under polarized light. He explained the construction of the peculiar form of electric lamp used in conjunction with the projection apparatus, in which the upper carbon is in the form of a revolving disc and the lower a thin finely-pointed rod. Mr. Nelson had never seen a projection microscope capable of giving a well-defined bright image on the screen, visible at any distance, with powers beyond a 2 in. or 1 in. The want of light with the higher powers was a serious drawback to projectors. An interesting paper was read by Mr. Nelson entitled: "On the Structure of Nodules in Pleurosigma and Climacosphenia," illustrated by photo-diagrams with the lantern. He thought a comparison of the tubes in the raphe and their junction by a minute canal in the central nodule, from specimens found in the Nottingham Deposit with recent Pleurosigmas, showed some evidence of evolution. Optically these minute tubes were at the limit of visibility and excellent tests of definition in objectives.

MICROSCOPICAL MANIPULATION.

Varnish.—The different colored varnishes can be very easily prepared by coarsely powdering sealing-wax of the

desired color, and dissolving the same in alcohol but it is advisable to use these varnishes for "finishing" purposes only, as they are, after some time, extremely liable to chip, and leave the glass. If, however, the best wax is used, the risk of this happening is lessened. An excellent black finishing varnish, free from brittleness, can also be made by well mixing gold size with a small quantity of lampblack.

Finishing Varnishes.—I have made excellent varnishes by well mixing on glass, with a spatula, japan gold size with the oil paints sold in compressible tubes for artists' use. Only mix the quantity required for immediate use. Any color can be got in this way. This will dry well, in say 12 hours, with a hard, level, washable gloss. In using the sealing-wax varnish recommended, I have found that the black is greatly improved by adding about twenty drops of carbon-black solution, got by dissolving about two drachms of carbon in a gill of methylated spirit, and that the red is improved by adding about 1 dwt. (troy) of vermillion to it. The red settles if the vanish be not used for about three hours, and therefore needs well shaking or stirring before use. Gentle warmth, though not necessary dries all the above varnishes much more quickly and smoothly than its absence.—J. H. Henderson.

Gelatin Culture Media.—The behavior of many sensitive organisms, particularly parasites, depends entirely on how the nutrient gelatin is compounded, and consequently this should always be stated. The melting point of nutrient gelatins increases as more gelatin is added. It decreases on addition of acids and alkalies and by long boiling. Grape sugar or cane sugar added to nutrient gelatin frequently restrains or entirely prevents liquefaction, while at the same time it stimulates growth. For this reason gelatin should be made with beef broth free from sugar. Owing to the fact that commercial gelatin contains acid salts, which are neutral or alkaline to litmus but retard the growth of many organisms, the gelatin media should first be rendered neutral to phenolphthalein, after which, if desired, it may be acidified with particular acids. A com-

mercial gelatin of uniform character and washed free from all inhibiting acid substances is a desideratum.—*Bot. Gaz.*

Gram's Method of Double Staining Micro-organisms of all Sorts.—This consists in using ½ cc. of a saturated alcoholic solution of gentain violet and 10 cc. of a solution of anilin-water gentian violet mixed. The resultant dye will be deeply colored and opaque. For the watery solution take alittle anilin oil in a test tube, then add pure water, shake, filter. The filtrate should be clear, not cloudy. A cover-glass containing the smear of any sort is rendered dry by contact with flame and dropped into a saucer cantaining the dye, specimen side down into the liquid where it may remain say four minutes. Holding the cover glass with forceps wash it thouroughly and immerse it for 4 minutes in a solution made of one gram of iodine dissolved with 2 grains of potassium iodide in 300 cc. of distilled water. Again wash in water and put it in 95 per cent alcohol which will take the stain out of the background but leave the micrococci or bacteria beautifully colored so that they can be readily viewed under one-sixth inch objectives. Prior thereto the cover-glass can be put into dilute eosin for an instant, not exceeding 25 or 30 seconds, which will give a contrast stain the back-ground making the objects stand out still more clearly. Some skill is needed to enable one to stop the alcoholic decolorization at the point where the background is cleared and before the objects loose their stain for in due time the alcohol would entirely decolor the whole specimen. Likewise avoid too much eosin, else after staining the background it proceeds to mix with and injuire the first stain. The eosin gives light pink color, a beautiful contrast to the violet-colored micro-organisms.

BIOLOGICAL NOTES.

Chalk.—In the proceedings of the Cotteswood Naturalists Field Club, XII, 3, 1898, Mr. Chas. Upton writes of the contents of a piece of upper chalk from Purley using the title "Chalk under the Microscope."

Observations Upon Spindle-Formation in Anthers.—Mr. A. A. Lawson at the University of California has recently spent some time upon this subject using the anthers of Cobæa scandens on account of their large size, large mother-cells and large nuclei.

The anthers were gathered and immediately fixed in the field. The following fixing fluids were used:

Alcohol, 95 per cent.
Chromic acid, 1 per cent.
Flemming's mixture, chromic-osmic-acetic, strong solution.
Wilson's sublimate-acetic.
Boveri's picro-acetic.
Corrosive sublimate, saturated solution in 95 per cent alcohol.

The best results were obtained by using Flemming's strong solution diluted with one volume of water. The material was washed in running water from six to eight hours. It was then carried through different grades of alcohol by means of an apparatus consisting of a tumbler with a cover and a glass funnel. The funnel was of such a size as to be supported in the mouth of the tumbler. A piece of parchment paper was folded and placed in the funnel in the same manner as for filtering. Ten per cent solution of alcohol was placed in the tumbler and the anthers were placed in water in the funnel. The mouth of the tumbler was covered and the material was thus allowed to remain for an hour or two. The alcohol in the tumbler was changed at intervals to 25 per cent, 50 per cent and 95 per cent solutions. By this means the effect of the rapid change from a weak to a strong solution of alcohol was obviated. The anthers were then thoroughly dehydrated in absolute alcohol. They were then placed in a mixture of bergamot oil and alcohol and then in pure bergamot oil. From the bergamot oil they were transferred to a mixture of bergamot oil and paraffin, and from this to pure paraffin, where they remained at a temperature of 55 deg. C. for twenty-four hours. Microtome sections of ⅔ microns in thickness were used.

Many stains were tried; especially iron-hæmatoxylin and Bordeau red, ruthenium red and thionin, etc., but the best results were obtained from Flemmings' triple stain, safranin, gentian violet, and orange G.

In the resting condition of the pollen-mother-cell the nucleus is quite large. It contains one or two large nucleoli which stain very readily with safranin and sometimes appear to be vacuolated. The chromatin, which is in the skein stage, stains blue with the gentian violet. As soon as the chromatin breaks up and forms the chromosomes it stains red with safranin. The chromosomes appear as small oval bodies which are invariably situated in contact with the nuclear wall. The largest number that was observed in polar view was twelve. The linin appears in the form of a lumpy or granular thread; it stains blue and is invariably connected with the chromosomes.

The cytoplasm now appears in the form of a clear reticulum. The meshes of this network, which can be traced from the nuclear wall to the cell-wall, appear to be smaller and radially elongated in the immediate neighborhood of the nucleus; but they increase in size as one follows them outwards and are comparatively large towards the cell-wall. Scattered irregularly through the cytoplasm are numerous small spherical bodies. These bodies have the appearance of oil-globules in the living cell, but after the cell has been killed in Flemming's fixing fluid they appear quite black.

SUMMARY.

The observations made upon the formation of the spindle in *Cobœa* may be briefly stated as follows: A granular substance gradually accumulates and forms a complete zone around the nucleus. This zone is designated perikaryoplasm. Upon the breaking down of the nuclear wall the linin of the nucleus and the perikaryoplasm form a network which occupies the central portion of the cell. This network grows out into several projections which become the cones in the multipolar figures. The spindle-fibres are formed by the elongation of the meshes of the network in the direction of the projections. The cones

elongate and become sharply pointed. They fuse in two groups and form the bipolar spindle in the same manner as that observed by Osterhout in *Equisetum*. The mature spindle is characterized by the great length and crossing of the mantle-fibres. The spindle-formation of the second division is identical with that of the first division. No bodies that could be identified as centrosomes were found in any stage of the process.—*Cal. Acad. Sci.*

DIATOMS.

Slides.—Strewn-slides of Diatoms can be obtained from P. Klavsen, Denmark, containing diatoms from 174 localities as follows: Numbers 1-44 are fossil, Nos. 51-174, recent. A collection of 100 of these slides can be had for $14.00.

1 Cement-stone i, Fur, Denmark, 2 Cement-stone ii, Fur, 3 Moler, Mors, Denmark, 4 Szt, Peter, Hungary, 5 Kekko, Hungary, 6 Szakal, Hungary, 7 Felso-Esztergaly, Hungary, 8 Simbirsk, Russia, 9 Ananino, Russia, 10 Kusnetzk, Russia, 11 Hainspach, Bohemia, 12 Chatovin, Bohemia, 13 St. Fiora, Italia, 14 Moron, Spain, 15 St. Monica i, Cal. 16 St Monica ii, 17 Redondo Beach i, Cal, 18 Redondo Beach ii, 19 St Maria, Cal, 20 San Pedro, Cal, 21 San Pablo, Cal, 22, Lompoc, Cal, 23 Los Angeles, Cal, 24 Richmond i, Va, 25 Richmond ii, 26 Petersburg, Va, 27 Nottingham, Md, 28 Popes Creek, Md, 29 Atlantic City i, N. J., 30 Atlantic City ii, 31 Beach Haven, N. J., 32 Hatfield swamp, N. J., 33 Newark, N. J., 34 Beddington, Me, 35 Hopkinton, N. H., 36 Mejillones i, Chile, 37 Mejillones ii, 38 Barbados, 39 Cement-stone i, Sendai, Japan, 40 Cement-stone ii, Sendai, 41 Abashiri, Japan, 42 Oamaru i, New Zealand, 43 Oamaru ii, 44 Wernamo, Sweden.

Marine. 51 Frederiksværk, Denmark, 52 Frederikssund, Denm, 53 Thisted, Denm, 54 Vejle, Denm, 55 Strib, Denm, 56 Fano, North Sea, 57 Sylt i, North Sea, 58 Syltii, 59 Husum, North Sea. 60 Cuxhaven i, North Sea. 61 Cuxhaven ii, 62 Marstrand i, Sweden, 63 Marstrand ii, 64 Corsica, 65 Cette, France, 66 West Falmouth, Mass, 67 Mobile Bay, Ala, 68 Appalachicola Bay, 69 Morris Creek, Conn., 70 New

Haven, Conn., 71 Trinidad, 72 Arica, Peru, 73 Monterey i, Cal., 74 Monterey ii, 75 St Crux, Cal., 76 Fort Ross, Cal., 77, Puget sound, Wash, 78 Port Townsend i, Wash, 79 Port Townsend ii, 80 Port Townsend iii, 81 Rodrigues, 82 Sandwich Islands, 83 Funafutti, South Sea, 84 Samoa Islands, 85 Swamp, Australia.

Brackish water. 101 Odense Harbour, 102 Odense Canal, 103 Seden i, Odense Fjord, 104 Seden ii, 105 Seden iii, 106 Lumby, Od, Fjord, 107 Klintebjerg, Od, Fjord, 108 Stige, Od, Fjord, 109 Munkebo i, Kjertinge Fjord, 110 Munkebo ii, 111 Horne Bay Funen, 112 Cuxhaven i, 113 Cuxhaven ii, 114 Heiligendamm, Baltic, 115 Usedom, Baltic, 116 Swinemunde Baltic, 117 Warnow River, Germany, 118 Aseleben, Saxony, 119 Hudson River, U. S. 120 Congo River, Africa.

Fresh Water. 131, Odense Rivulet, 132 Valby, Copenhagen, 133 Aalborg, Denm, 134 Lac Sarnen, Switzerland, 135 Ploener Lake i, Holstein, 136 Ploener Lake ii, 137 Haselbach, Saxony, 138 Golzern, Saxony, 139 Bienitz, Saxony, 140 Eilenburg, Saxony, 141 Chemnitz, Saxony, 142 Sachsische Schweiz, 143 Groeden, Tyrol, 144 Eisenach, Thuringen, 145 Platten Lake, Hungary, 146 Garnock River, Scotland, 147 Loc Kinnord i, Scotland, 148 Loc Kinnord ii, 149 Pawtucket, Rhode Island, 150 Godfrey's Bay, Mass., 151 Texas, 152 Erie Lake.

Plankton. 161 Limfjorden, Denm, 162 Little Belt, Demn, 163 Great Belt, Denm, 164 Northwest Reef, 165 Marstrand, Sweden, 166 Drobak i, Christiania Fjord, Norway, 167 Drobak ii, 168, Drobak iii, 169 Atlantic Ocean, between Norway and Iceland, 170 North Atlantic Ocean, 171 Atlantic Ocean, Bergen, 172 Bay of Bengal, 173 Baltic, 174 Ploener Lake.

BACTERIOLOGY.

Bacteriological Researches Upon Whooping Cough.—Dr. Otto Zusch observed twenty-five cases of whooping cough. The micro-organism he found and described is a short bacillus. It did not show any evidence of agglutination in the presence even of undiluted serum from whooping cough cases. While he does not believe that the bac--

terium he describes can be accepted as the specific etiologic factor, he does not hesitate to offer the following conclusions in support of its specific pathogenic nature : The apparent uniform occurrence of the bacteria in the sputum of whooping cough cases and their non-observance in sputa of a large series of cases of other affections. The pecular correspondence of the bacteriological findings with the clinical course. Furthermore, the experience that the greatest danger of transmission of the disease lies in the catarrhal stadium, *i. e.*, in a stage in which the bacteria are found in the purest state and largest numbers.

Influenza Bacillus.—Bacteriological examination of a case supposed to be one of pneumococcus infection revealed the fact that about ninety-five per cent of the micro-organisms in the smears were influenza bacilli. This is interesting when contrasted with two other cases giving symptoms of influenza, in one of which streptococci were found and in the other pneumococci, but no influenza bacilli. The latter were easily obtained from the infected lungs by streaking an agar plate first with blood from the rabbit and then with the exudate. From the sputa it is more difficult, and at least half a dozen plates should be made. The colonies are about half the size of those of the pneumococcus and were exceedingly faint. These bacilli grow in hæmoglobin or blood and occasionally make threads. They grow only at fairly high temperatures, and do not produce septicæmia in animals. They were not stained by Gram. As a rule, these bacilli do not grow at all secondarily without blood, and never to any extent.

A fair guess at the identity can be made from the large number of small bacilli present rather than from the grouping. No observer could absolutely distinguish the the influenza bacilli in the sputum alone. These bacilli grow best in pigeon's or rabbit's blood, and very little hæmoglobin was required. Sterililized horse's blood had also given fair results.—Dr. W. H. Park, N. Y. Path. Soc.

Typhoid Bacillus in Milk.—The germs being in milk before churning are found in butter three months later.

They do not multiply in butter if the butter milk has been well removed, but if left in this furnishes an excellent medium for multiplication. In sterilized milk, typhoid bacteria can exist for upwards of four months. Inoculated into freshly drawn milk they have been found after three months. Inocculated into sour milk they take complete possession thereof and became almost a pure culture.

Seeds of Bacteria.—Commonly called spores, they grow one in each organism and have dense shells or walls protecting their germs. To see them they must get stained of a color different from the color of the bacterial body. We therefore stain the whole object red by covering the cover-glass preparation, but not the glass, with carbolic fuchsin solution or anilin water fuchsin, dry it in over a hot flame, cover again, dry again, till a deep red color has been imparted to the whole including the spores. With immersing in dilute alcohol we can now dissolve out the color from all but the spores, since it will adhere to them much more persistently than to the rest, using the lens to see the condition of affairs from time to time. Methylene blue will now color the whitened parts quickly before it can penetrate the spores and we have deep red spores showing on the light blue field—the body of the organism. This staining business is really a very fascinating occupation, and gives the clue to the diagnosis of all microbic diseases.

MEDICAL MICROSCOPY.

Alcohol.—Psychopathological and new microscopical researches are constantly narrowing and dispelling theories of the use of alcohol both as a beverage and a medicine.—Dr. J. D. Crothers.

Faulty Rum.—This is the name given by spirit distillers to spirit containing an organism which injures it and causes annual loss of great amount. The micro-organism Coleothrix methystes, has such lust for sugar that it braves the untoward environment of a liquid containing 70 per cent alcohol. It transforms from coccus to rod, coccus to

filament, and filament to coccus, rendering its identity still open to research.

DRUG AND FOOD ADULTERATION.

Microscope in Study of Drugs.—All of our colleges and schools of pharmacy are utilizing the simple and compound microscope and giving a more or less thourough training in the study of botany (particularly), preparatory to the study of crude and powdered drugs, foods, etc. The microscope is not only sufficient, but absolutely necessary in some cases in determining the purity of a drug, food or spice. This instrument is safe only in the hands of him who is thoroughly trained in the sciences, the subjects of which are involved. The products of the animal kingdom can only be examined authoritatively by the zoologist; those of the plant kingdom by the botanist, etc. Recently a sample of black pepper was submitted which was supposed to have been adulterated with cayenne pepper. A chemical examination gave no clue to the problem. But by means of the microscope the adulterant was detected with certainty. Some years ago a fruit jelly was upon the market, which was sold at an unusually low price. In was naturally supposed to be adulterated ; but what with was not known until by means of the microscope the presence of a diatom (Arachnoidiscus ehrenbergii) was revealed. The next question was where and upon what does this diatom grow? It was found that it grew upon certain seaweeds in the waters near Japan, and not upon fruit tree in France, and this led to the conclusion that the jelly was made from this seaweed.

MICROSCOPICAL SOCIETIES.

Royal Microscopical Society.—At the annual meeting on Jan. 18, after the report of the council for the past year and the treasurer's statement of accounts had been read and adopted, the following were elected for the ensuing year :—President, E. M. Nelson; Vice Presidents, A. W.

Bennett, G. C. Karop, The Hon. Sir Ford North, J. J. Vezey; Treasurer, W. T. Suffolk; Secretaries, Rev. Dr. W. H. Dallinger, Dr. R. G. Hebb. The president then delivered the anual address. The first portion was a review of the work of the past year, in the course of which he congratulated the society on its improved position; the second portion was a paper on Dispersion, in which he discussed some formulæ necessary in constructing achromatic lenses; diagrams and tables in illustration of the subject being thrown upon the screen. The mathematical calculations involved in questions of dispersion and refractive index not being things that can be readily grasped by any person who has not previously given much attention to the subject. Mr. Nelson put before them a method of measuring refractive indices by which the calculations could be made much more easily than had before been possible; now it was only necessary to measure two lines in the spectrum, and apply his formula to get the result.

NEW PUBLICATIONS.

The Microscope; By Jabez Hogg.—The fifteenth edition of this work which first appeared in 1854 has just been issued by Geo. Routledge and Sons, London. Price, 10 s. 6 d. The present edition is greatly enlarged from former ones and contains an immense amount of information upon general subjects. It does not, however, pretend to cover medical microscopy except by giving an account of a few of the best-known pathogenic bacteria including B. anthrax and B. pestis. It is admirably illustrated.

Coccospheres and Rhabdospheres.—Both these are, by G. R. Murray and V. H. Blackman, now placed among the algæ and described as "free, unicellular bodies, provided with an outer covering of calcareous plates, free from overlapping, or readily separable from each other, the plates characterized by symmetrical excrescences or markings." They are placed in the family Coccosphaeraceæ. The Coccospheres are: C. pelagica and C. leptopora and the Rhabdospheres are either R. tubifera or claviger.

Atlas of Bacteriology.—Slater & Spitta, London, 1898. It contains 111 original photomicrographs and 134 pages of text. An introduction gives the methods of photography. Photographs are given of the two newly discovered Bacillus pestis bubonicæ and Micrococcus melitensis. They are non-motile and free from flagella. Many cover-glass specimens are illustrated, also gelatine plate cultures of B. mycoides and B. coli communis and agar cultures of Streptococcus pyogenes and Diplococcus pneumoniæ under a low power of the microscope.

MISCELLANEOUS.

List of Second-Hand Books For Sale.

(Price Does Not Include Delivery).

Babcock. Vignettes from Invisible Life.	75
Bailey. Microscopical Observations made in So. Car., Ga., and Fla.	60
Baker. Of Microscopes' and the Discoveries Made Thereby. Broken.	2 00
Beale. The Microscope in its Application to Practical Medicine.	1 50
Beale. Disease Germs.	25
Blackham. Angular Aperture of Objectives for the Microscope.	50
Brocklesby, Amateur Microscopist.	60
Carpenter. The Microscope and its Revelations. 1856.	50
Same. Phil. 1856.	75
Davies. Preparation and Mounting of Microscopic Objects.	50
Deems. Urinary Analysis. New.	15
De Fonvielle, Les Marveilles du Monde Invisible. 115 Vignettes. Nice copy.	65
Dolley. Technology of Bacteria Investigation. New.	80
Dubief. Manuel Pratique de Microbiologie.	1 75
Fort. Traite Elementaire d'Histologie.	50
Frey. Compendium of Histology. 1878.	1 00
Same. Better condition. 1876.	1 00

Frey. Histology and Histochemistry of Man. 1 25
Frey. The Microscope and Microscopical Technology. 3 50
Gluge. Atlas of Pathological Histology. 1 00
Goring & Pritchard. Micrographia; Essays on Microscopes, Micrometers, etc. Broken. 2 00
Gosse. Evenings at the Microscope. 75
Griffith. Elementary Text-Book of the Microscope. 1 00
Griffith & Henfrey. The Micrographic Dictionary, edited by Griffith, Berkeley & Jones. 1883. 10 00
Same. 1856. 5 00
Harley. Histological Demonstrations. 75
Hassall. Microscopic Anatomy of the Human Body. 2 00
Same in German. 1 50
Heitzmann. Microscopical Morphology of the Animal Body. 2 50
Hogg. The Microscope; its History, Construction and Application. 1 00
Earlier editions of the above. 50
Klein. Elements of Histology. 50
Kolliker. Elements d'Histologie Humaine. Fine copy. 1 00
Kolliker. Manual of Human Histology. 75
Kolliker. Manual of Human Microscopical Anatomy. 75
Lankester. Half-Hours with the Microscope. 60
Leidy. Parasites of the Termites. 25
Marsh. Section-Cutting. New. 25
Miller. Practical Microscopy. New. 1 00
Microscopical Journal and Structural Record. Rare. 4 50
Microscopic Miscellany; being selections from the Microscopic Journal 1847. 2 00
Microscopic Photographs, 30 large photographs of Hair and Wool, made by U. S. War Dept. Rare. 4 00
Nageli und Schwendener. Das Mikroskop. 1 75
Needham. Nouvelles Decouvertes faits avec le Microscope. 1 00
Peaslee. Human Histology. 50
Phin. How to use the Microscope. New. 20
Phin. Practical Hints on the Microscope. New. 15
Pritchard. Microscopic Cabinet. Small. 1 25
Same. Boards. Broken, 1 pl. stained. 75

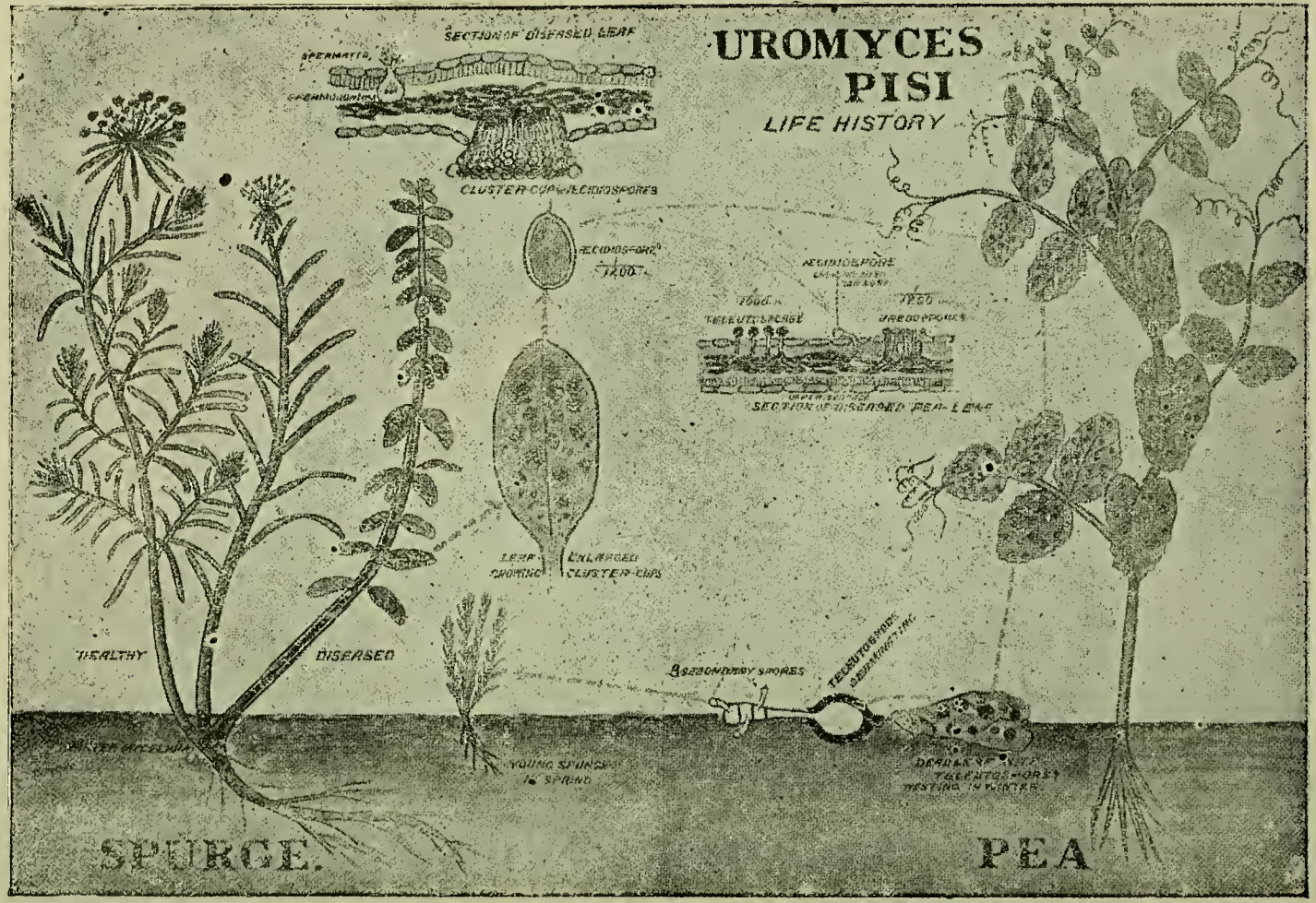
Same. Microscopic Illustrations of Living Objects.	2 00
Same. 1838.	1 00
Prudden. A Manual of Practical Normal Histology.	50
Quekett. Lectures on Histology.	2 00
Quekett. On the Microscope.	75
Rindfleisch. Text-Book of Pathological Histology.	1 00
Robin. Du Microscope et des Injections.	50
Robin. Traite du Microscope.	1 25
Ross. The Microscope. New.	25
Satterthwaite. Manual of Histology.	1 50
Schenk. Grundriss der Normalen Histologie des Menschen.	1 00
Seiler. Compendium of Microscopical Technology.	50
Smith. How to See with the Microscope.	1 00
Sommerville. Molecular and Microscopic Science.	2 50
Stowell. Students' Manual of Histology.	1 00
Stricker. Manual of Histology.	3 50
Van Heurck. The Microscope. New.	5 00
Van Phelsum. Historia Physiologica Ascaridum.	1 00
Von Duben. Treatise on Microscopical Diagnosis.	50
Wood. Common Objects of the Microscope.	75
Same. 1866.	60
Zirkel. Microscopical Petrography. Cloth.	5 00
Same. Paper.	4 50

The foregoing has been copied from catalogue of A. E. Foote, and Company, Philadelphia, Pa. to whom correspondence may be addressed.

School.—The third year of Microscopy, in the clinical laboratory at the Johns Hopkins medical school has been one of marked success. The laboratory cost $10,000 and has accomodations for 160 students.

Card.—Qualitative, Quantitative and microscopical urinalyses, urinary calculi, gonococcus, urine for diagnosis of typhoid, sputum for bacillus tuberculosis, analysis of water, vinegar, milk, tests for arsenic in wallpaper, etc.—*W. H. Ohler, Portland, Me.*

Moore's Bacteriology.—"It would be difficult to find anywhere in the world, in the same number of pages, as many important and useful suggestions."—A Critic.



An infected Spurge. An infected Pea-plant. Passage of Æcidiospores from spurge leaf to pea leaf. Germination of teleutospores producing secondary spores and inoculation of young spurge by these secondary spores.

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CONTENTS.

A Plea For the Study of the Micro-Fungi.

GREENWOOD PIM.

The domain of natural science is now so vast that no one person can deal in any detail with more than a small section or sub-section if he wishes to do any serious work, and not to be merely a scientific butterfly, flitting about from one thing to another. Hence it follows that specialization becomes inevitable, and this is true in microscopical science as in any other department, and the possessor of a microscope must choose the subject at which he desires to work, and stick to it; but he must, of course, acquire sufficient knowledge of the immediate kindred subjects to be able readily to distinguish his own specialities from those which most nearly approach them.

To those whose taste lies in the direction of botanical

Entered at the post-office as second-class matter.

rather than zoological investigation, and who may be in doubt as to what group to take up, I would submit the claims of the smaller Fungi—those which in general require the microscope for their determination, as contrasted with the larger forms which are readily recognized by the naked eye or hand lens, and which only need the higher powers for the elucidation of their minute anatomy; as in the phanerogams and higher cryptogams. The microscopic fungi present many points of advantage over their larger brethren. They are extremely varied in structure, but some of them are to be found almost everywhere where organic substances exist: on living leaves; on dead branches, twigs, leaves and stumps; on jam and paste; on old shoes; on chemical solutions; some even on living, but unhealthy fish (salmon disease) and on insects. In general, they are easily preserved as herbarium specimens, and in mounting for the microscope need but little exceptional apparatus or skill in manipulation. Many are exceedingly beautiful, being surpassed in this respect by few, if any, other microscopic objects. Again, although England and Scotland have been fairly explored by hunters of both macro- and micro-fungi, I think few will assert that these countries are by any means exhausted; while, with the exception of two or three small districts, Ireland is a *terra incognita*, and a good deal still remains to be done in America. Hence there exists among these lowly plants a far better chance of coming across forms new to the World's Flora, or even to science, than is the case with other divisions of the vegetable kingdom, such as mosses, algæ, liverworts, etc.; and only those who have experienced it know the pleasure of discovering even a humble mould which has not been hitherto recorded.

Given a fairly good microscope with 1 inch and $\frac{1}{4}$ inch objectives or their equivalents, the remaining necessaries are the usual glass slips and covers, a scalpel, a razor, a

forceps, a few needles and a bottle of Dean's medium. In very many cases it is sufficient to pick off a small portion, say of a pustule of Puccinia, and place it in a drop of water, put on the cover-glass and flatten out, moving the glass to separate the spores, when the main characteristics will be easily made out. To get an idea of the entire structure, and its relation to the nidus on which it is growing, a section of the leaf should be made. This can readily be done by placing the leaf in a slit piece of elder pith, and cutting both leaf and pith with a sharp razor. With a little practice it will be found that fairly thin sections can be made without much difficulty. The asci and sporidia of the Sphæriacei may be obtained by crushing the conceptacles under the cover-glass. Moulds of the Aspergillus nature are not so easily dealt with. Their spores are attached very loosely, and the whole character is dependent on the attachment and grouping of these. Hence, if placed directly in water, the spores would be washed away, and the specimen become valueless. This difficulty can be overcome in a great measure by placing the piece intended for examination on the glass slip dry, adding a drop of absolute alcohol or acetic acid, and then a drop of Dean's medium. If glycerine jelly be used, the alcohol sometimes precipitates the gelatine in a cloudy form, but with Dean's medium there is less trouble, and the alcohol gets rid of air bubbles, which are otherwise very annoying. In specimens mounted without alcohol, bubbles can be got rid of by boiling gently over a spirit lamp, but this cannot be done with moulds. When the specimen is not wanted permanently, a mixture of glycerine and water makes a good temporary mountant; in this it will remain for a long time without drying up.

I now propose to give a very brief outline of a few of the principal groups of Micro-fungi.

Uredineae and Ustilagineae.—These are the Rusts, Brands and Smuts which are found on corn and many

other plants. They are true parasites, occurring on living leaves, and are characterized by a great development of spores in proportion to the vegetative portion or mycelium, and were formerly called Coniomycetes, or Dust-fungi, for this reason. As examples, we may take Phragmidium bulbosum (rubi), which is exceedingly common on the bramble in summer and autumn, and forms little sooty patches on the under side of the leaf. Under the microscope it is seen to consist of oblong bodies divided into four or five sections, and having a kind of handle at one end; these are the "teleutospores." Mixed with the teleutospores will probably be some globose warted yellow bodies, formerly placed in a separate genus (Lecythea), but now known to be only another stage of the Phragmidium, and called uredo- (or brand) spores. Other species occur on roses—often doing them much injury—on raspberry, barren strawberry, etc.

In the closely-allied genus, Puccinia, which forms the rust of wheat, and of which other species are found on a great variety of wild and cultivated plants, the teleutospore has only two compartments, and in addition to the uredo-spores which occur on the same plant as the teleutospores—usually earlier in the season—a third form of fructification is met with in many cases, which is so unlike the Puccinia that it was placed in a separate order and genus, called *Æcidium*, popularly *Cluster Cups*. These are generally found on entirely different kinds of plants from the Puccinia, and form groups of tiny cups, usually yellow or white, with fringed margins and yellow spores within. They are now described as the "æcidiospores" of the Puccinia. One of the commonest is that found on leaves and fruits of the gooseberry; in the former the leaf is red above, and the cluster of cups beneath yellow, forming a very pretty low-power object for the microscope. We have thus a most remarkable alternation of generations, for the spores of the berberry

Æcidium if sown on wheat plants will give rise to the wheat Puccinia, and the teleutospore of the latter will produce the berberry Æcidium. This has been proved by numerous experiments by Plowright, De Bary, and others, not only in the case of the wheat Puccinia, but in many other species.

Various species of Puccinia may be met with on violets, grasses, plums, beans, willow-herb, primroses, thistles, ground ivy, box, periwinkle, and very many other plants. Æcidium forms occur on colt's-foot, primrose anemone, berberry, willow-herb, butter-cup, dandelion, nettle, etc.

The Smuts, Ustilagineæ, are usually intensely black and very generally occur in the floral organs, stamens, ovary, seeds, etc , while the Uredineæ are mostly brown and yellow, and chiefly affect the leaves and stems of the host plants. The best-known example—Smut of oats—may be found in almost every cornfield in autumn, the parasite converting the ear into a shrivelled mass of sooty powder. The conidia or spores are exceedingly numerous and very small. Another common, but easily overlooked species is the Bunt of wheat. This is entirely enclosed within the ear, and is not seen till the seed is crushed which, instead of affording white flour, gives only a blackish evil-smelling dust. Its conidia are much larger than in the smut of oats, and delicately spinulose. They give rise on germination to curious secondary spores. Other smuts are met with on the goatsbeard, scilla, carices, scabious, silene, stems of buttercups, etc.

Perisporiacei.—This is another group of true parasites of which the Mildew of the vine and of the rose are the best known, if not very typical examples, inasmuch as their fructification is rarely seen. In their early stages most of the species produce a white bloom on various leaves, consisting of a well-developed mycelium, from which arise jointed threads ; these readily break apart

at the joints, and are capable of reproducing the plant; the vine and rose mildews seldom get beyond this stage. In other cases, however, spherical bodies, called conceptacles, are found amongst the mycelium; these usually have appendages which are very various in form; they also contain asci,or sacs, each ascus enclosing the spores. The appendages are often very curious and beautiful. In the mildew of the garden pea they are long flexuous threads. In Phyllactina guttata, found on the hazel, ash, etc., they are needle-shaped with a bulbous base. In the mildew of the sycamore and maple (uncinula), they are divided at the extremity and recurved, resembling the sign for the constellation Aries in astronomy; in that of the poplar and willow they are curved into a spiral. Microsphæria grossulariæ, common on gooseberry leaves, has the appendages divided dichotomously at the tips and so on. The mouldy appearance is readily visible to the naked eye, and the conceptacles, if present, can be seen with a pocket lens. In mounting, one or two should be crushed so that the number of asci in the conceptacles, and the number of spores in each ascus can be ascertained. A fragment of a leaf, with conceptacles in situ,makes a pretty opaque object, while some must be detached and examined by transmitted light and a somewhat higher power to make out the details as to appendages, etc. A few forms referred to this group are saprophytes, growing on dead straw, paper, etc.

In the *Peronosporeae* we have a third and very different group of parasitic fungi, although somewhat resembling those described in the last section. Like them the mycelium forms a delicate bloom on the leaves, usually on the underside, but it also ramifies extensively in the parenchymatous tissue, the fertile hyphæ finding their way out through the stomata. They then usually become somewhat branched, each branchlet bearing at its tip a simple ovoid or rounded conidium. This conidium

readily falls off, and in a drop of water the contents become divided into about eight zoospores, which escape and swim freely by means of their cilia. Soon they come to rest, and if in a suitable nidus, such as the leaf of the plant they prey on, germinate and insert a hypha-tube through a stoma; this ramifies through the tissue and very soon reaches maturity. Thus in moist weather the plant is reproduced with great rapidity. The two best-known examples of this group are the potato disease, Phytophthora infestans, and the white rust of cabbages and other crucifers, Cystopus candidus, but other species are commonly found on lettuces, onions, various umbelliferæ, clover, etc. In many instances a resting-spore is formed in autumn in the decaying leaves and stems. This developes a hard, sometimes rugose, coat, and in this stage remains over the winter, and as the weather becomes warmer, germinates and restarts the cycle. Resting-spores are found in many species of Peronospora, but so far are unknown in the potato blight.

The "damping off" of seedlings so well known to gardeners is due to various species of Pythium, which belongs to a neighbouring group. If some cress seed be sowed very thickly in a pot, some of the young plants will soon be found to bend over and often break off. If one of these weaklings be placed in a drop of water, a crop of Pythium will soon be developed.

Somewhat allied to these are the curious Saprolegniæ, which are usually found on animals, insects, etc., in water, one species causing the well-known "salmon disease." In these we have two forms of fructification, zoosporangia, which give rise to swarm-spores, and oosporangia, containing oospores, which are fertilized by spermatozoids produced in antheridia, which are branchlets specialised for the purpose.

In the black and white moulds, found on all kinds of decaying substances, we have a very great variety of

forms, formerly, and to some extent still, classified under the order Hyphomycetes, in allusion to the great development of their hyphæ or thread-like vegetative system, and with them were ranged in older works most of the Peronosporeæ. Many of the Hyphomycetes are now known to be forms in the life-cycle of higher fungi of the ascomycetous order, but for those whose development has not been as yet certainly traced out it is still a convenient resting place. The black moulds or Dematiei occur mostly on dead wood and stems; Mucedines (white moulds) on almost every conceivable organic substance, such as leaves, herbaceous stems, jam, fruit, bread, paste, leather, etc. In both series we usually find an abundant mycelium, light or dark colored as the case may be, from which arise the fertile hyphæ, bearing the conidia, as this type of spore is commonly called. The manner in which these conidia are arranged largely determines the genus and species of the individual under consideration. Thus we may have them in tassels as in Penicillium (blue moulds) when the hypha divides somewhat palmately prior to the formation of the conidia, and also in Aspergillus when the hypha ends in a swollen portion which bears the conidia. They are borne on irregular branches in Botrytis and Polyactis; in tufts arranged in a racemose fashion in Botryo sporium, a beautiful snow-white mould, common on decaying herbaceous stems. In all these they are more or less globose and unilocular, but they are pear-shaped and septate in Dactylium, very long and with many transverse septa in Helminthosporium, while in Macrosporium they are septate both longitudinally and transversely, giving rise to the structure known as "muricate." In fact, the variations are almost endless, and it is very difficult to convey an accurate idea of their structure by words without figures.

Another group, also popularly called moulds and which grow in similar situations,and very often on dung,are the

Mucorini. A pot of jam or paste is thickly studded with apparently very long slender pins, each with a small round head. This is Mucor mucedo. The round head is found to be a sporangium or capsule filled with spores, which readily germinate in a suitable medium, and give rise to an abundant mycelium which again produces the sporangia in a very short space of time but under cirtain circumstances we may find a very different form of reproduction, similar to what is found in many algæ, and called zygospores. They originate thus: two neighboring branches of the hyphæ approach each other tip to tip, become swollen or club-shaped; a septum is formed cutting off the terminal portion of each. The portion so cut off is called a *gamete*. The two gametes are at first separated by their respective cell-walls, these soon disappear and one large cell is left suspended from the two original branches. The wall quickly thickens and assumes variously warted or spiny appearance externally and has the property of retaining its power of germination for a long time. Through the genera Pythium and Saprolegnia already referred to the Mucorini approach more or less closely the Peronosporeæ.

Space will admit of notice of but two more sections of microscopic fungi—the Phacidiacei and the Sphaeriacei. Both these occur in general on dead leaves, stems, or wood, while a few are parasitic on living plants, on grasses or living insects, which latter, however, they ultimately kill. All have spores borne in asci and contained in perithecia or receptacles very like those in the Perisporiacei.

In the Phacidiacei the perithecium opens by valvular teeth, in the Sphaeriacei by a central pore; of the former a familiar instance may be found in the Rhytisma acerinum which forms black patches so common on sycamore leaves, the fruit being perfected in spring, as the leaves lie decaying on the ground. Other, such as Stegia

ilicis and Trochila lauro-cerasi, are to be found on almost every dead holly or laurel leaf.

In the Sphaeriacei the conceptacles may be quite naked, as in many species of Sphaeria, or more or less immersed in a stroma or bed, which may be incrusting the branch as in Diatrype ; formed into masses of various shapes as in Xylaria (the candle-snuff fungus), Hypoxylon, and other genera. In Nectria, one species of which is exceedingly common on dead stems of currant, etc., the perithecia are usually clustered together and of a rich red color, resembling, under a low power, a basket of strawberries. The spores, too, are very various : some are simple, hyaline, and shortly ovate or rounded,others of various degrees of length, and one- or many-septate, and colored ; some drawn out into veritable needles.

Some of the Hyphomycetes, as already mentioned, are known to be conidial fruit of certain ascigerous fungi ; a third form of fructification is met with in the form of minute perithecia containing naked spores, formerly grouped under Sphaeropsidei ; in a fourth, long, glutinous tendrils, consisting of myriads of simple spores are met with, while in some cases—Sphaeria herbarum, for instance—no fewer than five forms, once considered distinct genera and species, are now united as different stages in its cycle of existence.

Thus it will be seen that there is a great field open to the investigator in working out the life history of these organisms, which, though lowly and seemingly insignificant, are in many instances of great economic importance from the injuries they cause to valuable crops and trees. Such are the potato-blight, wheat-rust, vine-mildew and many more. As to reference books, Dr. Cooke's "Microscopic Fungi" (Rust, Smut, Mildew and Mould) is of great assistance to the beginner. The earlier editions fo it were written when the relations between Puccinia and Æcidium, Peronospora and Cystopus, etc., were scarcely

known, but probably the later editions have been brought up to date. For the Uredineæ and Ustilagineæ the student may consult "Dr. Plowright's Monograph," and for most of the other groups—except the last two, on which I know no modern work in English—Mr. Massee's "British Fungus Flora." With reference to plants injurious to trees and crops, we have Dr. W. G. Smith's "Diseases of Field and Garden Crops," and especially the English edition of Tubeuf's "Diseases of Plants induced by Cryptogamic Parasites."

In conclusion, I wish to say that the groups have not been dealt with in any sort of systematic order. They have been taken rather in a kind of biological sequence, according to the nature of the substances on which they live. I have used the word spore throughout as indicating the reproductive body, no matter how it has been formed—whether naked, in asci and so forth. Within the limits of such an article as this it has been impossible to touch more than the extreme fringe of the subject, which to treat in detail would occupy not one but several large volumes. My leading idea has been to stimulate persons desirous of doing some microscopic work, and in doubt as to what branch to choose, to select this most fascinating study, and to leave them, not satiated, but rather like Oliver Twist, "asking for more" information.—*Ill. Ann. Mic.*

On The Exhibition of Live Animals at Soirees.

MARCUS HARTOG.

The exhibition of Polyzoa, Hydrozoa, Rotifers, Crustacea, etc., has always been a matter of difficulty, and the following details of a method which avoids the constant need of watching to prevent evaporation, etc., may be found of interest. The animals are mounted in a hanging drop of water on the under side of the cover-glass,

luted on to a cell of paraffined millboard. In this condition I have preserved Lophopus and other Polyzoa for forty-eight hours, including two nights' exhibition. The following details may be found useful.

The cell is composed of a piece of millboard about 2″ × 1¼″; in this a circular hole, ⅝″—¾″ in diameter, is punched by a wad-punch, as sold by the gunsmiths. The cell is then immersed in a bath of melted paraffin candle, so long as bubbles rise; it is then placed on a glass slide, about 4″ × 2″, and left to cool: the side of the cell which bears the burr from the punch must, of course, be uppermost, or else it will not lie flat and stick to the slide; a number of these may be prepared at the same time, as they keep for years, and travel very well. When the time comes the cover-glass is laid on a flat surface, and the weed bearing the objects is placed on it; a needle may be used to arrange it, if necessary; or the drop of water containing swimming organisms is gently put on it with a pipette. The margin of the cover must then be wiped with a corner of the handkerchief or a piece of thick blotting-paper, by a succession of circular movements, each over a short segment of the circumference; it is then left for a little till the moisture dries off from the wiped edge, and it appears quite clean and shiny; then, if necessary, a little more water may be added in the centre with a dropping-tube, a fine pipette, or even a pair of fine forceps. The cover is next pushed to overhang the edge of the flat surface on which it has been lying, taken up with the fine forceps (which must be *quite dry*), and dexterously turned over and lowered on to the prepared cell so that the drop is well over the centre of the hole. The edge of the cover must then be luted on; the instrument I use is a coarse hairpin twisted into a ring near one end, produced beyond the turn for half an inch, and bent at a right angle, the ring serving as a reservoir of heat. With this it is easy to melt shreds of paraffin so as to seal the

cover to the cell, both fast and hermetically. It is best to seal at two opposite points first, so as to fix the cover at once and then fill up the intervals. Objectives up to the Zeiss D (low angle $\frac{1}{6}''$) are available.

Besides the other advantages of this system, we may note that the animals are evidently much more at home in this prison than in any of the ordinary devices. The shyest tubicolous Rotifers and Polyzoa come forth readily, stay well exposed, stand a good deal of motion without shrinking back, and when they do withdraw return into view with the least possible delay; and free swimming animals may be so confined by limiting the size of the drop as to keep them in the field under relatively high magnification. I am free to admit that the method is more troublesome than putting between two pieces of glass, a thick one and a thin one, but with the paraffin cell, once done, all is secure for the evening, the only possible dangers being those that all microscopic mounts are liable to.—*Ill. Am. Mic.*

Uniformity and Accuracy.

M. I. CROSS.

To the working microscopist it is a great advantage, amounting almost to a necessity, that the objectives produced by the different makers shall interchange in the nosepiece of his microscope; but many of us have found that it has been impossible to screw home some objectives we have obtained until the thread has been eased, and have at once cast blame on the manufacturer. He, however, has not hitherto been altogether at fault, for no doubt he has worked to his standard gauges supplied by the Royal Microscopical Society many years ago, and is entitled to aver that his lenses are screwed to the "universal" thread and that others must be wrong.

Now this "universal" thread has for years been a misnomer, the gauges issued by the Royal Microscop. society

having been slightly variable, and admittedly imperfect.

Recognizing the desirability of establishing once for all a standard size, the society recently placed at the disposal of opticians and others who might wish to become possessed of such tools, accurately adjusted steel screw gauges. These are so constructed as not to interfere "with the interchangeability of previous object glasses and microscope nosepieces which have been *correctly* made to the original standard," but by means of minute changes in the diameter "a slightly larger margin for error in individual lenses" is allowed for.

Microscopists would imagine that opticians would have gladly made use of a means which would have enabled their clients to derive so much convenience and benefit, but on enquiry it will be found that so far from this being the case, nothing has been done to give practical effect to the recommendations of the society, while the firm which is noted for using the most abnormal "universal" thread has shown no inclination whatever to work to the new gauge. The committee appointed last year to consider the question of this gauge was composed of as practical a set of men as could be selected for the purpose, yet from want of action on the part of manufacturers, the effort of the society seems likely to be a futile one. Wake up, opticians! do all you can to help those who pay your dividends—it will be profitable in the long run.

There are several other ways in which opticians could help microscopists. One matter is of first-rate importance, and that is the adoption of an universal size or sizes for eyepieces. This has been urged on many previous occasions, and here again it is the optician who is at fault, for so long ago as the year 1882 the Royal Microscopical Society recommended the use of two sizes only, one with a diameter of fitting of 0.92 in. for students' instruments, and the other 1.35 in. for microscopes of

large size. Who can say how many different gauges are used by the various manufacturers for eyepiece sizes? Yet the two sizes recommended would meet every want. The two features named above are much-needed reforms, and the only way in which they can be brought about is for workers to stipulate for the society's sizes and accept no other.

When these alterations have been effected there are plenty more matters that are worthy of attention, such as making the different eyepieces of a series to work in the same focal plane ; having all eyepieces engraved with their initial magnifying powers rather than such absurd letters as A, B, C, etc. Then it would not be a practical impossibility for all the brass boxes in which object glasses are issued to be of uniform size or sizes, and for object glasses to be corrected to one definite thickness of cover-glass, which would in addition permit of the correction collars of all objectives being divided alike, so that values should not have to be ascertained for each individual lens. If all these reforms were effected before another Annual were issued, many a microscopist would have been enabled to do better and more accurate work than hitherto. So much for uniformity, now for accuracy.

On testing an apochromatic object glass stated by the makers to have a numerical aperture of .95, some months ago, I found that it was scarcely .87. I returned it, and received an admission of the want of aperture, but notwithstanding long and weary waiting, the firm—a very distinguished one—has not been able to produce a lens of the full aperture. This is a trivial matter compared with some I have examined where the over-statement has exceeded 20 per cent. It must be admitted that a slight variation may occasionally occur, and in more than one instance I have found an object glass to possess a larger aperture than that attributed to it. It is, however, an unfortunate fact that in the competition which has arisen

in recent years to give large apertures at a moderate cost, makers have been so unscrupulous as to state the apertures of their objectives to be far in excess of that which they actually possess. This is fraud pure and simple, and it is a temptation which all opticians should have principle enough to withstand. Associated with this misrepresentation question is another of only slightly less importance ; one is, in fact, the outcome of the other. Many objectives are stated to be of a certain focal power, but on testing, it will very frequently be found that they magnify considerably more than they should do. Thus a 1-6 in., which should yield a magnification of 50 diameters at an image distance of ten inches, will probably prove to magnify about 70 diameters. It is obviously easier to give a larger aperture with an increased magnification in the objective, but it is no satisfaction to a worker to purchase an ostensible 1-6 in. lens of, say, .85 N.A., to find that it is actually a 1-7 in. of .80 N.A. or thereabouts. It is imperatively necessary that the powers and apertures of objectives be stated as nearly as possible.

Uniformity and accuracy are more than desirable in connection with the microscope, and the opinions expressed in this short note are those of a large section of working microscopists in all parts of the world.—*I. A. M.*

Magnesium Light in Photo-Micrography.

PROCELLA.

I have practiced photo-micrography for the last twenty-five years. I began a considerable time before the introduction of the modern dry plate in 1879-'80. Collodion was the sensitive medium then used, and as my business duties only permitted the necessary leisure in the evenings the practice was pursued under considerable difficulty—the chief one being the illuminant.

As my experience may be useful to others, I propose to describe very shortly what it has been and in what way I have succeeded in overcoming the difficulties incident to the subject. My apparatus is a very simple one and has been all constructed with my own hands. The base-board of the apparatus is of yellow pine about 3 feet long, about 8 inches broad and 1½ inches thick. I find yellow pine much more rigid than a harder wood,and it is much lighter. The camera is a quarter plate one, with a bellows having an extension of about two feet. The end of the camera carrying the dark slide is ridgidly attached to one end of the board, while the other end of the camera, which is attached to a sliding front, can be moved forward and fixed by a pinching screw at any distance within the limits of the bellows extension. The top of the base-board at each side has narrow strips screwed on within which the front of the camara slides. The camera front has a flange screwed with the same thread as the Dallmeyer half-plate R. R. and carries a tube about 2 inches long which fits it. The microscope is one which I specially made myself for the purpose. It is mounted on two brass trestles which are rigidly screwed to a board which slides within the guides on the top of the base-board. The piece is about a foot long, and it again can be pinched firmly at any position on the base-board. The base-board for this purpose having a groove reaching within 6 inches of each end. Beyond the microscope there is an upright board of thin wood which has an aperture in it of an inch in diameter opposite to the optical axis of the microscope. This aperture is used for adjusting the size of the image wanted, and for focussing by means of a lamp of any kind placed behind it. A small wooden shutter about 3 inches long, whose shape is iike that of a vertical section of a sugarloaf rotates at the narrow end upon a screw nail while another screw allows it to fall into position when the lamp is removed,

In the centre of the broad end of the shutter a piece of brass tube with an aperture of about 3-16ths of an inch is screwed, and this tube when the shutter is in position is exactly opposite the optical axis of the microscope. This adjustment is easily made by means of a low power object glass and eye-piece before the shutter is fixed, and when this is done in my case with a fixed microscope, all work is ended. Now, when about to take a photo-micrograph, the microscope and camera are moved along the base-board, with the lamp behind the one-inch aperture, the shutter being swung aside on its nail out of the way, until with the object glass used one is satisfied with the magnification and has obtained a sharp focus. The front of the camera is then pinched firmly down and so is the board on which the microscope is carried. The focus is again examined to see that all is right, a plate is put in the dark slide, this end of the camera covered with a dark cloth, the lamp removed and the small shutter is turned into position, the shutter of the dark slide is withdrawn, and we are ready for an exposure. I do not turn down the lights in the room. Suppose I am using a Zeiss 35 mm. apochromatic as the objective with a camera extention such as to get 30 inches between the object and the sensitive plate, I push about three-quarters of an inch of magnesium ribbon through the aperture in the small brass tube in the shutter and set fire to the ribbon by means of a match, and this three-fourths of an inch of magnesium ribbon, which will barely take three seconds to burn, will give me a dense negative. I use no condensers with low powers. With this illuminant they are not necessary. The light proceeds from a small point and the rays reaching the object are nearly parallel. Sir Henry Roscoe, in his book on "Spectrum Analysis," states that this light is rich in purple and violet light, the very qualities which affect the sensitive plate most. I have used magnesium now for over ten years, and have con

structed more than a dozen apparatus similar to that I have endeavored to describe for the public laboratories and for private teachers in the city in which I reside. Magnesium ribbon is easily lighted if the end is dipped either in paraffin oil or spirit of wine. To give another illustration of the power of the light. I recently was asked to produce some photo-micrographs of sand, one of these being sand from the desert near Cairo, the other singing sand from one of our western islands. I took two 3 × 1 slides and spread a very little gum on them, then sprinkled the grains of sand on the gum. I placed the slides on the stage of the microscope with a piece of black paper behind, and using Zeiss 70 mm. apochromatic, I obtained, by means of the gas in the room, as sharp a focus as possible. I then, after the apparatus was in order, placed a board alongside the camera to receive the magnesium oxide as it dropped. I took a short piece of brass tube and pushed about two inches of magnesium ribbon through it, lighted it, and keeping it about a foot from the slide, I burnt about eight or ten inches of the ribbon by pushing the ribbon through the tube as it was consumed. I used no condenser, and in this way I obtained two very fine negatives. Lantern slides have been made from these and they excite the admiration of everyone who sees them on the screen. The light and shade in the prominences and hollows in the sand grains are beautifully rendered. They are at present being reproduced by a photo-mechanical process in the transactions of a geological society. I have taken photo-micrographs of such diatoms as Navicula lyra and N. splendida by means of Zeiss 4 mm. apochromatic without any condenser by simply burning about six inches of ribbon.

I cannot admire the persistent way in which photo-micrographers will insist upon using oil lamps, even though they are assisted by every possible optical accessory, when they have at their command, if they choose to use it, a

light so very effective as magnesium. The rapidity and power of its action, its cleanliness, and the ease with which it can be used is all in its favor. Why waste minutes of valuable time with oil lamps, when a second or two will do the same thing with magnesium? With the above mentioned 70 mm. objective when I am photographing a transparent object I usually have to place two separate pieces of obscured glass between the light and the object, as otherwise, however small a piece of ribbon I burn, my plate would be overexposed. I do not need to allude to any of the other methods of illumination as I have practised this one now for so long and find it so well adapted to the end in view by its simplicity and cheapness, that I regard every other, except sunlight, as inferior to it in every way. I invariably use Ilford Ordinary or Empress plates, as from the photo-micrographs I made of the granulation of the silver in these plates which were exhibited at a meeting of the British Astronomical Association two years ago, I found the silver in a finer state of division in them than in those of any of the other makers.—*I. A. M.*

Cleaning Glassware.

[From V. A. MOORE'S Bacteriology.]

It is necessary that the glassware employed should be thoroughly cleaned before it is used. Several special methods have been suggested for this purpose but the one frequently employed by chemists seem to be the most easily handled and quite as efficient for general use as the more elaborate, specialized processes. It consists in applying the chromic acid cleaning mixture after washing the tubes and flasks with water. It is sometimes necessary to employ other methods for cover-glasses which are to be used in staining bacteria where a mordant is required. Clean all of the glassware, test tubes, fermen-

tation tubes, flasks, Petri dishes and reagent bottles. Put the slides and cover-glasses in the cleaning mixture,they can be rinsed and wiped later.

Test Tubes. Wash these carefully with soap and water using the test tube brush. After washing stand them in a glass jar (aquarium) and fill them to within about 2 cm. of the top with cleaning mixture. After it has acted for from 4 to 10 minutes (it is better to stand longer) pour it out of the tubes into the bottle originally containing it. Rinse the tubes thoroughly in tap water until all color disappears and then in hot water and drain them, using individual drainage board from locker. After they are dry, wipe the outside of the tubes with a slighty dampened cloth.

Fermentation tubes. Treat these in the same manner as the test tubes excepting in the use of the brush which must be omitted.

Flasks. Wash the flasks thoroughly with soap and water. Then fill them with the cleaning mixture and allow it to act for at least 10 minutes after which it can be poured back. Rinse the flasks thoroughly in the same manner as the test tubes and drain them. When dry the outside should be wiped with a damp cloth.

Petri dishes and reagent bottles. Thoroughly wash the Petri dishes and reagent bottles in hot soap suds after which rinse them separately in hot water. Wipe the Petri dishes dry with a cloth and drain the bottles. The cleaning mixture need not be used.

Slides and cover-glasses. Drop the cover-glasses singly into a glass jar containing cleaning mixture and allow them to remain there for 24 hours or longer. Pour off the cleaning mixture and rinse the cover-glasses until all of the color disappears, then cover them with alcohol until needed when they can be wiped with a soft linen cloth or with lens paper. Treat the slides in the same manner as the cover-glasses. They can be wiped directly out of the rinsing water. The slides can be cleaned satisfactorily by washing them in a strong, hot soap suds, rinse in water and wipe.

Cleaning and culture apparatus. Place the tubes, flasks, or Petri dishes containing old cultures in a water bath,

cover them with water to which add a little sal soda (about an ounce to the gallon of water) and boil for 20 minutes. Pour off the water and empty the tubes after which again boil them for 5 minutes in clean soap and water. Then wash and treat with the cleaning mixture the same as the new tubes.

Formula for chromic acid cleaning mixture. Dissolve 80 grams of potassium dichromate in 300 c. c. of warm water. When all of it is dissolved and the solution cooled add it slowly, with constant stirring, to 460 c.c. concentrated sulphuric acid with constant stirring, storeing the mixture in a glass-stoppered bottle.

A method for cleaning cover-glasses for flagella stain. For this work the ordinary method of cleaning cover-glasses is not sufficient. After being cleaned by the ordinary method, boil the cover-glasses in an agate cup or glass beaker in a 10 per cent solution of caustic soda for five minutes. After cooling rinse the cover-glasses thoroughly in distilled water after which place them in a beaker and cover with a 1 per cent solution of hydrochloric acid, heat to the boiling point, and allow to stand for several hours (over night or longer). Then pour the acid off and rinse the cover-glasses several times in distilled water and finally in alcohol. Wipe them out of alcohol as they are needed.

EDITORIAL.

We wish to call special attention of friends, physicians, and microscopists to a new and easy method of blood staining, p. 163, which will prove of great importance in diagnosing disease and especially request that those to whom we take the pains to mail copies will report upon its use as compared with the old methods formerly employed.

Color of Primitive Man.—A writer in *Knowledge* gives an article on this subject illustrated with Photo-micrographs but he does not commit himself as believing man was either white, brown or black but gives data from which the reader may form his own conclusions.

Pineal Gland.—Dr. Campbell of Liverpool has described

the macroscopical and microscopical characters of this organ, prepared slides and made drawings. He examined a large number of these glands. In all these was either a definite central cavity (a ventricle) or else a collection of specialized tissue resembling neuroglia. Abnormal conditions appeared in the pineal glands of insane patients.

Varnishes and Labels.—Many things will do:—Sealing wax dissolved in methylated spirit, copal varnish, gold size, printers' varnish mixed with any pigment of the desired color. Pigments can also be ground up in French polish, and used for the purpose.

MICROSCOPICAL MANIPULATION.

Fixing and Staining Blood.—Absolute alcohol is the most reliable fixative known. Eosin and Methylene blue are the standard stains for demonstrating all the ordinary characteristics of normal and abnormal blood. But eosin under certain circumstances cannot be made to stain certain cell granules. This has led to combination stains such as the Ehrlich-Biondi and Ehrlich's later triacid, but they are troublesome to use and require special fixing methods. If to alcoholic eosin solution or alcohol soluble eosin is added methylene blue, brilliant grass-green crystals are precipitated. When methylene blue is added to a watery solution of eosin or to water-soluble eosin amorphous and dark metalic-green crystals result. Consequently preparations have been put on sale consisting of eosin methylene blue mixtures designed to avoid these precipitates. The first named crystals are very sparingly soluble in cold alcohol but more so in hot alcohol from which they can be recrystalized. This shows them to be a deffinite compound body. Both these kinds of crystals melt at 227 degs. C. taken on the surface of mercury. Because of their being so poorly soluble in alcohol these crystals are not useful as a blood stain without previous fixation of the film. Dissolved in chloroform or aniline oil, they give dark violet colored solutions. Watery solution gives amorphous precipitates of the two dyes which is very sparingly soluble in water,

but in alcohol it gives a blue solution with strong green fluorescence. It is more soluble in methyl alcohol. Dr. Jenner in the Clinical Laboratory of St. Thomas' Hospital, London, has adopted this solution for fixing and staining blood. He makes it of equal parts of a half per cent to one and a quarter per cent solution of Grubler's water-soluble eosin, yellow shade, in distilled water and of a one per cent solution of Grubler's medicinal methylene blue also distilled in water. These are mixed together in an open basin (not in a flask) and thoroughly stirred with a glass rod. Preferably, let the mixture stand 24 hours, filter, dry the residue either in the air or in an oven. It can be dried at 55 degrees C. without harm. When dry, scrape off the residue from the filter paper and powder it. Shake this up with distilled water and wash in a filter. The washing will be of a thin dirty-purplish color. Again dry, powder and store in bottles. When wanted for use, shake up one-half a gramme of the powder with 100 cubic centimeters of pure methyl alcohol and filter. This stain may be equally well made by dissolving the eosin and the medical methylene blue directly in absolute methylic alcohol and mixing them in proportion of 125 cc. of .5 per cent solution of eosin with 100 cc. of .5 per cent solution of methylene blue. Or prepared solution may be purchased from R. Kanthack, 18 Berners Street W. London, or from American dealers. This stain is a single solution, easily made, keeps well, will fix and stain blood fiims in two minutes. It will demonstrate, with perfect clearness, with rapidity, certainty, and in well marked contrasts the red blood discs, the blood platelets, the nuclei of white blood cells, the fine granules of the polymorpho nuclear leucocytes, the coarse oxyphil granules, the basophil granules, and when present erythroblasts, bacteria, malarial parasites and filariæ. It is a simpler and clearer method than any heretofore published. The differential staining results from the decomposition of the compound substance by the various elements of the blood, those parts which are acid combining with the basic coloring matter and those that are basic combining with the acid part. That the stain is readily so split up

may be seen by adding weak acids or weak alkalis to such a watery solution of it as can be made—acids turning the solution blue and alkalies changing it to a pink, the stain acting as a delicate indicator. The same result comes from heating such weak watery solutions which turn blue on warming but turn back to the original color on cooling.

Cover-glass preparations are made in the ordinary way except that the glasses must be absolutely clean and free from acid or alkalis, and on them as soon as the extremely thin films are dry a few drops of the staining solution is to be poured, no previous fixation being required. Keep the supply of clean cover-glasses in absolute alcohol. Cover the specimen with a watch glass to prevent evaporation and consequent precipitation. In from one to three minutes, the stain should be poured off, the specimen rinsed in distilled water if possible till the film has a pink color. This will occur in 5 to 10 seconds. If filtered water is not available, use other but immerse only momentarily and dry between filter-paper or rapidly over flame. Dry it high up over a flame or filter by agitation in the air. Mount in xylol balsam. Under the microscope, the red discs will be of a terra-cotta color ; the nuclei of the white corpuscles, blue ; the platelets, mauve ; the granules of the polymorpho-nuclear white cells and of myclocytes, red ; the granules of the basophils (mast cells), dark violet ; bacteria, filaria, or malarial parasites blue. If the specimen is washed in unfiltered water, the red colors are more pronounced ; the blue of the white cell nuclei will be almost washed out while the dark blue of the smaller hyaline white cells (lymphocytes) stand out in strong contrast to the now pale nucleus.

Multiple Images in the Cornea of a Beetle's Eye.—In a large number of text-books on the microscope it is stated that if the cornea of a beetle's eye be placed on the stage of a microscope, and an object be placed between the source of illumination and the cornea, that object will be seen reproduced in every facet of the cornea. The experiment is an exceedingly interesting one, but there are

very few people who know how it should be carried out. The cornea must be flattened, not left in the globular condition of life. The simplest way to exhibit the effect is to cut a cross out of brown paper, about ¾'' long, place this on the mirror of the microscope and focus the facets of the cornea in the usual way with a ½'' objective. You then—and this is the important stage on which the result depends—gently rack the objective upwards, causing the structure to appear to go out of focus, at the same time moving the cross on the mirror by means of a pointed stick of wood. It will be evident where it is best to stop raising the objective, for the cross or the stick will be seen in the facets. It then only remains for the cross to be so set on the mirror that it appears in the centre of each of the facets. There are many other ways in which the effect can be produced, a very pretty one being to throw a brilliant light on the face of a friend who sits at the side of the microscope, and so arranging the mirror that the reflection of his face falls upon it and is again transmitted to the cornea. Also by a little scheming the second hand of a watch can be seen in each of the facets. When well shown, these experiments always create astonishment and interest. A little practice soon enables one to do them with facility.

MICROSCOPICAL SOCIETIES.

Quekett Microscopical Club.—367th meeting Friday, March 17, at 20, Hanover-square. Mr. Rousselet read a paper on "Torchosphaera," an extraordinary rotifer, not as yet found in England. This animal is a sphere of about the size of Volvox globator, and two species are known, one with the ciliary zone at the equator, thus dividing it into two equal halves, and another with the zone nearer the pole, which is thus unequally divided. The organs are contained in the lower hemisphere, and very clearly visible. A mounted specimen was exhibited under the microscope. Mr. Soar read a note on a water-mite, a species of atax, which he believed to be new. Mr. Lewis Wright, as-

sisted by Mr. Russell Wright, gave an exhibition with the projection microscope, showing a series of specimens with various powers of animal and vegetable histology, insect parts etc., also diatoms with dark-ground illumination, and other objects with the lieberkuhn. Mr. Wright said his main purpose had been to show the advances made in projection since his former exhibition in the mathematical theatre of University College some fourteen years ago. Progress, in the main, was due to the increased rigidity of the instrument, permitting better focussing, the use of condensers adapted to the aperture of the objectives employed, better objectives, and, last but not least, a vastly improved screen. He had hoped to have used the electric arc as the source of illumination, but it was found that although there was plenty of current available in the mains to the building, the fuses fitted were insufficient to stand it, and, therefore, he had to put up with gas, and, as a result, could not employ the higher magnifications he had intended. On Saturday, April 8, there will be a meeting, by permission, at the Royal Botanic Gardens.

Philadelphia Moss Chapter.—A new science club, has recently been organized for the study of general Bryology. Weekly meetings are held at the Acadamy of Natural Sciences, which will soon be alternated with field meetings. The object being to augment the Academy's collection of Mosses and Hepaticae.

The officers of the Chapter are— President, Professor A. F. K. Krout ; Vice Pres., Professor Wm. Findlay ; Secretary and Treasurer, Alexander Mac Elwee ; Corresponding Secretary, Mrs. Josephine D. Lowe.

Active students in Cryptogamic Botany, and especially of the Archegoniatae, are eligible to membership in the Chapter.

The Royal Microscopical Society.—The first president (1840-1) was Professor Owen, afterwards Sir Richard Owen, K. C. B., D. C. L,, M. D., LL. D., F. R. S., etc., whose scientific achievements are known throughout the world. At the first annual meeting on the 15th of February, 1841,

the society consisted of 177 members, of whom no less than twenty-two were Fellows of the Royal Society, and included such well-known names as Thos. Bell (professor of zoology at King's College), Birkett (of Guy's), George Busk, F. R. S. (president 1848-9), Sir James Clarke, John Edward Gray (keeper of the zoological department of the British Museum), John Lindley, Ph. D., F. R. S. (president 1842-3), John Kippist (the librarian of the Linnæan Society), the Marquis of Northampton (then president of the Royal Society), Sir John Tomes, Erasmus Wilson, and Joseph Jackson Lister, F. R. S., who has been described as "the pillar and source of all the microscopy of his age." In passing, it may be noted that it was on January 29th, 1840, that the society adopted standard sizes for the glass slips for objects, 3 x 1 in. and 3 x 1½ in., which sizes are universally employed to-day. As the list of subsequent presidents is read over, one cannot but be struck by the fact that they were all eminently practical men; some of their names are so familiar to us on account of their work that it seems impossible to dissociate them from present day microscopy, such, for instance, as George Jackson, M. R. C. S. (1852-3), who gave us the well-known Jackson form of microscope; Dr. William B. Carpenter, C. B., F. R. S. (1854-5), whose book, recently edited and revised by Dr. Dallinger, is the standard work on matters microscopical (it was during Dr. Carpenter's presidency that the standard screw for objectives was fixed and introduced); John Thomas Quekett, F. R. S. (1860) and others. Professor Huxley was a member of the council in 1857, and contributed his first paper to the Society's proceedings during the presidency of Dr. Arthur Farre, F. R. S. (1850-1).

"The society was established for the promotion of microscopical and biological science, by the commuication, discussion and publication of observations and discoveries relating to (1) improvements in the construction and mode of application of the microscope, or (2) biological or other subjects of microscopical research."

How perfectly the society has adhered to the lines laid

down, can be seen on reference to its Journal, which holds a high position amongst the leading scientific publications. In it are to be found original papers, written by Fellows, which have contributed materially to the advancement of knowledge in matters microscopical. It has also for many years been the centre of communication of information regarding new discoveries and processes in biology, subjects which have often been closely linked with the material well-being of the world at large.

The society has always been especially strong in the mechanical and optical sections of microscopy. Details peculiar to English microscope stands, which must eventually be more fully recognized by Continental Manufacturers—such, for instance, as the tripod form of foot, the centring substage and fine adjustment from same, the rackwork draw-tube, the improvement of the fine adjustment and numerous other details which make for perfection have been nurtured into prominence and acceptance by the society. In the optical section every fresh effort has been encouraged the substage condenser has been evolved from a makeshift to a system which is as carefully made and needs as skillful employment as the objective, while the introduction of wonderful excellence in objectives of moderate cost has been fostered and practically compelled by the constant criticisms and encouragement of the Fellows. As a result we have lenses at our disposal only slightly inferior to the apochromats, which have enabled many men to do original and reliable work without being handicapped by the enormous expenditure that was necessary for lenses a few years ago.

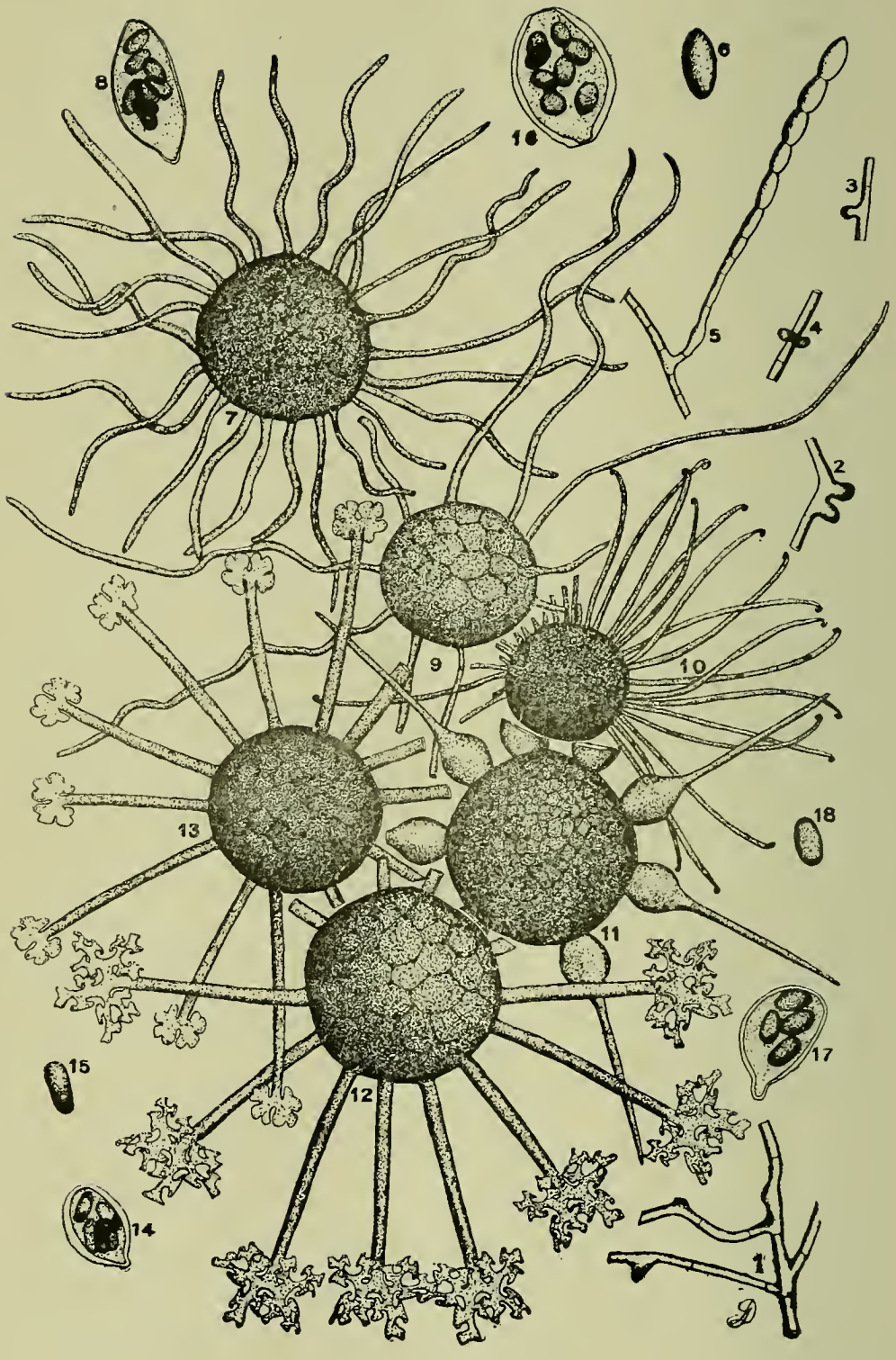
To maintain the best traditions of a society which has done such yoeman service in the past is a great responsibility, and calls for constant activity on the part of its council and members. This list of names of the officers which heads this brief notice will afford a guarantee of the maintenance of its vitality and eminence, but this depends in no less degree on the individual Fellows than on the president and council. Communications of investigations undertaken, new theories, and progress that has been

made in any branch of microscopical work, always receive a courteous hearing from the society, and frequently valuable assistance is afforded by hints from the ripe experiences of the Fellows. The Journal, which is issued bimonthly to the members, contains, in addition to the transactions, a summary of current researches in zoology, botany and microscopy throughout the world, and is an invaluable record for workers. The extensive library and instruments are always at the disposal of members, and the rooms at 20, Hanover Square, are open daily.

Representing, as it does, microscopy in its scientific aspect only, the membership is necessarily limited, and the very high standing of the society has rather tended to discourage workers from applying for admission to its ranks, but all really interested microscopists are entitled to claim its advantages by becoming Fellows. The "toy" microscopist, who uses—or rather misuses—his instrument for curiosity and amusement, is not eligible, but any person who may be desirous of gaining a better understanding of the theory and methods of employment of the most fascinating instrument ever devised, or wishes to be au fait in eurrent scientific microscopy, will have no difficulty in finding a Fellow who will nominate him. The secretaries of the society are always glad to render assistance to such persons. The society should have on its roll every earnest worker, and it is the duty of such to afford his best support to the society, not alone for his individual benefit, but for the good of microscopy generally and the vast interests that are interwoven with it.

NEW PUBLICATIONS.

The Microscope.—Simon Henry Gage. This is the Seventh Edition with the enlargements and the matter rewritten regarding refraction, numerical aperture, filar micrometers, imbedding and sectioning by the paraffin method. The title-page is changed to read ; "An Introduction to Microscopic Methods and Histology." Ithaca, New York, 1899.



PERITHECIA AND DETAILS OF POWDERY MILDEWS.

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CONTENTS.

The Powdery Mildews.

[FRONTISPIECE LOANED BY ASA GRAY BULLETIN.]

Following the plea for the study of micro-fungi in our last number appropriately comes this beautiful plate which was drawn by David Griffiths to illustrate his article in the Bulletin and which editor T. A. Williams has kindly loaned. As very few of our readers see the Bulletin which is now in its seventh volume, (published bimonthly at fifty cents a year at Takoma Park, D. C.) the illustration will have all the value of an original contribution.

Authorities.—In addition to the authorities cited last month, by Pim, Griffiths names: Saccardo's Sylloge Fungorum, the North American Pyrenomycetes by Ellis and Everhart; Parasitic Fungi of Illinois by T. J. Burrill; Uncinula by F. D. Kelsey; papers by R. A. Harper and by F. D. Kelsey.

Technique.—A hand lens is useful in making collections of affected leaves. Having gathered pea leaves affected by mildew, soak in a 5 to 10 per cent solution of caustic potash for 15 minutes and wash. This will enable you to remove the mycelial film from the leaf if you scrape a spot lightly. With scalpel or forceps pull off the film and mount in water, straightening out all folds. Use low power and then higher powers to examine with.

There will be seen the threads of mycelium as in fig. 1; the swellings called haustoria as in figs. 2, 3, 4. It was these by which the mycelium anchored itself to its host and sapped its vitality. From the horizontal mycelium grow the conidia-bearing hyphae as in fig. 5. The outermost ripens and drops off freeing a spore (fig. 6).

Later in the season similar treatment and a mount in dilute alcohol will reveal some perithecia, or little black specks (figs. 7, 9, 10, 11, 11, 13). Different genera have perithecia of different kinds and numbers of cells in which asci are produced.

Contents of the Plate.

Figs. 1 (x 340), mycelium.
Figs. 2, 3, 4, (x 340), haustoria.
Figs. 5 (x 340), 6 (x 480), conidiophore and conidial spore.
Figs. 8, 14, 16, 17 (x 340), asci.
Figs. 7, 10,11 (x 170), 9 (x 340), 12, 13 (x 480) perithecia.
Figs. 15, 18, (x 480), ascospores.

Species Shown.

Erysiphe graminis D C. Figures 1-8 (mycelium, haustoria, conidiophore, conidial spore, perithecium, ascus).

Sphaerotheca castagnei Figs. 9,16 (perithecium, ascus).

Uncinula salicis (DC.) Winter. Figs. 10, 17, 18 (perithecium, ascus, ascospores).

Phyllactinia suffulta (Reb.) Sacc. Fig. 11 (perithecium, ascus, ascospores).

Microsphaera alni (DC.) Winter. Figs. 12, 14, 15 (perithecium, ascus, ascospore).

Podosphaera oxycanthae (DC.) DeB. Fig. 13 (perithecium).

ORGANS EXHIBITED.

PERITHAECIUM.—The six prominent objects (fig. 7, Erysiphe; fig. 9, Sphaerotheca; fig. 10, Uncinula; fig. 11, Phyllactinia; fig. 12, Microsphæra; fig. 13, Podosphaera) all with round bodies having different kinds of appendages are perithecia of different species, and inside of each perithecium are one or more asci (shown in figs. 8, 14, 16, 17). In Sphaerotheca (fig. 9), and Podosphæra (fig. 13) is only one ascus; bnt in Erysiphe (fig. 7) and Microsphaera (fig. 12) there are several asci.

APPENDAGES OF THE PERITHECIA.—Those of Sphaerotheca (fig. 9) and Erysiphe (fig. 7) are similar to mycelium (see fig. 1) but they differ in the number of asci contained within. Those of Uncinula (fig. 10) are coiled at the tips; those of Phyllactinia (fig. 11) are swollen at the base; those of Microsphaera (fig. 12) and those of Podosphaera (fig. 13) are both branched at the tips but they differ in the number of asci borne within. Thus the perithecia furnish means of distinguishing these six genera.

ASCUS.—One in each perithecium, Sphaerotheca (fig. 16) and Podosphaera. Several in each perithecium, Erysiphe (fig. 8), Microsphaera (fig. 14), Uncinula (fig. 17) and Phyllactinia.

ASCOSPORES.—Microsphæra (fig. 15), Uncinula (fig. 18).

Collecting will naturally be easier as the season progresses so that by August or September many kinds of mildews can be seen readily. The collected leaves can

be preserved by usual herbarium methods. Get freshly attacked leaves as well as advanced specimens. Label and preserve careful data regarding time,place, host-plant etc., as in coarser botanical work. The material can be stored up for microscopical study during the winter when fresh objects are scarce or entirely wanting.

Action of Twenty Per Cent Watery Solution of Alcohol on Brain Tissues.

EPHRAIM CUTTER, M. D. & G. B. HARRIMAN, D. D. S.

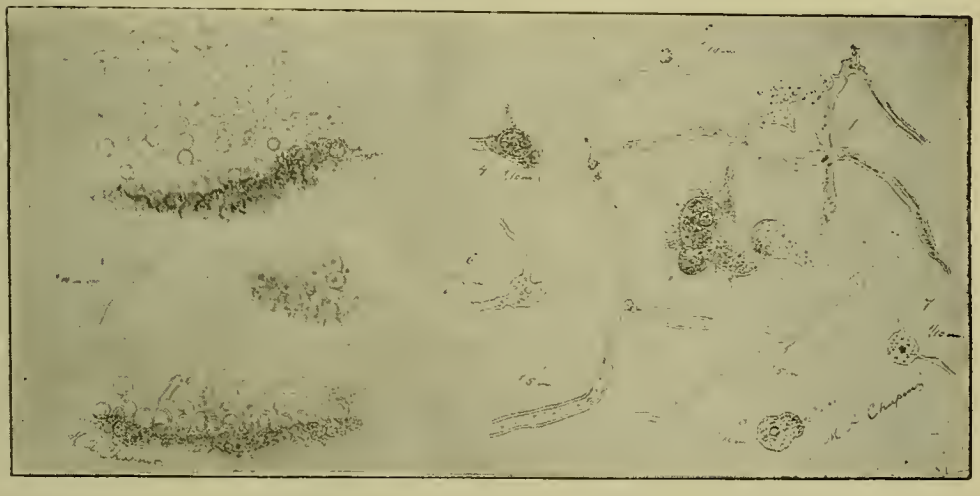
This can only be told fully by vivisection of a brain drunken and by vivisection of a brain in health. Unless criminal laws are altered to permit human vivisection the comparison cannot be made. In this line a wish was expressed that Guiteau's brain might have been utilized and make his crime contribute to the stock of our knowledge. No doubt such opportunities for comparison might be had on battle field or ship but such positions are not convenient places in which to study. Hence we resorted to the expedient of studying fresh brains of calves, cortical portions, with and without contact with 20 per cent alcohol in water. As far as possible allowances were made for post-mortem changes.

The most marked morphological changes were found—to wit, the nerve fibers became contracted and granular with ragged outlines. The mass was mottled with irregularly shaped glass-like spots, surrounded by fields of amorphous granules and especially beautiful dissections of multipolar ganglionic nerve cells. These were very characteristic and impressive as showing where the primal action of the cerebral nerve centers begins and showing the nearest physical agency that we can trace to the bodily home of the soul. We regret that we did not photograph them.

The figure on the right shows the 24-hour dissection of

alcohol in the cortical part of the brain of a sheep just beheaded. One inch objective and $1\frac{1}{2}$ inch ocular were used. At 1 is Plexus of nerve filaments; double outlines; contents clear and granular escaping at one broken end; nuclei at angles of filaments. At 2 nerve filament with bulbous expansions and double outlines. We used on this the one-tenth inch objective and a $1\frac{1}{2}$ inch ocular. At 3 is shown unipolar ganglionic cell dissected out by alcohol. At 4, 5, 6, bipolar nerve cells and at 6 they are discharging contents.

At 7 is a tripolar cell. In a like drawing deposited in the archives of the Victoria Institute, London, there is a



DRAWING BY MRS. MARY A. (CHAPIN) SMITH.

beautiful quadripolar cell. Indeed the whole figure is a better showing than this is. At 8, larger ganglionic nerve cells with nucleus and nucleolus contents granulated by alcohol.

On the left there is represented a sheep's brain as above kept 48 hours in the dilute alcohol, showing the neurine or white substance of Schwann in the act of exuding from the granulated condensed mass of peripheral substance. The neurine appears in the form of loops with double walls that at their finials break up into double walled globes, some with nuclei and some with none, ad-

hereing together but pushing outwards until some appear perfectly free and independent as if they were new cells just nascent into a new life. In the left ecnter is a terminal filament exuded with a finial globe magnified with a 1-10th inch objective and ocular the same. The adjacent figures were made with the 1-5th inch and same ocular.

It was impossible to represent the extreme delicacy and transparency of the exnded substance. We dont attempt to explain this. We simply show what transpired but it was unexpected to note such a kinetic forming power exerted by alcohol.

New York, March 12, 1899.

Preparing, Cutting, Staining, and Mounting Sections of Botanical Tissues.

MARTIN J. COLE.

There is no branch of Practical Microscopy more interesting than the study of the structure of vegetable tissues, a marvellous variety of which can always be readily obtained, and I now propose to show how easily such specimens can be prepared.

HARDENING AND FIXING.—The stems, roots and leaves should be gathered, cut into small pieces about half-an-inch long, and placed in a small bottle of methylated spirit; change the spirit every day until no color comes away from the tissues. They may then be cut into sections or kept for any length of time.

SECTION CUTTING.—Sections of the soft stems and roots may be cut by hand with an ordinary razor. Hold the specimen between the thumb and forefinger of the left hand. Keep the finger straight, so that its upper surface may form a rest for the razor to slide on. Take the razor, hold it firmly in the hand, and keep the handle in a line with the blade, and draw it through the tissues from heel to tip towards yourself. While cutting, keep the razor

well wetted with methylated spirit, and as the sections are cut place them in a saucer of clean water. When specimens are too small to be held by the hand, they may be embedded in carrot. Take a piece of carrot, about an inch long, cut it in half longitudinally, place the tissue between the two halves, and tie together with some twine, then proceed to cut the sections as before. Hand cutting is all very well for rough work, but it is very difficult to get good even sections, and I would suggest that all students should have a good microtome of some kind.

Screw the microtome firmly to a table, and with a large brass tube punch out a cylinder of carrot to fit into the well of a microtome. Cut this in half longitudinally, and with one of the smaller punches scoop out enough space in one half of the carrot to take the specimen ; then place the other half of a carrot in position, and make sure that the specimen is held firmly between them, but of course, it must not be crushed. Now put the cylinder of carrot and specimen into the well of the microtome and cut the sections. While cutting, keep the knife and the surface of the microtome well wetted with methylated spirit, and as the sections are cut place them in a saucer of water.

In order to investigate the structure of a plant stem correctly, it is necessary to have a longitudinal section as well as a transverse. To obtain this, cut off about $\frac{1}{8}$ inch of the stem transversely, and place it horizontally in the groove of the carrot, with its flat ends against the walls of the cavity, then place it in microtome and cut sections. When the specimen has an irregular surface it must be imbedded in paraffin. Take some paraffin wax and melt over a water bath. Place the specimen in the well of the microtome in the desired position, pour in enough paraffin to cover it and allow it to cool, then cut sections as before.

BLEACHING.—Vegetable sections usually require bleaching before they can be properly stained. Chlorinated soda is used for this purpose. Take of dry chloride of

lime, two ounces; of common washing soda, four ounces: and distilled water, two pints. Mix the lime in one pint of water and dissolve the soda in the other. Mix the two solutions together, shake well, and let the mixture stand twenty-four hours. Pour off the clear fluid, filter through paper, and keep in a stoppered bottle in a dark place, or cover the bottle with black paper.

Place sections to be bleached in a bottle of distilled water, and soak them until all trace of spirit is removed, pour off the water and add bleaching fluid, cork up well and let it stand for from one to twelve hours, according to the nature of the specimen. As a rule, the tissue should be quite white, but some parts of sections will never lose all their color, for instance, Rhizome of *Pteris aquinila*; in this case, as soon as the hard black schlerenchyma turns yellow, stop the bleaching. Pour off bleaching fluid, add water, and keep on changing the water until all trace of smell of chlorine is removed, then give a final wash in distilled water, and proceed with the staining, or the sections may be bottled up in methylated spirit until required.

STAINING.—Sections of ovaries and young stems that do not contain much woody tissue, should be stained in hœmatoxylin.

Hoematoxylin	30 grains.
Absolute alcohol	3½ ounces.
Distilled water	3½ "
Glycerine	3 "
Ammonia alum	30 grains
Glacial acetic acid	3 drams.

Dissolve the hœmatoxylin in alcohol, and the alum in the water; then add, to the latter, the acetic acid. Mix the two solutions together, and let the mixture stand for at least a month before use. This stain is rather troublesome to make. It is better to buy a bottle ready made, as most of the leading opticians supply it.

1. Add about 30 drops of the solution of hœmatoxylin to an ounce of distilled water, and stain the section for fifteen to twenty minutes.
2. Wash well in distilled water.
3. Soak for a few minutes in ordinary tap water until the color becomes blue.
4. Place in strong methylated spirit and dehydrate for at least ten minutes.
5. Place in clove oil to clear for about five or ten minutes.
6. Mount in Canada balsam.

Most stems, roots and leaves can be double stained with the following staining solutions :

BORAX CARMINE.

Pure carmine..... 1 dram.
Liq. ammonia..... 2 drams.

Dissolve the ammonia and add twelve ounces of saturated solution of borax in distilled water; filter and keep in a stoppered bottle.

1. Place the section in a little of the above stain in a watch glass for about five minutes.
2. Wash well in methylated spirit.
3. Take of hydrochloric acid, 1 part; and of methylated spirit, 20 parts. Mix together and soak the section until it becomes of a bright scarlet color; if over stained, until the excess of stain is removed.
4. Wash well in methylated spirit.
5. Make up an alcoholic solution of Grubler's acid aniline green in methylated spirit, about two grains to an ounce of spirit, filter and immerse the section for ten to fifteen minutes.
6. Wash well in methylated spirit; if over stained with the green, soak until the excess of color is removed. The different parts of the specimen should be distinctly visible to the naked eye—woody tissues, green ; parenchyma, red.

7. Dehydrate in methylated spirit.
8. Clear in clove oil.
9. Mount in Canada balsam.

MOUNTING IN CANADA BALSAM.—Take three ounces of dried Canada balsam and dissolve in three fluid ounces of best benzole, filter and keep in a stoppered bottle.

Clean a glass slide, take up a little balsam with a glass rod and place a few drops on the centre of the slide, take the section out of the clove oil on a lifter and place it in the balsam on the slide. Clean a cover-glass, and with the aid of a pair of fine forceps, carefully put the edge of the cover into the balsam, ease it down so that no air bubbles may be included in the mount. When the balsam has completely covered the under surface of the cover-glass, press gently on its upper surface with the point of the forceps, this will squeeze out any excess of balsam, and set the section quite flat. Now put away for about twenty-four hours to dry.

When the balsam has dried, take a soft camel's-hair brush with rather long hairs, and with a little methylated chloroform carefully wash away the exuded balsam from around the edge of the cover-glass, drain off the chloroform and allow the slide to dry by exposure to air only. When dry, place the slide in a turntable and run on a ring of some good shellac cement. I have found an enamel used for bicycles answer very well. As sold, it is rather too thin for microscopical work, but this difficulty is easily overcome by allowing it to evaporate for a few days. Having applied a ring of cement, it must be allowed to dry for about twelve hours, then take a piece of soft rag and some turpentine, and carefully wash away any trace of balsam and chloroform, dry with a clean cloth and apply a second coat of enamel.

Notes on Microscopy.

J. H. COOKE.

INJECTION STAINING.—Professor Robertson, M. A., of St. Andrew's University, finds that there are many plants which do not admit of the use of the filter pump for injection-staining or of treatment by the well-known method of Von Hohnel. For such specimens, he suggests an alternative method, which he thinks will be found useful, both to private workers and for classes. A piece of india-rubber tubing, eight or more feet in length, is securely wired to the end of a glass funnel. The apparatus is fixed at a convenient height, and a compressor clip is affixed to the free end of the tubing. A section of the stem to be injected is cut smooth and round, and is securely wired in the lower free end of the tube. A weak aqueous solution of fuschin is poured into the funnel, the compresser clip is removed, and a beaker is placed below to receive the drippings. After a few hours it will be found that much of the solution has passed through the conducting elements, staining them *en route*. The stem is then affixed to a second apparatus and treated in a similar manner with a weak solution of picric acid. The second treatment darkens the stained elements and fixes the tissue. If the specimen is intended for sectioning, it should finally be placed in ninety per cent alcohol for a few days, after which it will be ready for the microtone.

CULTURE CELL.—The following method of making a culture cell whereby the various stages in the propagation of the diatomaceæ may be directly observed under the microscope is both simple and effective. It was first suggested by Dr. P. Miquel, and has since been used by him in his laboratory with considerable success. To a glass slip cement a glass ring out of the side of which a small piece has been cut. Cover this with a cover-glass.

Through the orifice introduce the cultivating media containing a small quantity of a culture of, say, *Nitzschia longissima*, and expose it to the light. In the course of time the interior of the cover-glass will be covered with a beautiful growth of the frustules of the diatoms. This cell must, of course, be stood upright on its side. Cells may also be prepared by making the orifice in the cover-glass.

CLEANING.—To clean cover-glasses and slides, immerse for one hour in a mixture made by adding to a saturated aqueous solution of potassium bichromate about one-eighth of its bulk of strong sulphuric acid. This will also be found useful for cleaning glasses that are intended for use as colored screens.

FIXING.—Herr K. Koninski suggests a new gelatin-formalin method for fixing paraffin sections to the slide. The plate is first covered with a film of gelatin by the usual method, and, when this has set, ribands of sections are arranged on it. The plate is then gently warmed until the gelatin has liquefied. Remove the superfluous gelatin with bibulous paper and allow the preparation to set. To render the gelatin firm and insoluble, the plates are immersed in pure formalin for ten minutes. Thus prepared, the mounts are sufficiently hard to resist the action of boiling water.

CEMENT.—The following will be found to be a useful cement for making live-cells and zoophyte troughs, and for cementing the glass of aquaria. Dissolve gum elastic and thoroughly rub in with it a sufficiency of white lead and linseed oil varnish to form a paste.

FLUID MOUNTS.—The last number of the Journal of the Quekett Microscopical Society contains a resume by F. Rousslet, on "Micro-Cements for Fluid Mounts." The author has obtained his best results by first giving a coat of pure damar in benzole, then a coat of a mixture of damar and gold-size, then three or four thin coats of pure

gold-size at intervals of twenty-four hours, and finally a thin coat of Ward's brown cement.

Oil of Cassin.—The high refractive index of oil of cassin, and the fact that it dries hard enough to make permanent mounts, renders this reagent particularly useful to microscopists. It has an index of 1.6016, and clears from eighty per cent alcohol.

Residue.—To determine the character of a residue, place a small portion on a glass slip and saturate it with a drop of water. Note the general characters of the particles with the aid of a one-inch or a half-inch objective. At the end of the glass slip, place a drop of a ten per cent solution of sulphuric acid, and at the opposite end a small piece of blotting paper. Diffusion will take place, and if any particles of carbonates be present, they will reveal themselves by effervescing. Quartz grains, clay, and carbonaceous matter will remain unaffected.

Colored Screens.—For the purpose either of increasing the contrast between different colored objects in a specimen, or of reducing it, there are useful adjuncts to the equipment of the microscopist and the photo-micrographer. To obtain perfection of definition and of contrast either for visual or micro-photographic work, the use of partial monochromatic light is essential when working on bacteria and similar subjects; and the following plan for making reliable screens may, therefore, be of assistance to those who are engaged on this branch of study. It was first suggested by Mr. Wall to Dr. Spitta, the author of "Photo-micrography." Coat a patent plate with a two and a half per cent solution of albumen; and, when dry, pour over it one hundred and seventy minims of an eight per cent solution of gelatine, level and allow to dry. To make red, orange, green, or violet screens, soak the prepared plates in a one per cent solution of crysoidine, aurantia, napthal green, and methyl violet respectively. For yellow screens, soak the

plate in a solution of twenty grains of picric acid, dissolved to saturation in absolute acohol,two ounces of water and a little ammonia.

MYCETOZOA.—Dr. Caspar O. Miller has developed an elaborate method of studying the mycetozoa by means of which he expects ultimately pure cultures will be obtaine. Bacteria are present in all cultures, and he is, therefore, now giving his attention to the influence that these have on the growth of mycetozoa. The plan of working that he most favors is as follows :—Well wash a handful of hay until the water is colorless, and then leave it to soak for about twenty-four hours. Pour off this water, filter it and dilute with fresh water until it is of a white wine color, after which add two per cent of milk to the infusion. Filter again and sterilize for future use. The hay is then cut up and placed in Erlenmeyer flasks, sufficient to fill them two-thirds full. Just cover the hay with water and sterilize for fifteen minutes, and repeat this process the following day with fresh water. This water is then poured off, and sufficient of the previously prepared infusion of hay and milk is added to cover it to a depth of one centimetre, after which the flasks are sterilized in a steam sterilizer for ten minutes on three successive days. They are then ready for use. The cultures were transplanted by means of a sterilized pipette. The results of the experiments were communicated to the *Journal of the Microscopical Society*, Vol. XLI., and all who are interested in the aseptic cultivation of mycetozoa would do well, therefore, to refer to that journal for the detailed description which it contains.

EMBEDDING.—The following method for embedding small bodies, such as spores and pollen grains for sectioning in large quantities for class purposes, has been successfully adopted in the botanical laboratory of the University of Ohio. The spores are placed in a small test-tube and treated as for paraffin embedding. When the

material is ready the tube is filled with paraffin,and after the spores have sunk to the bottom the whole is quickly cooled. The paraffin soon hardens. The tube is then broken, leaving the paraffin cast, which, with a little trimming, is at once ready for the microtome.—*Knowledge.*

General Characters of the Fungi.

G. MASSEE.

Fungi were differentiated as a group before the evolution of terrestrial (dry land) vegetation, and as a combination of necessity, convenience, and gratitude, lived in the first instance as parasites upon various members of the group of plants from which they were evolved, the seaweeds or algæ. A small section, the conservative party, have retained this habit up to the present day.

All fungi are parasites in the sense of requiring organic food, some being satisfied with dead matter, others attacking living organisms; many are capable of utilizing with equal satisfaction living or dead matter as food.

In primitive aquatic forms the entire fungus is buried in the body of the host on which it is parasitic, the one form of reproductive bodies, which are of sexual origin, and closely resembling those of the ancestral algal type, being liberated on the decay of the host, and dispersed by water.

The evolution of terrestrial vegetation afforded an opportunity for the extension in space of fungi; and the extent to which they availed themselves of the opportunity may be estimated from the fact that at the present day no fewer than 45,000 species of terrestrial fungi are known, whose distribution is equal to that of the higher forms of vegetable life on the presence of which their existence depends, no fungus having deviated from the primitive parasitic mode of life.

Fossil wood from rocks belonging to the carboniferous

period affords ample evidence of the presence of parasitic fungi belonging to the primitive type indicated above. Neither do the members of the animal kingdom altogether escape, unmistakable evidence of the presence of parasitic fungi in the remains of ancient corals having been recorded; and even at the present day various species of insects serve as hosts for highly evolved types of fungi.

In one of the earliest groups of terrestrial fungi, the Peronosporeæ, the evolution of a second form of reproduction was perfected, known as the conidial or summer form of fruit. The ancestral sexual fruit produced in the tissues of the host was retained without modification.

The conidial form of fruit is developed as follows. The members of the Peronosporeæ are parasitic on living plants, attacking more especially the foliage; the vegetative mycelium remains inside the host, and after ramifying and accumulating an amount of reserve material gives origin to immense numbers of specialized branches, which either push through the epidermis or through the stomata into the air, and there produce myriads of conidia asexually formed, very minute reproductive bodies.

The object of producing conidia outside the host was for the purpose of utilizing the available terrestrial means of spore dissemination. In early times wind and rain would be the dispersive agents, and with the advent of groups of insects having a taste for nectar or being attracted by brilliant colors, we find groups of fungi, as some of the Ascomycetes—*Claviceps*, *Sclerotinia*, etc.—and all the Phalloideæ, offering the attractions of nectar, smell, and brilliant colors in various combinations, for the purpose of effecting through the unconscious agency of insects the diffusion of conidia.

The conidial phase of reproduction in most fungi corresponds to what are popularly known as moulds and mildews, and all such were at one time considered as dis-

tinct species, each having its own generic and specific name.

The special function of the conidial form of reproduction is to enable the fungus to extend its geographical range; as already stated, the conidia are exceedingly minute, are produced in immense numbers, and in rapid succession during that period of time when the host-plant is in full foliage and active growth; are readily disseminated by wind, rain, insects, birds, and other agents, and are capable of germination the moment they are mature; consequently those that happen to alight on the foliage of a suitable host-plant enter its tissues, and within a few days form a new centre of disease from which conidia are liberated to continue the extension of the species.

As a rule parasitic fungi are only capable of growing on host-plants that are allied to each other; hence from the above account it can be readily understood how rapidly it is possible for a fungus epidemic to spread, more especially where numerous plants of the same species are growing in close proximity, as is the case with most cultivated crops. All fungus epidemics are due to the rapid spread of the conidial form of the fungus.

It is important that the significance of the conidial form of reproduction should be clearly understood, for although evolved very early after fungi passed from their primordial aquatic home and took possession of the dry land, and in the earliest forms to be considered as subordinate to the older sexual form of reproduction, nevertheless the acquisition of a second mode of reproduction proved to be a step in the right direction, and has continued to become more and more highly evolved, until finally in the highest and newest types of fungal evolution we find the conidial mode of reproduction alone remaining, the sexual form of fruit having been completely arrested.

Now returning to the Peronosporeæ: after the conidial

form of the fungus has run its course, and the infested leaves or other portions are killed by the fungus and have fallen to the ground, the mycelium in their tissues continues to grow, and gives origin to numerous sexually produced reproductive bodies called oospores from their mode of origin; also termed resting-spores because they require to remain in a resting or passive condition for some time before they are capable of germination.

During the winter the leaves containing resting-spores may decay and completely disappear, but the oospores remain unchanged on the soil until the following spring, when they germinate and give origin to minute conidia which are distributed by wind, and those that happen to be deposited on the young leaves of a suitable host-plant germinate at once, enter the tissues, and within a few days give origin to the conidial form of reproduction once more.

The function of resting-spores is to enable the fungus to tide over that period during which the host on which it is parasitic is not in a condition of active vegetation.

Following the Peronosporeæ in the sequence of evolution, we come to the enormous group of fungi, in the broader sense known as the Ascomycetes; and throughout the assemblage conidial, and what may be termed the sexual methods of reproduction are present. However, in the Erysipheæ, one of the oldest sections of the Ascomycetes, the resting-spore phase is still the result of a sexual act, whereas in the Sphæriaceæ and other sections, although the fruit producing resting-spores is morphologically similar to that of the Erysipheæ, it is in reality asexual in origin; traces of the sexual organs are present in various sections, but are functionally effete, and in others have become quite rudimentary.

Finally, in the most modern and highly differentiated group of fungi, the Basidiomycetes, there is no vestige remaining of the originally sexual mode of reproduction,

the continuance of the species depending entirely on the production of asexual conidia, aided by various vegetative methods whose gradual evolution progressed during the period of decadence of the sexual method of reproduction.

The last-mentioned group, including the well-known and universally distributed "toadstools," mushrooms, puffballs, etc., illustrates the highest phase of evolution of the conidial form of reproduction; which, contrasted with the delicate mould-like form first evolved, shows a remarkable amount of differentiation, being always comparatively large, often gigantic, and sometimes woody and perennial.

Among vegetative modes of reproduction two forms are worthy of special mention. Sclerotia are solid aggregations of mycelium of variable size and form, depending on the species to which they belong. In New South Wales, Victoria, and Queensland, certain irregularly globose bodies varying in size from a cricket ball to that of a child's head, dark brown externally, whitish and marbled within, and of a woody hardness, are fairly abundant in the soil in certain districts, and are known locally as "native bread." Berkeley, recognizing the fungus nature of these productions, bestowed on them the name of *Mylitta australis*, but was unable to indicate their affinity owing to the absence of reproductive organs. Quite recently these structures have been proved to be the sclerotia of a fungus called *Polyporus mylittæ*, Cke. and Mass. Many other species of fungi produce sclerotia, some of which are not larger than a pin's head.

Sclerotia are formed from the vegetative mycelium of the fungus, and functionally are of the same value as the various kinds of resting-spores, remaining for a time in an unchanged condition—sometimes for many years—and eventually giving origin to a new plant, either directly, or from mycelium or conidia, which are first formed.

Rhizomorphs differ from sclerotia in being long, cord-like bodies formed also from the mycelium. These structures radiate from the point of origin in all directions in the soil, at a depth of a few inches below the surface. When the tip of a rhizomorph comes in contact with the root of a tree it penetrates the tissues, where a dense mycelium soon forms, feeding upon and eventually killing the tree, after which the above-ground fruit of the fungus is developed, new rhizomorphs meanwhile radiating from the new centre of development in search of other victims. *Armillaria mellea*, a very common British "toadstood," spreads rapidly by means of subterranean rhizomorphs, and proves very destructive to various species of forest trees.—*Q. C.*

On the Structure of the Nodules in Pleurosigma.

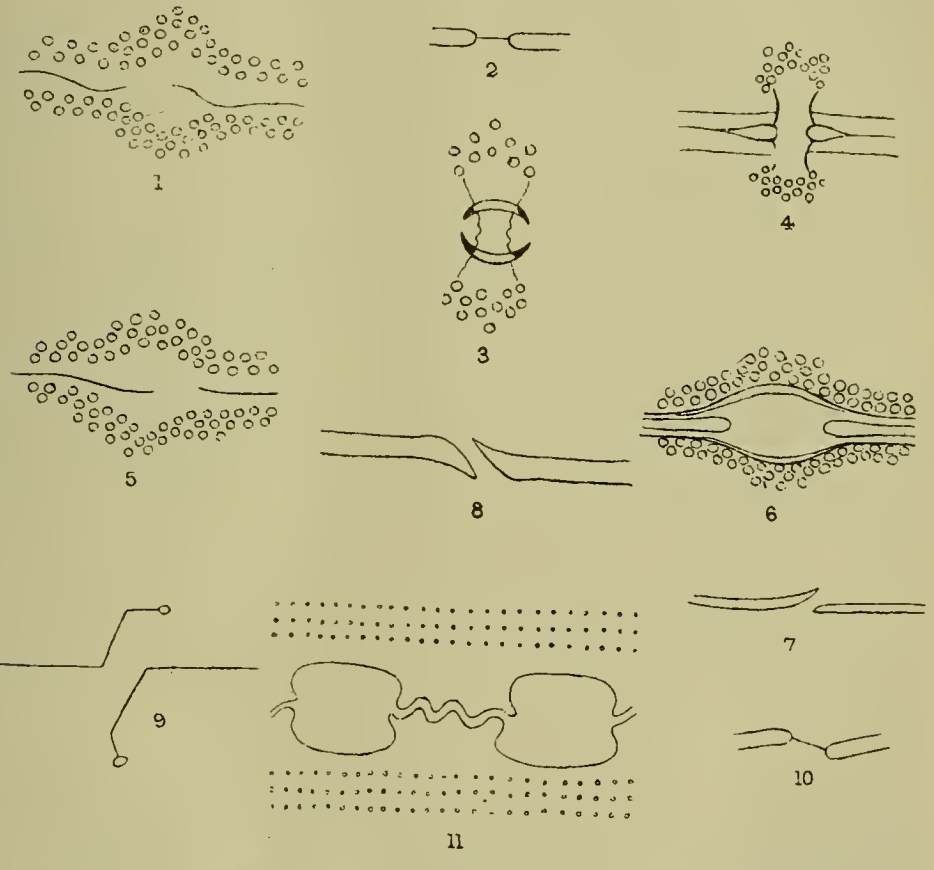
EDWARD M. NELSON.

While examining the Pleurosigmæ in the Nottingham (Maryland,) deposit I was much struck with a marked difference in the structure of the nodules when compared with those of recent forms. Now, as the Nottingham deposit belongs to the Middle Tertiary Period, it cannot fail to be interesting to compare these with other fossil and recent forms.

Fig. 1 shows the raphæ pipes entering the nodule of a Nottingham Pleurosigma, as seen from the outside of the valve. You will notice that both ends of the pipe, after making a slight dip, terminate with their extremities pointing directly at one another. Fig. 2 is a very highly magnified picture of the extremities of the pipes; they are joined by a very fine thread-like structure, which by the way is an excessively difficult image. Passing on now to the nodule itself, we come to fig. 3 or an inside view at the highest focus; fig. 4 is the same at an intermediate focus; focussing down still lower we come to an

inside view of fig. 1, which being similar to it renders another figure unnessary.

Turning now to a P. angulatum, we have in the outside view at fig. 5 the extremeties of the pipes very differently arranged. Here we see the left-hand pipe slightly bent down, while the right hand one is considerably turned to one side. Passing on to an inside view, fig. 6, we also find a totally different structure : the nodule has become oval, with the raphæ pipes running a little way



into it at each extremity. Focussing down, we come to the inside view of fig. 5, there being no intermediate image, as was the case in the fossil form.

In figure 10, the ends of the raphæ pipes of a Nottingham Pleurosigma are shown greatly magnified,and it will be seen that one side exhibits a tendency to turn down, this slight displacement being just perceptible. Care must be exercised always to examine a specimen that is

lying flat, for if it is tilted fallaceous appearances will be present; it is also far better to examine these structures from the outside, because an inside view usually presents more difficulty.

It may be said that this is only a matter of slight variation, such as is to be met with in all the diatomaceæ; but twenty-four varieties of Pleurosigmæ have been examined, and not a single instance of the ends of the pipes pointing to one another has been observed. The following is a list of the twenty-four varieties of Pleurosigmæ:—

P. formosum, decorum, elongatum, strigosum, rhombeum, convexum, quadratum, angulatum (3 varieties), æstuarii, naviculaceum, balticum (3 varieties), strigilis, attenuatum, hippocampus (2 varieties), acuminatum, scalproides, affine, eximum, affine var. fossilis (Richmond).

In addition to these P. fasciola and littorale were examined, but the structure in both these forms is so minute that it could not be traced. P. spectabile has such a deep nodule that the structure on the upper surface could not be seen by an inspection from the inside; a valve mounted the other way up was not at hand.

The ends of the pipes in P. formosum are shown in fig. 8; they are not very easy to make out, for although it is a very large pleurosigma it has nevertheless a rather small nodule. P. decorum is merely a replica of P. formosum. P. rigidum is a very difficult one to observe: it has one end of its raphæ pipe straight, the other comes straight towards it, and then turns very slightly to one side.

In exterior shape the Maryland Pleurosigma and P. affine var. fossilis Richmond are very similar to the P. angulatum, met with in common spread slides, but in P. affine the extremities of the valve are blunter and more like P. rigidum.

With regard to the sigmoid curve of the raphæ, that of P. rigidum is the straightest, P. affine is straighter in

the centre and curves more rapidly at the extremities, while in Pleurosigma affine var. fossilis, the Maryland pleurosigma and Pleurosigma angulatum, the curve of the raphæ has a more regular sweep throughout. In fact, these three diatoms—viz. P. affine var. fossilis, the Maryland pleurosigma and P. angulatum may be considered identically the same as to their exterior shape, the only differences between them being in the nodules and in the coarseness of their perforated structures. P. affine differs from these three in the bluntness of the extremities of the valve, in the sweep of the curve of its raphæ, in the excessive coarseness of its perforated structure, and in the oval shape of the perforations. The termination of its raphae pipes follows P. angulatum, but the shape of the nodule is like P. affine var. fossilis and the Maryland pleurosigma. As diatoms are named neither from the termination of their raphæ pipes, nor from the shape of their nodules, but solely from their exterior form, it is difficult to understand why the P. affine var. fossilis should have been named a variety of P. affine and not of angulatum.

With regard to the perforated quincunx pattern all over the valve, that on the Maryland Pleurosigma is coarser than that on P. angulatum, but in other respects it is very similar. That of P. affine is coarser than that on any other Pleurosigma I am acquainted with, and curiously the perforations are, as just stated, oval. That on P. affine var. fossilis, however, is finer and less oval than that on P. affine. The structure on the P. affine is so coarse that it can be easily resolved by any lens of .45 N. A. aperture, with axial cone illumination, but the same lens will barely show striæ on a P. angulatum with oblique light. An apochromatic 4 mm. for the long tube will show the bent raphæ pipes in P. affine (balsam), P. affine var fossilis (balsam), and in P. angulatum (dense medium), but it will show neither those in P. angulatum

(dry mount), nor in the Navicula firma var. hitchcockii (balsam), to be mentioned presently, although this last is a striking object with an oil immersion $\frac{1}{8}$. With regard to the different forms of nodules they may be divided into four groups. 1st, the Maryland forms (fig. 3 and 4). 2ndly, the angulatum form (fig. 6). 3rdly, the balticum form, which outwardly resembles the angulatum form, but its raphae pipes enter farther into the nodule, and they have distinct knobs where they turn down into the thickness of the silex, also the perforated structure is displaced to the right and left of the nodule. 4th and lastly, we find a nodule intermediate between the angulatum and balticum forms.

In the first group we have the Maryland Pleurosigma, affine, affine var. fossilis, rigidum, rhombeum, convexum, naviculaceum.

In the second P. angulatum, formosum, decorum, elongatum, strigosum, quadratum, aestuarii, acuminatum, littorale, fasciola.

In the third various varieties of P. balticum.

In the fourth P. attenuatum, strigilis, hippocampus, eximum, scalproides.

In hippocampus the ends of the raphæ pipes are equally turned aside, and being strong are very easy to observe; there are also small knobs like balticum.

Among the Naviculaceæ there are in Moller's Type Plate six varieties of N. firma, which have the extremities of their raphæ pipes turning in opposite directions. That of N. firma var. latissima, now known as Nav. tumescens, was admirably figured by Mr. Karop and described by me. The diatom there is called a Pinnularia, but I have since been informed that it is N. tumescens. These pipes come down the raphæ to the nodule, and then they spirally descend, with a right and left hand twist respectively, through the thickness of the silex into a chamber, which opens into the inside of the valve by

the aperture, as heretofore alluded to. The smallest of the six forms of N. firma examined is named var. hitchcockii, and it has the most diverging pipe ends of all: so much is this the case that, instead of finding their way into the central nodule, they pass outside it into a primary areolation on either side of the nodule (fig. 9). Now this diatom, as well as the N. tumescens, are fossils, so it would appear that these Naviculaceæ had put on this peculiar adaptation, while the Nottingham Pleurosigmæ were only thinking about it.

These observed facts naturally give rise to the following questions. It is obvious that the Maryland Pleurosigma is a very old form, so also is the P. affine var. fossilis: as these have straight or nearly straight raphæ pipes, and also nodules of the first group, may not these structures be taken as indications of early types? If this is the case, may we not conclude that the varieties of Pleurosigmae named in the first group are survivals of this old type, and may not those mentioned in the second group be later forms? Thus, for example, may we not consider P. rigidum as the most perfect survival of the oldest type of Pleurosigma yet known, because it has the same form of nodule and straighter raphae pipes than any of the more recent forms?

This is one of the most beautiful and interesting, as well as instructive, of microscopic structures. The principal view of the valve shows an elongated isosceles triangle, having three bands running its whole length. The outer bands are sieve-like structures; the minute holes being closer together in the transverse than in the longitudinal direction.

In common parlance it would be said that the longitudinal striae were finer than the transverse; the transverse striae vary, however, being finer at the wide end of the valve, where they count 53,300 per inch, and coarser at the small end. This agrees with the law of diatom for-

mation in circular forms, viz., "immature at the centre, mature at the periphery"; the growth of circular forms being from the centre outwards. The climacosphenia grows from the base or small end towards the apex or wide end.

The middle band has coarse structure at the rate of 33,500 per inch. It takes a fine 12 mm. apochromatic to dot the outer and finer bands near the apex or large end in a balsam mount, with axial cone illumination. When this diatom is seen sideways it shows the interesting structure alluded to above; this consists of a helical pipe passing through some bulkheads, which divide the diatom into several compartments. As we come to the compartments near the wide end, or older part of the valve, we find that the pipe closes up and the bulkheads become solid silex.

Mr. Rousselet has appropriately suggested that this structure is a useful test for 12 mm. apochromatics, and similar objectives. With such glasses, however, the helical tube appears only like interlocking teeth, it requires more aperture to develop its full beauty.—*Q. Club.*

EDITORIAL.

A Field of Labor.—Attention of microscopists is invited to the unsolved questions regarding algæ. What are the changes that occur between the time when algæ disappear and reappear again? Are not some algæ mere stages of growth of other algæ, if so which? Systematic observation during cultivation of such plants as Porphyridium Oscillaria, Tetraspora, and Schizogonium are recommended. What are the life cycles of the marine algæ, like Laminariæ, which can be cultivated from spores?

Wanted.—Earth containing diatoms from Redondo Beach for a European Subscriber who offers cash, or, in exchange, Hungarian diatomaceous material from St. Peter.

MICROSCOPICAL APPARATUS.

Nosepiece.—The inner screw on Reichert's and Leitz's objectives is that of the Hartnack stand. By taking out the stop, these objectives can be used on any old Hartnack stand without the necessity for an adapter. I do not think this is generally known.—J. B. Nias.

Fixing and Hardening Apparatus.—An ingenious Westerner has cut up glass tubing into 3 centimetre lengths and made little buckets of them by turning out rims while melted. Over this a cloth bottom is made fast by tying and to the other end is attached a suspender for handling it. In the bucket, place small sporanges, flower buds and other vegetable tissues to be hardened. Immerse the bucket in the hardening liquid.

Critical Illumination.—To secure this, place the instrument horizontal. Project the thin edge of a flame along the optical axis of the condenser, so that the image viewed with a one-inch objective shall be sharply defined in the same field of view as that occupied by the objects when under observation. Then substitute for the one-inch a sixteenth, a twenty-fifth, or a fiftieth. With slight readjustment of the achromatic condenser, the field of view will be brilliantly illuminated and the most flagellate organisms defined with great sharpness.

Microtome Knife.—Professor T. D. Briscoe of Marietta College has made an addition to his student microtome to offset two difficulties. The pressure of one's hand on the knife block caused the knife to change the thickness of the slice being cut; and second, in case the block of tissue presents structure harder in one part than another, the knife could not be carried steadily through the whole of a section. The knife block should be pulled in a perfectly horizontal direction, with no chance of any greater or lesser pressure downwards on the way, and by adding a lever with the power-arm five or six times as long as the arm attached to the knife-block a steady motion can be secured.

Light.—The position of the carbon points in an electric

arc lamp may be so regulated by hand that by reference to cross-wires on a glass screen, the source of light may always be kept in the same place. A small area of great intensity is obtainable by setting the carbons in an oblique position.

MICROSCOPICAL MANIPULATION.

Red Stain.—The hæmoglobin granules in the lamprey, frog, hens and guinea pigs may be made visible by use of saturated solution of neutral red in 0.8 per cent sodium chloride.

Preservative.—Alcohol decolorizes sponge specimens but a solution of formalin-glycerine will not. Use three parts pure glycerine to two parts of a 3 per cent formalin.

Examination of Steel.—The structural character can be shown by mounting specimens ¾ inch in diameter on a slip. Grind down the surface on oil stone, then on a Belgian oil-hone, then polish on chamois leather stretched over a block of wood charged with peroxide of tin. Do not use emery, a crocus paper, range or wheels charged with any cutting powders.

BIOLOGICAL NOTES.

The Marine Biological Laboratory At Wood's Holl.—The Twelfth Session of the Marine Biological Laboratory will begin on June 1st, and will continue for four months. This session promises to be the most successful in the history of the Laboratory. The courses of instruction heretofore offered will be maintained by an exceptionally strong staff, and three entirely new courses have been added, including Micro-chemistry. There will be:

A Course of Lectures on Plant Morphology and Physiology.

First Week, July 5–12.—Erwin F. Smith, 'Bacteria'; D. T. MacDougal, 'Physiological Subjects'; Douglas H. Campbell, 'The Evolution of the Sporophyte in the Archegoni-

ates and Flowering Plants.'

Second Week, July 12–19.—Miss Clara E. Cummings, 'Lichens'; L. M. Underwood, 'The Evolution of the Hepaticae'; Rodney H. True, 'Plants and Poisons.'

Third Week, July 19–26.—H. J. Webber, 'Spermatogenesis, Development of Embryo Sac, and Fecundation in Gymnosperms'; C. O. Townsend, 'Physiology of the Plant Cell.'

Fourth Week, July 26–August 2.—J. M. Macfarlane, 'Plant Irritabillity'; G. F. Atkinson, 'Higher Fungi.'

Fifth Week, August 2–9.—J. M. Macfarlane, 'Physiomorphology of a Few Angiospermic Orders'; Henry Kraemer, 'The Unorganized Contentsof Cells of Plants.'

Sixth Week, August 9–16.—D. M. Mottier, 'Cytological Studies on the Pollen and Embryo-sac of Angiosperms'; D. P. Penhallow, 'Paleobotany.'

BACTERIOLOGY.

Systematic Examination of Water Supply.—During the years 1897 and 1898, cultural examinations of the water supplied to the Denver city mains were made with greater or less regularity. Bacteriological examinations were also made of several supplies at their sources, but it was impossible to test any one of these supplies individually, as they were delivered to the consumer, since the water supplied by the Denver Union Water Company constitutes a mixed supply. In the examinations made at the Marston Lake filter plant, in the water both before and after filteration, gas-producing and acid-generating bacteria were frequently found. Among some of the species isolated may be mentioned the bacillus proteus vulgaris and the bacillus coli communis. These bacteriological examinations show clearly that however excellent the style of filters may be for removing the coarser organic and inorganic impurities of a water destined for domestic consumption, yet they are absolutely not qualified to remove those microscopic plants which are the lowest forms of vegetable life and are generically known as bacteria.

DIATOMS.

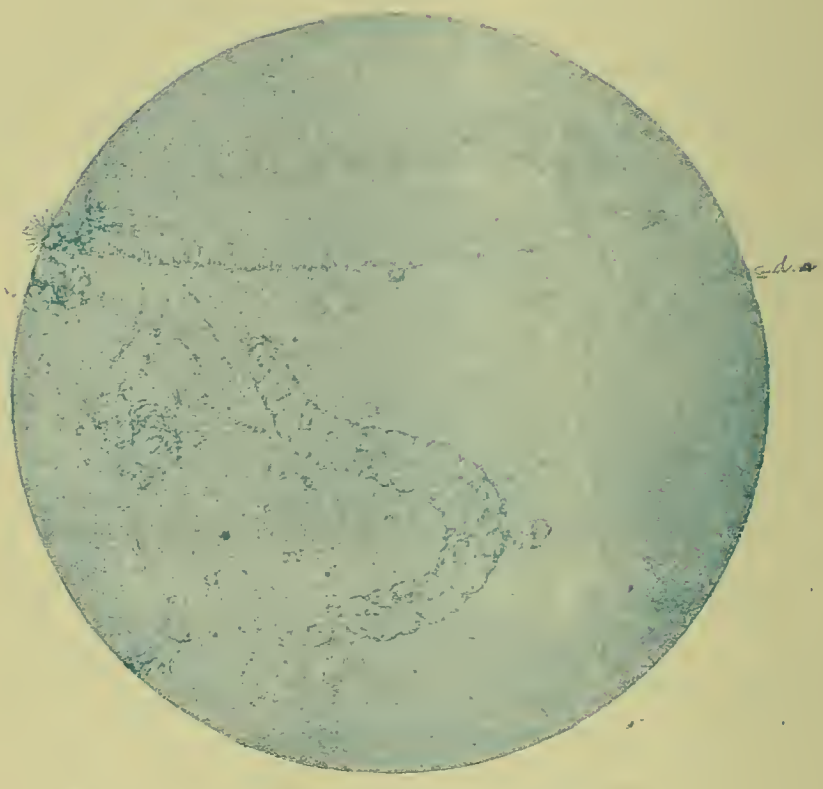
The Marking on Diatoms.—Whipple's work on the Microscopy of Drinking Water, says:

"The valves of most diatoms are marked with lines or points. In many cases the lines may be resolved into series of points, pearls, beads, or striæ, when a higher power of the microscope is used. The variations in the number and size of these points and their uniformity in different individuals of the same species make them convenient objects for testing the resolving power of microscopes. The variation in the number of these striæ may be seen from the following table:

	Number of Striæ per Millimeter.	
	Longitudinal.	Transverse.
Epithemia ocellata, Kz.....	800	430
Navicula major, Kz.....	850	630
Navicula viridis, Kz.....	2400	720
Navicula lyra.....	850	1,000
Cymbella navicula, Ehr.....	1,200	1,500
Pleurosigma angulatum, Sm..	1,580	2,100
Synedra pulchella, Kz.....	680	2,150
Navicula rhomboides.....	1,700	2,700
Amphipleura pellucida, Ktz..	3,400	3,700 to 5,200.

NEW PUBLICATIONS.

The Prang Standard of Color.—This is a book giving in print 1176 different standard color-fields, arranged in monochromatic and polychromatic scales on 7 plates, 10 x 14 inches, under a simple and direct system of nomenclature, permitting the precise designation of any color and indicating at the same time its composition. Supplementary text explains the work and its use. Colorists, Art Teachers, Art Students, Manufacturers, and users of colored materials, Stamp Collectors, Color Printers, etc., should possess this work. Price 50 cents, published by Louis Prang, Boston, Mass.



A Spherical Rotifer.

See page 222.

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CONTENTS.

About Stands and Accessories.

JOHN W. TATHAM, M. D.

We hear a great deal nowadays about a very common type of instrument to which the title "student's microscope" has been accorded, with more liberality than justice, as it seems to me. The instrument of the class to which this name has been given, consists invariably of a small stand, approximating more or less closely to the Continental model, with a short tube, of small diameter, a stage inconveniently near the table, and where a condenser is included, this is generally of the so-called Abbe chromatic form, which is either made to slide in a rigid

fitting or else supported on a modified form of sub-stage.

The ingenuity which has been expended on this type of stand is remarkable; most, though not all, of our English firms having competed with one another to produce the most attractive instrument at a given price. But if the type itself is not everything that can be wished, it would be unfair to blame the opticians, for they have simply catered for a public want. The demand comes mainly from the teachers in the laboratories of our medical schools, who seem almost with one accord to have capitulated to the Continental fashion, and to have advised their students accordingly.

Now it must at once be admitted that the better forms of small stand are very handy to use, and that they allow of the manipulation and examination of specimens in fluid whilst lying horizontally on the stage.

There is no doubt that they serve their purpose well, and it would be hopeless, if indeed it were desirable, to attempt to displace them by instruments of larger build. It should always, however, be insisted upon that instruments of this type must be constructed as simply and as rigidly as possible. They should be fitted with a large, firm stage, preferably of glass or of some other material that will resist the action of corrosive and other fluids; and sufficient space should be given between the pillar and the central opening of the stage to allow of the use of large culture plates, and of ordinary apparatus for dissection. There should always be a substage fitting, perfectly centered to the optic axis of the microscope tube; but it is doubtful whether it is worth the expense to mount the illuminating apparatus for instruments of this kind on a centering and focussing substage. Such instruments as these should be provided in all cases with a double nosepiece. This type of microscope is common in the English market at the present time, and appears to meet with general approval. Several instruments of like

description carefully and substantially made, have been submitted for the inspection of the Club during the year: for example, the "Fram" microscope of Messrs. Watson, the "Scientific-Student" microscope of Messrs. Beck ,and some others. But I have occasionally observed, especially of late years, a tendency to increase both the complexity and the costliness of these instruments, and this to such a degree as to render them unsuitable for the purpose for which they were designed, and which alone they are fitted to serve—namely, that of dissecting, or laboratory working instruments. I have met with stands, not exceeding ten inches in height, and fitted with binocular bodies, triple or even quadruple nosepieces, elaborate mechanical stages, swinging so-called "turn-out" substages intended to carry an achromatic or other condenser, paraboloid, spot lens, polariscope, and a host of other apparatus such as would be suitable only for a full-sized, complete stand of the English model. I confess that when I see small stands of this class encumbered with milled heads and other projections, both above and below the stage, and overweighted with complicated and costly accessories, I am at a loss to understand how they can be used to any good effect by the average student. It cannot, therefore, be too strongly asserted that instruments of the kind I have now been considering are, in their nature, working or mounting instruments only, and that any attempt to raise them to a higher position, by the addition of elaborate mechanical appliances, can only result in failure and disappointment.

But there is another type of instrument, seldom met with now, but which in my earley days used to be called the Student's microscope, and, in my opinion, much more appropriately. The stands referred to were of full English size, and of first-class material and workmanship. They were supplied by their makers in a plain, elementary form ; being fitted, in the first instance, with noth-

ing more than coarse and fine movements to the body, simple square stage and sliding bar; but they possessed the very obvious advantage that they were capable of being built up into instruments of great completeness, according to the wish or means of the purchaser, and having been originally designed accordingly, they were not overbalanced by subsequent additions. Thus a mechanical stage, with universal movements, a rack-and-pinion focussing substage, with centering and rotating adjustments, could be adapted, binocular bodies could be added if desired, and all other apparatus required by the most accomplished microscopist. I believe that I am not awarding praise unfairly when I say that the firm of Ross & Co. were the first to introduce this excellent plan, which was subsequently adopted for a brief season by most of the other London opticians. I purposely bring into prominence what is now—unfortunately, as I think—a matter of ancient history, because I am confident that the present rage for a so-called complete microscope of the small Continental pattern will eventually pass away. I believe, further, that at no distant date the instrument for the study, as distinguished from the laboratory, will be a solidly built English stand of full dimensions, mounted on a heavy tripod base, and differing little in general form from that type which many of us in this Club know so well how to appreciate. To meet the present fashion of constructing objectives without correcting collar, it is necessary that such an ideal instrument as I am projecting should be fitted with a draw-tube capable of considerable extension by rack and pinion. The substage also should, I think, receive more attention than is now generally devoted to this important part of the stand. It should be much more substantially constructed, and its movements should be more delicate and steady than is the case at present with any but the most costly instruments. It must be remembered that the substage of a full-sized

stand has to support and focus, without tremor or oscillation, a considerable weight of apparatus. My own substage, for example, fitted with an achromatic condenser, which by the way, is always in position, weighs not less than 1 lb. 6 oz. Unless, therefore, the mechanism which sustains and moves this load be substantially and accurately constructed, it is obvious that the strain will inevitably disarrange the necessarily delicate adjustments of the instrument.

In my opinion, which has not been formed without consultation with experienced workers in this metal, it is in the construction of the substage and the apparatus supported thereby, as well as in that of the double or triple nosepiece,that aluminium,as a much lighter substitute for brass, seems to promise to be useful. That substages, and indeed complete microscopes of great perfection, can be made of this metal, or rather of one of its alloys, has been abundantly shown by Messrs. Swift at one of our recent meetings; and although opinions may differ as to the advisability of constructing any but a portable stand, like the one shown bv Mr. Swift, exclusively of aluminium, it will, I think, hardly be contended that anything but advantage could result from the use of this metal in the manufacture of the substage and the apparatus which it supports.

Turning now to the optical portion of the microscope, there is no doubt that within the last few years most important advances have been made towards perfection. Even during the last twelve months, or at any rate within the last two years, more than one optician has added a contribution to the resources of the microscopist. By the use of several different varieties of Jena glass, opticians are daily vieing with one another in the perfecting of apochromatic objectives without the use of fluorite in their construction. Only recently a new glass of 1-10th inch focal length and of 1.30 N.A. has been constructed

by Leitz, of Wetzlar. Several of these glasses, constructed for the long as well as for the short tube, have found their way into this country, and from the specimens I have seen I am bound to speak highly of their performance. When used with a malachite green screen, the best of these glasses gives an image of remarkable brightness, capable of standing fairly high eyepiecing, without appreciable loss of definition. This glass is sold at a very moderate price: in fact, it is, as far as I know, the cheapest glass of the kind hitherto produced. Another objective, which, in its present state of perfection, is new at any rate to me, deserves special mention here. It is a 1-12th of hard glass, specially constructed for use in hot climates, by Reichert, of Vienna. It has an aperture rather greater than that of the 1-10th first spoken of—perhaps of about 1.35. This objective also is one of exceptional merit; it is a very strong resolver, and produces an image of great purity and brilliancy, which bears eyepiece amplification remarkably well. It has, however, in my judgment, the important defect that it is adjusted only for a very short tube. If this objective could be corrected for the ten-inch tube, and made to work as well on this as it does on the short tube, I am sure that it would be found to compare favorably with the majority of fluorite apochromatics, although the latter cannot be purchased for less than double the price which is at present charged for this objective.—*From Presidential Address Before the London Quekett Club.*

Notes on Microscopy.

JOHN H. COOKE.

AGAINST RUST.--In biological laboratories, where the microtome is in constant use, it is of importance that the edges of the microtome knives should be kept in as perfect a condition as possible. To prevent dulling of the edge, which results from oxidation, the knives should be kept immersed in a one per cent solution of carbonate of sodium. This treatment will not only prevent their rusting, but will also render them perfectly asceptic.

YEASTS.--Dr. Buchner's recent discovery that the alcoholic fermentation set up by the yeast plant, is due to the chemical power. of an amyloyte acting in a manner similar to digestive ferment, caused a sensation among those who had hitherto held that the action of yeast could not be dissociated from the living plant. To demonstrate the action of this ferment, the yeast cells should be thoroughly disrupted by grinding them up with quartz sand, and, subsequently, be submitted to a pressure of about five hundred atmospheres. Some few cells may escape destruction in this process, and these should be searched for with the microscope, and either disrupted or removed. The resultant powdered mass will act similarly to untreated yeast, thus showing that the fermentative action hitherto attributed to the living cell is, in reality, due to a fermentative enzyme.

The nucleus of the malarial parasite has been demonstrated by Ziemann with the aid of a new stain, consisting of a mixture of a 1 per ct. watery solution of rectified methylene-blue and a 1 per cent watery solution of eosin.

OBJECTIVE.—At a recent meeting of the Royal Microscopical Society of London, Mr. E. M. Nelson exhibited a new objective by Zeiss, called a "plankton-searcher," a low-power water immersion objective, designed for use in examining living objects in water.

FIBRES.—Vegetable fibres may be distinguished from animal fibres by treatment with iodine and dilute sulphuric acid. The former take a characteristic color,either yellow or blue, while the latter do not. The re agents should be prepared as follows :—Dissolve one part of potassium iodide in one hundred parts of distilled water, and add an excess of pure iodine, so that the solution shall always remain saturated. Mix one part of distilled water with three parts of sulphuric acid, and, when cool, add two parts of Price's glycerine. Both re-agents should be kept in glass-stoppered bottles, and as they are liable to change they should be occasionally tested on known fibres. These re-agents, applied under the microscope, afford a means of determining the species from which the fibre is derived. To do this, some cells should be separated, extended on a glass slip,and slightly moistened with glycerine. The length and breadth may then be determined with a micrometer, and note should be made, at this stage, of the shape and degree of taper of the cells. Allow a small drop of the iodine to flow under the cover, removing any surplus with blotting paper. As soon as the iodine has penetrated, apply the sulphuric acid in the same way, carefully watch the results, and compare them with the action of the re-agents on known fibres.

SECTION-LIFTER.—A cheap and effective section-lifter may be made by hammering out flat the end of a copper wire of one-eighth inch in diameter and four inches long. The hammering should be done on a smooth iron, and when it is of the requisite width it may be trimmed with a pair of scissors and smoothed on a whetstone.

ROCK POWDER.—Miss Catherine Raisin writes: "On certain structures formed in the drying of a fluid with particles in suspension," in which she described the many interesting forms that the powders of various rocks exhibit when mounted in water and dried. These forms are

classified. Some suggestions are made as to the possible explanation they afford of the origin of many of the structural characters of rocks in nature.

VARNISH.—Excellent finishing varnishes may be made by well mixing on glass artists' oil paint with gold size. Only sufficient for immediate needs should be mixed at one time. Sealing wax, dissolved in alcohol, is also useful, but only the best wax should be used, otherwise the varnish is liable to chip and leave the glass.

WASHING.—Dr. G. H. Bryan suggests the following device for the more effective preparation of desmids, diatoms, and other minute mounting material. The removal of all traces of acid necessitates frequent washings and decantations of the residues. It is, therefore, important that the vessel in which these operations are performed should hang vertically, otherwise much of the residue rests against the sides of the vessel and gets carried away during the decantation. Dr. Bryan gets over this difficulty by suspending the test-tube by a U-shaped piece of wire across the base of which an india-rubber ring is fixed. The test-tube is placed in the ring, the elasticity of which allows the tube to hang perfectly freely. The precipitation of the diatoms is hastened if the tube be set swinging slightly. The device will also be found useful for washing and collecting the insoluble residues of limestones and other rocks.

CROSS-SECTIONS.—Cross-sections of fibres may be obtained by glueing together a mass of the fibre, bedding in paraffin or by rolling them like a cigarette in a piece of sheet wax. When cut, the mass of mixed sections is placed in benzole or alcohol, when the wax soon floats on top and may be poured off.

MOUNTING.—The following method of mounting in balsam on the cover, and backing up with black varnish, will be found useful for such objects as foraminifera, minerals, seeds, etc. Cement a thin glass-cover to a slip by

applying a little balsam to the edge of the cover, and accurately centre it on the turntable. Place and arrange the objects on a thin coating of balsam in the centre of the cover. When dry and set, the objects may be completely covered with balsam and put into the dry oven until hardened. If the objects are white a layer of Brunswick black is now laid all over the balsam ; if the objects are black white zinc cement should be used. In either case care should be taken to lay them on in thin coats, and allow to dry in the open air before the next layer is applied. Now remove the glass-cover from the slip by slightly heating it. It may now be turned over and mounted on the cell designed for it. Gelatine dissolved in water, with enough alcohol added to liquefy it from the jelly state, is a good preparation for fastening the cover to the cell.—*Knowledge.*

The Celloidin Process of Section-Cutting.

H. WALKER.

There is some diversity of opinion as to the relative merits of celloidin and paraffin for imbedding purposes. The latter is, undoubtedly, the most universally used, and, in the hands of an expert manipulator, produces very brilliant results. I have usually found that, when one begins with paraffin, it is rarely discarded for any other substitute, and the same applies pretty much to the use of celloidin. Still, there are exceptions, and the student must be guided pretty much by individual experience. Thus, I have found that when there is a chitinous integument of more than average hardness, as in some bees, it is very apt to break away from the surrounding celloidin, and the entire section will often drop out. It has not sufficient hold upon the polished surface, and, under the impact of the knife, pulls away. For such preparations, I recommend paraffin. Again, though very penetrative in

a thin condition, it is not so in its more viscid form, and it is necessary very frequently to cut the material small, and open up closed cavities wherever possible. Still, for insect preparations, cytological, and embryological work generally, it is by many good workers preferred to paraffin, provided time is no object, as the process of infiltration is much slower.

Celloidin is prepared from pyroxylin, and is usually sold in the form of hard, horny, transparent shavings or chips, but when obtained from Grubler's Laboratory is in flat, milky-white, semi-translucent cakes, of the consistency of cheese. In this condition it smells strongly of ether, and, on exposure to the air, shrinks and dries to a horny consistency. It is perfectly soluble in ether or absolute alcohol, and in some essential oils, but not in the slightest degree in rectified spirits. The objects to be cut are prepared by infiltration, and for this purpose the celloidin must be dissolved in equal parts of absolute alcohol and ether, forming, practically speaking, a collodion. It should be of at least two consistencies—the one quite thin and watery, the other thick and syrupy. Nothing having a vestige of aqueous matter must come into contact with the solution.

Method of Preparation of the Object.—Cut the fresh tissue into pieces corresponding with the required size of the sections, and after fixation thoroughly dehydrate in absolute alcohol. This must be as carefully done as if paraffin were to be used. The thorough expulsion of all aqueous matter must be complete. The object may then require passing through a bath of equal parts of ether and absolute alcohol,if of a particularly obstinate nature; but this will not be necessary with the average run of material. And I may as well say here that the material may be stained previously or not : but it is much preferable to do the staining after cutting. The reason is not far to seek. It is then easy to stain the object success-

fully, and to observe the selective action of the reagent. If stained previously, picro-carmine or hæmatoxylin are to be preferred. I shall have more to say about this part of the process later.

Infiltration.—The objects may now be immersed direct from the alcohol to the celloidin solution, using the thinner of the two, in which they may remain any length of time ; the longer the better.

Specimen tubes 3in. by 1in. are useful, and need not be kept tightly corked, for the gradual evaporation of the solvents will thicken the celliodin, and bring it nearer the desired consistency. See that all air-spaces in the specimen are opened, and, as the solution diminishes by evaporation, add a little of the thickest grade until the whole is of the consistency of treacle. As to the time that the specimen should remain in the celloïdin bath, everything depends upon its nature. Small flies, insect-heads (for preparation of the eyes), and spiders, from a week to a month ; muscle, skin, liver, kidney, lung, embryo of chick, amphioxus, marine worms, three to five days ; very small or easily-penetrated objects, such as marine sponges, twenty-four hours.

Congealing the Mass.—While infiltration has been progressing, prepare a number of discs of close, hard-grained wood, from ½in. to ¾in. diameter, and ½in. thick. Soak these thoroughly in the celloidin solution—in fact, treating them exactly as described in the foregoing paragraph, and then allowing them to dry in the air. Take a slip of ordinary thin writing-paper and make a thimble, having a wood disc for a closed end, and gumming the overlap with a narrow strip of gum arabic solution, but do not gum it to the block in any way, so that it can afterwards be rolled off without difficulty. When the gum is dry, dip the thimble in thin celloidin, and pour enough thick solution in to cover the bottom for the depth of ⅛in. Half-fill one of the specimen tubes with rectified spirits, or

better still, 70 per cent alcohol, and, with a pair of tweezers, gently lower the paper thimble, or mould—for such it is—into the alcohol. Leave it until the celloidin has turned milky white, and, in point of fact, returned to the original consistency of the cake celloidin. The object of doing this is to prevent the specimen when put into the mould from touching the wood, and so enabling the operator to cut sections down to the uttermost layer, which would obviously be impossible if it rested on the bottom. If the wood had not been previously soaked in solution, multitudes of bubbles would rise when submitted to the action of the spirit, and become imprisoned in the congealed mass, rendering it spongy and yielding to the knife. Now remove the mould from the spirits, dry off the alcohol, put a drop or two of thin celloidin on the floor of the cylinder, lay the specimen carefully thereon, seeing that it stands isolated, and pour enough thick solution over to well cover it. At this stage, provided the whole is of a good stiff consistency, I have transferred it at once to rectified spirits; but in every case the precaution must be taken of exposing it beforehand to the air for a short time—half an hour is not too long, so as to allow a skin to form, which will prevent the alcohol acting too rapidly and breaking up the mass. The celloidin becomes more and more opaque, finally, in the course of an hour, turning white and waxy, when the paper may be easily peeled off, and the whole, with the wooden disc attached, and which must on no account be detached, can then be kept indefinitely in spirits until required for cutting. Bolles Lee recommends that the imbedded object be kept for some days under a tight-fitting bell-jar, allowing fresh air to have accsss daily, before transferring to the alcohol bath, but I have never found it necessary. Other methods have been recommended for hardening the mass, upon the successful issue of which the after cutting largely depends—chloroform, for example, which not only

hardens but makes the mass quite transparent; and some workers go to the extent of freezing the congealed mass during cutting. I have tried all methods, and recommend the student to do the same, selecting the process he finds in his hands the most successful. If any difficulty be experienced in obtaining colloidin, common collodion will give the same results, and the method of preparation does not differ from that given above. The two materials are almost identical, but the former is more convenient for use. Let me caution the student against the adoption of methylated spirits. As ordinarily sold, it contains mineral naphtha, which renders it quite unfit for histological work. Rectified spirits is the safest, or alcohol of 70 per cent. If the imbedded objects are not required for immediate use, the paper can be left on and particulars written thereon with a lead pencil.

Cutting.—Very good work can be obtained with the Cathcart microtome and a good razor. It is not within the province of this paper to deal with the machine employed—it is taken for granted that the student has had experience in this direction. But a microtome must be used that will permit the flooding of the mass with alcohol, or enable it to be cut under the fluid. The instrument mentioned is cheap, costing some $4 without the ether spray, and I have cut many thousands of sections with it. The celloidin block with its imbedded object must be clamped in the vice (which is where the wooden base comes in handy), and trimmed to a square form, but not approaching too close to the specimen itself. A truncated pyramid offers the greatest resistance to the knife, for celloidin is elastic, and is liable to give under the impact of the cutter. It is to overcome this that the mass is sometimes frozen. It is an absolute essential that both the knife and the mass be kept well wetted with alcohol, and the sections as cut must be floated into saucer of the same. The squared block should be set in the

microtome so that it will encounter the knife cornerwise, and the cutter must be used with a combined forward and transverse movement. If it is desired to mount the sections serially, they must be laid with a spatula on a glass slip kept wetted with alcohol in rotation as cut, and touching each other, which brings us at once to the making of serial sections.

Serial Sections.—Celloidin is particularly suitable for this department of microscopical work, and the method is surprisingly simple and effective. Transfer the sections as cut to a shallow, flat porcelain tray, the bottom of which is just covered with 7oz of alcohol, or they may be laid on blotting-paper similarly wetted. Take every care that they do not float about and mix,and when some forty to fifty have been cut, arrange in a long ribbon on a 3 by 1 slip wetted with alcohol, and almost the full length of the slip. Let the squares of celloidin just touch each other, lifting into position with a fine copper or aluminium spatula. With a glass dipper allow a drop or two of absolute alcohol, to which has been added the slightest trace of water to trickle over the sections; and after draining it off, with a second dipper drop a little very thin collodion or celloidin solution over them, spreading it thinly with the glass rod. This is quite simple, and the sections are not easily displaced.

When the entire ribbon has been thus covered, and the solution has been skinned, transfer the whole to the 70 per cent alcohol bath, when in a minute or two the long ribbon can be lifted with a scalpel, trimmed, and peeled off. Proceed in the same way with the others until a consecutive series of ribbons is obtained. These may be cut in two, and arranged in the form of a rectangle, one row below the other, measuring $1\frac{1}{2}$ by $3\frac{1}{4}$ if intended for ordinary slips, or 2 by $1\frac{1}{4}$ if for the wider glass. With the dipper, cement the strips together in one compact film, exactly as described for the ribbons, transfer to

the alcohol bath, carefully strip off the film, and trim it round with the scalpel. The film must be removed from the glass, or the air imprisoned beneath will prevent its satisfactory mounting. In any case, it must be detached for staining.

Staining.—The stain *par excellence* is Delafeld's hæmatoxylin, and as it is well-nigh impossible to purchase this valuable reagent properly prepared, I append the classical formula :—Make a saturated solution of ammonia alum in distilled water, and to 100 cubic centimetres add 1gr. of the finest hæmatoxylin crystals dissolved in 5c.c. of absolute alcohol. Place this mixture in an uncorked bottle and expose to daylight for a week, then add 25 cc. of pure glycerine and a similar quantity of methylic alcohol. Shake thoroughly together, and expose to light until the solution has deepened to an intense claret color, keeping well corked ; filter ; in a month, it is ready for use. It decomposes in time, but may be refiltered.

The action is rather peculiar, insomuch as the film itself does not very readily take the stain, which is more than I can say for any other. Picro-carmine hopelessly dyes it, the anilines do the same, and if any such are required to be used, the object must be treated before imbedding. Use a weak solution and stain slowly—the action is far more selective, and can be checked at any stage. Slightly overstain the preparations. The proper strength is about five or six drops of the above solution to the watch-glass of water. Wash the sections and transfer to a good bulk of the stain,and turn the film occasionally. Ten to twenty minutes is usually ample ; then wash in water, and transfer to a saucer of weak acetic acid. This decolorizes the film completely, but must not be allowed to act too long, or the color of the sections will suffer. The celloidin often retains a slight violet tint, in itself not at all detrimental.

Clearing and Mounting.—Unlike paraffin, celloidin

does not require dissolving out. It possesses the rare quality of becomming as clear as glass, and quite invisible in the finished slide. First dehydrate on a slip by running slightly-diluted absolute alcohol over the section, and drawing off at one corner of the slide. It is preferable, and, in case of serial sections, absolutely necessary, to lay a thin cover-glass over the preparation, and allow the alcohol to run between by capillary attraction. The dehydration need not be very thoroughly carried out if the cleaning is done with carbolic acid, which I strongly recommend. The white crystallized acid must be used, and, if manipulated in the following manner,no disagreeable effects will result. Never allow it to touch the skin —it burns badly ; and, above all, do not leave it about, but keep it under lock and key. Take a common flat spirit bottle, nearly fill it with hot water, and, after corking tightly, lay it on its side.

On this place the slide, with the covered section, and plenty of the strong alcohol between the glasses. Strew a few crystals of carbolic on, say, the left edge of the cover ; they will instantly melt, and by the application of a bit of blotting-paper to the opposite edge, the oily liquid may be drawn under. The milky-white celloidin rapidly becomes beautifully transparent, and when all trace of cloudiness has disappeared, the cover may be removed, and the now stiffened section transferred to a covered pot of xylol. Use the xylol freely to get rid of the carbolic, for I have known it to alter the color of the staiu to a warm brown if not removed. If the clearing is carried out without using a cover-glass, the section wraps and twists badly. It occasionally happens that the sections stick to the slip or cover. This risk is minimized by keeping the film moving slightly, but with ordinary care there is not much risk of breakage from this cause. Various oils may be used for clearing ; oil of cedar-wood, for example. This is very slow in its action,

but simple to use. After dehydration, pour enough over the cover, or lay the whole slide in a bath of oil. It will creep between the glasses in a night usually. Cajeput oil may also be used, but clove oil dissolves the celloidin. The sections may be kept in a tightly-closed tube of Canada balsam dissolved rather thinly in xylol, and afterwards mounted in the usual way in a thicker grade of the same medium.—*English Mechanic.*

A Method of Making Type Slides for Opaque Objects with Removable Cover.

D. BRYCE SCOTT.

The first step is to prepare the photographed bottom on which the objects are fixed. This can, of course, be prepared to any size or pattern, according to the requirements of the mounter. For my own purposes I prepare a slide having its surface divided into 300 spaces of varying sizes, which is constructed thus :—

1. Take a piece of black cardboard 32¼ inches long and 11¼ inches wide. Scale off 20⅝ inches into 25 divisions lengthways, then scale off 6¼ inches downwards into 12 divisions, making the first division downwards the same breadth as the 25 divisions along the top, which will thus become squares. Gradually reduce the size of the remaining divisions downwards, until those in the last line are only ⅓ the breadth of those in the top line. Then number the spaces in the corner from 1 to 300. All the lines and figures are to be made with china white dissolved in water, with sufficient gum added to make the white adhere when dry. Reduce to ⅝ in. wide and 2 1-16 inches long. The printed photos are next pasted upon ordinary portrait photo cards, and burnished. They are then varnished by pouring a thin filtered solution of shellac over them, and allowing it to dry. Unless this is

done the slightest scratch will show on the photograph, which is very delicate, and will not stand much rough usage. The shellac preparation used by photographers to pour over tin types will be found best for the purpose, as it dries very quickly.

Cut the cards on which the photos are mounted to the standard size, 3 inches by 1 inch, taking great care to ensure that the background is exactly centred on the card.

2. Take a slip of dry and well-seasoned bay wood or mahogany, 3 inches long, 1 inch wide, and 1-16 inch thick (thinner or thicker if required). Glue over the strip on each side a piece of manila paper of good quality, using liquid glue, and dry under a flat weight.

When dry cut out the centre of the wooden slip, leaving a strip $\frac{1}{8}$ inch wide on each side, and 5-16 inch at each end. Then smooth down all the roughnesses with fine glass-paper, and blacken all over with lamp-black in alcohol. Add sufficient shellac to the alcohol to make the lamp-black adhere to the cell, but not enough to make a gloss.

3. Glue the photographed bottom to the wooden cell with liquid glue, and dry under a flat weight.

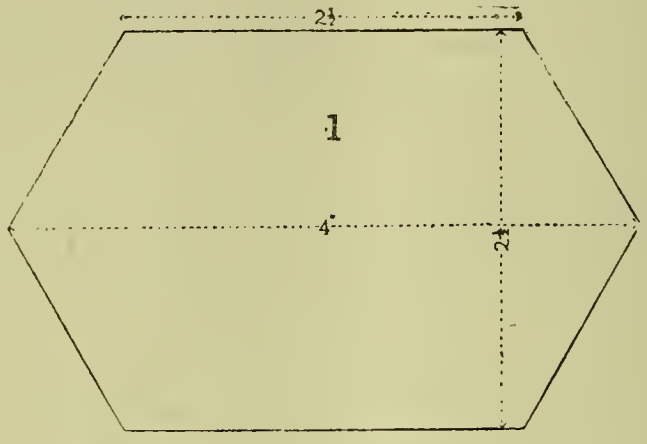
4. Place a thin glass slip, 3 inches by 1, of best quality, with ground edges, on top of the cell.

5. Take a slip of manilla paper, about 3 inches long by $2\frac{1}{4}$ inches wide, moisten with a sponge, wrap it round the cell and glass slip, and glue the edges on the back of the photographed bottom. Be very careful that no glue touches the glass-cover. All the manilla paper used in the preparation of the slide must be dampened but not soaked; the damp paper stretches and works better.

6. Take a strip of manilla paper, 1 inch by about $6\frac{1}{4}$ inches long, damp and glue it, and paste over the last strip. This strip will go right round the long diameter of the slide. No glue must be allowed to touch the glass cover where the paper goes over it at the ends.

7. Cut a piece of manilla paper to the size and shape of sketch No. 1. Damp and glue it, and wrap around the slide, folding the corners over neatly.

None of the edges of these three thick papers (paragraphs 5, 6, 7) must overlap, or the surfaces of the slied



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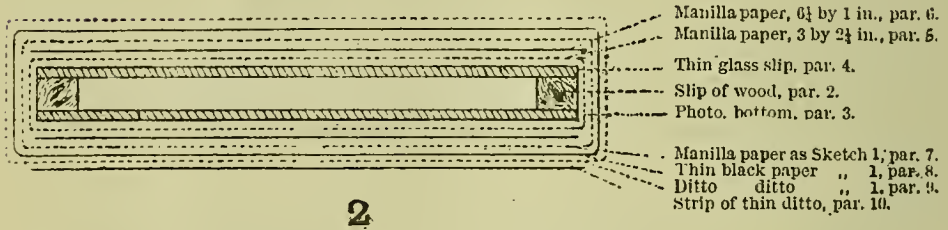

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will be uneven when finished. Trim the papers to the exact size when the liquid glue is on the paper.

8. Glue on a strip of thin black paper (black tissue) cut to the size and shape of sketch No. 1. Thin the glue with a little water, just thin enough to prevent the paper tearing when it is brushed on.

9. Glue on a similar piece of black paper, cut as in sketch No. 1, making them join on the opposite side of the built-up cell.

10. Cut a piece of thin black paper, 3 inches by 1, and glue over the last-made joint. Press the finished slide between two flat surfaces, such as a table and a piece of smooth hard wood, to squeeze out all the air cells in the moist paper. Put it away to dry for two or three days.

11. When the cell is dry, cut out the centre of the

surface on the side having the glass-cover (see paragraph 12), 2 and 3-16 inches long by $\frac{3}{4}$ inch wide, leaving about $\frac{1}{8}$ inch on each side and 5-16 inch on each end. Then cut off one end 1-16 of an inch on top and 1-16 inch on the end of the cell. This will allow the glass-cover to be pushed out through the slot thus made at one end of the slide. If there is no glue on the glass it will come out quite easily when you press it with your thumb and slide it towards the slot; or it can be pushed out from the opposite end with a blunt needle pierced through the paper. Do not use a sharp needle, or you will crack the slide. Before cutting the slot in the end of the slide, measure both ends accurately and cut the widest end, as the glass slips are frequently a little wider at one end than at the other. Grind the corners of the end to be pushed in on an oilstone: this will prevent the usually sharp corners of the glass from tearing the paper when it is pushed into place.

12. When building up the slide keep sketch No. 2 in front of you and work in the same way. By this means you will always know which is the top or glass-cover side, as the bottom is the side on which the last strip, 1 by 3, is pasted (paragraph 10).

13. Take the glass slip out, and replace it with a strip of mica or thin tin. Blacken the edges where cut, and put aside for an hour for the black to dry. Then replace the glass slip, and put aside in a warm place (not hot) for five or six days, to thoroughly dry and season. If allowed to dry without the glass slip being in place, it will be sure to warp.

A Spherical Rotifer.

WITH FRONTISPIECE.

A remarkable rotifer, Trochosphæra solstitialis, found in the Yangtsze Kiang river at Wuhu in August, 1892,by Surgeon Gunson Thorpe ; in the Illinois river in August, 1896, by Dr. C. A. Kofoid; was taken in a pond near Lake Erie in 1898. The anatomy of the animal is extremely simple and beautifully displayed, all the organs, usually so indistinct and closely packed together in rotifers, being here spread out and suspended in the transparent sphere in the most delightful manner. The ciliary wreath encircles the sphere above the middle, leaving the usual dorsal gap, and dividing it into two unequal segments, the larger oral segment containing all the organs,and the smaller aboral segment having nothing at all. Close below the wreath on the ventral side is the mouth and mastax (m); a long thin œsophagus leads into the alimentary canal, which is suspended in the centre of the sphere. This canal has about its middle a single small knob-like outgrowth or gland,makes half a corkscrew turn,and ends in a cloaca (cl) which apparently opens also on the ventral side. In all other rotifers without exception having a cloaca this organ opens on the dorsal side. Surgeon Gunson Thorpe was so impressed by this anomaly in his new species that, rather than admit that this was a new departure, he preferred to declare that the side on which both the mouth and cloaca open is really the dorsal side of the animal. But then the usual dorsal gap in the ciliary wreath and single dorsal antenna (d. a.), which are both very distinct, would in this animal be situated on the ventral side. However, there is a better way out of the difficulty, the cloaca is only apparantly ventral when in reality it is dorsal. In the rotifers having no foot there is no clear indication as to where the dorsal side begins ; the best explanation in this case is to say that the ven-

tral side ends just before the cloaca, and to call the whole tract, from the gap in the trochal wreath to the cloacal orifice, dorsal. In this way the cloaca and all other organs will appear in the usual position which obtains throughout the class.

The flat ribbon-like ovary (ov) is suspended between the body wall and the stomach (st) and opens by a thin walled oviduct in the cloaca. The lateral canals (l. c.) are attached on each side close to the walls of the sphere, and appear to open in a small contractile vesicle (c. v); their apparent connection with the nerve threads of the lateral antennæ (l. a.) does not appear to be real or organic, but only accidental. The red eyes (e), with crystalline lenses, are present, situated on the ciliary wreath in a line at right angles to the mouth. The nervous system is very distinct, and can be beautifully studied in this animal; the brain ganglion (br) is seen just above the mastax, sending long and short nerve-threads (n) across the body cavity to the dorsal and lateral antennæ, to the ciliary wreath, the eyes, and all other organs. Two long and two short gastric glands are attached at the apex of the stomach, and four or five pairs of small muscular bands (m. b.) are arranged just below the ciliary wreath, by means of which only this zone can be slightly contracted. In size and shape it is like a young volvox globator, with perfectly transparent integument.—*Q. C.*

EDITORIAL.

Adulteration of Insect Powder.—Two out of five brands of insect powder examined by E. J. Huber, were found to be adulterated with ground oxeye daisy flowers. Experimenting with this adulterant, he found it to be entirely devoid of insecticide properties.

A "Double Serum."—Dr. J. Honl (Wien. Klin. Rund.) has undertaken to produce a "double serum" for the treat-

ment of mixed infection with diphtheria and streptococcus bacilli by rendering animals first immune against diphtheria and then against the streptococcus bacillus. The serum prepared from the blood of such animals will, he expects, serve to render the patient who is treated with it immune to both the bacilli.

MICROSCOPICAL MANIPULATION.

The Microscopic Diagnosis of Trachoma.—Snydacker (Medicine, Apr., 1899) states that the classification of trachoma has hitherto been so loose and uncertain as to render definite lines uncertain. Any form of follicular inflammation of the conjunctiva which resisted treatment and persisted for any length of time has been regarded a form of the disease. Snydacker claims the etiologic factor of trachoma is constant, and its recognition only assures us of an accurate, certain and scientific diagnosis. In this view, he is not supported as yet, although his previous paper is receiving marked attention. According to Snydacker, trachoma is due to a capsulated diplococcus, 1½ to 2 microns in length and 5 microns in breadth, which is not decolorized by the Gram method of staining, and whose septum at times has an affinity for anilin stain, causing the diplococcus to simulate a bacillus. The organism is constantly present in the trachoma follicle and secretions, before astringent and antiseptic remedies have been employed. It is easily differentiated from all other germs, as none other answers to its description. The method employed in suspected cases of trachoma is briefly as follows: When enlarged follicles are found, one of these is expressed and its contents employed. If there are no follicles, the secretions can be examined, though these are very unsatisfactory. The expressed follicular contents are spread thinly and evenly over four cover-glasses; two of these are fixed ten minutes in absolute alcohol, two are fixed in a flame. One of each kind is now put through the ordinary Gram method of staining. The other two are stained according to the Gram-Weigert method, lithium carmine being em-

ployed as the counter-stain. In this way four slides are prepared by slightly different methods, whose results should coincide. Where lithium carmine is employed as a counter-stain the bacteria are shown as a deep violet on a bright carmine backgroung. As the bacteria are very minute, and at times scarce, it may be necessary to search a number of fields. Owing to the affinity of the septum between the diplococci for anilin stains, some of them simulate baccilli. Around a few of them the gelatinous capsule may be seen faintly. The heavily stained elliptical bodies are cells, repeatedly observed within the trachoma nodule, which resemble myelocytes.

Emulsions and Emulsifiers.—Each emulsion is examined under the microscope four times, the first time within twenty-four hours after making,the second time after four days, the third time after twelve days, and the fourth time after thirty days, the object of the repeated examinations being to ascertain if any changes took place in the size of the oil globules on standing. Changes do take place if the emulsion becomes rancid, but otherwise there is practically no change, as will be observed later. Decomposition or spoiling of the emulsions must be avoided during the period of the examinations, for otherwise the results will be untrustworthy. Spoiling or decomposition cao be prevented, where its occurrence is thought possible, by the addition of a few drops of formaldehyde solution.

In making certain microscopical examinations, a drop of water was placed on the slide, the bottle of emulsion was well shaken, a glass rod was dipped deep into the mixture, and the end of this rod was then touched to the water on the slide. The water diluted the emulsion so that measurement of the globules was quite easy. The agitation of the liquid was thought necessary to insure proper commingling of the globules. The use of the glass rod avoided touching the cork to the slide, which was found likely to have at its end some hardened, partially decomposed emulsion. Each specimen was given a number, and the results of the examination are given in the Bulletin or Pharmacy for June.

Staining Tissues.—Picro-anilin-blue is used for lymphatic glandular tissue and for nervous tissues. Five cc. of the saturated aqueous solution of anilin blue are added to 100cc. of a saturated aqueous solution of picric acid. Let the tissue remain but a few minutes in the combined solution. It is often better to use the solutions separately. Allow the tissue to remain in the blue solution until it assumes a pale sky-blue color; then immerse it in the picric solution for ten or fifteen minutes. The nuclei will have stained a bright green, and the surrounding tissue a pale pea-green. Like results are obtained in both fresh and "fixed" tissues. If the sections are to be mounted in balsam the picric stain is apt to be dissolved out by the alcohol during the process of dehydration. This can be overcome by dehydrating in alcohol in which picric acid has been dissolved. If the separate solutions have been used, the tissue may be carried direct from the blue stain to a one-half per cent alcoholic solution of picric acid, thus shortening the process.

BACTERIOLOGY.

Tuberculosis Among the Queen's Cows.—A tuberculin test was recently made at Windsor of a herd of forty shorthorn and Jersey cows belonging to the Queen, and most of them were found to react to the test. They were all apparently in good condition but as a result of the test, thirty-two cows appeared to be tuberculous, their temperature rising to 104 degrees F. or more; five cows appeared to be healthy, and three were doubtful. The whole herd was killed and the carcasses were examined at the Royal Veterinary College. Of thirty-four animals whose temperature had risen above 104 degrees F., thirty-three were found to be tuberculous. The remaining animal was not tuberculous, but had a diseased uterus. The rise in this case was sudden, and did not occur until after the twelfth hour. Of four cows which did not react, three were found to be free from tubercle, and the fourth had one small caseous gland in which tubercle bacilli were found. The

two remaining cows which were classed as doubtful were both found to be tuberculous. In the formation of the new dairy herd at Windsor, all animals purchased for it will be tested, and admitted only when they do not react.

MEDICAL MICROSCOPY.

Plague Serum as a Therapeutic Agent.—W. Symmers, of Cairo, gives a record of experiments made to determine the therapeutic efficacy of plague serum ("Centralb. f. Bakt."), carried out at the Serum Institute at Abbasieh, in Egypt. Cultures were made from the bacilli of bubonic plague obtained from Bombay, and experiments tried on guinea-pigs, white rats and mice, and horses. Cultures on agar or on bouillon were found to possess little virulence, and it was necessary to pass the bacilli through white mice before sufficient virulence could be obtained. Cultures from these were then made in neutral bouillon or agar agar, and measured quantities injected into horses, generally into the subcutaneous tissues of the neck. Local swelling and inflammation extending to the glands, and slight (febrile) rise of temperture followed, but in no case was there much constitutional disturbance. After repeated injections of sterilized bouillon or agar cultures at a temperature of 60 deg. C., or of the living bacilli—sometimes as many as fifteen injections being given—the jugular vein was opened, and the blood thus withdrawn was centrifugalized and serum obtained. The minimum fatal dose of live cultures being separately determined, quantities of serum were now mixed with these, and the mixture was injected into the peritoneal cavity of white rats. It was found that serum from the horse treated with live cultures gave but feeble antitoxic power, while the horse treated with sterilized agar cultures furnishes the strongest serum, and of this a quantity equal to ¼ c.cm. was sufficient to preserve white rats against the minimum fatal dose of the bacillus; smaller quantities of the serum proved insufficient. From all the experiments made it was concluded that the antitoxic power of the serum was

about equal to that obtained by Yersin at the Pasteur Institute, and comparable to the sera of cholera and of enteric fever previously prepared by Symmers himself at the British institute of Preventive Medicine. On the whole, it appears the sera in the present experiments did not have a sufficiently high antitoxic valve to warrant any hope of their being of therapeutic use in an actual epidemic of plague. It was suggested that for the latter purpose more virulent bacilli should be employed, and that the injections of cultures thereof should be carried out in large quantities and for longer times on horses, under which conditions a more effective antitoxic serum might probably be obtained to serve the purposes of practical treatment in actual cases of plague.—British Medical Journal.

The Cancer Microbes.—Dr. Roux, of the Pasteur Institute in Paris, disclaims all knowledge of Dr. Bra, the discoverer of an alleged microbe of cancer, and affirms his disbelief in the authenticity of the discovery. Another cancer microbe has been described by Dr. Plimmer, of London. He claims to have isolated certain micro-organisms which, he believes, stand in causal relationship to cancerous growths. These organisms possess great vitality, and multiply under conditions which prove fatal to most other pathogenic microbes. They are capable of cultivation; and inoculation of animals with these cultures, Dr, Plimmer says, is followed by the production of carcinoma. The organism differs in toto from the one described by Dr. Bra, of Paris.

MICROSCOPICAL SOCIETIES.

Royal Microscopical Society.—May, 1899. The president called special attention to two old microscopes. The first, which had been presented to the society by Mr. J. M. Offord, was signed "Adams," and was a very interesting model, which filled up a gap in the historic collection of the society. Its probable date was about 1785-95. The second microscope, which had been presented by Dr. Dallinger, was full of interest, and evidently constructed about the

end of the last century. It was the earliest example of a microscope with a rackwork limb in the society's collection. Dr. Hebb exhibited, on behalf of Miss Latham, two slides of blood which had been stained with methylene blue: one was of normal blood, which had retained the blue stain; the other was of blood from a diabetic person; but in this the blue had been discharged, probably by the action of the glucose which is present in the blood in this disease. The President regretted to have to inform the meeting that Prof. Lionel Beale had been taken ill, and was unable to be present to read his paper. Dr. Hebb read a letter from Mr. Bryce Scott, who said if any Fellows cared for West India dredgings, rich in Foraminifera, he would be pleased to forward them some. The President then, on behalf of the society, presented to Mr. T. H. Powell, an enlarged framed copy of the portrait of the late Mr. Hugh Powell. The President made a few remarks upon the theory and construction of eyepieces for the microscope. He then proceeded to explain the subject by means of diagrams upon the blackboard, and having replied to various questions, he said that at the next meeting it was hoped Dr. H. C. Sorby would read a paper on the "Preparation of Microscopical Specimens of Marine Worms," and that there would be an exhibition of pond life.

Quekett Microscopical Club.—The 369th meeting May 19, Dr. John Tatham, president announced that Dr. M. C. Cooke had, at the express desire of the committee, most kindly written a short history of the founding of the club.

Mr. C. L. Curties exhibited Reichert's electric warm stage, which permitted a constant temperature to be maintained for any length of time within 0.1 degree Centigrade. Messrs. Watson exhibited West's dissecting stage, for placing over the ordinary stage when the microscope was being used for the dissection or examination of objects in fluids. F. Enock, gave a lecture on the life-history of the Tiger-beetle, illustrated by a number of beautifully-executed slides, shown by the oxy-hydrogen lantern. It was a model of thorough, patient, scientific work, and the pictures were charming.

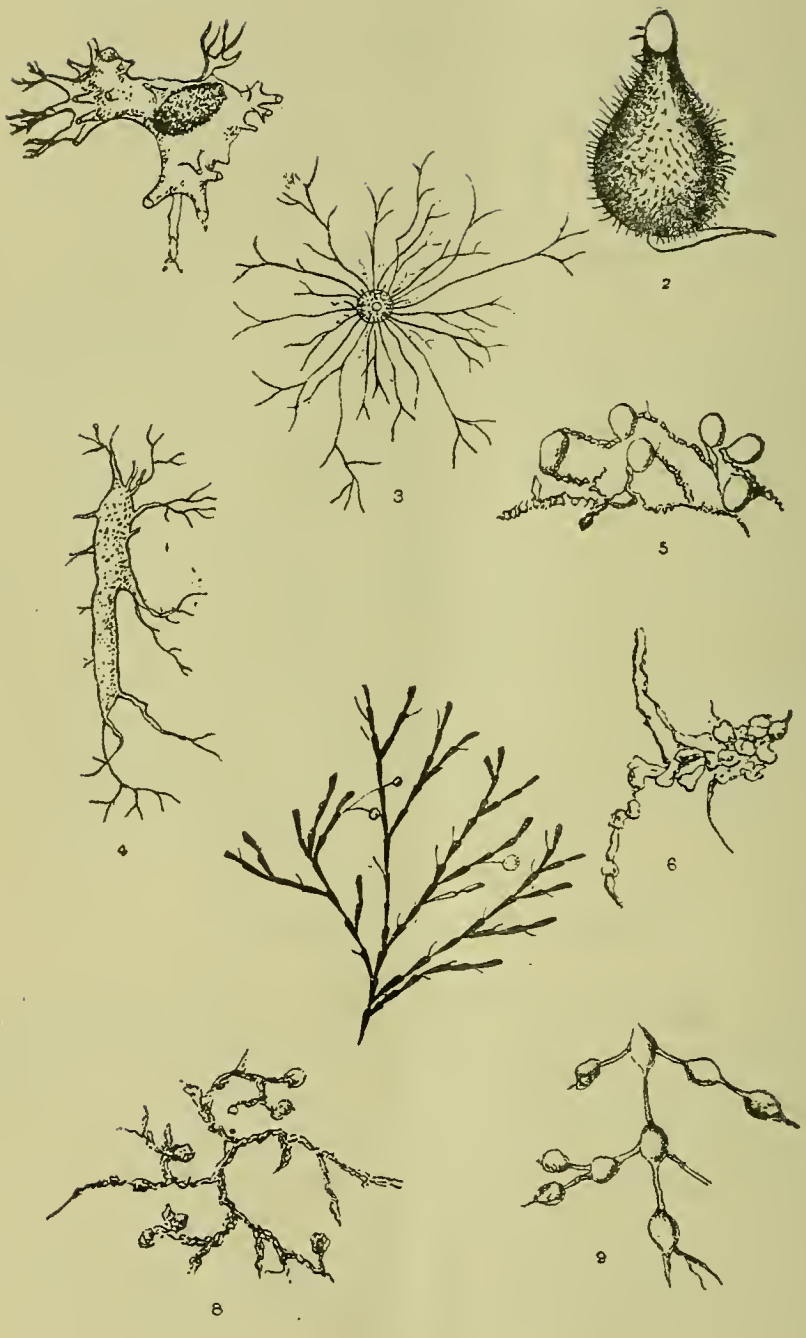
NEW PUBLICATIONS.

The Scientific Skeleton.—Samuel Blodgett, Grafton, N. Dak., 16 mo. 105 pp. 25 cents. The author in a quiet and modest way sets forth very many plausible objections to the received scientific teachings in regard to gravitation, evolution, tides, winds, movements of the heavenly bodies, etc. In contradistinction to the usual objector, he has no theories of his own to substitute. He prefers to say we do not know to saying such absurd things as he proves the fundamental theories of science to be. He deals with the allegations of such astronomers as C. A. Young, LL. D. in a way to command respect and thoughtfulness. Science is materialistic in the sense that it takes no account of any supposed intelligence inhering in matter. It would smile at the idea of the sun being an enormous personality, self-controlled, and accomplishing its purposes by means of the material sun which astronomy studies. Though Blodgett does not say so, he reasons as if a proper study of the sun's activities ought to proceed on a much larger hypothesis than that the sun is only matter in motion. Suppose man were studied solely as protoplasm in motion!

MICROSCOPICAL NOTES.

Bloody Water.—There exists a very small Infusorian, the Euglena viridis, which is capable of enormous multiplication and after spending its youth in green it changes at maturity to a beautiful crimson which deeply tinges the water in which the colonies live. This bloody water has been seen in the White Mountains and it is supposed that, by causing great numbers of these animals to grow by a psychic power, Moses and the Egyptian magicians produced the bloody waters described in Exodus 7 : 17-24.

Bleaching.—The peroxide of hydrogen (Marchand) may be used with advantage to whiten out the organs of small animals.



Blue-Green Algæ, Sea-weeds.

THE AMERICAN

MONTHLY

MICROSCOPICAL JOURNAL.

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CONTENTS.

Marine Microscopic Organisms.

W. H. HARRIS.

WITH FRONTISPIECE.

The organisms referred to are vegetable and are found in fragments of Molluscan shells and Foraminiferæ, in calcareous sponge spicules and particles of Polyzoa, in spines of Echinoderms. They are very small, live often at great depths and are studied with difficulty. They belong to the Thallogens. This subclass has three alliances, Algales, Fungales, Lichenales, and includes asexual or flowerless plants, without proper stems or leaves, mere masses of cells. Reproduction is by disintegration and so-

lidification of some part of their tissue. Seaward has classed them under Thallophyta as : 1. Peridiniales : 2. Cocospheres and Rhabdospheres; 3. Schizophyta (Chizophyceæ and Schizomycetes, the bacteria). They are small single-celled plants extremely low as to organization, reproducing by simple cell-division or by spores. In cases they contain chlorophyll and a blue-green coloring matter, hence called Blue-Green Algæ, Cyanophyceæ or Schizophyceæ. Those free from color are called Schizomycetes. Being exceedingly fragile, they break up into an unrecognizable mass with slightest pressure. It is necessary to release them from the shells of their hosts by a slow process of decalcification. The descriptions of 12 figured species are as follows ; figures 1-9 being in the frontispiece and figures 10-12 on page 237. The following data have been reported by W. H. Harris to the Quekett Club :

Lacuna fistulosa.—A small irregular-shaped species, occupying the superficial layers of the fragment ; the portion of cavity nearer the surface is beset with numerous short, stout, tubular processes, which reach the surface of the containing fragment ; sometimes tolerably large portions of shell are eroded, exposing the under side of the organism. They occur as isolated individuals, and are rare. Cebu, Phillippine Islands, 20 fathoms, and Auckland, N. Z., littoral deposit. Fig. 1 x 280.

Lacuna pubescens.—This is a robust flask-like form, thickly beset with moderately long appendages, which give the organism a hairy or fleecy appearance. There are at times one or two rather long filamentous processes given off from the bulbous portion. Undoubtedly it is a rather rare form. Java Sea and Macassar Straits, both 45 fathoms. Probably the majority of forms known as *Lacunæ* are merely a phase or condition in the life history of the plants, or possibly the inital stage of a filamentous species. Fig. 2 x 210.

Lacuna radiata.—The flask-like cavity of this species is rather deeply immersed in the containing fragment; the neck-like portion is considerably elongated and very small in diameter. The exterior surface of the globular portion is distinctly mammillated, and from each apex, branched or unbranched, very fine hair-like processes are produced, which reach the surface.

They usually occur separately, but two may become confluent; spore-like bodies having an amber tint are present in some instances; these escape through an eroded aperture, which destroys the neck-like portion of the organism. Widely distributed, but not very common. British material has furnished the best examples—notably Kenfig Pool, Bristol Channel. Found in shallow-water deposits. Fig. 3 x 210.

Lacuna radicans.—This species is very variable in form and may consist of a sample of unbranched central cavity, or they may assume various contours according to the number of branches they produce. The central cavity, however, is tolerably large, and gives off at frequent intervals, around the margin, very fine and freely branched filaments, usually decreasing in size towards their extremities. Always solitary. Not common Fig. 4 x 150.

Lacuna moniliformis.—This organism consists of a series of irregular-shaped Lacunæ connected by filaments, which, when fully developed, are freely branched and liberally provided with short excretory ducts. This is one of a few plants met with which appear to occupy an intermediate position between the genera Lacunæ and Achlyæ; the characteristic features of both are intimately combined and balanced. Very rare. Challenger Station No. 23, off Sombrero Island, West Indies, 450 fathoms. Fig. 5 x 300.

Achlya monile.—A small and very interesting organism; it is highly branched; the filaments in their early stage of growth have a distinctly septate appearance

(probably illusory), but as they mature this feature becomes more prominent by the enlargement into bead-like cavities,which are sometimes contiguous,at others slightly separated. The filaments are rather large in diameter for the size of the plant, and they have a tendency to erode the surface of the shell. Devoid of appendages. Very rare. Typical specimens have been found in recent material from Lagos Bay, S. Australia, and in Miocene deposit from Auckland, New Zealand. Fig. 6 x 300.

Achlya gracilis.—The distinguishing characters of this species appear to be more uniformly persistent than in many of the members of the group. The filaments may be divided into three distinct groups; which always occupy separate portions of the shell they invade. The contour of the plant is displayed at or near the surface of the nacreous layers; at frequent intervals branches are given off which descend vertically into the shell; in some instances these terminate in irregular-shaped cavities (to which further reference will be made), while others pass on until they sometimes reach the opposite surface,where they may terminate, or they may be deflected and return for some distance towards the original point of entry. These filaments may be simple and hair-like, or they may be slightly branched. The superficial aspect of the plant conveys the idea of rigidity, caused by the uniformity with which the branches divide. The filaments are exceedingly small in diameter, and at the point where they descend into the shell they appear to be jointed. The vesicles which terminate the short branches are generally contained wholly within the nacreous portion of the shell; in their perfect condition they are spherical, and are provided with an excretory duct, but they appear to speedily erode the shell and form irregular-shaped cavities; with the exception of the excretory duct referred to, the plant appears to be destitute of appendages for communicating with the surrounding water; in this respect

it appears to occupy an intermediate position between the group so provided and that which is quite destitute. Widely distributed, but rare outside the British area. Fig. 7 x 300. The figure exaggerates the diameter of the filaments.

Achlya fluxuosa.—This organism possesses nearly all the features of *A. gracilis*, but instead of having the rigid appearance of the latter, the superficial branches are beautifully curved, the individual plants are smaller and nearly circular in contour; it appears to quickly erode the surface of the shell, leaving continuous channels which present a serrated appearance at the margins. The cavities which terminate the shorter branches are irregular in form; some are nearly globular, others cylindrical, and occasionally they assume the form of little groups of filaments built up like a string of beads, like the reproductive bodies of *Penicillium glaucum*; but whatever their form, they occasionally appear to be thickly beset with short hair-like appendages. Comparatively common in littoral deposits of the Cornish and Devonshire coasts. Fig. 8 x 180.

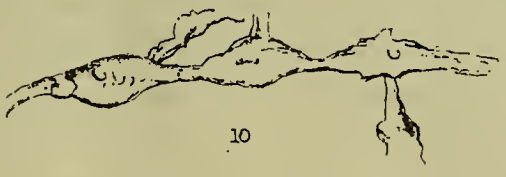
Achlya articulata.—A very well defined species, but rather difficult to detect owing to the plants being rather small and usually rather deeply immersed in the substance of the shell they invade. The filaments are composed of numerous elongated pear or club-shaped joints, articulated in a regular system, the bulbous parts being the growing ends. The joints more deeply situate appear to be destitute of appendages, but as they approach the surface rather long simple tubular processes are developed. The plants branch rather freely and in moderately thick shells; several whorls may be observed, causing the organism to assume the form of a bush in miniature. Very rare. Found at Challenger stations 127 and 187, and in littoral material from Whitesand Bay, Cornwall. Fig. 9 x 300.

Achlya modosa.—This species appears to be closely allied to *A. perforans*, the distinguishing feature being the frequent and nearly uniform expansion of the filaments into nodular areas, whence arise lateral branches; these are developed at all angles, and retain the same feature. The appendages or excretory ducts are fairly numerous; they consist of short, rather stout, tubular processes arising from the median line of the filaments. Not common, although widely distributed. Found at all depths down to 435 fathoms. Fig. 10 x 300.

Achlya reticulata.—This is a very distinct, robust, and exceedingly handsome species. It occurs in fragments of shell and foraminifera of the genus *Operculina*; when suitably placed for free development it usually forms a circular patch, frequently attaining the one-twentieth of an inch in diameter. The filaments are fairly large, but they are irregular in size; branches are produced at frequent intervals, which soon coalesce and form a perfect network of filaments arranged in graceful curves; the surface is very liberally furnished with short, rather stout appendages (excretory ducts), the apertures of which vary in form and size, giving the organism the appearance of being decorated with minute germs. Rather plentiful in material from Java Sea and Macassar Straits, 45 fathoms; but not found in any dredgings outside this area. Fig. 11 x 180.

Achlya flabelliformis.—This is a rather small organism, but its distinguishing features are very well defined. It may be described as consisting of two distinct parts—viz., a tubular portion, which gradually passes into an expanded part. The tubular portion frequently perforates the enclosing fragment, entering on one side and passing in an oblique direction to the other surface; occasionally it may reappear on the same side it entered, forming in its course the segment of a circle; but however it behaves in this respect, it always expands as it

reaches the surface, and ultimately assumes a fan-like form with a few growing points slightly in advance of the main body. The surface of the expanded portion is plentifully furnished with rather short, stout appendages; the tubular part is also provided with similar organs for some distance from the expanded part, but



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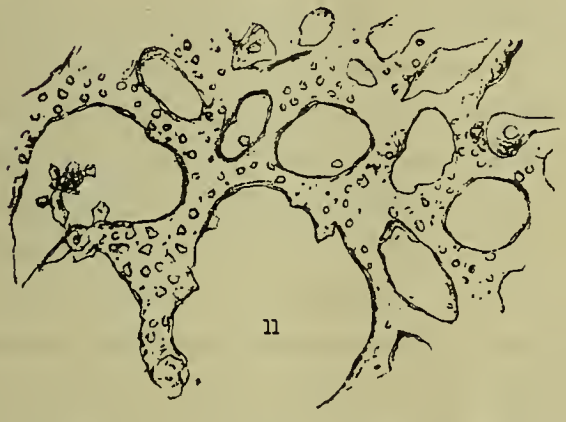

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more sparingly, the portion near the original point of entry being destitute. Very frequently the surface is eroded to some extent, thus exposing the interior of the organism. Rather rare. Appears to be a comparatively shallow-water form; coral-reef material has furnished the majority of the specimens. Fig. 12 x 300.

Merits of Different Objectives.

DR. PIERCE TYRRELL.

It is amusing to hear the miserable German or Continental stands praised so highly by college professors and men that never had a chance to use a first-class instrument made in America with a good mechanical stage. I have used a professional Bulloch stand for 25 years. It works as perfectly to-day as ever.

The mechanical stage is perfection itself. There is ample room below the stage for all accessories and a swinging mirror bar for the easy examination of urinary tubes and casts. No stand should be made without a swinging mirror bar to give oblique light. In the continental stands there is no room below the stage. If they have a condenser it is in a cramped position so that it is almost impossible to get first-class illumination from the mirror. The Jackson arm is preferable to all others because least liable to vibration. These views result from 33 years microscopical manipulation and use of the best Spencer and Tolles objectives. Herbert R. Spencer has never had a rival. There have been good and cheap German objectives but if you wish an objective which will always be free from the dry rot of apochromatic buy the best oil-immersion objectives made by Spencer, or, if you want a dry objective that is perfect, then buy Spencer's 1-5 or 1-8 inch of high angle. These have never had an equal in a long experience uninfluenced by the interests of any firm of manufacturers. I have used the Tolles 1-2 inch; 2-3 inch, 67°; 1-6 inch, N. A. 1.37; and 1-12 inch; also, Spencer's 1-4 inch; 1-15 inch; 1-25 inch; Bausch & Lomb 1-16 inch; and Gundlach 1-6 inch 180°. Manipulating all these for many years gives reliable knowledge of their relative merits.

Making Plate and Esmarch Roll Cultures.

V. A. MOORE.

The general principle under-lying the separation of bacteria by means of plate and roll cultures is to dilute the substance containing the bacteria so that the individual organisms will be separated from each other by an appreciable distance and then fixed in a solid medium where each organism can multiply into a growth or colony without coming into contact with any other organism

or colony. For this purpose agar and gelatin are used. Originally, Koch employed a rectangular piece of glass for holding the layer of medium and protected it from contamination by putting it under a bell jar. Later Esmarch introduced the "roll culture" method which was extensively followed, until the Petri dishes were introduced. Since that time they have been largely used in place of the Koch plate and Esmarch tube. On this account the plate cultures of to-day are usually made in Petri dishes. The roll culture, however, is occasionally made.

General Directions.—Make a series of 3 agar plates, one of 3 gelatin plates, and a series of 3 gelatin roll cultures (Esmarch rolls) from the bouillon culture of *Bacillus coli communis*. Place the agar plates in the incubator and the gelatin plates and rolls in a locker for that purpose.

Making Agar Plates.—Take three large tubes of agar, stand them in a water bath and boil until the agar is liquefied. Then cool by standing the tubes with a thermometer in a cup of water at a temperature of about 50 deg. C. As the temperature rises add a little cold water. When the temperature of the agar reaches that of the water and the temperature of the whole has lowered to 45 deg. C. the agar is ready for use. For convenience in labeling number the tubes 1, 2, and 3.

Place 3 sterilized Petri dishes on the leveling tripod and adjust it by means of a spirit level. With the wire loop proceed by the same method as followed in making bouillon cultures. Take one loopful of the bouillon culture and place it in an agar tube No. 1 and mix by carefully shaking it. Flame the wire and transfer two loopfuls of agar from tube 1 to tube 2 and mix as before. Again flame the loop and transfer 3 loopfuls from tube 2 to tube 3 and mix as with tubes 1 and 2. After the tubes are inoculated, pour the agar into the Petri dishes. In

doing this remove the plug, flame the mouth of the tube, and after quickly cooling, raise with the left hand the edge of the cover on one side of the dish sufficiently to allow of inserting the mouth of the tube, and hold it until the agar is poured and replace it immediately. Label, and number the Petri dishes to correspond with the dilutions in the tubes, thus, plate 1 is from tube one, plate 2 is from tube 2, and plate 3 is from tube 3. In making the dilutions it is important that the wire loop should be flamed after making each transfer.

MAKING GELATIN PLATES.—These are prepared precisely as the agar plates with these exceptions. (1) The gelatin is liquefied at a temperature of 45 deg. C. (2) The plates when made are to be kept in the locker the same as the gelatin stab cultures. (3) In hot weather it is sometimes necessary to put a piece of ice in the reservoir under the glass plate on the leveling tripod to congeal the gelatin.

The directions given above for making the dilutions are applicable only when the original culture is moderately clouded. If there are comparatively very few bacteria in the liquid a larger quantity of the culture will be necessary. If there are many more, as in turbid boullion or slant agar culture, it will be necessary to take a much smaller quantity for the first dilution. It is often desirable to make the first dilution in a tube of sterile water or bouillon instead of gelatin or agar, and to make two rather than three plates.

MAKING ESMARCH ROLL CULTURES.—For this purpose gelatin is ordinarily used as agar does not adhere readily to the sides of the tubes. It is sometimes used. Take the desired number of large tubes of gelatin, liquefy, inoculate, label and number the dilutions as in making gelatin plate cultures. Place a block of ice about 6 inches long in an agate iron tray. Melt a slight horizontal groove in the ice with a test tube containing hot media or water.

The inoculated tubes are tipped and rolled so that the liquid gelatin moistens the inside of the tube to within about a centimeter of the plug. Then roll the tube rapidly in the groove on the ice until the medium becomes solid. The gelatin should not come in contact with the plug. In rolling the tube the plugged end should always project beyond the ice.

A Few Words on Red Mites Found in Fresh-Water.

CHAS. D. SOAR.

Given a good microscope and an easily accessible pond, and what a field for research is still open to the student in natural history! What a lot of Nature's little secrets are yet left unread! In the vicinity of a good all-the-year-round pond a small local club would have sufficient material to keep it going always. If the club did not number many members, the pond would furnish enough different subjects to allow each member to study a different group. For instance, one member could take Rotifera, another the Entomostraca,another the Diatoms, etc., etc., and they would sometimes find their independent studies of great mutual assistance. Not a few of the great army of pond hunters (or puddle rakers as they are sometimes called in derision by the unthinking) are looking forward to the formation of a Fresh Water Biological Station in England, on a similar plan to that at Plon in Holstein, Germany. When this takes place, which we hope it will, we shall be in a better position to study the life history of various forms of aquatic life of which we are now so ignorant.

Now there is one form of pond life in particular to which I wish to draw attention, and that is Hydrachnide (Fresh-Water Mites). They are very beautiful both in color and form, and they are fairly common,but very few pond hunters, if they take one when collecting, know more

about it than that it is a member of the acarina. I have often ask microscopists when they have been collecting to kindly save me any water mites they might find of a particular genus, but they invariably answer "I only know the common red one," and are very much surprised when they are told that there are between twenty and thirty distinct species of red mites found in fresh water in England.

In England we know of twenty-six distinct genera, and seventy-two identified species, only two of which have been named by Englishmen—Thyas petrophilus (Michael) and Arrenurus novus (George). The red mites which I know, are distributed as follows: Arrenurus three, Piona one, Nesæa five, Limnesia, three, Hydrachna two, Hydrodroma two, Marica one, Diplodontus one, Eylais one, Bradybates one, Limnochares one, Thyas two. This list will no doubt be added to later on. I only wish in this paper to mention a very few of the red mites, chiefly to show the difference in the external structure of these beautiful creatures. To mention and figure all the known British red mites would take up too much space.

Water mites, as far as is known, generally deposit their eggs on the leaves and stems of water plants. The under side of the leaf of Anacharis often shows how the ova of a beautiful red mite with blue legs—Limnesia histronica (Hermann)—are deposited. The eggs are not red, but deep orange-yellow. After a number of days, the time varying in different species, the larva, which is hexapod, is hatched out. During this stage many if not all larvæ become parasitic on some other form of pond life A small red mite in the larval or parasitic stage, can be taken from the leg of a water-boatman Corixa Geoffroyi; it is very small, and one recently found had not taken up its abode very long, because the legs were still visible. When they have lived on their host for sometime and have grown very considerably, the legs havo

either disappeared altogether or have shrivelled up into a very small compass, being of no further use to the little creature. The last mentioned are, I believe, the larval stage of Limnesia histrionica. So little is known at present about the larval atages of water mites, that it is almost impossible to name the species correctly from the larvæ. After having spent sometime in the state just mentioned they become free swimming, with eight legs, and are now very much like the adults, so much so, that you can now tell with tolerable certainty the species to which they belong.

All of the members of the genus Arrenurus are hard skinned mites,the males only have tails,the females being without these appendages. Arrenurus tricuspidator (Muller), is a very brilliant red mite. It can easily be recognized by the peculiar formation of its tail. Arrenurus emarginator (Muller) is a very large red mite. I believe we shall have several more red mites to place in this genus later, because I have several red females that I cannot name until the males are taken.

The genus Hydrachna contains soft-bodied mites,which can be recognized by the mouth organs projecting as far forward as the palpus. In this genus we have two red mites. H. cruenta (Mull.), is easily known by the patch on the dorsal surface behind the eyes.

The genus Hydrodroma is also represented by two red mites, also distinguished for the patch on the dorsal surface. The genus Nesæa has several red mites. Of course, all fresh-water mites are not red, all colors being represented in these beautiful creatures more or less, but I have said enough to show that instead of anyone capturing the common red water mite it is much more likely that he has only captured a mite of a common color.—*Illustrated Annual.*

Recent Work in Clinical Microscopy.

H. A. L. RYFKOGEL, M. D.

Although no such important discovery as the Widal reaction has been made in the line of clinical microscopy during the past year, nevertheless a certain amount of work of permanent value has been accomplished. This has, to a great extent, consisted in the further application of methods previously evolved, such as the application of the Widal reaction to leprosy, the perfection of the bacteriological diagnosis of influenza, and the advancing our knowledge concerning eosinophil cells in sputum.

However, the year has not been wholly lacking in original results from investigations in clinical microscopy. Thus the cultivation of the bacillus of Ducrey, and the practical use of Sudan III as a fat dye, and as a stain for the bacillus of tuberculosis must be recognized as original work recently accomplished.

On hematological subjects much has been written and considerable work has been done. Perhaps the most important investigation of the year on the blood has been that of Simon on the significance of Neusser's granules. When Neusser in 1894 announced the discovery of these granules, he advanced the claim that their presence was pathognomonic of the uric-acid diathesis in all its protean manifestations. He excepted certain tuberculous cases, in which the granules were seen, and in these he believed their presence to be a good prognostic sign. Simon, quite contrary to these observations, found in the blood of every healthy person he examined abundance of these granules; further, he usually found them in the majority of diseases, whether uric-acidemia or not; in fact, the only cases in which he could not find them were a number of cases of malignant disease, one of acute gonorrhea and two of gastric ulcer. Thus it appears that the absence rather than the presence of these bodies may be of import.

Some additional cases of trichinosis have been reported during the year, and in every case an enormous increase in the number of eosinophil cells has been noted; in fact, it was in some instances, this increase that led to the diagnosis. It may now be laid down as a dictum that in every case with irregular fever and pains in the muscles a differential count of the white cells should be made and when a very large increase of the eosinophil cells is found, a diagnosis can be made. This disease has undoudtedly in the past been frequently overlooked,and this new symptom should greatly minimize the number of undiagnosed cases.

The literature of the year on the Widal reaction has brought forth no fresh knowledge, but the statistics advanced cannot fail to increase our confidence in the reaction as a diagnostic aid, and although a certain proportion of cases do not show the reaction, the consensus of the year's opinion from clinicians remains as before, namely, that if the test be made by a thoroughly competent and careful bacteriologist, the reaction,when present, is diagnostic. This is especially certain if the test be made with the serum and the dilution be not less than one to fifty.

The principals of this reaction have been applied by Spronck to the diagnosis of leprosy. Cultures of the lepra bacillus were obtained by inoculating neutralized potato with glycerin, with the leprous tissue, and transplanting the resulting growth onto Loeffler's gelatin horse serum. Cultivations, as thus carried on, somewhat altered the appearance of the bacillus, causing it to assume the appearance of the diphtheria bacillus rather than that of the bacillus of tuberculosis. The modified bacillus did not grow on meat bouillon,but flourished in fish bouillon. The serum of leprous patients readily agglutinates young cultures, whether grown on fish bouillon or on horse serum gelatin, and emulsified. The dilution is one to sixty,

to one to a thousand. The serum of non-leprous patients does not act in this dilution. This method will, if its reliability be confirmed, be of great diagnostic value in the early stages of leprosy.

The agglutinative serum reaction applied to the bacterium of Malta fever—Micrococcus miletensis—recently materially aided Musser and Sailer, of Philadelphia, in diagnosing the first case of this disorder so far reported in the United States. The disease occurred in an army officer who had returned from Porto Rico with symptoms slightly resembling typhoid and Musser and Sailer rightly suggest that many cases from Porto Rico and Cuba, reported as typhoid fever without the Widal reaction, or malaria without the plasmodium, may be really cases of this infection, and they suggest that all cases of "continued undulant fever" be tested for this special form of serum reaction.

Pappenheim and Fraenkel have found in cases of pulmonary gangrene large numbers of smegma bacilli, which in one case led to the diagnosis of pulmonary tuberculosis, whereas, at the autopsy, no tuberculous lesions were found. In Pappenheim's opinion, if the smears be decolorized with a solution containing absolute alcohol, one hundred parts, rosolic acid, one part, and methylene blue to saturation, no danger of error will be present. Fraenkel claims that it is only necessary to take special precautions in cases of putrid sputa, rich in fats and myelin. Sudan III, as will be mentioned further on, has also been used as a differential stain.

Carl Sternberg has cultivated the Boas-oppler bacillus, and finds that it grows in short forms on glucose agar, but becomes long and slender on maltose bouillon, and he advances the interesting theory that the short forms are often present in the gastric contents, and escape notice, but when certain chemical changes occur, through cancer or otherwise, the long, characteristic forms appear.

Some valuable investigations in the bacteriologic diagnosis of influenza have been conducted by Wyncoop, of the Chicago Health Department. He found that after some experience the Cannon-Pfeiffer bacillus could be readily recognized in fresh sputum, and cultures could be easily made on beef blood serum which contained a little hemaglobin. The method was made, in fact, almost as practical as the bacteriological diagnosis of diphtheria. In the course of his studies, Wyncoop found the bacillus in a number of other diseases, such as diptheria, scarlet fever and pneumonia. In all such cases the bacteriologic findings at once cleared up the meaning of symptoms that had before puzzled the clinicians. Thus, in some cases that at first appeared to be diptheria, a pure culture of the influenza bacillus, was found, and the subsequent course of the disease coincided with the bacteriologic diagnosis.

Sudan III has been used as a fat dye with excellent results, fat bodies, whether fat cells, or granules in cells, urinary casts, or bodies of bacilli, being stained a brilliant red. The fat bodies do not show the blurred appearance seen in osmic acid staining, but stand out with remarkable clearness. The dye has also been used as a differential stain for the bacillus tuberculosis. It acts by staining the fat prəsent in the bodies of the bacilli.—*Pacific Record.*

Notes on Microscopy.

JOHN H. COOKE.

EMBEDDING.—The embedding of soft tissues requires considerable skill on the part of the operator. But frequently, notwithstanding the greatest care, failure results, either owing to the variable nature of the medium used, or because the processes of hardening have been too protracted. Those who have experienced these difficulties should try the white of an egg as the embedding medium.

The process is as follows :—Make a small paper box, about a-half-an-inch square, and fill it with the white of an egg. Eliminate all alcohol from the material which is to be mounted, and embed it in the albumen. Expose the box and its contents to heat, and when hardened place it again in alcohol. If the sections are passed through oil of cloves with balsam, the albumen will become clear and transparent.

BACTERIOLOGY.—An illustration of the pitfalls that confront science students is given by Dr. Martin Ficker, a German bacteriologist. Among various sources of error in work with bacteria is the glass of the vessel used, as different kinds of glass impart varying degrees of alkalinity to water, and it is found that some bacteria, notably those of cholera, are favorably affected by alkalinity. This novel cause seems responsible for marked and important discrepancies.

MITES.—Freshwater mites do not, as a rule, make satisfactory mounts. They shrink and fade, thus losing in the preserved state the beautiful symmetry and colorings which render them, when alive, such charming objects for observation and study. The difficulty is the mounting medium. No formula is known which will give perfectly satisfactory results, but the following, if carefully prepared, will enable the microscopist to preserve his specimens, for some years at least, from bleaching and collapse :—Prepare three mixtures of distilled water and pure glycerine in the proportions of twelve parts, ten parts, and eight parts of water, respectively, to one part of pure glycerine, and to the last add a small drop of carbolic acid. Place the specimens in the twelve-part mixture and leave them for twelve hours, after which place them in the ten-part mixture and leave them for a similar period. They may now be permanently mounted in the third mixture. Solid glass cells are preferable to built-up ones.

LABELS.—The preservation of labels of reagent bottles is a frequent source of trouble to the working microscopist. A good varnish for this purpose may be made by macerating the following substances, and thoroughly shaking the mixture until all are dissolved:—Sandarac, sixty parts; mastic, twenty-five parts; camphor, one part; oil of lavender, eight parts; Venice turpentine, four parts; ether, six parts; and alcohol, forty-four parts.

TRACING PAPER.—It is frequently desirable to supplement the records obtained by photographing micro-sections with a drawing, and it would probably be oftener done were it not for the difficulty that many experience when using either the neutral tint reflector or the camera lucida. With a vertical camera and suitable tracing paper this difficulty disappears, and it is possible, after a little practice, to acquire a creditable amount of dexterity in representing the salient features of all objects that do not require high-power objectives for their delineation. In making such drawings it is a great advantage to be able to do them direct from the microscope, so that no transference or copying is afterwards needed. For this purpose, the following recipe for making a tracing paper, which can be re-converted into ordinary drawing paper after the drawing has been made on it, will be found to be both simple and effective:—Immerse any ordinary drawing paper in a mixture consisting of one volume of castor oil and three volumes of spirits of wine, and hang it up to dry in a warm room for two or three hours. Place a sheet in the focussing screen of the camera and make the drawing, after which place it in a bath of spirits of wine and allow it to remain there until the oil has been dissolved out. The paper will resume its former state and appearance.

WORKING ROCKS.—The isolation of the skeletons of siliceous organisms, foramens, and other small objects from rock specimens, may be readily effected by first dry-

ing the rock in air and then dropping it into a hot, saturated solution of Glauber's salts. On cooling, the processes of crystallization break up the rock mass.

MOUNTS.—Balsam mounts are apt to deteriorate, unless some means are adopted to prevent the access of air or moisture. They may be protected by running a ring of melted paraffin wax around the edge of the cover-glass, by means of the turn-table, and afterwards, when set, protecting this in its turn with a ring of finishing cement. A very beautiful and instructive preparation may be made by heating xylene balsam on a slide until the xylene has almost evaporated, and then adding a few crystal of sulphonal. The preparation should be gently warmed until the sulphonal melts and mixes with the balsam. The cover-glass is then put on. If perfect crystals are obtained this mount will show, with the aid of the polariscope, not only the most gorgeous colorings, but also perfect examples of the black cross.

POINTER.—An occular pointer is a useful accessory for class demonstrations when it is desired to indicate any particular structure or object in the field of view. To make one, cut a circle of cardboard of a size sufficient to fit easily in the ocular tube. Out of the centre of the circle punch a second circle, having a diameter slightly larger than that of the ocular diaphragm. Blacken this cardboard ring, and, with a little gum, fasten an eye lash to edge of the smaller circle so that the *cilium* shall project halfway across the opening. Remove the eye-lens of the ocular, and drop the cardboard ring into the tube so that it rests on the diaphragm. The *cilium* is now at the level of the real image, and the specimen on the stage of the microscope can be so placed that the pointer will indicate exactly any particular portion to which it may be desired to draw attention.

ALCOHOL is used by many microscopists both for killing their specimens and for preparing them for the labora-

tory and the museum. In the case of marine animals the use of alcohol in inexperienced hands is often attended with unsatisfactory results, and this for several reasons. On thick-walled animals, particularly those provided with chitinous envelopes, this reagent acts prejudicially on the internal organs,while in the case of the smaller crustacea it gives rise to precipitates in the body fluids which frequently prevent a satisfactory dissection of the parts being made. It has also been found in practice that alcohol tends to fix the sea-water contained in the organism, and thus, by forming a crust, prevents both the hardening and the staining fluids from penetrating the tissues. With proper precautions there are, however, few reagents which give better results. The method employed in the Marine Biological Laboratory at Naples is worth the attention of those whose efforts with alcohol have not always been so successful as they may have desired them to be. To kill, let us say, an annelid, the animal is transferred to a beaker containing sea-water. A few drops of alcohol are added, and this is repeated at short intervals until the animal expires. The tentative method of killing neither causes contraction nor distortion of the parts. The animal dies slowly, and, when dead, is so supple that it may be readily arranged in any desired position. The main point is to avoid the mistake, usually made, of killing in strong grades of spirit. After death the specimen may be passed through the various grades of alcohol in the usual way.

Honeydew.—The popular theory regarding "honeydew" is that it is an excretion from aphides. Mr. H. W. Brice has recently made a microscopical examination of the substance, and has arrived at the conclusion that the popular theory is fallacious. Under the microscope some thickly-coated leaves of the lime and sycamore revealed not more than three or four insects per leaf. These insects were removed, and an hour afterwards the beads of

honeydew were found to be more numerous and larger than when the leaves were first gathered. He concludes, therefore, that the leaves of some trees, under favorable climatic conditions, become surcharged with saccharine matter, and, the cells bursting, a copious exudation of "honeydew" takes place. The fact that the "dew" gathered from the sycamore and the oak is much darker than that from the lime is held to prove that it partakes of the nature of the tree and not of the insect.—*Knowledge.*

Two New Stains for the Gonococcus.

R. G. SCHNEE, M. D.

The chief diagnostic features of the gonococcus are as follows :

1. Arrangement in pairs, having the adjacent sides flattened or concave.
2. Relatively large size.
3. Found within the protoplasm of the pus cells around the nucleus.
4. Decolorizes by Gram's method, which is not true of other pus cocci.

Owing to the lack of uniformity in results by Gram's method, and the necessity of quite an amount of technical ability in its use, the following new stains are recommended for routine work. Although in well-marked cases of gonorrhea the gonococci are easily shown by staining with methylene blue, these stains are more rapid and more easily handled.

In cases of vaginitis and suspicious leucorrheal discharges, they meet a long-felt want. Where large numbers of other organisms are present and gonococci are relatively few in number, the methylene blue cannot be depended upon at all, and Gram's method is not satisfactory. In just such cases either of these stains, if used properly, will show the organism, if present, beautifully differen-

tiated. The preference would probably be given Pick's solution, as it will keep well and is more easily prepared. This is made and used as follows:

Ziehl's carbol fuchsin.....15 drops.
Concentrated alcoholic solution of methyl blue 8 "
Distilled water.....20 c. c.

Stain cold for 10 seconds, wash with water, dry, and mount. The gonococcus will be stained a deep blue, other bacteria a light blue, cell nuclei a still lighter blue, and protoplasm pink.

The second stain is recommended by Lanz and should be freshly prepared, though in our laboratories it has been used for six weeks after preparation. Formula:

Saturated solution of fuchsin in 2 per cent carbolic acid (aqueous).....10 c. c.
Saturated solution of thionin in 2 per cent carbolic acid (aqueous).....40 c. c.

Stain, without warming, for ¼ to ½ minute, and wash with water. The gonococci are stained by the thionin, the cell protoplasm by the fuchsin, and the nuclei by both colors at the same time.

If these solutions are allowed to act too long, the desired result is not obtained.

EDITORIAL.

Soap for Imbedding.—When time does not permit use of paraffin or collodion for imbedding plant tissues, use soap dissolved in equal parts of glycerine and 95 per cent alcohol. Pour into a warm, shallow dish and let it harden. Immerse the specimens first in a dilute solution of soap to secure thorough penetration and infiltration. Then imbed in a watch glass.

Credit.—We are indebted to Science-Gossip for many of the following items.

F. Shillington Scales, F. R. M. S. in Science Gossip says: Our object is to make this column as interesting and as helpful as possible to our readers, by keeping them informed of various matters of interest in the microscopical world, especially with reference to methods of work. We propose in future to lay greater stress, from time to time, upon the improvement of the microscope and its accessories. We desire, in fact, to make this column a valuable record of the progress of the microscope, of new or modified stands, objectives, apparatus, etc. The present keen competition amongst opticians, especially amongst the limited few who are in the front rank, and with whom workers are mostly concerned, gives rise to constant progress. It will be our endeavour to keep our readers advised on these points without fear and without favor.

MICROSCOPICAL APPARATUS.

New Achromatic Condenser.—Although the Abbe Condenser, or, as it is often called, Illuminator, is recognized as being suitable for most general practical work, mainly on account of its being easy to use, yet for more critical work a well eorrected condenser becomes a necessity. Messrs. Beck have brought out a new condenser in high-power work that is achromatic and has a numerical aperture of 1 N. A. This is the maximum that can be obtained without having the front of the condenser in immersion contact with the under surface of the slide, and its aplanatic aperture exceeds ·9 N. A. It is thus greatly superior to the ordinary Abbe Illuminator. The front lens is removable to make a low-power condenser. An Iris diaphragm and a swinging arm, with rotating fitting for colored glasses, or stops, are provided. A bracket is ingeniously added at the bottom of the mount, into which the optical portion may be screwed, and the whole condenser thus reversed. This is often a convenience. The price of the complete condenser is $15 but with Iris dirphragm only $12.

New Tripple Nosepiece.—The Messrs. Beck have also

brought out a new triple nosepiece in bright lacquered brass, the price of which is only seventeen shillings and sixpence. It is not perhaps quite so attractive as the ordinary trefoil-shaped pattern, but has the advantage of being dust-proof and cheap.

New Reversible Compressor.—It is made of ebonite, and consists of a lower and two upper plates. The lower plate contains an oblong thin glass, held in position by two screws, the two upper plates contain a projecting milled ring, which, when revolved, brings an upper thin glass in contact with the lower glass. The whole arrangement is easily taken apart and conveniently arranged, whilst it is eminently serviceable.

Dissceting Microscopes.—Messrs. Bausch and Lomb have brought out two new dissecting microscopes. The one consists of a small wooden case or box, 4 inches x 2 inches x 1½ inches. One end of the case and the top is removable, and serves as a cover. A small glass stage slides in a groove at the top of the box, and can be replaced by a glass stage with cell, opal glass stage or black glass stage. Beneath is a plane mirror, and an upright rod carries three lenses, magnifying from 5 to 25 diameters. The other dissecting microscope is very similar to Leitz' well-known stand, but the focussing is by sliding adjustment instead of by rack and pinion.

Circuit Stage "Van Heurck" Microscope.—Messrs. Watson and Sons have recently put upon the market a modification of their well-known "Van Heurck" microscope, which, by allowing more room between the stage and the body, gives complete rotation to the former. This is a step in the right direction. It has always appeared ridiculous hat costly and elaborate microscopes of this sort should ıbe, as we have seen them, sent out with mechanical stage, centering adjustments, graduated divisions for rotation, and even rack and pinion for the same purpose, yet incapable of rotation for more than, say, 270 deg., through the screw heads of the mechanical stage fouling the body of the instrument.

Oil Immersion Condenser.—Messrs. Watson and Sons have sent out a new "Parachromatic Oil Immersion Condenser. For an objective to work at its best, the aperture of the condenser should approximate to that of the objective. Though it is not every objective that will stand so large a cone of light, the best of our immersion lenses need a larger aperture of illumination than the 170 deg. or so, that can nominally be passed through them with an ordinary dry achromatic condenser. The total aperture of this condenser is 1.35 N. A., and its aplanatic aperture is between 1.25 and 1.3 N. A. The clear aperture of the back lens is 6-10 inch, and the power of the condenser is equivalent to an objective of ¼ inch focus. It works through a slip 1·75 millimeteres thick, and the makers state that to get the best results the lamp should not be less than 5 inches, or more than 7 inches from the back of the lens. With this condenser and a fine achromatic immersion objective of similar aperture, we have obtained results that closely rival the apochromatics. It is an essential part of the outfit of the student of bacteria, the diatomist and others. The price is so moderate as to bring it within the reach of most microscopists. The optical portion only, with universal objective thread, costs $26 whilst the whole mounted with iris diaphragm, graduated to show aperture in use, stops, and revolving carrier is $31.

Abbe Camera Lucida.—Messrs. Bausch and Lomb have also brought out a simplified form of this camera, in which the mirror and prism are enclosed in a mount and fixed, the mirror itself being reduced in size. The whole is attached to the microscope by a clamp, and can be swung aside as required; whilst it can further be adjusted in position so as to be used with various eye-pieces.

New Stage Table.—It was first devised as a rough stage for the microscope. That instrument being placed vertically, the table is brought over its stage, and admits work of the roughest character without danger to the microscope. Further, it can be heated by aid of a spirit lamp, and when hot enough placed over the microscope, thus act-

ing as a hot stage, extremely useful in watching crystilization, etc. The wooden hand-rests being non-conductors of heat, the hands of the operator can conveniently rest on them during manipulation, while the top plate is too hot to conveniently handle. Again, for utility in mounting purposes, it is far in advance of the orthodox four-legged brass table of our forefathers. Needless to say, this simple instrument lends itself to numerous purposes, known only to the working microscopist in his daily round. A sheet of brass ⅛ inch thick, 4½ inches wide, and 16 inches long, or of a size to suit the microscope, has an arc cut out of each end, leaving ½ inch each side for legs when the table is finished. From the centre of the 4½ inch by 16-inch cut a circular aperture 1 inch in diameter. At 3¾ inches, also at 5¼ inches, from each end, drill and counter-sink a 3-16 inch hole, ¾ inch from each side; that is four holes at each end of the sheet of brass. Then bend the sheet into an irregular octagon, the 1 inch aperture being in the centre of the proposed table-top, which, when finished, should measure 4½ inches by 4½ inches, take care that the counter-sinks are on the underside. Then take two pieces of apple wood ½ inch thick, the size of your sloping-table sides. Screw these to the sides, a piece of asbestos cloth intervening; neatly finish, and the day's work will never be regreted. Microscopists, not of the amateur mechanic fraternity, had better place the work in the hands of an optician, giving him the particulars as to height and width of microscope.—G. West, London.

MICROSCOPICAL MANIPULATION.

Cleaning Slides.—Immerse them bodily in a strong solusion of Hudson's soap-powder in warm water. Soak for an hour or two, wash well with changes of warm water, and finally, if necessary, with methylated spirit. Cover-glasses and slips for important work may require special methods.

Decolorizing Algæ.—Dr. H. C. Sorby has found that diluted formalin decolorizes algæ, and by means of this re-

agent has suceeded in so reducing the color as to show the natural coloring of such algæ in lantern slides, instead of exhibiting only the ordinary dark shadows.

Killing and Preserving Marine Animals.—The addition of a small quantity of menthol to the sea-water in which marine animals are kept, causes them to expand fully. They can then be preserved permanently in a four per cent formalin solution. By this method he has preserved Synapta and several species of sea anemones. He also suggests the use of dilute glycerine for killing some animals. Afterwards he removes this by water, and subsequently mounts in Canada Balsam. By this means he has been able to mount certain worms, so as to show the minute blood-vessels filled with the natural red blood.

Yellow Fever Germ.—It is now conceded that Giuseppe Sanarelli of Montevideo, Uruguay is the discoverer of the specific germ of yellow fever. Dr. H. D. Geddings of the Marine Hospital Corps has investigated in New Orleans and Havana and will report the Bacillus icteroides to be the germ. Sanarelli's serum was presented to the U. S. government two years ago and it has been tested in the South but not found to be of sufficient strength. This however, will be corrected.

Staining the Capsules of the Pneumococcus and the Bacillus Friedlaender.—(Lond. Lancet, 1898, II., p. 1262). The following combination gives a clear image, which photographs well: Dahlia, 0.5 gram; Methyl green (00 cryst.) 1.5 gram; Fuchsin, (sat. alc. sol.) 10 cc; Distilled water, 200 cc. Rub up the dahlia and the methyl green in a mortar with part of the water until dissolved, then add the fuchsin, and finally the rest of the water. Prepare the film in the usual way. Flood the cover-slip (or slide) with the stain, and hold over the flame until steam begins to rise. Then place aside for about five minutes, wash in water, dry, and mount in xylol balsam. Treatment of the film with acetic acid does not lead to good results. The above is a good stain, and gives good results with the bacillus typhi abdominalis, bacillus coli communis, and especially with the Klebs-Loeffler bacillus.

NECROLOGY.

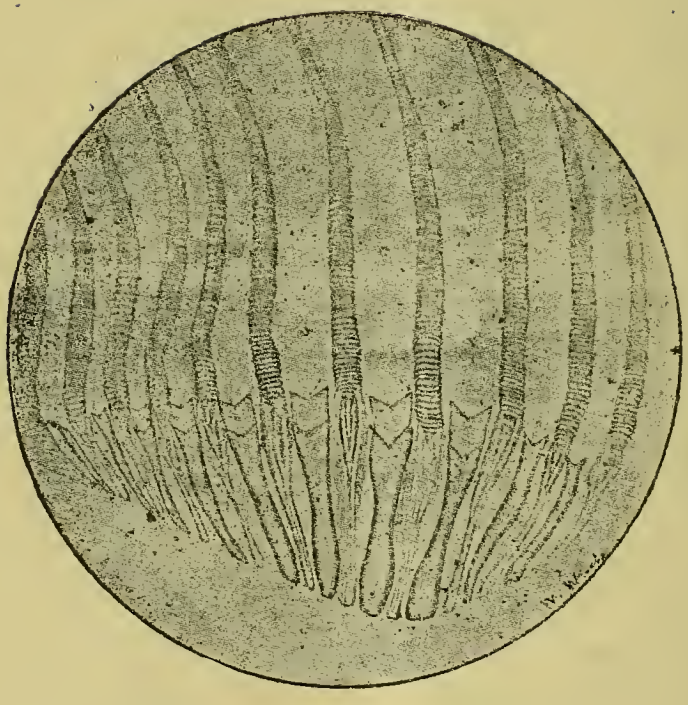
Mr. Jabez Hogg.—Mr. Jabez Hogg, the distinguished consulting ophthalmic surgeon, and the popular writer on the microscope, died suddenly at his residence, 102, Palace Gardens Terrace, Kensington, at the age of eighty-two. The youngest son of the late Mr. John Hogg, of the Royal Dockyard, Chatham, he was for some time a schoolfellow of the late Charles Dickens at a small school. From this preparatory school, Hogg passed to Rochester Grammar School, and leaving there at the age of fifteen was soon after apprenticed to a medical practitioner. The succeeding five years he passed in this employ, and studying medicine at the Hunterian School he entered as a student at Charing Cross Hospital, and in 1850 received his diploma as a member of the Royal College of Surgeons. Applying himself more especially to the study of the eye under all its aspects, he became a specialist in that subject, and for forty-five years, from 1850 to 1895, practised as an ophthalmic surgeon. He was for twenty-five years consulting surgeon to the Royal Westminster Ophthalmic Hospital, and also served in a similar position in the Hospital for Women and Children and the Royal Masonic Institutions, he being well-known in Masonry, in which he always took a deep interest, which obtained for him in 1867 from the Earl of Zetland the dignity of a Grand Officer of Grand Lodge. Mr. Hogg, before he had entered as a hospital student, had devoted himself to literary work, wri ing for some of the magazines, and preparing for publication a Manual on the Art of Photography, then in its early infancy.

NEW PUBLICATIONS.

Chas. Baker's New Catalogue.—Mr. Chas. Baker has sent us his new and enlarged catalogue which is a great improvement on former issues. Mr. Baker lists and illustrates not only his own well-known microscopes, but those

of Zeiss, Leitz, and Reichert. A great many slides are included. This firm has a slide-lending department. A noticeable feature is an exceptionally complete series of reagents and stains prepared by the most scientific methods. There is a special catalogue of the necessary apparatus for the study of the effects of malaria upon the blood, bacteriological outfits, etc. The preface states that microscopes are included that are suitable for engineering, brewing, baking, paper-making, and other industries.

Micro-organisms.—The Microscopy of Drinking Water, by G. C. Whipple, is published by John Wiley & Sons. While the importance of the chemical and bacteriological analysis of water has long been recognized, the study of the lower forms of animal life included under the term "microscopy of water" has received far too scant attention as a branch of hygiene. It is beginning to appear that quite as accurate estimates of the purity of drinking water may be based upon its microscopy as upon the somewhat uncertain and more laborious results of chemical and bacteriological analysis. On this account the appearance of an elementary work upon the forms of animal life commonly found in drinking water, and the significance attaching to their presence, is most acceptable to a large class of readers who are brought in contact with this subject. The present volume is specially adapted to the needs of the medical profession, as the practical use of the data presented requires only a moderate biological training. The first half of the work contains a great deal of useful information regarding the objects and method of microscopical examination of water, the factors governing the appearance of micro-organisms in water, the origin and significance of odors in water, and the method of storing surface water. The remaining chapters are devoted to an elementary description of the principal genera of micro-organisms, beautifully illustrated by twenty-nine full-page, half-tone pictures. The volume will fully repay careful reading, is sufficiently complete for an elementary book of reference, and should find a place in every medical library.



Teeth of the Blowfly.

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CONTENTS.

The Teeth on the Labella of the Blow Fly.

WALTER WESCHE.

WITH FRONTISPIECE.

One of the most familiar objects that is mounted as a slide for the microscope, is the proboscis of the Blow Fly (*Musca vomitoria*). Probably the most modest collection of objects contains a slide. It is used every day as a test for low powers, for flatness of field and sharpness of outline, and even familiarity has not diminished our wonder and admiration of this marvel of minute structure. But in the vast majority of cases, if a microscopist is asked to

show the teeth, a denial of their existence would be received, probably coupled with an assurance that there were no teeth, but some minute hairs on the edge of the labella which had been mistaken for them. It is a curiosity of microscopy that often, unless it is known what to look for, it is quite possible to miss seeing a structure or detail, from the fact that its focus is at a different distance from some near, yet more prominent object. This is precisely the reason, coupled with the fact, that the proboscis is usually looked at with low powers, why these escape observers; they lie at the base of the false or pseudo tracheæ, at a different focus, and the chitine of which they are composed being a light yellow and transparent, they are easily overlooked.

The teeth show between eleven of the false tracheæ, and six appear to be double, making a total of sixteen on each labellum. With the exception of one at the end furthest from the head, they have a cleft edge, thus securing two points to each tooth, and, one might suppose from this, are used for pricking or gently scraping a surface, possibly with the object of accelerating a flow of liquid. They are much thinner than those of the *Scatophaga*, and not round and tooth-like, but riband-like in appearance. The bases appear slightly rounded, and from these the teeth broaden and taper to a very thin edge—they might be compared to a row of chisels with cleft edges.

To see them a magnification of three hundred and fifty diameters is quite sufficient—a good quarter-inch power would do; the field at the back of the teeth is rather obscured by the chitinous support of the labellum, but if the bases of the false tracheæ are focussed, the characteristic edge of a tooth is probably seen, and that once found, the rest is easy. The length of the row of teeth is three two-hundredths of an inch.—*Knowledge.*

Experiments in Feeding Some Insects with Cultures of Comma or Cholera Bacilli.

R. L. MADDOX, M. D.

On looking over some old slides lately a few were found that related to the results obtained by experiments,made at two different periods in the year 1885, on feeding flies and other insects with cultures of living comma or cholera bacilli. Some of the mounts had been spoiled, being overrun by mycelial thread, the specimens having been mounted dry. Some of the specimens had been lightly stained, others were unstained. As none were figured or photographed, it occurred to me that three or four, if reproduced, might yet be of interest, if they were utilized to illustrate a very brief resume of the two articles published in the numbers for August and December of the Royal Microscopical Journal of the same year. Consequently four of the slides have been selected to illustrate by photomicrographs the following remarks :

The object with which the experiments were undertaken was firstly to ascertain if the comma bacillus was pathogenic to insects when fed in ordinary or diluted cultures on sugar ; secondly, to note if the dejecta contained any of the bacilli in a living state ; and thirdly,to find out if cultures could be made from such excreta.

Of course it was necessary to see microscopically if the ordinary dejections contained any curved bacilli. This was done by retaining such insects in captivity for some time before being fed with the cultures. These cultures, which were originally in agar-agar and gelatine media, had been very kindly given to me by Prof. E. Klein, F. R. S. Wasps, bees, *Eristalis* , the black beetle and the common blue-bottle were chiefly used in the experiments. The bee had to be discarded, as curved bacilli had been found in it by Mr. Cheshire, though I had not noticed any in the evacuations. One wasp was retained in captivity

some time, and seemed to me to be considerably affected by the food, but as two of the four illustrations refer to the blowfly, which is fairly hardy in captivity, one being set at liberty after forty days confinement, and fed for many days on the cultures, the remark will refer chiefly to that insect.

For the main particulars of the experiments I beg to refer to the aforenamed articles in the Royal Microscopical Journal. The insects were generally captured by placing a prepared tumbler over the insect, and then sliding stiff paper or card-board beneath and transferring the vessel to a clean and shallow saucer or plate of glass on which a square piece of glass was placed. This served to collect the dejections passed on it, and was easily removed to substitute another on which a small lump of sugar, dampened with the culture, sometimes diluted, was afterwards placed. The microscopical examinations were made after scraping up the excreta passed at various periods extending even to thirty-six hours and comprising thirty-one dejections, by a flattened needle, and mixing them with sterilized water on a cover-glass. There was one difficulty originated by this plan which I fancy led to many of the experiments being abortive, as many of the dejections were dried up, and the contained bacilli probably dead, or killed sometimes by the high temperature.

Before touching the details it may be as well to state that Dr. Grassi found in 1883 that flies which had fed on the ova of *Taenia solium* that had been kept in alcohol, passed dejections containing the ova; also that others which had fed on the ova of a *Tricocephalus* from a plate in the laboratory, carried and deposited the ova on pieces of paper placed in the kitchen. Dr. Grassi also found they could be carriers of the ova of the thread worm, *Oxyuris*. I think that lately experiments have been made of a more extended nature in the same direction with the plague bacillus, but unfortunately I have no data

to refer to. N. Davaine had also found that flies carry the contagion of infected blood, consequently my experiments only added another possibility to the list. I found that *Eristalis tenax* supported captivity fairly well, and as it breeds in sewers, I expected it might possess advantages for these experiments; but this was not the case, so the common blue-bottle fly was selected as the best, and the following remarks will apply chiefly to this insect. It may however be stated that the natural dejections of the *Eristalis* contained no curved rods, and after feeding on the cultures, only very few were seen in the evacuations. The cultures placed on aniline dyed sugar did not seem to particularly affect them, except to increase the oily globules in the stools. Some were allowed their liberty, while others were killed to examine the perivisceral fluid, when by staining many pale, motionless rod bacilli of four or five joints were noted, also a few rather large rods, but scarcely a curved bacillus could be found.

A female blowfly placed in captivity was firstly fed with sugar moistened with a watery solution of methyl violet for six days, and then seemed extremely feeble. It was then fed on sugar damped with a gelatine culture which though much broken down, contained abundance of commas, but fearing it might be unsuitable, I changed for an agar-agar culture not broken down. The fly at first fed freely on this, but later a male blowfly was also placed under the same tumbler. Both, after feeding off and on for six hours, furnished together six dejections. These, though much dried, furnished well marked, double or S-shaped bacilli, but without movement. The next day the flies were seen in coitus, and a little later the female was found dead. In the perivisceral fluid scarcely a comma could be found. The male was now kept by itself and fed from the original agar-agar culture. The daily examinations of the dejections did not lead to much, until about the seventh day a fair number of the crooked rods

were passed, some with a very sluggish motion, but short and dumpy in appearance. A day later, 18 dejections had been passed in the 24 hours; these contained little colonies of the commas, as well as single and double shaped ones. The perivisceral fluid of this fly was also examined, and in upwards of fifty fields only four curved bacilli were found. It is just possible that the few curved bacilli found in this fluid might have been carried in by the scissors used to make the incisions into the integument.

Another female blowfly was now made the subject of further experiments, as no commas were found in the normal dejections. It was fed on sugar dampened with agar culture that had been inoculated with prepared meat infusion. Curved bacilli being found in the dejections, some motile, an inoculation was made into a prepared meat infusion kept at 90 degrees F.; on the fourth day in four excreta thirty crooked bacilli were found, but three days later scarcely one could be seen. The culture was changed for another similar four days old and used to inoculate a fresh meat infusion, as the former was accidentally upset. This fly was sadly weak on its legs but strong on wing. This fresh culture was used to moisten the sugar.

The fly feeding from this freely, passed three liquid dejections. Only part of these were used to inoculate a clear meat infusion, which gave turbidity after thirty-two hours and yielded both long and short undulating rods, with only a few single commas, which a weak solution of aniline acetate rendered very clear. The fly, although much revived, could not crawl to the top of the tumbler, hence it was fed from the agar culture, and in two days thirty-one dejections were passed; they contained only a few curved rods, but the mixed dejections were used to inoculate a gelatine tube, as I had not yet succeeded in inoculating gelatine from the excreta. The tube was kept at room temperature 65 degrees Fahr.

Two days later there were fourteen evacuations semi-solid and one fluid; seven of the former were mixed with half of the latter and used to inoculate another gelatine tube. The other seven and a half were inoculated into another gelatine tube which was then heated to fluidity and poured out on four sterilized 3x1 slides, covered and kept at room temperature. On the third day these were examined and only one furnished amongst other growths the comma bacilli. The gelatine tube with the seven and a half dejections had on the third day a whitish raised warty-looking growth with no evidence of the track of the needle. This contained crooked rods of all degrees of curvatures even to a complete ring.

An inoculation into meat infusion from the same agar-agar culture when examined was found to abound with similar organisms. This was transmitted through the fly ; at first no curved rods could be found in the dejecta, but later on they yielded a fair number of comma bacilli. After seven days the fly was fed from the meat infusion culture, and passed some of the crooked rods; these were inoculated into a fresh meat infusion and in three days gave an abundance of bacilli, some in zo-ologœa masses, others free and motile.

The fly had now grown very weak, hence it was fed on plain meat infusion, on sugar, on fruit jelly and other things, and quickly regained strength. After having been in captivity forty days, it was given its liberty as no rods were longer found in the dejections. These experiments, troublesome as they were, show I think conclusively that the comma bacillus can be revived after passing through the digestive organs of the blow-fly, but if the dejections be dry, or the rods weakly or scanty, there is no great chance of a revival by the contamination of food, yet if fairly abundant, of strong growth and not too dried up, they may be able to spread disease.—*A. M. S.*

Questions in Regard to the Diphtheria Bacillus.

M. A. VEEDER, M. D.

There is no longer any question in regard to the identification of the diphtheria bacillus. That has been settled beyond dispute. There is much to be learned, however, in regard to its varieties, and their behavior under different conditions in and out of the body, and in association with other micro-organisms, as well as when present alone. For the purpose of such study there are two methods, each of which should supplement the other. These minute forms of life become known to us, not only as they appear under the microscope, but also by the behavior of the diseases which they produce. Thus the questions that force themselves on our attention during an epidemic become a guide for further microscopical study, and it is for the most part questions encountered in this way that it is now proposed to mention.

A very important question is that of the life history of the bacillus in the human throat under various forms of treatment, and without treatment. Since it has become customary with Health Boards to make the duration of quarantine depend upon the results of microscopical examination of cultures of bacilli from the throats of those having the disease and those exposed, there has been a tendency to concentrate attention upon this mode of propagation from individual to individual. There is no doubt that so long as the bacillus is present, even in the absence of all clinical symptoms of diphtheria, there is danger of conveying the disease, and measures of throat disinfection, and immunization of the person, and quarantine, should be persisted in until it is certain that all danger is past. Attention to detail is important in this connection. Disinfectant solutions for use in the throat require to be rightly applied at sufficiently frequent intervals, and in such manner as to reach behind the palate

and into the back of the nostrils, or they will fail no matter how well adapted by their chemical and physical properties to destroy the bacillus. In like manner the antitoxin, if used for purposes of immunization, requires to be of proper strength, and given early. It is safe to say that without the use of such measures, and quarantine of proper duration, diphthera will inevitably spread. But even when all these precautions have been employed thoroughly they may fail to eradicate the disease from particular localities. In other words the growth of the bacillus in the throat, whether in typical or atypical forms does not account for the manner in which diphtheria sometimes remains endemic in a particular household or neighborhood in spite of quarantine and throat disinfection. A very notable instance of this sort was reported at the Montreal meeting of the British Medical Association last year, and again at the American Public Health Association at Ottawa this year. In this case diphtheria has continued to recur in a state school in Minnesota at frequent intervals for ten years in spite of the most elaborate precautions. In the British Medical Journal for April 16th, 1898, at page 1009, it is stated that an atypical variety of the diphtheria bacillus, supposed to be the cause of the trouble in this school, was found to be confined strictly to inmates of the institution, with one exception of 24,000 examinations. In other words there was no endemic prevalence of anything of the sort in the town adjacent, or anywhere else in the state, so far as was known, except in this particular school. Presumably antitoxin, throat disinfection, and quarantine, were all employed with thoroughness commensurate with the interest that such a state of affairs, and its wide publication, would arouse, and yet the disease continued to recur.

It would seem evident in such a case that there must be some other method of propagation of the bacillus than in human throats, and that the culture medium, whatever

it may be, must be located somewhere on the premises, harboring and perpetuating the infection so that when destroyed within the body of every inmate, reinfection from without becomes possible again. The growth of the bacillus in media external to the body might very well originate atypical forms. But be this as it may, the writer as health officer and practising physician, has repeatedly been brought face to face with this very question as to the life of the diphtheria bacillus outside the body. As a rule when the disease has given evidence of a tendency to recur in a particular house or neighberhood it has been possible to find somewhere about the premises an accumulation of material obviously adapted to serve as a culture medium for this particular bacillus, and so situated that effluvia from it would surely gain access to those very persons who contracted the disease.

In any such case it is, as a rule, difficult to secure pure cultures of any particular bacillus that may be in question. The varieties present are more numerous than in the cultures from the throat so that the one wanted is lost in the crowd, and there may be admixture of much extraneous matter, if direct inoculation of the culture medium is attempted, so that it is difficult to get conclusive evidence. Thus far the best evidence attainable has been the immediate and complete disappearance of the disease, when the proper source of the trouble has been identified, and effectual measures for its removal by disinfection, or otherwise, have been adopted.

Still it is possible that definite information in regard to the life of the bacillus outside the body may be had experimentally. It should be determined for what length of time the bacillus remains alive not only in a single culture, but also in a succession of cultures, transferred from one to another. This may be done with the various media ordinarily employed for such purposes, or with saliva, or pus, or mucus, or other secretions from the body,

under varying conditions of temperature and moisture. Thus the development of atypical forms and changes in the virulence of the bacillus due to its mode of life outside the body may be detected by such a succession of cultures starting from a single one.

This is the laboratory side of the question, as yet unworked, except in desultory and fragmentary fashion. Leading up to it from the side of the practical work of the health board, is the identification of such culture material, and its proper disinfection, or destruction. In the experience of the writer a drain pipe that is rarely if ever flushed completely, and that is crusted over on the inside with partly dried filth is specially apt to form a medium for the retention and growth of successive crops of the diphtheria bacillus. Inoculation may occur in various ways, a little expectoration, rinsing the mouth at the kitchen sink, for example, may start the process. The bacilli implanted in an underground drain, or other receptacle that is constantly nearly dry, and never completely flushed, find these conditions very suitable for their growth. The temperature and moisture, and fresh accessions of organic matter from day to day are well adapted to bring about a series of cultures resembling substantially those from tube to tube suggested in the last paragraph. In such a case disinfectant solutions may run along the bottom of the drain leaving the top and sides untouched. Indeed in the case of a very large drain of this sort the writer found it necessary to generate chlorine in order to disinfect it completely. During continuance of infection there is constant liability of its diffusion by the partly dried material becoming detached and carried by the vapors arising from fermentation, or by access of air currents. An instance of this sort that came under the observation of the writer was in connection with a dry closet system, so-called, in a school building. The vaults containing the partly dried excretions

were in the cellar, and were cleaned only once or twice a year and never disinfected. Under these conditions an outbreak of diphtheria among the children appears to have been brought about by this material in the cellar becoming infected perhaps by particles of partly dried mucus containing the bacillus being carried down through the ventilating flues which were built so as to pass through these vaults. Infection once accomplished propagation of the bacillus on a large scale would ensue on the plan of plate cultures, there being accessions of fresh material suitable for the purpose daily. This being the case it would need only some failure of the ventilating apparatus to allow the vapors arising to find their way into the rooms most distant from the main ventilating shaft and it was in these rooms precisely that the disease occurred and spread. An effort was made in this case to secure cultures, but the difficulty was that the bacterial flora was too abundant, and the particular bacillus sought was lost in the crowd, as in other experiments of the kind with drain pipes and receptacles having the peculiarities indicated.

A very important point in connection with such prevalence of diphtheria as has just been indicated, is the occurrence simultaneously of much ordinary sore throat so-called, in which the usual form of the diphtheria bacillus appears to be wanting. It has occurred to the writer that some atypical variety of the bacillus, of greatly attenuated virulence, through a succession of cultures outside the body, may be responsible for this form of throat trouble, often spoken of at such times as sympathetic sore throat. I would regard this form of the disease, in connection with an outbreak of diphtheria, as clear evidence that it was becoming endemic in the locality; in other words, that cultures outside the body were in progress somewhere in the vicinity.

Mixed infection, or the association of diphtheria bacilli

with streptococci and other micro-organisms, is of great interest because of the increased danger to life, and because it may serve to explain at times the failure of the antitoxin which does not protect against other toxins than that of diphtheria. But these are points of interest to the practicing physician rather than the microscopist.

The fact that the diphtheria, like many other diseases, spreads in waves over extensive portions of the earth's surface, increasing very largely for a year or two, and then subsiding for a series of years, is usually referred to meteorological conditions modifying the virulence of the bacillus itself, or modifying the conditions on which its virulence depends. It may, however, be a question for study by the methods of modern microscopy. It is possible that the products of the bacterial activity may inhibit the growth of these organisms, in and out of the body, on a scale large enough to be evident at a glance in the statistics showing their epidemic prevalence. In other words even when practically left to themselves, as is the rule in many parts of the world, they do not increase indefinitely but exhaust the material susceptible to their attack, and perhaps in a measure originate their own antitoxines. In either case it is a question to be determined by the culture methods of the bacteriologist and microscopist, identifying atypical and modified forms of the bacillus, and their relation to the severity of the disease in particular cases, and its epidemic prevalence in general.

Another exceedingly interesting series of questions is as to why the bacillus attacks children in preference to adults, and certain tissues and parts of the body in preference to others. Considerable light has been thrown upon these very difficult and obscure phases of the subject by modern methods of study of embryology and comparative anatomy, bringing out what may be termed the developmental relations involved. This is the

special field of the histologist and microscopist, and it is likely to be exceedingly fruitful in the near future. Comparative pathology is the outcome; this is just beginning to be recognized as a part of the medical curriculum and is likely to answer many questions along the lines just indicated in this paragraph.—*A. M. S.*

The Detection of Blood by Means of the Guaiacum Reaction.

EDW. SCHAER.

The importance of a trustworthy means of identifying blood or blood constituents like hemoglobin, methemoglobin and hematin in numerous judicial cases and the value of a combination, on many rather difficult occasions, of the spectroscopic test and of the methods of preparing the characteristic crystals of hemin (as described by *Teichmann*, *Hoppe-Seyler*, *Brucke* and *Preyer*), with the "ozone-transferring" action of the coloring matter of blood towards guaiacum, has induced me, for many years to pay special attention to the last-named blood-test.

While others have mainly recommended to mix the blood solutions—(obtained by extraction of a fresh or old blood-stain with small quantities of water, either alkaline or acidulated with acetic acid)—under suitable conditions first with an alcoholic guaiacum solution and then with transferable oxygen in the form of hydric peroxide or of the analogous compound contained in old and isolated turpentine oil (for instance, the liquid of Hunefeld, v. i), and to observe the formation of the so-called "guaiacum blue," the method which I have proposed aims at the preparation of an intimate and durable mixture of the coloring matter of blood derived from the blood stain with guaiacum resin. This mixture may be conserved for an indefinite time as a "corpus delicti," and at every moment strikes a very intense blue color by contact with one or another of the liquids containing loosely combined

oxygen, provided that errors are cautiously avoided by check experiments. The proceeding, wanting but a short explanation in reference to the more explicit description in the paper quoted, chiefly consists in mixing the blood solution, obtained by extraction of blood stains, with an alcoholic solution of guaiacum resin (or, as it has been lately proposed by *O. Dobner*, with a similar but weaker solution of guaiaconic acid.) It is preferable, in this case, to use a solution of about 5 per cent of resin instead of the alcoholic guaiacum tincture (1 to 2 per cent) mentioned by Schonbein as the common reagent, in order to secure an easy secretion of resin in presence of small portions of blood. In this case a milky secretion results of the previously dissolved resin constituents, which, in these conditions, partly attract, fix and precipitate at the same time the dissolved or suspended coloring matter of blood (either in the state of hemoglobin and methemoglobin or of hematin). In this way a mechanical combination of the secreted resin with the said blood constituents is formed, which process reminds us in some way of the well-known method by which some ferments, lik pepsin, are secreted by means of an indifferent precipitate caused in the ferment solution and afterwards extracted. If, then, we separate the precipitated resin (or the above-mentioned constituent acting as reagent) by thoroughly dense filters (especially the newer "hardened filters" of commerce), the hematin compounds are fixed on the surface of the filter in extreme division together with the particles of resin. These filters, when well protected from light and air even during the filtration process and then cautiously dried in the exsiccator, may then be conserved for any length of time. But a small piece of them is wanted, to cause in a few moments an intense blue coloration in a porcelain dish or watch glass on white paper, after it has been moistened with a little spirit of wine, and then a small quantity of Hunefeld's

liquid (mixture of so-called "ozonized" turpentine oil with alcohol, chloroform and a little acetic acid) has been added. This process is equally applicable to bloodstains, and to the detection of blood in urine, and other similar objects, and may be used as well for the research of relatively fresh blood, as for that of old dry stains, owing to the fact already observed by *Schonbein*, viz., that the coloring matter of blood altered by exsiccation even in higher temperatures still shows in unimpaired degree the different "ozone-transferring" properties, and even seems to act more intensely in some respects, for instance, towards a mixture of peroxide of hydrogen and cyanine.

Since the publication of this modification in the methods of detection of blood by means of guaiacum, some observations on different points of solubility of the red-colored blood constituents, especially in dry blood, have taken place, which lead to new propositions concerning a reliable, very short and direct way for the detection of blood, and therefore may be communicated in this Journal, after having been briefly related in the pharmaceutical section of the annual meeting of German naturalists at Brunswick in 1897. By occasion of former studies and experiments on the physical and chemical behavior of chloryl hydrate, a special solvent power of highly concentrated, that is to say, 65 to 80 per cent aqueous solutions of the said compound has been observed, not only for several bodies already known, like starch, but also for various very different substances,like certain resins, coloring matters, stearoptenes and also albuminous matters, especially the coloring matter of blood. In fact, experience showed that blood stains which have become dry even for a long time on linen or other similar materials are extracted in a relatively short time by impregnation and contact with a chloral hydrate solutirn of about 70 per cent and more thoroughly dissolved than by any other treatment. Even blood stains

many years old may, by this operation—after a somewhat longer contact with the solution—be removed to such a degree that their trace is but hardly discernible on the linen. It may be observed on this occasion that the solution of the blood constituents by aqueous chloral hydrate is much facilitated if the blood spots have been previously wetted with small quantities of concentrated acetic acid. The use of this acid is not only admissible for itself, as the guaiacum-blue is not affected by it, but even offers a certain advantage concerning a reaction of control to be mentioned later on.

Inasmuch as guaiacum resin, as well as the guaiaconic acid, specially concerned in the formation of "guaiacum blue," are both easily soluble in the concentrated chloral hydrate solution, a simple method may be devised for the extraction of blood stains and the subsequent detection of the coloring matter of blood.

In fact, the guaiacum blood test can be prepared and managed on the simplest terms in this way, that first the colored spots in question, after moistening with a little acetic acid, are extracted either with a 70 per cent chloral hydrate solution or directly with a 1 per cent solution of guaiacum in aqueous chloral hydrate, containing 70 to 75 per cent of the latter. In regard to the fact that the resin constituents concerned in the subsequent reaction show a marked tendency for spontaneous oxidation—as is sufficiently proved by the well-known change of color in the air and light—this latter method, on the whole, seems less preferable than the first named, which consists in *first extracting the blood* by means of chloral solution and then *adding to the resulting blood solution* about an equal volume of the guaiacum chloral solution. If in this process the blood stain has been moistened with acetic acid previous to the treatment with chloral hydrate, the addition of guaiacum chloral solution to the chloralic extract of the stain to be tested for blood will permit a

control reaction, inasmuch as the casual presence of nitrites (as, for instance, nitrite of ammonia) in the respective stain would at once cause a more or less intense blue coloration of the mixture owing to the decomposition of these salts by the acetic acid, the nitrous acid coloring guaiacum, viz., guaiaconic acid blue even in high dilutions. Moreover, if the chloral extract of the stain contains only blood, the addition of the brownish-yellow guaiacum chloral solution to the pale red liquid derived from the stain will give a pale brown mixture exceedingly well adapted for a decisive zone reaction indicating the presence of hematin. To this blood guaiacum solution in aqueous chloral hydrate a stratum of the already mentioned turpentine solution of *Hunefeld* or of an adequate solution of hydric peroxide (*the indifference of which towards guaiacum tincture being previously stated*) is carefully added without mixing; then an intensely blue and rapidly increasing zone appears, with extraordinary sensibility, in the place of contact and diffusion of the two solutions, while by sudden mixture a less pure blue coloration of the liquid results. The method may also, in suitable cases, be so modified that the blood solution is first mixed with *Hunefeld's* liquid and then added to the guaiacum solution. This process can just as well be conducted to obtain a zone reaction or also a capillary reaction.

In cases of extraordinary small blood stains, so as to necessitate, in a certain measure, a microchemical operation, or where the respective residue of blood has to be tested on its natural place, it is advisable to digest the stain on a flat porcelain dish with strong chloral solution (v. s.), having first moistened with a small drop of acetic acid, and, after an hour's contact, to pour on the digested spot first a corresponding small quantity of guaiacum chloral solution, and then, after having thoroughly mixed, a few drops of the one or other liquid containing the

peroxide. With this method also a more or less intense blue coloration is seen to appear on the light-colored underground. Experience has shown me that even very old blood stains and exceedingly small parts of such may be identified in this manner, provided a sensible guaiacum chloral solution, prepared with quite fresh resin, and at the same time a liquid of *Hunefeld* or hydric peroxide solution of right composition and controlled in regard to their activity are used. A solution of *Hunefeld* suitable for the purpose may easily be prepared, mixing f. i. 15 c. c. of turpentine oil, exposed to light and air for a certain time (but which ought not to change directly blue in the guaiacum tincture), or 15 c. c. of a 3 to 5 per cent hydric peroxide solution, free from acids, with 25 c. c. of alcohol, 5 c. c. of chloroform and 1·5 c. c. of glacial acetic acid. The zone reaction surpasses the other older methods of testing by special purity of the blue color resulting from the formation of the so-called "guaiacum blue;" besides that this purer color is more durable, according to the fact that in the measure of progressive mixation of the active substances of the two layers (hematin as the oxygen-transferring body, hydric peroxide or essential oil as the source of oxygen and guaiaconic acid as the oxidable compound) small quantities of the intensely blue colored oxidation product are formed gradually. It is true, however, that the "guaiacum blue," especially in the presence of organic reducing agents, is not very stable, inasmuch as this compound, to use an expression of its first investigator, *C. F. Schonbein*, contains loosely combined, movable and active oxygen in the ozonide state.

The foregoing shows, as it seems, the usefulness of the modified guaiacum blood-test when cautiously applied; on the other hand, it cannot be denied that the reaction is liable to certain misinterpretations, in cases where some other organic or inorganic substances are present, instead of blood. It is scarcely necessary to mention in this place

the numerous compounds which, as, for instance, nitrous acid, free chlorine, bromine and iodine, chromic anhydride permanganic acid, peroxide of lead, the ferric and cupric salts, quinone, etc., directly color blue the guaiacum resin ; because first of all, many of these bodies are exempted *a priori* in the majority of materials submitted to the blood-tests, and secondly, because in their presence the liquid extract of a stain to be tested for blood would at once strike a blue color when mixed with a little guaiacum tincture *before the addition of Hunefeld's peroxide-solution.* In regard to these facts, the somewhat superficial notice of some text-books, viz., that the guaiacum blood-test is not reliable, "because many substances change guaiacum for themselves," cannot be taken as a warning against the use of the said method, since, certainly, no careful analyst will ever neglect to avoid mistakes by availing himself of the control-reactions indicated in each case! Yet, such substances of inorganic or organic origin, as share the "ozone-transferring" quality with the contents of the blood cells, viz., the coloring matter of blood, might in some single cases lead to a false interpretation of the guaiacum-blue-reaction. Among organic vegetable substances, bodies of the class of ferments may be named, as well as hydrolytic ferments (enzymes in the stricter sense of the word) chiefly so-called oxidizing ferments, as they occur in numerous parts of plants, especially in mushrooms and plant seeds, while among animal substances in the first line saliva, extracts of some organs, the contents of white blood cells and pus cells, etc., show analogous properties. These albuminous substances of the character of ferments, existing in vegetable and animal cells, and exerting in a more or less manifest degree a catalytic and, at the same time "oxygen-transferring" action towards hydric peroxide, strictly differ from the coloring matter of blood in that the last-named action is cancelled, or at least most strikingly

weakened by heating to 100° C., or also by contact with diluted hydrocyanic acid. In case the extract of a pretended blood stain contains such a substance of the class of ferments, instead of ingredients of blood, it will cease to show the guaiacum-reaction, even after a shorter digestion at the temperature of the water-bath, and also a control-experiment *with addition of hydrocyanic acid during the extraction* of the stain will give essentially negative results.

However, the avoiding of all possible mistakes becomes rather difficult in such cases, where the presence of even the smallest quantities of ferrous oxide or other ferrous compounds can occur, as, for instance, in the testing of suspected stains on rusty iron materials. If the rust, even in the absence of blood, contains small portions of certain ferrous compounds, viz., ferrous carbonate or other ferrous salts, they could, by extraction, be introduced into the filtered solution, even in case the latter had not taken up any ferric hydrate or basic ferric salt; yet such an extract of a stain, even with the slightest trace of ferrous oxide, would cause the guaiacum-blue-reaction after subsequent addition of guaiacum resin and hydric peroxide. A strict distinction of ferrous oxide and of the coloring matter of blood is not very easy in such cases, because the first-named compound manifests, even in the smallest quantities, the same intense "ozone-transferring" power as hemoglobin or hematin, which also contain iron. It will, therefore, form the object of further experiments to find out how the mentioned casual mistaking in the guaiacum-blood-reaction may be eliminated. On occasion of such further researches concerning the reaction discussed in this paper, the question would have to be treated, whether blood, which, after drying up in slow decomposition on certain materials, like stone, clay or rough metallic surfaces, and after disappearance of the organic substance by the action of air and water, leaves but rusty spots,

can generate in these conditions seizable quantities of ferrous compounds.

The above-described method of extraction of blood stains with concentrated solutions of chloral hydrate which, as is well-known by this time, are also good solvents for resins, has induced some experiments in order to ascertain whether, by using the process mentioned in the first part of this essay, chloral solutions containing blood and guaiacum may, by precipitation with water and subsequent filtration, give a resinous secretion containing blood constituents, showing the mentioned behavior and applicable to the guaiacum-blood-reaction after any time of conservation. It may here be stated, by the way, that the trials performed in this manner have but led to a moderately satisfactory result, probably because even a diluted chloral-solution still acts as a solvent on the resin in a low, but perceivable degree and besides, as I am induced to believe, because the coloring matter of blood is less easily precipitated by the secreting resin from a chloral-blood-solution than from a chiefly aqueous liquid. But, notwithstanding the loss of material caused in that way, by the use of this method resin-covered filters can be obtained possessing the properties quoted in the begining of this paper.

Lastly, it may be mentioned that—as it could be expected—the guaiacum blood-test executed with chloral solution is thoroughly applicable to a control-reaction, viz., to the chemical identification of the hemin-crystals, which are of high importance in judicial cases. A specially pure blue coloration is obtained, when, instead of the ordinary guaiacum-solution of guaiaconic acid in 200 to 500 parts of chloral-solution (v. s.) is used, and the reaction is observed in a glass tube as a zone-reaction. The guaiaconic acid, proposed as a substitute for the natural resin by *O. Doebner* in his interesting essay on guaiacum resin and "guaiacum-blue," is just as well liable to spon-

taneous oxidation in light and air with changes of color; and, according to my observations, its use is more convenient for the described zone-reactions than for experiments in watch glasses or dishes, where greater surfaces get into action. It is, moreover, obvious that this special experience cannot interfere with the certainly desirable use of the guaiaconic acid, as being the active constituent of the resin, in the numerous other guaiacum-reactions. I connot but feel convinced that the reactions with guaiacum resin have not, in all respects, met with the consideration they deserve, neither in general nor in medical and pharmaceutical chemistry, so I thought it advisable to publish this little contribution to the question in this convenient place.—*American Journal Pharmacy.*

STRASSBURG (GERMANY) PHARM. INSTITUTE UNIVERSITY.

Method for Preparing Nucleated Blood in Bulk for Class Demonstration.

T. E. OERTEL.

No book on microscopical technique which I have been able to consult gives a method for preparing blood in bulk. For class demonstration it is obvious that by having on hand ready prepared material the work will be greatly facilitated and a uniformity of result assured which could not be expected from the faulty manipulations of untrained students to whom blood is usually given for study early in their histological course.

It is much more convenient for the teacher to dispense his preparation from a small vial than to be compelled to make "smears" for a large class. "Smears" are also often unsatisfactory by reason of agglutination or crenation of the corpuscles, excess of serum and the formation of fibrin and much care is required in their proper fixation, by the usual method of heat, in order that the result be not disastrous.

These considerations led me to try and work out a method which would allow of the staining and keeping of nucleated blood in bulk ready for distribution to the class and so fixed that there should be but little distortion of the corpuscles.

The red blood cell is a delicate structure and some care in its manipulation is required.

If the steps of the method are strictly followed one may be confident of a successful issue.

Chloroform the animal selected ; a large frog is probably the most convenient ; open the thorax, puncture the aorta and allow the blood to flow directly into a small glass jar, with ground glass stopper, containing a one per cent aqueous solution of osmic acid. The solution should be largely in excess of the amount of blood, at least fifty times as great. The vessel is now closed and set aside for several hours in which time the blood cells will have become thoroughly fixed and hardened and have settled in a thin layer at the bottom.

Decant the supernatant fluid and add distilled water, gently agitating the vessel until the blood is thoroughly mixed with the water. Again decant after sedimentation has taken place or filter rapidly through very thin filter paper and wash off the filtrate in a small quantity of distilled water.

Next add Bohmer's haematoxylin diluted one-half with distilled water. Use no more of this mixture than enough to promote quick and thorough admixture with the water containing the blood. After a few moments staining filter as before, wash the filtrate from the paper by agitating in a large dish of distilled water and set the vessel aside for an hour or more in order that the nuclei of the cells may be well differentiated.

Dehydration is now accomplished by running the blood through various strengths of alcohol beginning with 70 per cent and ending with absolute, filtration or decan-

tation being practiced with each step. Care must be taken not to use too small a quantity of alcohol or the cells will not be well dehydrated.

Clear in carbol-xylol (carbolic acid one part,xylol three parts), allow the blood to settle in a large test tube or conical glass, draw off as much of the fluid as possible with a bulb pippete and add thin xylol balsam.

Keep in a well stoppered bottle and when wanted for use shake until the blood is thoroughly mixed with the balsam, with a small glass rod transfer a drop to a clean slide and superimpose a cover-glass. A neat and permanent preparation is the result.—*A. M. S.*

MICROSCOPICAL APPARATUS.

The Power Limit.—S. B. Twitchell (Ohio Med. Journal, January 1, 1898), discusses the resolving power of the microscope. Up to the present, Nobert's twentieth band,225,-190 lines to the inch, has never been resolved,and theoretically, with white light, only 146, 543 lines per inch can be distinguished. By utilizing, however, the shorter actinic rays and a photographic plate, theoretically 193,037 lines per inch should be resolved, that is effects beyond the possibility of ocular vision.

MICROSCOPICAL MANIPULATION.

To Cut Cells in Glass Slips.—A hole is bored through the glass very easily with a piece of copper tube running in a lathe, the end of the tube resting on the glass. The tube must be charged with emery powder and oil. A thin cover glass is then cemented on the slip, over the hole,with gold size, or ordinary gum may be used for this purpose and answers very well. I often confine a live insect in this way, and view it at both sides with a Lieberkuhn mirror or a side reflector. In this case I pasted the cover on very lightly with gum. Most insects possess a beauty when viewed alive which they lose after death.

Paste For Labelling on Tin.—The tin surface is first coated thinly with shellac after which there is no difficulty in making labels adhere. If a coating of shellac is then applied over the label, in which case white shellac should be used, the label may be washed.

To Determine the Genuineness of Ivory.—When put in concentrated sulphuric acid for twelve or fifteen minutes genuine animal ivory retains its white color, while vegetable ivory, from the phytelepas macrocarpra, assumes a pink color, which may be removed by washing with water.

How to Make Paper Waterproof.—The sheet is coated on both sides with a solution consisting of one part gelatin, four parts water, and one part glycerin. When dry, the paper is immersed in a 10 per cent solution of formalin. After this treatment the paper is said to become impervious even to steam.

BACTERIOLOGY.

A New Stain for the Bacillus Tuberculosis.—In view of the fact that Sudan III has been found a useful stain for fat in histological and pathological work, Marion Dorset has applied it to the tubercular bacilli with excellent results, as demonstrated by the excellent cuts with which his article is illustrated. The technique is carefully described in N. Y. Medical Journal, Feb. 4, 1899.

Mosquitoes and Malaria.—In the Annales de l'Institute Pasteur for February, 1899, Ronal Ross, of the Indian military service of Calcutta, presents the results of a lengthy series of examinations on this question. He states that the plasmodium may be found in the stomach of the mosquito; that it can grow there and can develope what have been called spore-threads. These may also be found in the poison sac of the mosquito, and from this may be conveyed to man in the sting. He thinks he has been able to follow the cycle throughout its various stages. He has been unable to show, by any yet developed technical procedures, the spore-threads in the poison sac, nor has he

been able to make preparations which definitely stain the plasmodia in the mosquito's body. He further states that the parasite of Texas fever and other fevers of Africa and other countries may be conveyed by other insects.

Staining the Capsules of Friedlander's Bacillus.—By Dr. J. J. Curry, (Jour. Exp. Med., IV., No. 2, p. 169). For staining the capsules, both in cultures and in cover-glass preparations made from the organs, the following method was used:

1. Cover the preparation (prepared without contact with water) with glacial acetic acid for a few seconds.
2. Wash off the acetic acid with a 1-per-cent solution of potassium hydrate.
2. Stain with aniline-gentian-violet solution for one minute without previously washing off the potash solution.
4. Wash off excess of stain quickly in water, dry thoroughly with filter-paper and over low flame, and mount the balsam.

If the specimen is stained too deeply it may be decolorized by washing lightly in ½-per-cent solution of acetic acid.

MEDICAL MICROSCOPY.

Tumor.—The committee subjected to microscopical examination the tumors from the scalp and the larger tumors of the skin of other regions in the well-known case of Mike Kelly, of Bellevue Hospital. These growths had all shown substantially the same characteristics, viz., that of a highly cellular fibroma, somewhat resembling fibro-sarcoma, but having a blood supply which was hardly sufficient for a tumor of the latter class. In the small nodules from the small intestine the pigment had been found lying between the peritoneal and muscular coats, and not at all within the cells of the tumor growth proper. These tumors had been slightly different in some respects from those on the external surface of the body, but essentially the same in structure. They contained a good deal of involuntary muscle, apparently derived from the coats of the

intestine. These growths were made up of connective tissue containing a very large number of connective-tissue cells, some of these cells being apparently proliferating. No evidence of neuroma or of connection with nerve fibres were found. Sections of the tumor tissues were exhibited under the microscope at New York Pathological Society, May 10, 1899.

MICROSCOPICAL SOCIETIES.

Royal Microscopical Society.—At the last meeting, Mr. E. M. Nelson, the president, exhibited an old ⅛in. objective, made by Andrew Ross, which had been presented to the Society by the Master of the Rolls. It was a very rare form of objective, constructed probably about the year 1838, and possessed a very primitive form of lens ajustment. A special interest was attached to it because it formally belonged to the father of the donor, Prof. John Lindley, the second president of the society (1842-1843). The president also exhibited a new coarse adjustment which Messrs. Watson had made in accordance with a suggestion contained in his paper, read before the society in March last. It showed that, with a loose pinion, it was possible to have a rack coarse adjustment that would work without "loss of time." A paper by Mr. Jas. Yate Johnson, entitled "Notes of Some Sponges Belonging to the Clionidæ, obtained at Madeira," was taken as read. Six slides of speculæ, &c., in illustration of the paper were exhibited under microscopes. The President called the attention of the Fellows present to an exhibition by Mr. Beck of parts of various wildflowers shown with low powers. This was the last meeting of the session, and the president announced that the first meeting after the vacation would be on October 18.

NEW PUBLICATIONS.

Transactions of the American Microscopical Society.—Edited by Henry B. Ward, Lincoln, Nebr. This is Vol.

XX of the series and, issued in July, 1899, relates to the meeting of August, 1898. It is rather larger than the previous volumes and contains much matter not read at the meeting. The meeting itself was unfortunate in the loss of its president and sickness of the vice-president. The secretary was also kept away by sickness. Indeed, this is the first we have heard that any meeting occurred. The volume contains a lot of bibliographical matter and biological classifications which could not be published in any other way and which will be of much value to a very small number of persons. The matter of general interest to microscopists is perhaps not so great as sometimes but all in all the volume is a useful contribution to scienfific literature. We trust another volume may be collected during the coming year whether there is a good meeting or not. It is intended to hold a meeting August 17, at Columbus, Ohio, just prior to the meeting of the American Association for Advancement of Science.

Urinary Analysis and Diagnosis by Microscopical and Chemical Examination. By Louis Heitzmann, M. D., Pp. xvii—253. 108 illustrations. New York: William Wood & Co., 1899. The feature of this work that commends itself in particular to the practitioner is the stress it lays upon urinary microscopy. Chemical uranalysis, indeed is not neglected, but the bulk of the volume is devoted to microscopic examination as a means of diagnosis. The concluding part of the book, which is devoted to this sort of diagnosis, is a model for clearness and for straightforward presentation. The text is enriched with many illustrations which have been prepared from drawings. Without exception they are clear and instructive. At first sight it is hard to imagine the claim that a new work upon uranalysis should have to our recognition, for in a few branches of medicine are there so many excellent works already, but we must confess that in Dr. Heitzmann's book we find much to admire and a good deal that the others do not contain.—Sci.-Gossip.

MICROSCOPICAL NOTES.

Algæ.—Mrs. H. M. Jernegan of Edgartown, Massachusetts, furnishes mounted and pressed sea mosses for scientific collections.

Objectives.—Dr. Pierce Tyrrell who has examined hundreds of dollars worth of objectives for the late W. H. Bullock and others corrects his article on page 239, fourth line, from "tubes and casts" to "renal tube casts." He says that his Abbe condenser is anything but achromatic.

Microscopical Preparations.—Mr. Abraham Flatters, of 16-18, Church Road, Longsight, Manchester, has new catalouges of his well-known microscopical and lantern slides. The catalogue of microscopical slides includes both botanical and zoological susjects, and we would call attention to a series of 48 slides, especially arranged to meet the requirements of pharmaceutical students, and sold at the very moderate price of a guinea. We have had an opportunity of examining these, and found them uniformly good, while some are really excellent, such as slides showing karyokinetic division in developing tissue, a section of the root of Phajus grandifolius, showing cell contents, sections of the male cone of Pinus silvestris, and of the fertile spike of Selaginella martensii. Many of the sections are double stained, and one of potato is worthy of notice as being stained with Mr. Flatters' "Gossypimine" stain, which differentiates starch granules most beautifully, and appears to be practically permanent. The stain itself can be obtained from Mr. Flatters direct, as well as other mounting stains, cements, and requisites, amongst which we may mention a new elastic black cement for finishing slides with one ring. The catalogue of lantern slides represents very fully zoology, botany, geology, physical geography, and such special subjects as insect metamorphoses, evolution, mimicry, etc. It is unusually complete. The slides, both lantern and microscopical, are sold at the modest price of 6s. per dozen, and are in no way inferior to those at double the price elsewhere.

Trap Ravine Near O'Rourke's Quarry Where Diatomes Are Found.

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CONTENTS.

The Existence of Bacillaria in the Basalt of New Jersey.

ARTHUR M. EDWARDS, M. D., F. L. S.

With Frontispiece.

I write to emphasize the finding of shells or lorica of Bacillaria in the trap rock of New Jersey, the grey almost black, solid, hard rock of the Orange mountains, since they have not heretofore been recognized in trap rock anywhere. Incidentally thereto I will refer to the origin of the trap rocks for I think that they have been incorrectly referred to an earlier age,—the Newark. It

was seen to be igneous, and melted by volcanic heat, then cooled to rock. Fossils, (organic remains) were not looked for.

I have studied the rocks of the Palisades, which come up on the right bank of the Hudson river. They appear as a perpendicular crest two hundred feet high and make a very striking feature to the accompanying scene when viewed from vessels on the river. This ridge runs from below Stoney Point, N. Y., to a point near Jersey City, N. J. Farther south, it appears again on Staten Island (where the Rahway river empties) and disappears. This forms a first range of mountains. The second range is the Wahchung or Orange mountains. The third range is parallel to it but has not assumed a local name. They appear to be of the same rock notwithstanding.

The Palisades are known to be intrusive traps, that is to say were thrust INTO the Newark sandstone. The others are extrusive, being poured out UPON the Newark sandstone. They were both forced up from below in a melted state and I doubt their being formed in the same period as the Newark sandstone.

In boyhood I went with Charles M. Wheatly for minerals to where they were driving the old Harlem Railroad tunnel through the granite and the Erie Railroad through the Palisades. Besides gathering minerals I saw the trap rock in the Palisades was polished and grooved by ice most likely, and the grooves came from the northwest. The volcano trap was marked by big haxagonal crystals often more than three or four feet across and stood up perpendicular. This can be seen on the Hudson River, and better still at O'Rourke's quarry in Orange, N. J. On the side of the gray trap ridge is the green serpentine as seen at the old Elysian Fields and on the west side is the red Newark sandstone. Then I went to dwell upon the red sandstone, the Juro-triassic of Dana, the Newark of Russell. I was near the trap and could

often get to it by trolleys or by walking. In O'Rourkes' quarry there is the best development of trap crystals. The silica is also in crystals, amethyst, smoke quarts and calcedony and opal. When studying what looked like a vein of quartz that is found in Orange I came across a bed of clay that looks as if it had been washed down the rocks from above. The apparent vein is perpendicular and over a foot in diameter. It is pulverulent and through it are the crystals of amethyst and smoke quartz. I easily dug it out,as the quartz is pulverulent and crushes in the hand. The quartz is deposited from solution in water and then forms crystals of amethyst and other things. The vein or fault being thus left open by the forcing of the sides apart may be filled from below or above. In this case it was not filled from above because only a thin layer of gravel was there. It cannot be filled from below by the forcing upward of the Newark sandstone for it contained different Bacillaria that are found in other rock above or below. Now this clay, which was stained reddish with iron, I dug out, took home, examined and washed it with ammonia in the usual manner. In it were Synedra ulna, Melosira granulata and others more scarce with spicules of Spongia. The Synedra and Melosira were distinct although not common. The clay had been deposited in fresh water, and had been laid down most likely in a lake. The Bacillaria in the clay of the trap rocks of New Jersey are thus established.

The trap rock was of volcanic origin melted into a tar-like substance and ejected upwards through the Newark sandstone. The clay in the faults, the accompanying silica, the chalcedony, smoke quartz and opal point to water, (not very hot) being present. Most likely the crystals or trap were formed in the melted rock which solidified. But it looks as if it was erupted after the Newark sandstone was formed, in fact after the mud that formed the sandstone began to be made into solid rock.

This seems to be the genesis of the trap, and the Bacillaria were formed after the trap, perhaps in the Eocene age.

Meeting of the American Microscopical Society.

COLUMBUS, OHIO.

This society was formed about twenty years ago by persons who had broken up the microscopical section of the American Association for Advancement of Science and then launched a separate organization. It has until this year appointed its meetings in cities contiguous to where the parent society was to meet. All this time, people of wisdom have known that separation was very unwise and have urged the society to go back to affiliation with the A. A. A. S. This year for the first time it has done so. It took the complete failure of the meeting last year to bring the Society to its senses, but we congratulate it upon the spirit of wisdom which appointed the meeting at Columbus. From local papers we gather the following account of what took place there.

Thursday August 17.—A meeting of the executive committee was held in the Park hotel, devoted to preparation of a report to the society, which met in the afternoon in Biological Hall, at the State university. The visitors were welcomed to the city by Dr. W. O. Thompson, president of the university, and the response was made by President Krauss. Secretary Ward presented the annual report, following which papers were announced to be read and discussed as follows, but only two were presented.

"A New Spencer One-twelfth Objective," by Henry R. Howard of Buffalo.

"An Apartment Incubator for Student Use," by Veranus A. Moore, Ithaca, N. Y.

"Experiments in Antisepsis," by P. A. Fish, Ithaca, N. Y.

"Some Essential Methods for Young Laboratory Instructors in Bacteriology," Raymond C. Reed, Ithaca, N. Y.

"The Methods Employed in a Study of the Chiasma of Bufo vulgaris," by B. D. Myers, Ithaca, N. Y.

Dr. R. H. Ward, of Troy, N. Y., spoke upon "An Experiment in Difficult Resolutions." A paper was read by Dr. Veeder, of Lyons, N. Y., on "The relation of Cancer to Defective Development." He dealt with the present relations of microscopy with tuberculosis, and in his paper presented a cure that will work a revolution in medical treatment for such diseases if the experiment proves successful. The cure is simple, being a process of freezing out tuberculosis with cold air. He states, however, that the disease is not curable in cases too far advanced.

V. A. Latham, of Chicago, read a paper on "Aniline Stains in the Detection and Diagnosis of Diabetes." Professor Gage, of Cornell university, described useful and valuable appurtenances and instruments for laboratory work. Dr. H. B. Ward, of Lincoln, Nebr. discussed recent movements toward the organization of a bibliography of science.

The president of the society, Dr. Krauss, closed the session with a recitation of some notes he had made on recent progress of investigation of the blood in German laboratories.

Thursday Evening.—The Annual Address of the President, Dr. W. C. Krauss of Buffalo was read. Subject: "Some Medico-Legal Aspects of Diseased Cerebral Arteries."

Friday Morning.—Professor Krauss again presided and about thirty persons were present.

A nominating committee was appointed to select officers: Professor Gage, Messrs. Kuehne, Pflaum, Smith, and Mrs. Gage. The papers presented were technical and of special interest to scientists only. The program included the following papers: "Microscopical Animals From the Fresh Water of Lousiana," by Professor J. C.

Smith, New Orleans; "The Structure and Classification of Diatoms, Dr. Chas. E. Bessey, Lincoln, Nebr.; the "Structure of the Soft Palate," W. F. Mercer, Ithaca, N. Y.; "Construction of a New Stand for Microscopical Work," Dr. A. G. Field, Des Moines, Iowa.

Friday Afternoon.—A symposium on the use of the microscope by teachers and individual workers was presented, Professor Gage, of Cornell, contributing a paper on "Histology," Professor Bleile, of Columbus, one on "Physiology and Bacteriology," and Professor Bessey one on "Botany."

Friday Evening.—Mr. J. F. Stone of 694 East Broad street, gave an informal reception to the society, when the microscopists were entertained with views of the Grand Canon of the Colorado.

Saturday Morning.—The society elected officers and adjourned. The new officers are: President, Dr. A. M. Bleile, Columbus; first vice president, C. H. Eigemann, Indiana university; second vice president, Dr. M. A. Veeder, of Lyons, N. Y.; secretary, H. B. Ward, Lincoln, Nebr; treasurer, J. C. Smith, New Orleans; custodian, Magnus Pflaum, of Pittsburg.

Achromatics versus Apochromatics.

EDMUND. J. SPITTA.

It has been said that "seeing what you know and knowing what you see" are two very different things. By this is meant that it is far easier to recognize special characteristic features in anything with which you are familiar, than to discover new details about the object for which you are neither mentally prepared nor have practised your eye to see. This is especially true with the microscope and applies very strongly in determining the relative value of lenses by different makers, but perhaps more especially so when a definite decision has to be arrived at

—which gives the better image the achromatic or the apochromatic? By the tutored eye small differences in definition, powers of resolution, blackness of shadows, flatness of field, etc., are all at once easily recognized; but to the less experienced it becomes a tax of much greater proportions and often necessitates a regular training of the eye to thoroughly appreciate. The same remark of course applies to the comparison of photographs produced by the different lenses—unless you know what to look for and how to look for it. It is the aim then of this article to assist those less acquainted with the subject to do this with a little practice for themselves.

Before however approaching the actual subject itself, it is necessary first to touch upon the theoretical difference which underlies the manufacture and construction of these different classes of objectives, not from a mathematical point of view, for that is not within the scope of this paper, but in what it is hoped may be considered a practical manner.

Probably every reader of this journal knows full well what is meant by the word spectrum—the rainbow effect that is produced by a beam of light passing through a prism of glass. Such an experiment most of us have been the unconscious witnesses of when we noticed the colors produced by the sun shining on one of the pendants of the glass lustres which often used to adorn old-fashioned mantlepieces. The actual path of the ray of light passing through the prism is in exageration, shown in text-book figures, which everyone must recognize who has even glanced at a textbook on light. It will be seen, first, that the *im*-merging beam of white light is broken up into several *e*-merging rays which are now all of different colors; and secondly, that these emergent pencils, although their edges are continuous one with another, do not overlie each other but appear fairly well separated, the violet being the most bent, then the blue, next the

green, followed by the yellow, whereas the red is the least bent of all. This difference in bending of the rays, it is well-known, is entirely due to the difference in the respective wave-lengths of the individual colors. If we pursue our experiments a little further, we shall find that the more dense the glass becomes of which the prism is made, so much the more usually are the colors bent as well as separated out, such separation being technically known as "dispersion." But this is not all. When comparing the dispersion produced by different kinds of glass, that is, glass in the manufacture of which different minerals are used, we notice another peculiarity and it is this, that although two glasses may not perhaps differ so very largely in density, yet prisms made with them do not produce the same kind of spectra. One glass, for example, will spread out the violet end and yet bunch together the red and yellow, whereas another will largely spread out the red and the yellow and afford but little dispersion of the colors in the violet end of the spectrum. Such peculiarity is rightly called the "irrationality of the spectrum," and the colors introduced from this cause in an image are said to result from the non-elimination of the "secondary spectrum."

Let us now consider for a moment what all this has to do with our subject? To fix our ideas we must first bear in mind that an image of the illuminant is formed by each color, so that if the sun had illuminated the prism in our previous experiment, with suitable arrangements we should have seen an image of that luminary in each color. Then, secondly, as a simple lens is nothing but a congeries of approximated prisms varying only in size and shape, so it is not difficult to understand that along its axis there would have been represented a consecutive series of sun-images in all the colors of the rainbow, not one of which would have lain in the same plane as any other.

In theoretical achromatism then for color, what the op-

tician of course would wish to do is to be able to make all these colors bring their respective images to one and the same focus on the axis of the lens, irrationality of the spectrum being for the moment disregarded. In the manufacture of achromatics however, this is an absolute impossibility, and the optician has to be content, so to speak, with combining certain colors for visual purposes to give the best resulting image—yellow or yellow-green for seeing with, and yellow and blue-violet for photographing with, leaving all the others outstanding. Prof. Abbe some years ago gave a large amount of attention to the theoretical aspect of the case and came to the same conclusion as others had done before him, that no improvement could be effected in achromatism, especially in the elimination of the secondary spectrum, until the optician was provided with new kinds of glass possessing properties at that time only dreamt of in theory. At length, however, Jena glass was introduced, which gave to the world many of the kinds of glass that had been wanted, thus creating quite a revolution in lens-making. The words "many kinds" are used, because for the present they do not seem able to make a description having the same peculiar properties possessed by the mineral "fluorite" up to the present so absolutely necessary in the construction of the apochromatic. Having the new kinds of glass at command, Prof. Abbe originated his celebrated system of Apochromatism, which, he says, differs essentially from all others hitherto designed. Apochromats realize simultaneously two conditions not hitherto fulfilled by any other optical combination: (1) By the union of *three* colors in *one* point of the axis which enables the optician to eliminate the secondary spectrum; and (2) the correcting of spherical aberration for *two* different colors in contradistinction to the hitherto usual correction for *one* color only in the brightest part of the spectrum.

To fix our ideas then, let us put the matter another way.

With the achromatic the greatest sharpness of the image hitherto formed was limited to one color, or two at the most, of the light transmitted (yellow or perhaps yellow-green for visual lenses and blue-violet for those used in photography), while all the other rays of the spectrum gave more er less confused images, appearing as colored images (fringes surrounding the sharp image), and partly as a general haze spread over more or less of the entire field. With the new system, however, the images are nearly, if not absolutely, sharp for *all* colors, hence the the quality of the finite image whether visually or photographically considered *is independent entirely of any special color of the illuminant.* It is easy therefore to understand the great value of these lenses in defining difficult structures and how it is that they give such sharp and excellent photographs, let alone such white and colorless images of objects like diatoms. Again too, Professor Abbe pointed out that in achromatics color correction was only obtained for one zone of the objective, the others being more or less defective, but that in his apochromatic system the chromatic aberration was corrected equally in *all* parts of the field. That affords the reason why the resulting definition is so fine because the image produced by each color is said to perfectly coincide with that of the rest.

But there are yet two other points of interest which must be mentioned and they are:—(1) That owing to all these perfections mentioned so much more *light is obtained.* Dr. Dallinger has stated that an ordinary achromatic of the best type would only pass 140 parts, but an apochromatic, 225 out of a possible 300. (2) That also owing to the perfection of their performance the apochromatics permit the use of eyepieces of extraordinary power without producing what is technically known as a "rotten image." An achromatic will only permit the use of an eyepiece having at the most the initial magnification of about 4 to 6, but so perfect is the performance of

the new system that it is easy to use eyepieces magnifying 8 to 10 diameters, often 18, and in special cases 27, with rare instances of even 40. The evolution of thought is so peculiar that a question is often here asked: "If this property for bearing high eyepieces be true, and certainly it is, what is the use of making high power objectives at all seeing that so much magnification can be done at the eye-end with eyepieces? To reply to this must be our next subject.

In the olden days or up to about the year 1874, power *per se* was all that was thought to be effective in making an object distinct, and its details plainly visible. It is not a little strange to think that up to that date there appears to have been an entire absence of knowledge, even by experts, both in theory and practice as to the real optical principles that enable one to see an image produced by the microscopic objective. But in 1877 or thereabouts, Prof Abbe gave to the world his great diffraction theory which has inaugurated an entirely new epoch in both the theory and construction, as well as the adaptation and use of microscopical lenses. But before this time, however, as knowledge has grown and experience accumulated, it had come to be very slowly recognized that a "something" beyond simple magnification affected the defing power of objectives, and at last it was in a great measure traced to the fact that the reason why one lens *defined* so much better than another, although of the same initial magnifying power, was due to the fact that one had a *greater aperture than the other*. Hence to increase the aperture rapidly became the aim of the optician. But here it should be distinctly stated that the aperture, or numerical angle of *the objective* must not be confounded with the still older idea that excellency of result depended on the angle of obliquity at which the light *emerged from or to the object*, involving as it did some specially assumed property of a special kind in the obliquity taken as such, for Prof. Abbe

made a most careful and profoundly scientific enquiry into the matter, and found that there was no basis whatever for such an assertion. No, it had formed on wrong conceptions, and wonderfully did he expose and demolish the theory in a manner too long to enter upon here in a short article of this nature. *The* angle is that formed *in the ob jective.*

To put the matter in quite another way, perfect definition is wholly and absolutely dependent not upon the obliquity of the rays to the object, as before believed, but in truth upon the obliquity they bear *to the axis of the microscope.* To repeat then, to increase the *aperture* of the objective is found to be the means for improvement of definition. But Prof. Abbe went further into the matter and found that increased aperture seemed to admit "*a something*" more than the true dioptric image, and this "*something*" he ultimately discovered by a series of elegantly and well-contrived experiments to be nothing more or less than the diffraction rays which proceeded from the object itself. Pressing the subject to its logical conclusion, he found that the more of these rays that were admitted to the optical combination, i. e., the greater the numerical apeture of the objective, the more the similarity that would exist between the object and its image.

We are now in a position to reply to our question, and the reply is simply this :—The higher the defining power of the objective, the greater is its numerical aperture (usually written thus "N. A."), and as the definition depends on the greatness of this aperture, so it directly follows, that the higher the power used the better the final result. Seeing that achromatics, especially the higher power ones, cannot without greater difficulty be made with such high numerical aperture as those built on the apochromatic system, so the former cannot on this ground —if on no other—be expected to give the results that the new system affords ; still, on the other hand, it must not

be omitted to be said that owing to the introduction of the new Jena glass, ordinary achromatics have been most wonderfully improved and some of the most recent lenses have reached a perfection hitherto thought impossible ; such for instance as the new one-inch, by Ross, N. A. ·3 ; Leitz one-and-a-half inch and his tenth ; a new twelfth by Beck ; No. 6 by Reichert, and others. This statement is of importance to the student, but still there is no getting away from the fact that by the use of apochromatic objectives of the highest order, we verily believe all the great discoveries of the present and probably of the future will be made.

We have now to compare the performance of the achromatic and the apochromatic lenses visually and photographically.

It is presumed the reader knows the value of employing what is called "*critical light*," and how to obtain it ; lest, however, such should not be the case, the following is the method of obtaining it : Critical light is said to be obtained when the substage condenser and the objective are both in focus on the object. This is easily done as follows :—first focus the object in the usual manner and then rack the condenser up or down until the edge of the flame of the lamp is distinctly seen in the field accurately focussed. To those unaccustomed to this class of illumination it may appear objectionable, but no lens can be said to be performing at its best except under these conditions. The light, if the objective be of low power, may be too intense ; if so, the Iris diaphragm may be closed until the image loses its flooding of light, so long as the image does not suffer in definition, because, cutting down the light by the iris will lower the N. A. of the objective, but seeing that the low powers have much less aperture than the high ones, a certain amount of contraction of the iris will do no harm and will much improve the image. Great care must be exercised, however, when at-

tempting to do this with high powers, for fear as before explained, of reducing the N. A. of the objective, which of course means spoiling its definition ; should the light still be too intense with low powers, however, then smoked glasses or monochromatic ones should be interposed between the illuminant and the condenser.

Lastly it is important that the condenser should be suited to the objective so far as relates to its N. A. Theoretically, both should have the same aperture, but practically, no objective will stand this except perhaps when looking at or photographing bacilli : but on the other hand it is necessary in order to obtain good results not to use the condenser with too low a N. A.

As before stated, a small amount of cutting down by the iris is justifiable under certain conditions, and most authorities set the maximum limit at about a third of the diameter of the back lens of the objective, excepting when employing the microscope on bacilli. Then lastly, to obtain the best results, certainly when photographing with the microscope, an achromatic condenser is a necessity, and the use of an apochromatic one is better still. Messrs. Powell & Lealand have recently brought out an excellent dry apochromatic condenser of ·95 N. A., whose performance leaves little to be desired. It may seem as if these remarks on the condenser were uncalled for considering the nature of the subject under consideration ; but attention is called to all these details as it is positively certain no objective will perform at its best, unless the greatest care be exercised in the application and suitability of the condenser and the arrangement of the light.

Before actually comparing lenses, suitable test objects should be chosen and carefully studied. The Proboscis of the blow-fly for low powers is good, but besides that a Podura Scale and some finely marked diatoms must be obtained ; and lastly, if it can be possibly procured, a really good specimen of the Amphipleura pellucida mount-

ed in realgar. The observer should make himself a master in readily obtaining "critical light," and in illumination generally, for the relative excellence of one glass over another may very largely depend on a fortunate illumination. Then too he must possess himself with an unbiassed mind, remembering to compare objectives so far as possible of similar N. A., and not to be content with a desultory off-hand examination, but rather that he should examine the performance of each lens step by step as the programme given below proceeds. He must dispossess himself too of any extravagant expectation, for this may beget a belief that certain performances ought to be present which perhaps may be even theoretically impossible with the aperture employed; and on finding them absent, such absence of the ideal may induce a strong and unconquerable feeling of disgust that adds a heavy load to be overcome before coming to an unbiassed final judgment.

Let us take now an inch achromatic and an inch apocromatic to compare their performance, using ordinary eyepieces for the former objective and compensating ones for the latter, and use the proboscis of the blow-fly as the test object. The programme suggested—although it is not intended to be exhaustive—may be conveniently arranged as follows:—

1.—Flatness of field.

2.—Blackness of shadows in the image.

3.—Brilliancy of the illumination.

4.—Resolving power.

5.—The absence or presence of colored fringes around minute objects such as fine hairs, or dots in diatoms.

1. Flatness of Field.—By this is meant that after focussing the centre of the object, the edges of the field, if the specimen reaches so far, are equally sharp. The antithesis of flatness of field is called roundness or curvature of field, these two terms being in this paper con-

sidered synonymous, although some writers do draw a difference between them. It will be seen that the flatness is ofttimes of larger area—reaching further to the edges in the achromatic than with the apochromatic, but that the definition in the centre is superior in the apochromatic to that in the achromatic image. In some "inches" by first-class makers, such as in the new inch by Ross .3 N. A., and one by Leitz & Reichert, it is not easy without close attention to discern any inferiority of image to that presented by the apochromatic, for the improvement afforded by that type of lens does not become so very evident when compared usually with excellent achromatics using low eyepieces, until a little higher power is employed, although apparent in photography. But note one thing, that the curvature of the field and the consequent loss of definition at the edges presents this difference in the two cases. In the achromatic no amount of refocussing will render the periphery sharp, but with the apochromatic the slightest turn of the screw renders the edges of the field as good as the centre—hence the mind can rapidly acquire a mental picture of the whole image by a touch of the screw. With the achromatic, however, if the edges be required to be examined, that portion of the specimen must be brought to the centre of the field before focussing renders the definition as good as possible.

2. Blackness of the Shadows or Dark Portions of the Image.—Notice whether the dark ribs, or tracheæ as they are called, of the proboscis are really black, and see which lens renders them the more so. Look carefully to see if there be a haze over the whole image like a veil which no management or adjustment of the light, condenser, or otherwise will get rid of except by sacrificing the definition of the objective by closing the iris diaphragm. The apochromatic to the practised eye will here show a superiority.

3. Brilliancy of Image.—Here a good deal of prac-

tice is necessary, as much depends on the actual illumination of the moment; but rapid change of the lenses nearly always affords a ready method of perceiving the superiority of the apochromatic.

4. Resolving Power.—This is directly proportional to the N. A. of the objective. By resolving power is meant, speaking popularly, the power possessed by the lens of breaking coarse details into still finer ones, and of rendering the fine details so distinct as to merit the term of being "picked out." The black markings of the tracheæ should with the inch look picked out and so sharp and clean-cut, that they ought to give the impression that they are drawn with a pen on paper. Remove now the proboscis and place on the stage a large diatom, such as an Arachnoidiscus, and notice how the fine markings are shown by each lens, and how white and colorless is the image in the apochromatic.

5. Absence of Color Fringes.—Around and about the markings of the diatom, and between the striæ or between the dots, there will be seen a certain amount of color fringes with the achromatic, which are entirely absent in the apochromatic owing to the elimination of the secondary spectrum. Return now the proboscis slide and look at the large hairs and note the difference in their fringe-like appendages.

The same specimens will do when comparing the images produced by two half-inches, each being of similar aperture, when it will be found the contrast in the images will be more marked. Use also a diatom with well-marked striæ such as a Navicula major. When comparing two 1-6ths, a podura scale may be also employed. The light wants careful arrangement to use this test. The markings should look "punched out," and very black with a central white streak; diatoms should be tried for color fringes, especially the Navicula. An eighth and a twelfth immersion require much more careful testing. A podura

scale should show the white centre to the note of exclamation as it is called, with a narrow constriction near its broad end, and its point as a fine straight line extending some long way down in the black. Diatoms possessing the finest markings should be now resorted to, comparing the quadrilateral dots in the Surirella gemma, the hexagonal markings in the Pleurosigma angulatum, the canaliculum in the Pleurosigma balticum described by Dr. Van Heurck, and the small central portions of the Aulacodiscus sturtii. To these may be added by some authorities the resolution of the close rulings in Nobert's lines. When using oblique light with a condenser of high N. A., the lines in Amphipleura pellucida, should stand out well defined, if the specimen be well marked. In all these tests the performance of the apochromatic is unapproachable by the achromatic, especially in the case of photography with them. It must be here remarked that all high-power objectives, according to a great authority in lens construction, can be made for special objects to give much more excellent results than those lenses corrected for general purposes, but then the objective is so restricted in its application that it necessitates others being obtained each for its special use, which involves great expense. By this is meant a lens constructed to show bacilli with a flat field may not perform so well for resolving the fine markings on diatoms; and one specially corrected to separate lines with "oblique light" may often not be such a good "all-round" lens as another which does not perform under these circumstances quite so well. This constitutes a great difficulty when comparing the performance of different lenses. When using ordinary achromatics for photography, a great falling-off in results is evident at once unless they are specially corrected for the purpose. Of late years, however, objectives have been made which give very fair results as photographers with good performance as visual lenses, and

it is with these that we compare the best results of the achromatic with the performance of the apochromatic.

Space will not allow explaining what is meant by penetrating power, which is of course the reciprocal of the resolving power, or how the numerical aperture can be measured by the Abbe apertometer; neither can room be found to show how definition may be affected by bad centring of the lenses by which is meant the irregularity of the alignment in the optic axes, the parallelism of their planes or in the setting of their planes at right angles to the optic axis; neither can the reader be afforded information upon the effects produced in the performance of a lens by using a solid cone of light from the condenser or a hollow one; axial illumination or the other form to which the term "oblique light" is usually applied, these remarks are somewhat foreign to the comparison of achromatics with apochromatics, and would more properly be found in a brochure on lens testing, or in a text-book on the microscope.—*Ill. An. Micr.*

Amœba and Their Differentiation from Body Cells.

DR. FEINBERG, (FORTSCHRITTE DER MEDICIN XVII., No. 4.

It is frequently very difficult, indeed often impossible, for even a trained eye to distinguish an animal or human cell from a free amœba. The author mentions two ways by which the distinction can be made with certainty, *viz.*, by cultivation and staining. He used a solution of common salt of different degrees of concentration, in which one or several organic substances were placed. Upon these the amœba developed within about three days with extreme rapidity. After about 8–14 days the substances were overgrown and infiltrated with amœba, and even the fluid contained numerous protozoa. Such cultures the author was able to preserve for nine months, and after this time obtained fresh cultures by re-inoculation upon new

media. Bacteria-free cultures were never secured by this method.

In stained amœba, the author states that the staining of the nucleus is absolutely distinctive. Von Leyden compares its appearance with that of a bird's eye. It consists of a central spot (chromatin), surrounded by a sharply defined, clear zone. This never occurs, says the author, in a human or animal cell. Hence, by this feature alone, one can tell whether the object observed is a body cell or an amœba.

For staining the amœba found free in the culture fluid, the author employed the following method:

A drop of the fluid was placed upon a cover-glass and a drop of serum added. On fixing with sublimate-alcohol, the albumin in the serum coagulated, and fixed the amœba in the state they were in at the moment. The preparation, was then well washed with iodin-alcohol after the method of Schaudien (*Sitzbr. d. Acad. d. Wiss.*, 1896, xxxix), stained for 24 hours in very dilute hematoxylin solution, and sufficiently decolorized. In this method the nucleus is more intensely stained than the protoplasm, and is surrounded by a delicate, sharply-defined white zone. Such a picture characterizes the amœba as such.

The encysted amœba were treated as follows:

Small particles of the organic substance which had served the amœba as a nutrient, were hardened in absolute alcohol. They were next embedded in paraffin, and cut in the ordinary way. Methylene-blue and eosin were used for staining. The protoplasm also shows a fine retiulate structure, which can be distinguished from that seen in body cells by its more delicate meshes; besides, the reticulum noted by Flemming in human and animal cells is not brought out by the methods of staining used by the author in these researches.

Miscellaneous Notes on Microscopy.

J. H. COOKE, F. L. S., F. G. S.

To Neutralize Balsam.—Canada balsam, one of the most useful of resinous media, is usually slightly acid. In some cases this is a drawback, but for mounting sections stained with carmine, or injected with carmine gelatine, or Berlin blue gelatine, it is advantageous. The balsam may be neutralized by mixing a little carbonate of soda with the thinned solution before it is thickened. The soda settles after a few days and leaves the balsam clear and neutral. Carmine will diffuse, and blue will fade in the neutral balsam

Covers.—Thin mica plates are suggested as making good covers for such objects as fish's eggs, frog's eggs,&c. On account of the flexibility of the mica, the egg is less liable to be crushed than with a glass cover, and if the effects of pressure on the development of the egg are to be studied, the mica cover may be easily manipulated.

Bone Section.—The preparation of a bone section so as to show the lacunæ, canaliculi and deep seated cells, may be quickly effected by the following method :—Take a fresh bone, and with a strong, sharp knife cut off a thin shaving. Immerse the section in carmine dissolved in ammonia, the ammonia being first neutralized by acetic acid. The walls of the vessels which penetrate the lacunæ and canaliculi are by this means stained crimson, and the true structure of the bone is thus rendered visible.

Rock Sections.—A solution of gum-dammar in xylol is more suitable than Canada balsam as a mounting medium for rock sections.

Luminous.—To render micro-photographs self-luminous soak them in castor-oil to make them transparent, and dust them over with powdered sulphate of baryta or sulphite of lime. When dry mount them on cardboard of a suitable size with starch paste. The phosphorescence of the salt lights up the photograph.

Drying.—Drying oils in every form, such as gold size,

paint, etc., becomes hard by oxidation, and not, as is generally supposed, by evaporation. The drying process is frequently a long one. Where time is an object, as in class demonstration, it may be expedited by placing the slides in a small chamber (a porcelain dish answers admirably) and passing over them a stream of oxygen obtained by the decomposition of potassium chlorate.

Killing Objects.—A saturated solution of bichloride of mercury, to which has been added one per cent acetic is an excellent medium for killing specimens of the protozoa on the slide or cover preparatory to mounting. In some cases a hot solution is useful, as being more rapid in its results, and producing little or no deformity of the cells.

Dimensions.—It is a pity that amateur microscopists do not more fully recognize the necessity of recording, to some uniform and convenient scale, the dimensions of the microscopic objects that they make their study. This want of uniformity not only detracts from the value of the work done, but it also renders the work of comparison of the drawings of the objects laborious and unreliable. By operating on some object of known size it is easy to ascertain what arrangement of the microscope, and of its objectives and ocular, is necessary to obtain an image with the camera lucida of any required size. Having determined on a given amplification it should be adhered to, and a scale be made corresponding to this amplification. The actual dimensions of the object might then be readily ascertained by applying this scale to the various parts of the image or drawing. The value of some such uniform method as this is self evident.

To Fix Objects.—To prevent sand grains and other loose objects from being forced out from under the cover-glass when mounting, Mr. H. C. Sorby recommends that the objects should be well mixed with weak gum and water, the gum to be of such a consistency as to make it easy to separate the grains and spread them uniformly over the space which will afterwards be covered by the cover-glass. The water is then allowed to evaporate slowly. Much of the

gum may collect around the margin, but by properly regulating the quantity originally added, enough will remain under the larger grains to hold them so fast that they will not be squeezed out in the excess of balsam.

Ink.—Dr. Marpmann, of Leipsic, has recently published the results of his microscopical examinations of sixty-seven samples of ink used in schools. Most of these inks were made with gall nuts, and contained saprophytes, bacteria, and micrococci. Nigrosin ink taken from a freshly opened bottle was found to contain both saprophytes and bacteria. Red and blue inks also yielded numerous bacteria. In two instances he succeeded in cultivating from nigrosin ink a bacillus which proved fatal to mice in four days. This ink had stood in an open bottle for three months, and the inference to be drawn from the enquiry is that ink used in schools should always be kept covered when not in use.

Stain.—According to Nocht, the success of the nucleus stain of Romanowsky, a mixture of eosin and methylene blue, depends upon the presence of certain impurities in the methylene blue. To obtain the best results the use of polychromic methylene blue is suggested, as the essentials for the formation of the nuclear stain are more frequently met with in this than in any other. Before using, its alkaline reaction should be neutralized with acetic acid, and the solution should then be mixed with ordinary methylene blue until it is clear and blue. Finally dilute the fluid with a one per cent aqueous solution until a reddish tinge is apparent near the edges. Macerate the preparations in this for some time, and if too much stain is taken up decolorize with dilute acetic acid.

Colored Leaves.—The causes of the coloring of leaves in autumn has formed the subject of a series of investigations by Mr. E. Overton. The leaves that turn red he classifies under two heads, those which remain throughout the winter, and those that fall soon after their change of color. Microscopic examination show that in both cases the palisade cells, and the cells that line the air-chambers

of the leaf, are charged with a red cell sap of the nature of glucosides. The cultivation of Hydrocharis morsusranœ, of Utricularia, and many other land plants, in a weak solution of glucose, confirmed his deductions. In each case the leaves assumed a rich reddish-brown tint.

Chromic Acid.—For general botanical work the most useful killing and fixing agents are solutions containing chromic acid. Prof. C. J. Chamberlain, gives some valuoble notes on the strengths of the solutions used. For spirogyra, fern prothallia, and similar objects, he suggests a solution made up of chromic acid, two grammes ; acetic acid, one cubic centimetre ; and water, ninety-seven cubic centimetres. If plasmolysis takes place, weaken the chromic, or strengthen the acetic, since the chromic has a tendency to produce contraction, and the acetic to cause swelling. Too large a proportion of acetic acid, however, may cause distortion, and hence it would be better to weaken the chromic acid.

Fixing Tissues.—Referring to the time that should be allowed for the fixing of tissues in chromic solutions, Prof. Chamberlain has found that twenty-four hours should be the minimum even for the most delicate objects. It is now well-known that zoologists allow fixing agents like Muller's fluid and Erlicke's fluid to act for weeks before the material is passed on to the next stage, and it is therefore questionable whether the time which is usually allowed by microscopists when using chromic acid solutions is not much too short. Sixteen to twenty-four hours is the time usually allowed ; but Prof. Chamberlain's experiments show that the material is better able to withstand subsequent processes if it has been kept in the fixing solutions for two or three days. More rapid penetration, and consequently more immediate killing, can be secured if the reagent is kept at a temperature of from thirty degrees to forty degrees Centigrade.

Wickersheim's Preserving Fluid.—This is not commonly used owing to the poor preparations that have been put on the market. Animal and vegetable bodies impreg-

nated with it retain their form, color, and flexibility in the most perfect manner. The objects to be preserved are placed in the fluid, and left in it for from six to twelve days, after which they are dried in the air. The ligaments remain soft and movable, and the animals or plants remain fit for anatomical dissection and study for long periods. The formula for the fluid is as follows ;—Dissolve one hundred grammes, alum, twenty-five grammes common salt,twelve grammes saltpetre, sixty grammes potash, ten grammes arsenious acid, in three thousand grammes boiling water. Filter the solution,and when cold add ten litres of the liquid to four litres of glycerine and one litre of methyl alcohol.

Preservative.—For the preservation of arachnids and myriapods the following mixture is recommended :—Glycerine and Wickersheim's fluid one and a-half ounces each, and distilled water three ounces,the whole to be shaken and thoroughly mixed and added to thirty ounces of ninety-five per cent alcohol. He considers that alcohol that has been previously used for preserving mites and spiders is preferable to pure alcohol, as the former already contains some of the fats dissolved out of the specimens. This liquid preserves the coloring of the specimens, and keeps them flexible.

Pond Animals.—For five months Dr. Marsson concentrated his attention on the study of the variations of the animal and plant life of the plankton of the Leipsig ponds, one result of which has been the discovery of many new and interesting—though anomalous facts. Two ponds, separated only by a road, never contained the same forms. Volvox aureus was found in abundance in the pond on the south side, but not a single specimen could be found in that on the north. Synura uvella was found in the one, in September,in great quantities,and none at all in the other. Both ponds afforded similar conditions of depth, character of soil, light, and plant growth, and swans and other water birds frequented both. On the 20th of May, Tintinidium fluviatile made its first appearance in a pond, and on the 26th it formed the largest constituent of the plankton, after

which it disappeared and did not return. In April, Codanella lacustris appeared in this same pond, then it disappeared entirely, and was first found in other ponds in August.

Objectives.—To many microscopists the terms "one quarter inch," or "one half inch," as applied to their objectives, convey the idea that when in focus, the object is at a distance of a quarter inch, or a half inch, from the front lens. They confound the equivalent focal length of the objective with the working distance. As a matter of fact, the latter is always considerably less than the former. The determination of the working distance of an objective is a point of considerable importance, and therefore all microscopists should make themselves familiar with the method of calculating it. The following simple device will be found useful for estimating the working distance of objectives that are not higher than one-twelfth inch. Make a long thin wooden wedge, ten centimetres in length along the base, and twenty millimetres in perpendicular height. Focus a diatom on a glass slip without a glass-cover, and then carefully push the wedge along the glass slip until it touches the objective. The thickness of the wedge at the point of contact will represent the working distance of the objective.

Picro-carmine.—Prof. Leroy gives the results of his experience in the use of picro-carmine as a counter-stain for bacteria in tissues. As a rule this reagent is somewhat uncertain in its effects, and it is therefore suggested that, to obviate risk of failure, the tissues should be first treated with logwood picro-carmine, and finally stained by Gram's method of bacterial staining. As a counter-stain, alum carmine alone gives only a nuclear stain and leaves the cytoplasm practically untouched. Better results can be obtained by first staining in alum carmine or borax carmine, then carrying the section through the regular Gram process, and lastly leaving the section for half a minute in a solution of sodium sulp-indigotate 0.1 gramme, and carbolic acid, five per cent aq. sol. one hundred cubic centimetres, after which follow on with alcohol, creasote, and balsam. By this method the nuclei will stain red, the cell

bodies apple green, and the bacteria purple (if gentian violet be used in the Gram's solution).—*Knowledge.*

MICROSCOPICAL MANIPULATION.

Removing Air Bubbles From Microscopic Mounts.—B. S. Proctor tells in the Pharmaceutical Journal of a simple method he has sometimes found useful for removing air bubbles in mounting microscopic objects. The method is thus described: "Take a small syringe of the well-known pattern having a glass barrel, vulcanite mounts and leather packing to the piston. These usually work so nearly air-tight that if the piston be drawn up while the nozzle is closed with the finger it will spring back to its original position. Unscrew the top and remove the piston, close the nozzle with a fragment of beeswax, and half fill the barrel with water: into this drop the section or tissues to be treated. Then replace the piston and screw on the top. The syringe being inverted and the plug of wax removed, the air is to be driven out of the barrel by raising the piston till the water begins to flow out of the nozzle, close the aperture with the finger and lower the piston. A partial vacum is thus formed, and the air rapidly escapes from the cells of the tissue, collecting in the point of the syringe. By removing the finger and raising the piston, the liberated air is forced out; this may be repeated several times so long as air is given off. The same mode of operating is applicable to objects to be mounted in Canada balsam if oil of turpentine be used instead of water and the objects to be mounted are quite dry before immersion in the turpentine."

Double Staining.—Ziemann proposes (Centralhalle fur Bact.) an elaboration of Romanowski's staining process, by which chromatin is colored carmine-red, or carmine-violet, and protoplasm blue. The author uses a mixture of a 1 per cent methyl blue solution, 1 part, with 0.1 per cent eosin solution, 6 parts. The staining is performed in concave well slides, which are covered to avoid evapora-

tion. Any pellicle which forms on the surface is removed with blotting paper. The staining is accelerated by the addition of 25 per cent of borax to the methyl blue solution. The time required for staining blood preparations is about half an hour. On the addition of borax, however, only ten minutes is necessary for organisms of low development, without borax fifteen minutes. Flagellates take longer than blood. The objects are fixed either by being passed thrice through a flame, or are immersed for half an hour in absolute alcohol. Flagellates are fixed by immersion in hot sublimate alcohol (saturated aqueous sublimate solution, 2 parts, absolute alcohol, 1 part), and washing with 63 per cent iodine alcohol. The results with bacteria are not yet satisfactory.

BIOLOGICAL NOTES.

Parasites of House Fly.—A parasite is extremely common on the house fly in Bermuda. It is I believe a species of Trombidium. Those that I noticed could move rapidly over the body of the fly, and when disturbed concealed themselves under the halteres. At other times they appeared to usually attach themselves to the abdomen of the host. The parasite was of a red color. Another curious parasite which I observed on the house fly in Bermuda was a small red ant. This creature attaches itself to the tarsus of the fly by means of its mandibles. It was a matter of common observation to see flies on the wing with these small ants attached. On one occasion I disengaged the ant and placed it on a table. It remained quiet until a fly came within suitable distance, when it made a rush, and was carried off clinging to the leg of the fly. I believe the reason of attack was made for the purpose of finally eating the fly. The ant held on until its host became exhausted, and then attacked a more vital spot than the foot, and killed it. These ants eat the soft parts out of a dead cockroach in a very short time. The Empusa muscæ is a well-known fungoid parasite of the housefly. The fungus "rests" during the hot weather in Bermuda, but during the cool

weather it decimates these insects. I believe that I succeeded in reducing the numbers of flies in my house by placing bodies of some dead from Empusa in a suitable cage. I then introduced many healthy ones. In a few days these became infected, and I let them out in order to communicate the disease to others. The result was that the walls of the rooms were soon covered by flies dead from the Empusa disease. Mr. A. D. Michael, F. R. M. S., etc., if I remember rightly, once told me that it was his custom to style the common house-fly a "menagerie in miniature," because of the number of parasites to which it acts as host.—H. A. Cummins, Major R. A. M. C., 29 Nightingale Place, Woolwich.—In Science-Gossip.

NEW PUBLICATIONS.

Mr. J. J. Browning's Catalogue.—Mr. Browning's catalogue contains many things of interest to microscopists. His micro-spectroscopic apparatus is well-known and requires no detailed notice here, but amongst the now numerous pocket aplanatic lenses in the market we may mention his Platyscopic lenses which were amongst the earliest in the field, and still hold their own. A useful novelty is Mr. Browning's small micro-camera, concerning which we hope to give our readers further information after a practical trial of its efficiency. Amongst microscopes we may mention the "Iris," fitted with sliding coarse adjustment, micrometer screw fine adjustment, draw-tube, diaphragm plate, and tube for sub-stage apparatus.

Zeiss' New Catalogue.—Carl Zeiss' Catalogue for 1898 is worthy of the reputation of the firm. Beautifully printed and bound, excellently arranged, and completely illustrated, it is more than a catalogue in virtue of the practical explanatory remarks interspersed in the text. Of the eminence of this firm, pre-eminent as makers of the famous apo-chromatic objeetives, it is unnecessary to speak, but their catalogue gives detailed information with regard to their stands, objectives, eyepiaces, and accessories, and should be in every worker's hands.

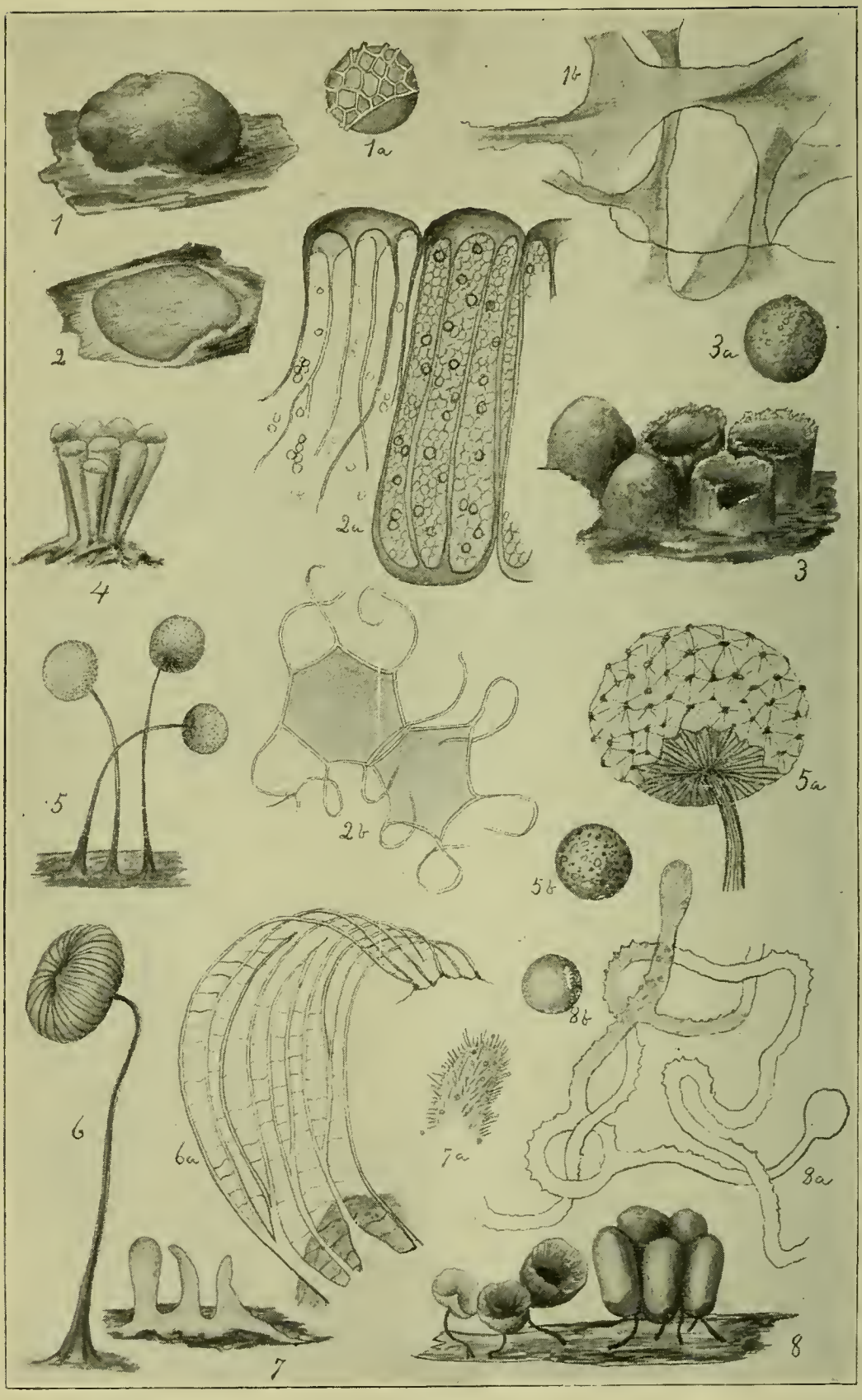
Mr. J. J. Hicks' Catalogue.—Mr. Hicks, of Hatton Garden, is well-known as a maker both of microscopes and objectives, and his catalogue therefore scarcely requires detailed notice. We may mention what appears to be a comparatively new model in his "Histological" microscope, which is fitted with rack-work coarse adjustment and micrometer-screw fine adjustment, stage with the Nelson type of horse-shoe opening, swinging understage fitment for condenser, and claw-shaped stand. It is sold at a price that brings it well within the reach of students.—*Sci. Gossip.*

The North American Slime Moulds.—Thomas H. Macbride, Professor of Botany in the University of Iowa. Macmillan Company, New York, 1899, 8vo., 231 pp., 18 plates, $2.25. This is a list of the Myxomycetes with technical description of each. The material described has been brought together in the university herbarium by aid of botanist from Alaska to Panama. The descriptions conform to European authorities a list of which is given in the preface. Schrader in 1797 first separated the slime moulds from the other fungi into a separate family. Fries in 1829 made a valuable summary of all then known but the microscopical technique of his time did not suffice for discrimating delicate forms. Spore measurement and microscopic details were brought out first by Dr. De Bary, 1859, and Rostafinski, 1873. A myxomycete is a chlorophyl-less organism whose vegetative phase consists of a naked mass of multinuclear protoplasm called plasmodium; reproduced by spores which are either free or more commonly enclosed in sporangia, and which on germinating produce ciliated or amœboid zoospores, whose coalescence gives rise to the plasmodium.

MICROSCOPICAL NOTES.

Slides.—For the benefit of amateurs who find difficulty in obtaining good objects at a moderate rate, I offer first class slides, at cost price (3d. each). Material, lists, and information free. Dealers not supplied. Frank P. Smith, 15, Cloudesley Place, Islington, London, N.

PLATE I



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CONTENTS.

The North American Slime-Moulds.

T. H. MACBRIDE'S NEW BOOK.

With Frontispiece.

Professor T. H. Macbride of the State University of Iowa has made a descriptive list of all Myxomycetes hitherto described from North America including Central America and the MacMillan Company of New York has brought out in a nice octavo volume of 230 pages accompanied by eighteen plates of which our frontispiece represents the first. The explanation of the plate is as follows:

Enteridium splendens. Æthalium, natural size, fig. 1; a spore X 1400, fig. 1, a; capillitium of same X 420, fig. 1, b.

Dictydiæthalium plumbeum. Æthalium, natural size,

fig. 2; sporangia and spores, X 50, fig. 2 a; persistent apices of the peridia, fig. 2 b.

Lindbladia effusa. A group of sporangia X 30, fig. 3; a single spore, X 1400 fig. 3 a.

Tubifera furruginosa. Group of sporangia, X 5, fig. 4.

Cribraria dictydioides. Three sporangia, X 15, fig. 5 a single sporangium showing reticulate thickening, X 60, fig. 5 a; a spore, X 1400, fig. 5 b.

Dictydium cancellatum. Sporangium X 30, fig. 6; par of the peridial wall, seen from within, X 84, fig. 6 a.

Ceratiomyxa fruticulosa. Three sporiferous pillars, X 40, fig. 7; tip of a single pillar, X 84, fig. 7 a.

Hemitrichia stipata. Sporangia, X 6, fig. 8; capillitium of same, X 750, fig. 8 a; a single spore X 1000, fig. 8 b.

The following paragraphs are quoted from the introduction to this volume which should be in the hands of every microscopist for use in identifying species:

The Myxomycetes,or Slime-moulds, include certain very delicate and extremely beautiful fungus-like organisms common in all the moist and wooded regions of the earth. Deriving sustenance, as they for the most part do, in the decomposition-products of organic matter, they are usually to be found upon or near decaying logs, sticks, leaves, and other masses of vegetable detritus, wherever the quantity of such material is sufficient to insure continuous moisture. In fruit, however, Slime-moulds may occur on objects of any and every sort. Their minuteness retires them from ordinary ken; but such is the extreme beauty of their microscopic structure, such the exceeding interest of their life history, that for many years enthusiastic students have found the group one of peculiar fascination, in some respects, at least, the most interesting and remarkable that falls beneath our lenses.

The Slime-moulds present in the course of their life history two very distinct phases: the *vegetative*, or growing, assimilating phase, and the *reproductive*. The former is in many cases inconspicuous and therefore unob-

served; the latter generally receives more or less attention at the hands of the collector of fungi. The vegetative phase differs from the correspondent phase of all other plants in that it exhibits extreme simplicity of structure, if structure that may be called which consists of a simple mass of protoplasm destitute of cell-walls, proteid in form and amœboid in its movements. This phase of the Slime-mould is described as plasmodial and it is proper to designate the vegetative phase in any species, as the *plasmodium* of the species. It was formerly taught that the plasmodium is unicellular, but more recent investigation has shown that the plasmodial protoplasm is not only multi-nuclear but karyokinetic; its cells divide and redivide, as do the *reproductive* cells of plants and animals generally. Nevertheless, in its plasmodial phase, the Slime-mould is hardly to be distinguished from any other protoplasmic mass, may be compaired to a giant amœba, and justifies in so far the views of those systematists who would remove the Slime-mould from the domain of the botanist altogether, and call them animals. The plasmodium is often quite large. It may frequently be found covering with manifold ramifications and net-like sheets the surface of some convenient substratum for the space of several square feet.

The substance of the plasmodium has about the consistency of the white of an egg; is slippery to the touch, tasteless, and odorless. Plasmodia vary in color in different species and at different times in the same species. The prevailing tint is yellow, but may be brown, orange, red, ruby-red, violet, in fact anything but green. Young plasmodia in certain species are colorless, many have a peculiar creamy tint difficult to define. Not only does the color change, sometimes more than once in the course of the life history of the same species, but it may be the same for several forms, which in fruit are singularly diverse indeed, so that the mere color of the plasmodium

brings small assistance to the systematist. In fact, the color depends no doubt upon the presence in the plasmodium of various matters, more or less foreign, unassimilated, possibly some of them excretory, differing from day to day. In its plasmodial state, as has been said, the Slime-mould affects damp or moist situations, and is found during warm weather in humus, on piles of rotten leaves, straw, but especially on and in the wet tissues of rotten stumps and logs. In such a situation the protoplasm spreads over all moist surfaces, creeps through the interstices of the rotting bark, spreads between the cells, betpeen the growth-layers of the wood, runs in corded vein-like nets between the wood and bark, and finds in all these cases nutrition in the products of organic decomposition. Such a plasmodium may be divided, and so long as suitable surroundings are maintained, each part will manifest all the properties of the whole. Parts of the same plasmodium will even coalesce again. If a piece of plasmodium-bearing wood be brought indoors, be protected from desiccation by aid of a moist dark chamber, not too warm (70° F.), the organism seems to suffer little if any injury, but will continue for days or weeks to manifest all the phenomena of living matter. Thus, under such circumstances, the plasmodium will constantly change shape and position, can be induced to spread over a plate of moist glass, and so be transferred to the stage of a microscope, there to exhibit in the richest and most interesting and abundant fashion the streaming protoplasmic currents. As just indicated, the plasmodia follow moisture, creep from one moist substance to another, especially follow nutritive substrata. They seem also to secure in some way exclusive possession. I have never seen them interfered with by hyphæ or enemies of any sort, nor do they seem to interfere with one another.

The plasmodial phase of the Slime-mould, like the hyphal phase of the fungus, may continue a long time; for

months, possibly for years. The reason for making the latter statement will presently appear. But however long or short the plasmodial phase continue, the time of fruit, the reproductive phase, at length arrives. When this time comes, induced partly by a certain maturity in the organism itself, partly no doubt by the trend of external conditions, the plasmodium no longer as before evades the light, but pushes to the surface, and appears usually in some elevated or exposed position, the upper side of the log, the top of the stump, the upper surface of its habitat, whatever that may be ; or even leaves its nutrient base entirely and finds lodging on some neighboring object. In such emergency the stems and leaves of flowering plants are often made to serve, and even fruits and flowers afford convenient resting places. The object now to be attained is not the formation of fruit alone, but likewise its speedy desiccation and the prompt dispersal of the perfected spores. Nothing can be more interesting than to watch the Slime-mould as its plasmodium accomplishes this its last migration. If hitherto its habitat has been the soft interior of a rotten log, it now begins to ooze out in all directions, to well up through the crevices of the bark as if pushed by some energy acting in the rear, to stream down upon the ground, to flow in a hundred tiny streams over all the region round about, to climb all stems, ascend all branches, even leaves and flowers, to the height of many inches, all to pass suddenly as if by magic charm into one widespread, dusty field of flying spores. Or, to be more exact, whatever the position ultimately assumed, the plasmodium soon becomes quiescent, takes on definite and ultimate shape, which varies greatly, almost for each species. The Slime-moulds were formerly classed with the gasteromycetous fungi, puff-balls, and in description of their fruiting phase the terms applicable to the description of a puff-ball are still employed, although it will be understood that the struct-

ures described are not in the two cases homologous; only analogous. The sporangium of the Slime-mould exhibits usually a distinct *peridium*, or outer limiting wall, which is at first continuous, enclosing the spores and their attendant machinery, but at length ruptures, irregularly as a rule, and so suffers its contents to escape. The peridium may be double, varies in texture, color, persistence, and so forth, as will be more fully set forth in the several specific descriptions. The peridium blends with the hypothallus below when such structure is recognizable, either directly, when the sporangium is sessile, or by the intervention of a *stipe*. Associated with the spores in the sporangium occurs the *capillitium*. This consists of most delicate thread- or hair-like elements, offering the greatest variety both in form and structure. The threads composing the capillitium are not to be regarded, even when free, as cells, nor even of cellular origin; they are on the other hand, in such a case, simply shreds and strands of the original plasmodium, portions that have not been used in the formation of spores, and are accordingly modified in such wise as to be useful in spore-dispersal. The capillitium threads may be solid or hollow, they may occur singly or be combined into a net, they may be terete or flat, attached to the peridial wall or free, simple or adorned with bands or spires and knobs in every variety, uniform or profusely knotted and thickened at intervals, and burdened with calcic crystals.

The germination of the spores ensues closely upon their dispersal or maturity and is unique in many respects. The wall of the spore is ruptured and the protoplasmic content escapes as a zoospore indistinguishable so far from an amœba. The amœboid zoospore is without cell wall, changes its outline, and moves slowly by creeping or flowing from point to point. At this stage many of the spores assume each a flagellate cilium, and so acquire power of more rapid locomotion. The zoospores, whether ciliate

or not, thus enjoy independent exlstence and are capable of continuing such existence for sometime, assimilating, growing, and even reproducing themselves by simple fission, over and over again. This takes place, of course, only in the presence of suitable nutrient media. Nevertheless the spores of many species germinate quickly simply in water. A drop suspended in the form of the ordinary drop culture on a cover-glass affords ample opportunity. In the course of time, usually not more than two or three days, the swarm spores cease their activity, lose their cilia, and come to rest, exhibiting at most nothing more than the slow amœboid movement first referred to. In the course of two or three days more, the little spores begin to assemble and flow together; at first into small aggregations, then larger, until at length all have blended in one large creeping protoplasmic mass to form thus once again the plasmodium,or plasmodial phase with which the round began. Small plasmodia may generally be obtained artificially from drop cultures. Hay infusions, infusions of rotten wood, etc., may sometimes give excellent results. The spores of *Didymium crustaceum* were sown upon a heap of leaves in autumn. An abundant display of the same species followed in the next June; but, of course, the intervening phases were not observed. The most satisfactory studies are obtained by plasmodia brought in directly from the field.

The cellulose of the Slime-mould looks toward the world of plants. The aerial fructification and stipitate habit of the higher forms tends in the same direction. The disposition to attach themselves to some fixed base is a curious characteristic of plants, more pronounced as we ascend the scale; but by no means lacking in many of the simplest, Diatoms, filamentous Algæ, etc., and it is quite as reasonable to call a Vorticella or Stentor, by virtue of its stipitate form and habit, a plant as to call Slime-mould an animal because in one stage of its history it resembles

an amœba. The whole life of the organism in any case must be taken into account. At the outset plants and animals are alike; there is no doubt about it; they differ in the course of their life histories. The plasmodium is the vegetative phase of the Slime-mould. It needs on cell-walls of cellulose, no more than do the dividing cells of a lily-endosperm: both are nourished by organic food and resort to walls only as conditions change. The possession of walls is an indication of some maturity. In the Slime-mould the assumption of walls is indeed delayed, but merely delayed. Walls at length appear, and when they do come they are like those of the lily; they are cellulose. The Myxomycetes may be regarded as a section of the organic world in which the forces of heredity are at a maximum, whatever those forces may be. Slime-moulds have in smallest degree responded to the stimulus of environment. They have, it is true, escaped the sea, the fresh waters in part, and become adapted to habitation on dry land; but nothing more.

About 400 species of Slime-moulds have been described. Saccardo enumerates 443, inclusive of those denominated doubtful or less perfectly known. These 443 species are distributed among 47 genera, of which 15 are represented by but a single species each,—monotypic. In the United States there have been recognized about 200 species. Of those here described, some are almost world-wide in their distribution, others are limited to comparatively narrow boundaries. The greater number occur in the temperate regions of the earth, although many are reported from the tropics, and some even from the arctic zone.

If the *Plasmodium malariæ* be indeed called a Slime-mould, and be, as is alleged, the promoting cause and agent in malarial fever, then the group entire suddenly springs to most unusual interest in the attention of all mankind. Aside from the injurious tendencies possible or real of these two, I know not that all other Slime-

moulds of all the world, taken all together, affect in any slightest measure the hap or fortune of man or nation And yet, if in the economic relations of things, man's intellectual life is to be considered, then surely come the uncertain Slime-moulds, with their fascinating problems proffered still in forms of unapproachable delicacy and beauty, not without inspiration.

Collection and Care of Slide-mould Material.

Specimens may be taken at the appropriate season in almost any or every locality. Beginning with the latter part of May or first of June, in the Northern states, plasmodia are to be found everywhere on piles of organic refuse: in the woods, especially about fallen and rotting logs, undisturbed piles of leaves, beds of moss, stumps, nor less in the open field where piles of straw or herbaceous matter of any sort sinks in undisturbed decay. Within fifty years tree-planting in all the prairie states has greatly extended the range of many more definitely woodland species, so that species of Stemonitis, for instance, are common in the groves on farms far into Nebraska and Dakota. In any locality the plasmodia pass rapidly to fruit, but not infrequently a plasmodium in June will be succeeded in the same place by others of the same species, on and on, until the cold of approaching winter checks all vital phenomena. The process of fruiting should be watched as far as possible and, for herbarium material,allowed to pass to perfection in the field.

Specimens collected should be placed immediately in boxes in such a way as to suffer no injury in transport; beautiful material is often ruined by lack of care on the part of the collector. Once at the herburium, specimens may be mounted by gluing the supporting material to the bottom of a small box. Boxes of uniform size and depth may be secured for the purpose. Some collectors prefer to fasten the specimen to a piece of stiff paper, of a size to be pressed into the box snugly, but which may be re-

moved at pleasure. Every pains must in any case be taken to exclude insects.

For simple microscopic examination it will be found convenient to first wet the material with alcohol on the slide, then with a weak solution of potassic hydrate, to cause the spores and other structures to assume proper plumpness. A little glycerine may be added or run under the cover if it is desired to preserve the material for further or prolonged study. For permanent mounting nothing in most cases is better than glycerine jelly. As a preparation, the material should lie for some time in Hantsch's fluid, opportunity being given for evaporation of the alcohol and water. When the material shows the proper clearness and fullness, it may be mounted in jelly in the usual way. Kaiser's formula gives beautiful results. After mounting, the preparation should be sealed with some good cement, as Hollis's glue.

Preparation of Certain Special Media.

V. A. MOORE.

In studying the properties of bacteria it is desirable to cultivate them on a number of different media. Bouillon, agar, and gelatin are most commonly used, but others are necessary in determining the cultural peculiarities and important biochemic properties of the organism in question. The cultivation of bacteria upon these media may be regarded somewhat as a test, to determine the presence or absence of certain properties. Thus, for example, will the species in hand coagulate the casein in milk, produce gas in media containing saccharose, grow on potato, etc.? The number of these tests which have been used and called important is large. A few species of bacteria require a particular kind or kinds of media for their diagnostic or most differential growth. Among these are those of glanders, diphtheria and tuberculosis.

General Directions.—Prepare for culture media, 5 tubes of potatoes, 5 tubes of milk, 5 tubes of litmus milk, 5 tubes of glucose agar, 5 tubes of glycerin agar, 4 fermentation tubes of bouillon containing glucose, 4 containing lactose, and 4 containing saccharose.

Preparatin of Potato for a Culture Medium.—Select medium sized potatoes, thoroughly wash and cut out with a cutter made for this purpose, a cylinder 3 to 4 cm. long (oblong pieces cut with a knife will do quite as well). Ordinarily 2 cylinders can be cut from each potato. The inclined surface is obtained by cutting out the potato projecting above the frame of the cylindrical knife. All of the skin must be removed. Wash the potato cylinders in cold, running water for some 5 minutes (a longer time is preferable) and place them in test tubes of the proper size (large or small according to size of cutter used), and add about 1 c.c. of water to each tube. Sterilize them by discontinuous boiling or steaming for 20 minutes each day for three consecutive days. Wipe, label, and store.

Preparation of Milk for a Culture Medium.—Place about 100 c.c. of fresh milk in a beaker in the ice box and allow it to stand for from 10 to 15 hours. Then carefully remove all the cream. It is well to filter the milk through a thin layer of absorbent cotton to remove any masses of cream. The reaction should be tested and if strongly acid it should be rejected or made 1.5 per cent acid to phenolphthaleni by the addition of n 1 sodium hydrate. Distribute the skimmed milk in small test tubes (7 c.c. in each) and sterilize by discontinuous steaming in the same manner and for the same length of time as the potatoes. Label and store in locker.

Preparation of Litmus Milk for a Culture Medium —This is prepared the same as the milk medium with the addition of enough of an aqueous solution of litmus to impart a decidedly blue color to the milk. Sterilize, label and store the same as the milk.

PREPARATION OF GLUCOSE AGAR.—Prepare 100 c.c. of agar. Reserve one half of it for glycerine agar and to the other half add 1 per cent glucose. Dissolve the powdered glucose in about 5 c.c. of boiled,hot water before adding it to the liquid agar. After thoroughly mixing distribute it in small sterile test tubes. Sterilize, label, and store the same as ordinary agar.

PREPARATION OF GLYCERINE AGAR.—Take the balance of the agar prepared above and add 5 per cent of pure glycerine. Thoroughly mix it with the liquid agar, after which distribute it in tubes. Sterilize, label, and store as ordinary agar.

PREPARATION OF GLUCOSE BOUILLON.—This is used in the fermentation tube. Take 100 c.c. of peptonized bouillon and add 1 gram of pure grape sugar (glucose). After it is dissolved and thoroughly disseminated through the bouillon by stirring or pouring, distribute the bouillon in the fermentation tubes, filling completely the closed branch and the open bulb about half full. Sterilize it by discontinuous steaming for twenty minutes each day for three consecutive days.

PREPARATION OF LACTOSE BOUILLON.—This is prepared by adding 1 per cent lactose to the peptonized bouillon. It is necessary, however, that the bouillon used does not contain muscle sugar. Bouillon free from muscle sugar can usually be obtained by macerating the meat for from 12 to 18 hours at a low temperature. After adding the lactose and thoroughly mixing it in the bouillon, sterilize label, and store.

SACCHAROSE BOUILLON.—This is peptonized bouillon to which 1 per cent sacchrose has been added. It is prepared from bouillon free from muscle sugar, in the same manner as lactose bouillon.

Evolution of Decay.

ARCH C. HART.

I believe decay to be a natural force which acts through media upon all material bodies, with the effect of changing their identity. I do not consider it a process of a force, nor as the result of the action of a form of energy, but as a force or form of energy that is only known to us by its manifestations through matter—effecting changes from a state of soundness or perfection to one less sound or perfect. I would classify it on the same general basis as gravitation, or any of the other great forces of nature. Gravitation existed and man used its power long before Newton formulated the law of gravitation. Men did not understand how the force acted.

So with the force decay. For ages men have recognized and used this power and depended upon its action for their existence. In these living bodies are we not dying to live, and living to die? But to prove that this force acts with other forces in making men and worlds grow old would be difficult. The lack of demonstration, however, makes it no less a fact. All things are growing or decaying; advancing in integration or disintegration. The change from perfection to one less perfect, to my mind, results from the action of a force, and we term it decay. Surely life is a force. Why then is not decay a force?

Fire, air, light, electricity, acids, alkalies, salts, alcohols, oils and water I take to be some of the important media through which decay acts in effecting change of identity seen in material bodies. In citing water as one of the most important media I have done so because it is one of the most universal of all compounds as well as the greatest of Nature's solvents and cements. Science has already proven that upon water for many of their combinations depend the animal, the vegetable and even the

mineral kingdoms. The cell holds imprisoned within its walls water of combination. The crystal holds within its angles water of crystallization. I do not think any argument is necessary to prove the importance of water as a medium for the action of decay.

In explanation of the action of many chemical compounds, chemistry, to my mind, teaches that many of the acids, alkalies, salts, alcohols and oils are antiseptics or germicides chiefly owing to their relative affinity for water. To say a substance is germicidal because it kills germs is like calling pistols homicidal because they kill men. Such an answer is not sufficiently scientific. How do antiseptics and germicides act as a class? What are the principles involved? Bacteria, we have already learned, will not grow on tissues that have been treated with solutions of certain strength of bichloride of mercury, nitrate of silver, chloride of gold, sulphate of copper, chloride of tin, formaldehyde, the essential oils, and many of the alcohols, acids and alkalies. For example, in the treatment of gonorrheal ophthalmia, the cause of as much blindness as any one disease, after having thoroughly cleansed the eye by repeated douchings of lukewarm sterilized water, there is instilled several drops of a two per cent solution of nitrate of silver, which, if used in time, will suffice to stop the disease.

How these chemicals act so as to prevent the growth of bacteria is due to their ability to harden albumen and render it insoluble to the action of bacteria; they were powerful in preventing decay just in proportion to their ability to form insoluble albuminates with the various tissues of the body.

How they harden albumen and render it insoluble is explained as follows: Chemistry teaches us that nitrate of silver and the list of germicides already named, likewise alcohol, acids, alkalies and oils, are constantly demanding water. That in many instances when applied to tissues

they cause a shrinkage or expansion beyond recognition. That the changed appearance noted in the tissue is due, in part at least, to the altered condition of the water in the tissues. The nitrate of silver, for example, has so changed the water of combination that the bacteria cannot penetrate the film thus formed with the tissues, and the bacteria in the tissue have either had sufficient water removed from them to cause their death, or else have become so confined that they cannot get the water necessary for their proper growth. The soil has in reality become hardened, insomuch as it is now insoluble to their digestive action, and might with truth be called antiseptic.

In proof of the importance of rendering the water in the tissues inaccessible to bacteria, and that the way the cells render themselves germicidal is by removing the water from the bacteria themselves, or else so placing the water of the tissues as to be insoluble to the digestive action of bacteria, it is necessary to explain why foods are preserved from the action of bacteria by cooking, drying, freezing, salting, sugaring in alcohol, acids, alkalies and oils.

In cooking foods, boiling, frying, baking, etc., the water is driven out of them, so that they shrink quite beyond recognition. Bacteria, when present, of course, are generally killed, and those that may get lodgment do not grow well, owing to the decreased quantity of water now present in the tissues. Freezing solidifies the water in the tissues, and while they remain frozen the water is inaccessible to bacterial growth. Drying removes the water of combination in part, so although the tissues may be covered with bacteria, the bacteria do not grow; not because the bacteria are dead, but because they cannot get the water necessary for their growth. The proof that it is only water that is needed, anyone knows who has had experience with dried foods that have become damp. In the use of smoke as applied in curing hams, bacon, etc.,

the heated air removes water from the tissues, and in its penetration carries into the tissues creasote and the other active agents that have an affinity for water. The creasote, etc., removes the water from the tissues, and especially from the surface, and because of a more immediate contact forms a layer insoluble to bacteria. Salting or sugaring are similar. The salt abstracts water from the tissues. Salt is sprinkled over fish as they are packed in vessels for preservation without the addition of any brine, as they are said to make their own brine—the salt literally squeezes the water out of the bodies of the fish. Any child knows that eating sugar will make him thirsty.

When we eat salty foods or much sweets we remove the water from our tissues, in reality cause a fever and thirst, just as if we were ill with some disease characterized by an increase of temperature. The sugar or salt enters the tissues and removes the water in part, and this increases the demand on the part of the tissues for more water. So with alcohol.

Bacteria, we have already learned, are only able to thrive when liberally supplied with water. There are other conditions necessary, as temperature, rest, light, etc. I mention water, as it is one of the conditions I believe we are able to control sufficiently to prevent their growth.

When a patient is down with typhoid fever or any of the fevers that owe their development to bacteria, the patient suffers with thirst and fever. Why? I believe it is because the growth and development of bacteria is consuming such enormous quantities of water that the system is over-drained, and the patient, if his tissues have not sufficient vitality to hold the water in combination, dies often simply because the bacteria have used up all the available water.

Physiologists speak of cells becoming specialized in their work of elaboration in the body. They mean that the cells divide up the work of the body just the same as

a body of men divides up the work of a community. By reason of this division of labor they are able to do their work better; they are specialists. To illustrate: the liver is an organ whose cells are specialized in the secretion of bile, glycogen, etc.; while the periosteum might be called an organ having cells specialized in the secretion of bone forming materials. So when the bones of the old are fractured they do not readily knit, because the cells specialized in the secretion of bone-forming compounds have almost entirely disappeared. It is the ability on the part of cells to become specialists, capable of doing one thing extremely well, that imparts to the organism immunity from certain diseases. The cells, learning that bacteria tend to remove the water from them, endeavor to fix the water in themselves. They may do this by the elaboration of some chemical compound that has a greater affinity for the water of combination than have the bacteria. Be that as it may, the bacteria cannot get the water necessary for their growth.

I believe if it is through a change in the main compound of the body that it becomes possible for disease to manifest itself, whether it be an atrophy or hypertrophy of the tissues resultant from the action of bacteria, chemical or natural forces, the more stable we can fix, seal or combine the water in the tissue, the more effective will be its power of resisting decay.

From my experiments with the teeth I believe it is possible to fix, seal or combine the water in the teeth so as to be inaccessible for a time to bacteria.

It has already been proved that certain conditions did confer immunity for a time to the digestive action of bacteria; that some teeth were less susceptible than others; that decay oft times is checked and the layer of decalcified dentine rendered immune to the further action of bacteria; that while the cracks in the enamel and the spaces between the cells are large enough for the entrance

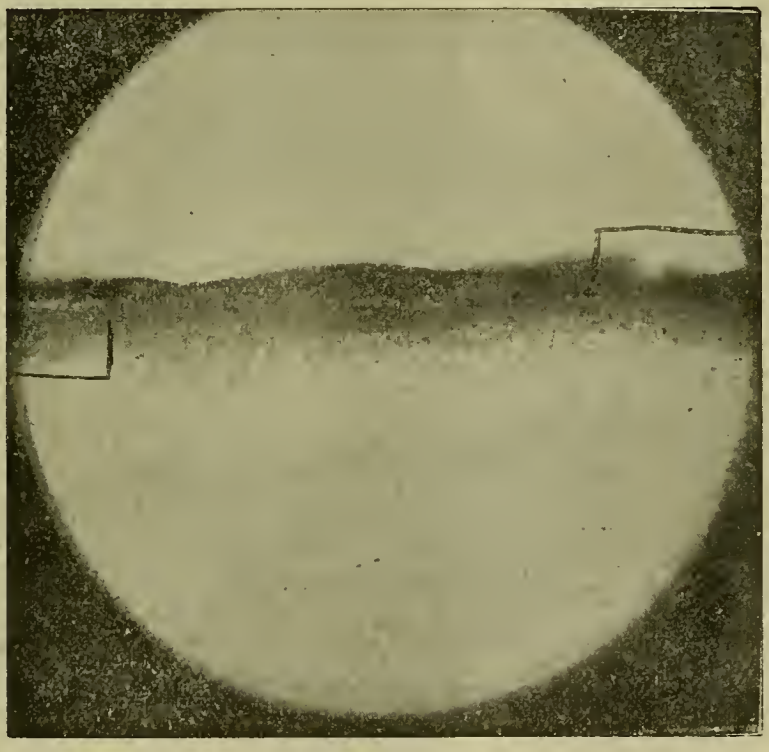
of bacteria there is often no decay of sufficient importance to attract attention, unless the enamel or dentine be examined by the aid of the microscope and differential stains. Then the surface enamel and dentine will show bacteria adhering, and even penetrating for a considerable distance the structure of the tooth.

The above figure shows the penetration of nitrate of silver into enamel partly decomposed by bacteria. *a*, Immune layer of enamel deeply stained. Taken from a young lady's mouth in which white decay was rapidly destroying the teeth. This tooth was treated three years ago to nitrate of silver without filling. Decay stopped in all teeth thus treated. Tooth crowned.

The extent of decomposition thus produced by bacteria may be as I have stated only visible through the microscope or differential staining. (See fig. 1, a : and fig, 2). For example, when a tooth presenting the white chalky

spots of beginning decay is treated with a solution of nitrate of silver, the extent of decay can be readily seen.

This partially decomposed surface containing millions of bacteria, I believe, can be made to take up substances capable of forming a layer immune to bacterial growth. So I have given the term "immune layer" to any layer of enamel or dentine that has become sterile to the growth of bacteria. This layer is partly due, in some mouths, to a protective film of bacteria that has become stained, and remains adherent to the surface of the teeth.



Section of enamel showing immune layer that has been produced by the action of formaldehyde or formalin upon the enamel. Hardened in alcohol 50-per-cent formalin 10-per-cent while still warm. Stained with Rubin S eosin and oil of cinnamon. The staining brings out the depth of penetration or the fact of bacteria. Under direct sunlight the bacteria can be seen penetrating the enamel at points indicated.

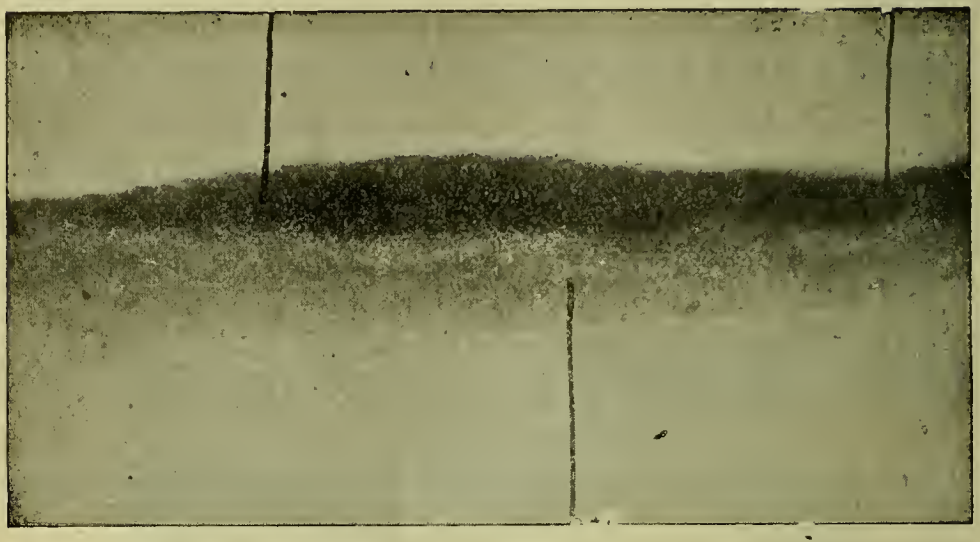
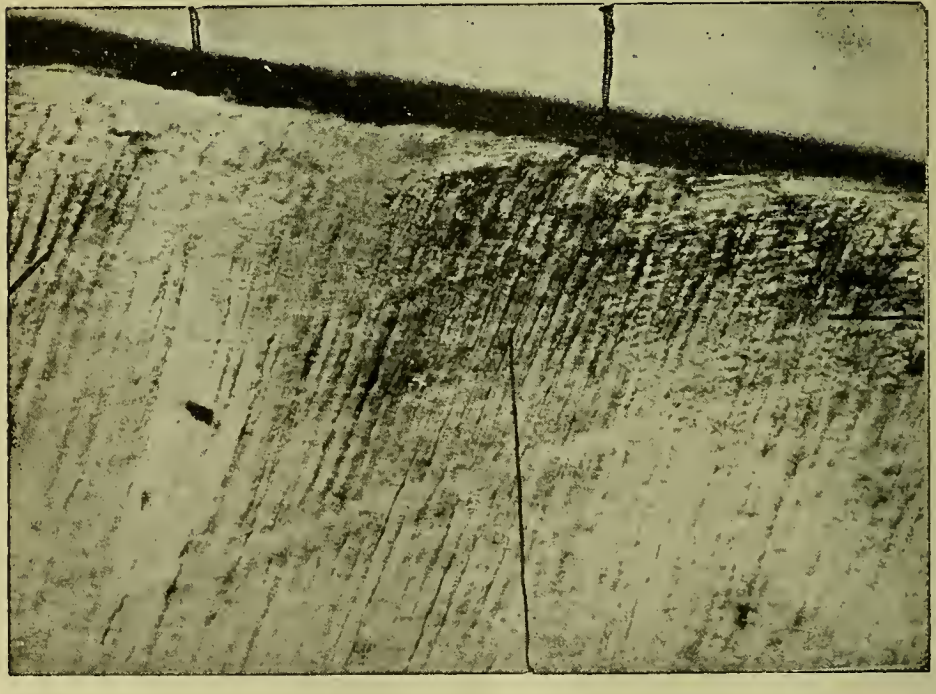


Figure 3 is the same section as figure 2, under a higher power. Shows coating of acid-producing and enamel-destroying bacteria, on top of figure; on lower side depth of penetration, stained by eosin.



I have observed many mouths in which there was no decay, or where decay had ceased ; and occasionally get-

ting such teeth for minute examination, I have found the outside layer of enamel and dentine to be stained with various compounds. The exact chemical nature of these compounds I have not been able to prove to my entire satisfaction. On scraping off this protective stain, the the microscope reveals it to be chiefly bacteria, and because of this, I am inclined to believe that certain bacteria have the power of protecting the teeth, just a lichen growing on rocks protects them from disintegration.

This shows penetration of tobacco stain in the enamel. Tooth lateral incisor from man aged 53. Has used tobacco since he was a boy. Mesio-approximal cavity with tooth-structure black from infiltration of stain. Cavity has been there for years, never became any larger, so he never had it filled. Tooth evidently immune to the action of bacteria.

The bacteria causing this peculiar discoloration I have observed growing near the gum margin,and in places difficult of cleansing, especially on the lingual and approximal surfaces. Their growth on the teeth will be noticed as a

peculiar black or reddish line, and when present ti is quite impossible to find decay. From this it would seem that there are certain bacteria which of themselves have the power to confer immunity from attacks of those bacteria that cause the destruction of the teeth.

In mouths where the decay is slow the decayed portion is always discolored. The cause of this discoloration is an important question, and in many mouths one quite difficult of solution. In many instances I have found the decoloration is tobacco stain (see p. 341). This, I observed, had penetrated quite deeply the enamel and dentine, and, by the aid of direct sunlight reflected by the mirror up through the Abbe condenser, and the specimen under the microscope, one could see many bacteria which had taken on the stain from tobacco. This would suggest that the discoloration seen in decay is partly due to bacteria having become stained.

The use of direct sunlight in the examination of specimens stained to show bacteria will make visible bacteria as far as they have penetrated the tooth structure. This idea came from observing how a ray of sunlight would make visible the bacteria and particles of dust in a dark room. I immediately made use of it in my study of specimens of teeth under the microscope. It was not necessary to grind them so thin to show bacteria penetrating both enamel and dentine.

This immune layer is sometimes due to the cracks and spaces in and between the cells having become filled with insoluble compounds, as is seen in teeth stained with tobacco or nitrate of silver, chloride of gold, sulphate of copper, etc., similar to the manner that oil covers the surface of water and retards its evaporation. In other words, the water already in the enamel, dentine and bacteria has attracted compounds that have a greater affinity for the water in the tooth substance than have the bacteria. So decay stops for a time, simply because the bac-

teria cannot grow down in the enamel, not because the hole is too small, but because the water in the tooth has become too insoluble or inaccessible to the digestive action of bacteria. The remains of the dead bacteria actually serve as a filling and protection against the entrance of other bacteria.

We carry into actual practice the application of the principle, viz: protecting the water in enamel and dentine by causing the tooth, and the bacteria as far as they have penetrated the tooth, to take up certain substances dissolved in water, like formaldehyde, nitrate of silver, chloride of gold, sulphate of copper, chloride of zinc, chloride of tin, and a whole host of substances of a like nature that I believe have the power of protecting the water in the tissues from the growth of bacteria.

Formaldehyde is used about as follows: After cleansing the surface to be hardened with pyrozone (three per cent medicinal) make several applications of the formaldehyde, varying in strength from two to forty per cent (forty per cent being full strength as it comes to us from the shops) to the cavity, carious surface, and healthy portion of the tooth and teeth under the rubber-dam, from ten to forty minutes. The cavity is then dried out and coated with a saturated solution of paraform in chloroform, to which has been added sufficient hard Canada balsam to make the solution a thin varnish. into this, after waiting for the varnish to nearly dry, may be burnished amalgam, stuck gold, gutta-percha or cement.

Formaldehyde should never be applied to the surfaces of the teeth, except the rubber-dam be in position, fitted evenly around the necks of the teeth, so that there shall be no holes whereby the mucous surfaces of the mouth may become exposed to the action of formaldehyde, as it produces an ugly slough. In the application of the formaldeyde it is important to have the surfaces of the teeth free from all adhering colonies of bacteria, so as to be

sure to kill all bacteria that have penetrated the enamel or dentine.

When once the people fully realize that bacteria alone are the cause of their soft, chalky teeth, and that it is possible to so harden the teeth that they will resist the solvent action of bacteria, then the gilded and glaring signs "painless extraction" will have to come down. Dentistry will become a profession loved by mankind, and to be called a dentist an honor.—*Pacific Medical Journal.*

Miscellaneous Notes on Microscopy.

JOHN. H. COOKE, F. L. S., F. G. S.

Cleaning Diatoms.—The addition of a little ammonia to the water in which diatomaceous materials are being washed facilitates the removal of slimy, gelatinous products, cleanses the diatoms, and hastens their settlement to the bottom of the vessel.

Pulverizing Rock.—To extract siliceous organisms from rock fragments, heat the rock to about one hundred degrees Fahrenheit, and then plunge it into a boiling solution of soda sulphate. This salt takes up water as it crystallizes, and the rock, therefore, readily pulverizes under its influence.

Diatom Mounts.—Balsam of tolu, from which the cinnamic and benzoic acids have been removed by prolonged boiling in a large quantity of water, is an admirable mountant for diatoms. It should be dissolved in rectified benzine, filtered, dried, and finally dissolved in alcohol or chloroform. The refractive index of the balsam thus prepared is 1.72 when dry.

Double Stain.—Methyl-green is an effective double stain for all vegetable sections. When applied to fibres, whether of pure cellulose or of lignin, it produces a deep green if the fibre has been mordanted before staining; while a light green is produced when the order of stain and mordant is reserved. Apply in strong aqueous solution.

Amœba.—For the preparation of permanent mounts of

living amæboid organisms, the following method, first suggested by Certes, will be found to give satisfactory results. To thirty cubic centimeters of the water containing the living amœbæ, add about one cubic centimetre of osmic acid solution (one per cent). After settling for a few hours wash the deposit, concentrate, stain, and mount in distilled water containing a trace of osmic acid.

Yeast Cultures.—To demonstrate in yeast cultures the formation of alcohol as a result of the growth of the plant in a sugar solution, the following method is less troublesome than the ordinary qualitative tests, and much more satisfactory. To the yeast culture add a few drops of iodine solution,and then enough KHO solution to destroy the color of the iodine. Iodoform will be produced and can be recognized by its characteristic odor.

Insect Preservation.—The proper preservation of soft-bodied organisms is one of the chief difficulties that the working microscopist has to contend against. The number of preserving media is great, but there are few of them that are at once as simple and effective as that suggested by A. E. Verrill for preserving insects in their natural forms and colors. The solution consists of two-and-a-half pounds of common salt and four ounces of nitre dissolved in a gallon of water and filtered. The specimens should be prepared for permanent preservation in this solution by being previously immersed in a solution consisting of a quart of the first solution and two ounces of arsenite of potash in a gallon of water.

Sponges.—To kill sponges extended, the specimen is placed in a glass jar filled with water, and the following solution is added drop by drop at intervals of one minute:—Methyl alcohol, ten parts; salt water,ninety parts; natrium chloride, six-tenths of a part. If the specimen does not retract after forty-five minutes, pour some hot sublimate on quickly. To preserve specimens for sections, put them in one per cent osmic acid for two minutes and then successively in five, ten, twenty, thirty per cent alcohol up to ninety per cent, harden in absolute alcohol and imbed in paraffin, stain with borax carmine and hæmatoxylin.

Plankton Nets.—The distribution of aquatic life from season to season throughout the various zones of the plankton in the larger masses of water of the globe is an object of study which has received considerable attention at the hands of investigators during the last decade. The greatest difficulty that has been experienced has been the need of apparatus upon which the operator can rely to work satisfactorily under all conditions and at all times of the year. The nets that are usually used cannot claim these advantages, inasmuch as they are always liable to damage and loss when working on a rocky bottom, and during the winter months, when the plankton yields its most interesting results they are inadmissible in northern latitudes owing to floating ice. The accuracy of the results, too, leave much to be desired, as with the net it is not possible to determine exactly the volume of organisms actually present in a given quantity of water, nor is it practicable to determine the various groups that are characteristic of the many vertical zones or strata of water of which the plankton is constituted.

Plankton Pump.—For critical work, nets have had to be discarded, and in place of them Dr. H. B. Ward has used during his recent investigations on the Great Lakes, a light weight force pump which he calls the "plankton" pump, and which can be carried about and operated by one person. The cylinder of the machine is eleven inches long by three and a half inches in diameter, and it has a capacity of three hundred and forty-seven and a half cubic inches per stroke. It is essentially an ordinary force pump, save that it has very finely ground check valves, to which. it is believed, the accuracy of the working of the apparatus is largely due. The pump is connected with the water by a hose one and a half inches in diameter, the lower end of which is adjusted to the various vertical zones of water by means of an attachment to a floating block. Most gratifying results have attended the use of this apparatus. It is possible to measure with great accuracy the amount of water filtered. Collecting can be carried on without any disturbance of the water, and water can be drawn from any

stratum, thus enabling the investigator to examine in detail the vertical distribution of the plankton.

Rubber Stoppers.—Dr. H. A. Hagan has made careful records for the purpose of determining the durability of the rubber stoppers which are used in vials containing microscopic objects in alcohol. From an examination of some seven thousand vials with rubber stoppers, two-thirds of which had been in use for from ten to twelve years, he comes to the conclusion that less than one in a thousand gives out every year after twelve years' use, and in the first six years probably only one out of two thousand. Stoppers of large size keep much longer than those of small size. American rubber stoppers are all made of vulcanized indiarubber, and have the disadvantage of forming small crystals of sulphur about the stopper, which becomes loosened and attach themselves to the specimens. It is supposed that pure rubber stoppers used for chemical purposes would not present this disadvantage, which may be obviated, however, or very much reduced, if the stoppers are washed or soaked in hot water for an hour or two before being used.

Hardening.—The usual processes that are adopted for the hardening of brain and similarly soft tissues, frequently result in failure by reason of the pressure of the tissues on the hard surface of the containing vessel, and the consequent mis-shapening of the lobes and convolutions, or the rupturing of the delicate superficial membranes. To prevent this, Prof. W. C. Krauss, of Nebraska University, has devised a hardening receptacle which obviates most of the disadvantages of the old method besides being exceedingly simple and inexpensive. An oval tin pail, of from four to six quarts capacity, with an ordinary handle and a tight fitting cover, has a series of ten hooks soldered on the inside about one inch from the top of the rim. The convex surface of the fresh brain is covered with a sheet of cotton, and over this is placed a piece of netting. The whole is then lowered gently into the pail containing the hardening fluid, and suspended in it by means of the hooks. The brain thus undergoes hardening in all directions at once,

and does not lose its normal symmetry. The cotton protects the convolutions from being ruptured.

Holoscopic Eyepiece.—Many workers have in their battery of objectives samples of both the achromatic and apochromatic types, having their favorites of both series. But this has necessitated two sets of eye-pieces, the Huyghenian for the former, and the Compensating for the latter; but this will no longer be necessary, because in the "Holoscopic" eye-piece of W. Watson & Sons we have an eye-piece which has been specially devised for both purposes. It consists of two tubes, the outer one carrying the field lens, and the inner, or telescopic one, having fixed to it the eye lens and diaphragm. Carefully selected appropriate glasses are used for the lenses, and when the telescopic tube is pushed in as far as it will go, the eye-piece is an under-corrected one, suitable for work with achromatic objectives. As the tube is drawn out, the eye-piece becomes increasingly a compensating one, possessing the so-called over-correction associated with the compensating eye-pieces which are used with the apochromatic objectives. This eye-piece yields really beautiful images, and not only does it obviate the necessity for two sets of eye-pieces, but it gives to the worker a power of adaptation which he has not hitherto possessed. Very few of the apochromatic objectives have the same amount of under-correction, consequently with the fixed eye-pieces the over-correction is frequently too great. With the "Holoscopic" eye-piece, the over-correction can be exactly adjusted to the objective, and a divided scale is engraved upon the draw-tube, so that the position may be recorded. It is made in the following magnifying powers:—

For the six-inch tube length, 5, 7, 10, and 14 diameters.

For the ten-inch tube length, 7, 10, 14, and 20 ,,

The cost is very little greater than that of the Huyghenian eye-pieces.—*Knowledge.*

BIOLOGICAL NOTES.

L. H. PAMMEL.

History of a Tuberculous Herd.—Dr. H. L. Russell (Bull. 78: Wisconsin Agricultural Experiment Station) gives some interesting facts concerning the history of a herd of tuberculous cows which was under his observation for several years. Calves from tuberculous mothers as well as progeny from non-reacting animals have been allowed to suckle several of the reacting animals; also healthy young cattle have been in contact with the affected herd in a stable and pasture to see if they would acquire the disease by ingestion or inhalation. In no case was the disease contracted. Frequent microscopic examinations were made of the milk and in not a single case could tubercle bacilli be found. The bacilli were always found in milk where small quantities of tuberculose sputum had been added to the milk. Milk from the animal is not a frequent source of danger, though from a precautionary stand-point to public health it should be pasteurized.

Bacterial Treatment of Sewage.—Pidgeon (Jour. Royal Agricultural Society, III. 10: 249) discusses the bacterial treatment of sewage as it is now carried on in some of the cities of England. At Exeter where a septic system of sewage purification has been adopted for the treatment of sewage derived fpom St. Leonards, a suberb having a population of 1,500 people and a flow of sewage averaging 54,000 gallons per 24 hours, good results have been obtained. During the first year's experience at Exeter the scum on the septic tank varied in thickness from four inches in the winter to one inch in summer. The effluent from the septic tank was suitable for land treatment. It is stated that no sludge is produced from such substances as paper, rags, or even feathers as they disappear in the septic chamber. The nitrifying filter occupies a space of 400 square yards while the septic chamber has an area of 130 square yards. This makes a total of nearly one-ninth of an acre. Without the use of chemicals the excreta of 1,500 people

are dealt with by Nature's scavengers. The disposal of sewage in this way is certain to assume great importance in all of the smaller cities and towns throughout the United States. The successful workings of any of these plants are therefore of great interest at this time.

Capsule Bacilli In Nasal Mucosa.—Simoni (Centralb., Bakt Par. 1 Abth. 25 : 625-31) says that the capsule bacilli are not infrequent in the nasal mucosa of man and animals and from a study of the biological and morphological characters of these bacteria he has been forced to the conclusion that they are all varieties of a single species of which the Pneumobacillus is the chief representative, and that Frisch's Bacillus is an extreme representative of the same type as well as the Bacillus mucosus.

Bacillus of Distemper of Dogs.—Dr. Jess (Centralb. Bakt. Par. 1 Abth. 25: 541-46. 1899) claims to have discovered in the nasal discharge of dogs suffering from distemper a bacillus which is 1.8–2.3 microns long .6–.9 broad. The Bacillus was also found in the blood and internal viscera of the animal. The organism is provided with a single flagellum at one pole. Infection of pure cultures produced fever, discharge from nose and eyes, diarrhoea and in some cases death after three days.

Plant Protection From Bacteria and Fungi.—Dr. Bokorny) Biol. Centralb. 19 : 177-185) gives an account of the various substances which serve to protect plants against fungus and bacterial enemies. The most widely distributed of these are tannins but it seems that 1 per cent of tannin is necessary to give the plants immunity against the attacks of fungus and bacterial enemies. Bacteria apparently offer less resistance to tannin than to moulds. Salts of oxalic acid are not poisonous to the lower fungi. The essential oils are very effective in affording protection to plants from the attack of parasite fungi.—L. H. Pammel.

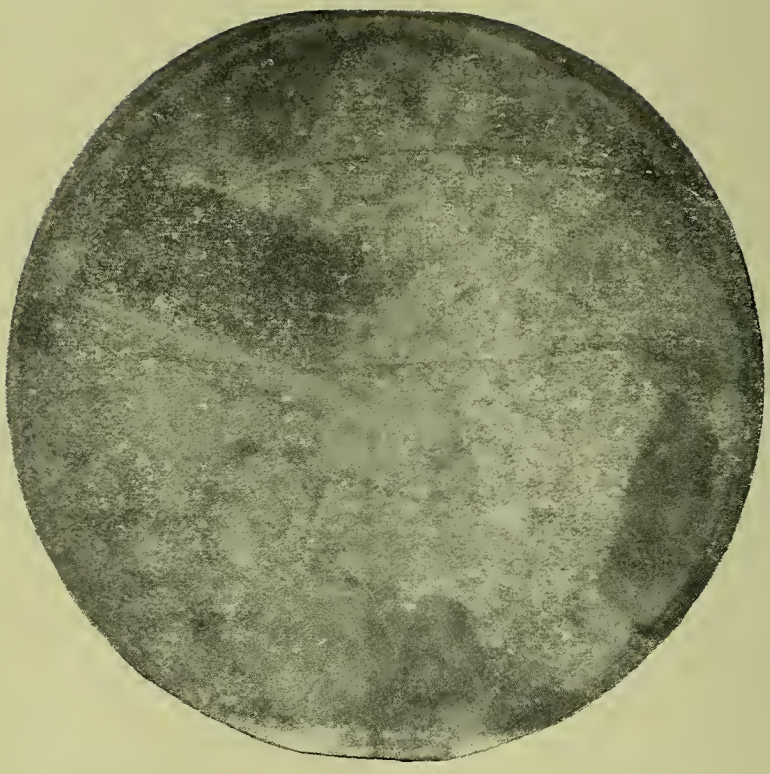


Fig. 1. Wort gelatine plate culture (mould).

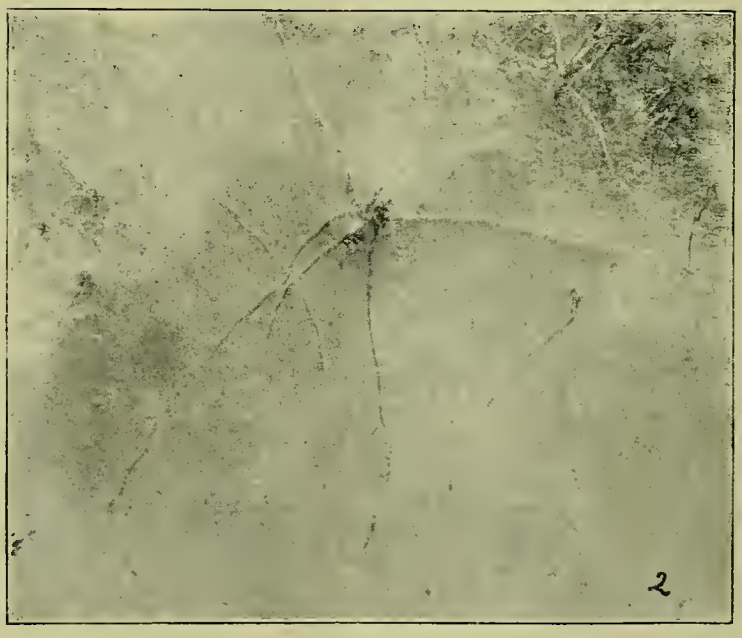


Fig. 2. Germination of conibia in miost chamder.

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CONTENTS.

Aspergillus Oryzæ.

K. E. GOLDEN.

With Frontispiece.

This is a mould which is of much practical interest by reason of its zymotic activity, since it secretes a diastatic ferment, and also for the claim which has been made that under certain conditions of growth, it is convertible into a yeast, and that, like most yeasts, it can give rise to alcoholic fermentation. It would constitute, in fact, if all claims made for it were true, a good working basis for an entire distillery. It has been used by the Japanese for centuries in one of their important fermentation industries, that of sake brewing, though like many other ferments

used in early times, its true nature was not understood.

In the manufacture of sake, rice is steamed and then mixed with some rice which is covered with the mould, or else the rice is sown with the spores. The spores germinate in the moist and warm air, forming a much-branched mycelium which penetrates to all parts of the grains. The mycelium in developing secretes a diastatic ferment, which acts on the starch, first liquefying it, then changing the liquefied starch to sugar. The formation of spores is avoided by adding quantities of fresh grain from time to time, and mixing the fresh grain with that which has been inoculated. The addition of fresh grain is repeated several times, the mass thus formed of grains and mould being given the name "taka koji." The koji is mashed with about three times its volume of fresh steamed rice and four times its volume of water, and then allowed to stand at a temperature between 20° and 30° C. After some days the mash clears, from the saccharification of the starch, and a spontaneous fermentation sets in. This fermentation is due, however, to a different organism from A. oryzæ. It is presumably on account of this fermentation that the mould has been erroneously called Japanese yeast. The fermentation goes on for two or three weeks, and at the end of that time the liquid is filtered. The resulting liquor is clear, pale yellow, and contains about thirteen per cent of alcohol.

The mould has not been well-known in this country until recently, though it has been known in Europe, and has received considerable attention from European botanists for about twenty years. In later years very enthusiastic claims have been made in regard to its physiological action, it being claimed that in the growth of the mould, "crystals" of diastase were formed in the filaments, that it was also so active and certain in its action as an alcoholic ferment, that in time it would entirely supersede yeast in the fermentation industries.

The material for the following experiments was some of the so-called "original" material, obtained from Takamine. This original material is a portion of koji, which was grown without any special precautions to keep it pure. Pure cultures were made from this material, and were also used.

A. oryzæ is a mould of a yellowish green color when seen in the mature stage. The color varies with the age of the plant and also with the medium upon which the plant is grown. Favorable solid media are bran, rice,and

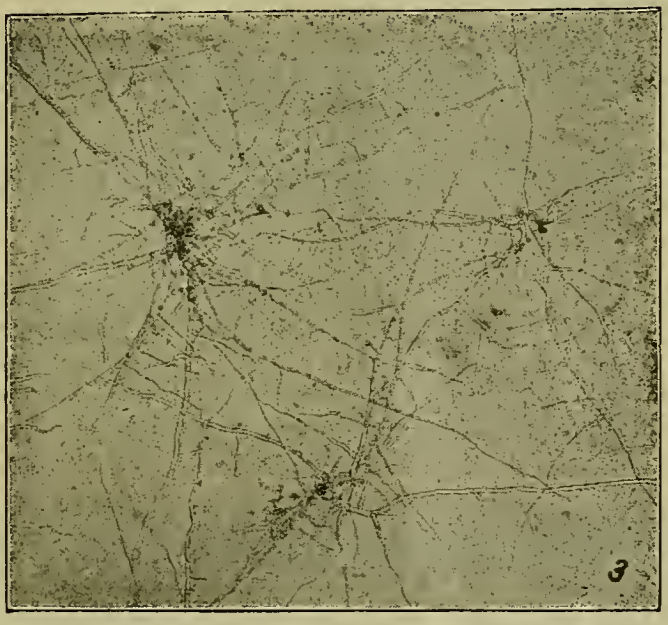


Fig. 3. Germination of conidia in a moist chamber.

wort geletine. On rice and wort gelatine the young growths are of a light yellow green, the color being due to the numberless conidia formed. As the growth ages, the color changes to dark olive green. On plate cultures the mycelia are usually in colonies, due to the massing and germination of a number of conidia in one spot; as a result, the plate presents a very irregular growth appearance (frontispiece, fig. 1). On bran the color of the young growth is much darker than that of the same age on rice, and in all growths the color is brownish olive to dark

brown. In very old growths not a trace of green appears.

The mycelium is a mass of fine, fleecy filaments, very much branched, and containing numerous septa. Wehmer states that the branching and septa were not easily seen,

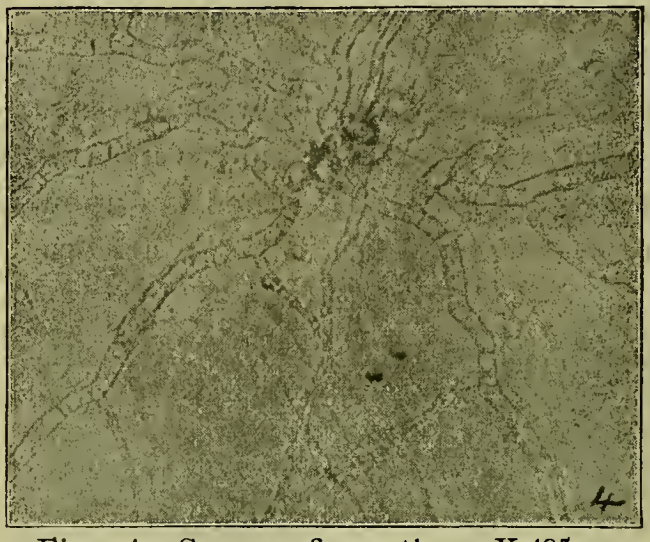


Fig. 4. Same as figure three, X 495.

except with high magnification, but I had no difficulty in seeing both features with low powers (Figs. 2, 3). These were taken from gelatine-plates cultures. The magnification is 75 diameters. In young growths the filaments

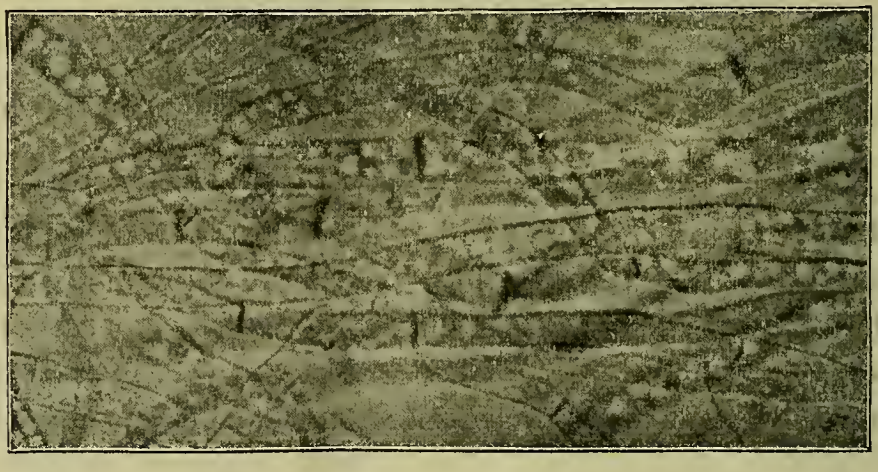


Fig. 5. Filaments from Wort gelatine plate culture, x 95.

are filled with a finely granular protoplasm, which becomes much vacuolated as growth proceeds. The filaments vary much in diameter even in the same culture,

the main filament being large, while the branches taper, sometimes these being extremely fine. In old cultures the filaments become very large, thick and rough-walled. They are always colorless.

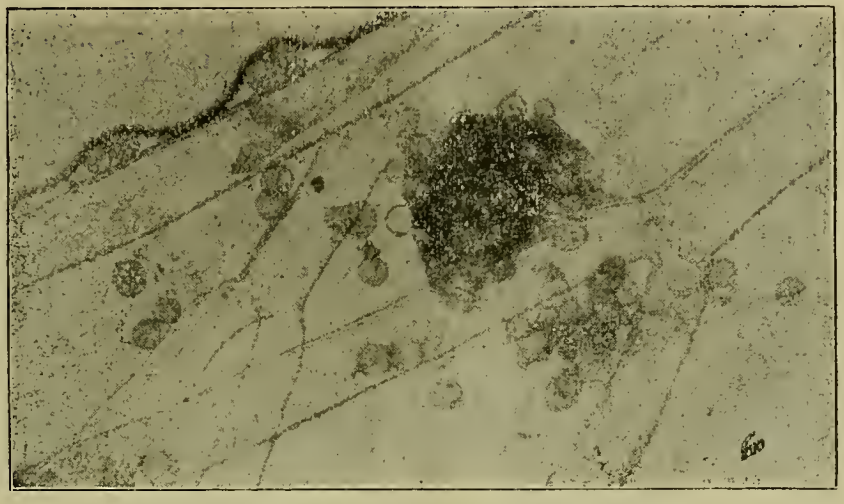
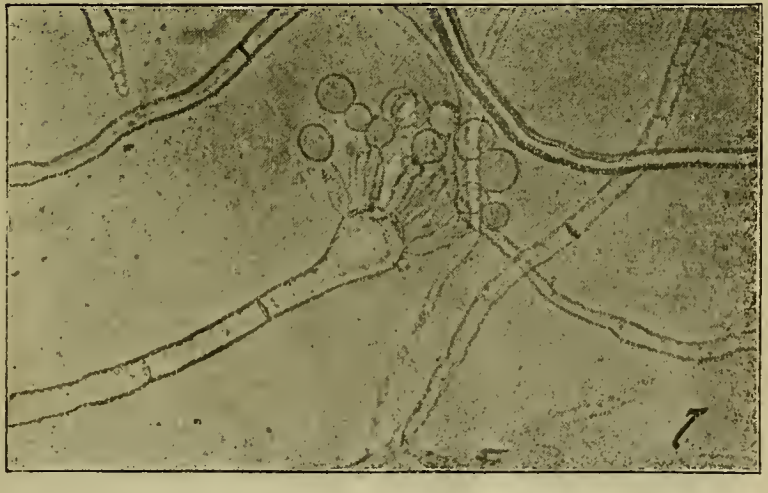


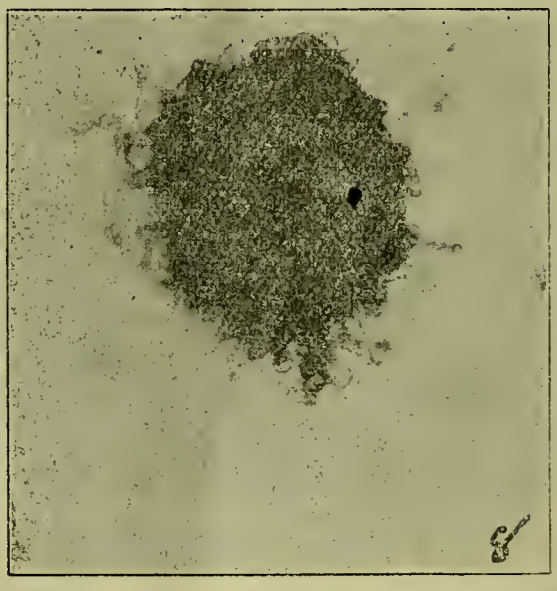
Fig. 6. Same as figure 5 but X 495.

The conidiophores, figs. 7, 8, 9 can be distinguished from the mycelial hyphæ as they gradually enlarge to the spherical end. The length varies to such an extent that any figures would not mean anything. The conidiophores are



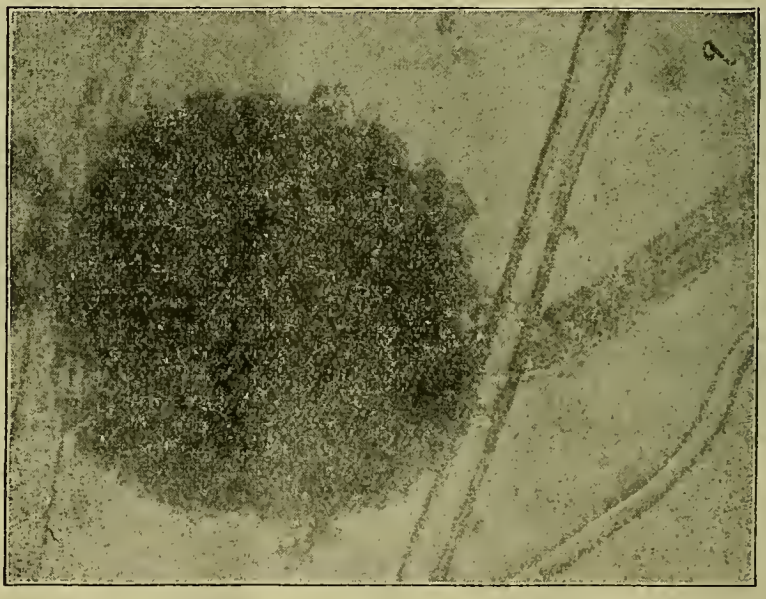
sometimes short branches at right angles to the filaments from which they arise sometimes so long that their connection is somewhat difficult to determine. Busgen gives

the length of the conidiophore as .5mm., Schroter, 1 mm., while Wehmer merely states that they vary in length. They become much enlarged in old cultures, the



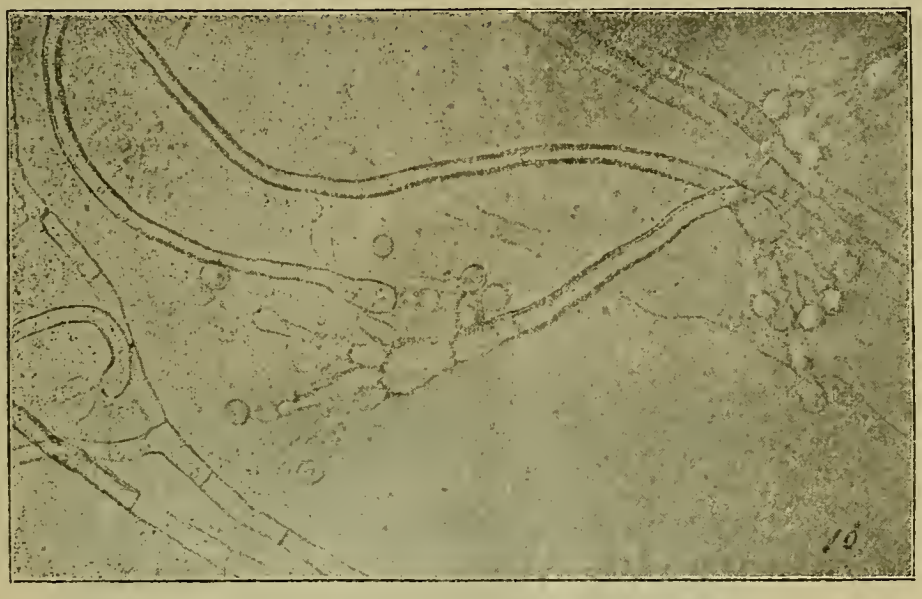
walls become very much thickened and also roughened.

In young growths the sterigmata are short and regular, and vary from a few in number to sufficient to com-

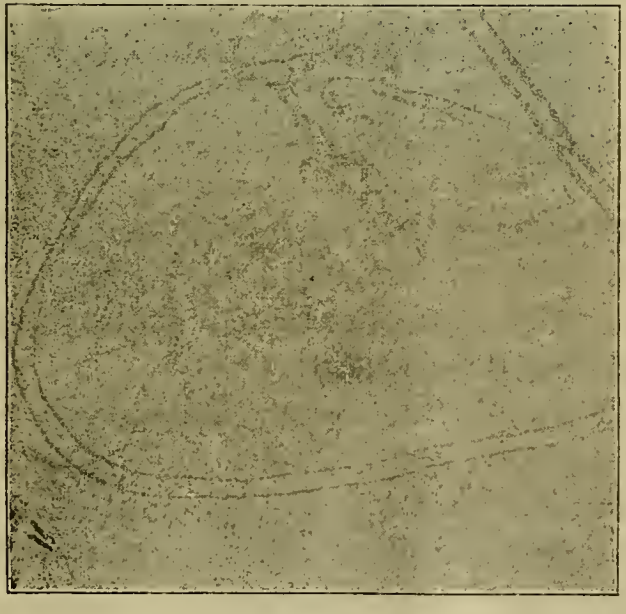


pletely cover the spherical head; but in older growths, especially when submerged, they become septate, some-

times a sterigma developing into a conidiophore, which on its end again developes sterigmata. These peculiarities are found in moist chamber developments, figs. 10, 11.



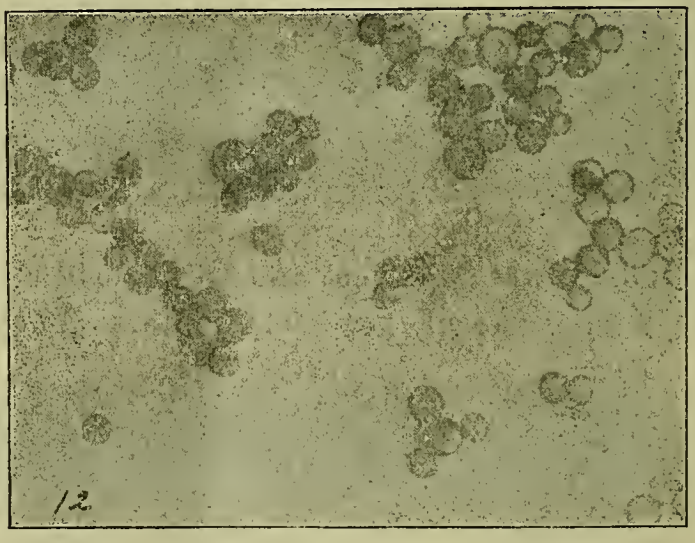
The conidia are spherical and are formed by an abstriction from the ends of the sterigmata. They are color-



less and smooth-walled when first formed and when grown submerged, but very soon develop a yellow color, which darkens to a green, and when old, olive and brown. As

soon as the conidia developed in the drop in a moist chamber reach the air, the walls thicken irregularly and assume a fine watery appearance. Fig. 7 shows the submerged head, fig. 12, older conidia grown in the air. No pictures could be taken that give an adequate idea of the number of conidia formed in a chain, as in their growth they extend so far beyond the plane of the water drop that it was impossible to focus them. And again they are so lightly held together that any attempt to mount them under a cover-glass causes them to separate.

The formation of conidia is the only method of repro-



duction known ; no perithecia have been observed, though they have been mentioned by the earlier investigators, but this has come about through the erroneous designation of the fungus as a Eurotium.

Physiology.—To determine if the mould were capable of causing alcoholic fermentation, the mould spores were sown in ten per cent solutions of maltose, dextrose, lactose; and sucrose, also wort, all in fermentation tubes. No gas was generated nor was any alcohol formed. The mould, however, grew much better in dextrose and maltose than in lactose and sucrose. The lactose growth remained meager, but the sucrose was merely slower, finally reach-

ing the same extent of growth as the dextrose and maltose. To test the action in bread, cultures in wort were made of the mould and also of a yeast which gives a vigorous fermentation. After these had grown for five days, sponges were made in which the yeast and mould were used and equal quantities of the other ingredients. In one set the yeast was used alone, in another the mould alone, while in a third the yeast and mould were used together. The sponges were allowed to ferment, then kneaded into dough, and again fermented, at the end of which time they were baked. The yeast sponge fermented most vigorously, the yeast and mould much slower, while the mould sponge showed but very little change. The yeast and mould together took an hour longer than the yeast alone to reach the same degree of fermentation. The loaves from the yeast were of sweet taste and odor, and even-grained. Those from the mould were soggy and heavy, had a sweet odor, but left a sharp aftertaste. The loaves from the yeast and mould were very like those from the yeast, but also left the sharp aftertaste, though this was not unpleasant. Four persons having no knowledge of the constituents of the loaves, selected the ones made from yeast alone as being the best bread.

In testing the germinative power, cultures were made in wort, wort gelatine, Pasteur solution with the four sugars, lactose, dextrose, maltose, and sucrose from inoculating material that varied in age from very young through different periods to one year and eleven months, and which had been grown upon rice, bran, wort gelatine, wort, and Pasteur solution containing the different sugars. The results show that the germinative power lessened with age, but a more important factor than age was that of the original medium in which the culture had been made. Some of the growths from the wort gelatine plates had entirely lost their germinative power, while others were weakened. Wehmer states that the age of the in-

oculating material made no difference in germinative power, neither did the medium upon which it had been grown.

For ascospore formation young conidia were sown upon gypsum blocks in the usual way for obtaining yeast spores, and in about a mouth's time rounded masses of protoplasm, resembling yeast spores, were formed in some of the cells, though no cell-wall could be determined for these spore-like bodies. The same spore-like bodies were formed from the protoplasm in mycelial filaments undergoing the same treatment.

No experiments were made directly to determine the diastatic action, as work upon this has been done quite extensively by chemists. So far as any experiment would show, there was no indication that A. oryzæ has the power of causing alcoholic fermentation, nor of being transformed through any conditions whatever into a yeast, neither can it be used effectively in bread-making.

Formaldehyd Disinfection.

The hygienic laboratory of the Marine Hospital Service has conducted extensive experiments in disinfection with dry heat and formaldehyd in a vacuum chamber to determine the quantity of formalin (40 per cent solution of formaldehyd gas) and degrees of heat required for disinfection in one hour or less. Hitherto 12 to 48 hours have been required for efficiency under the low percentage of gas secured. A new apparatus has been made by the Kensington Engine Works, Phila., which holds as high a per cent of gas as desired in an airtight chamber. It has a capacity of 1680 liters, with vacuum apparatus, formaldehyd generator and ammonia generator. The cylinder contains a rack on which disinfecting articles are hung. The generators are copper or iron boilers containing copper coils,with a capacity of 3.21 liters. The coils connect

with a steam boiler and the generators communicate with the disinfecting chamber by small pipes.

METHOD OF OPERATING.

Steam is turned into the jacket until a temperature of at least 80° C is obtained within the chamber. The articles to be disinfected are placed in the rack or car which is rolled into the chamber, the door is closed and secured, and a vacuum of 15 to 20 inches obtained. By this time, steam having been allowed to course through the coil within the generator containing the formalin mixture, there should be a pressure of 40 to 60 pounds. The heat in the chamber has been gradually increased, and the thermometer should register nearly or quite 90° C. With the above pressure and temperature, the valve between the generator and chamber is opened just enough to permit the escape of gas in sufficient quantity to cause the pressure to gradually decrease.

At the end of ten or fifteen minutes all the gas has probably been expelled and the valve may now be closed. Steam is kept slowly coursing through the jacket in order to maintain a constant temperature of 90° C., or more. A higher degree of heat, while doing no harm, does not appear to increase the efficiency of the formaldehyd, but it is certainly diminished at a lower temperature. The vacuum will usually fall seven or eight pounds, and possibly more, according to the quantity of gas entering the chamber. At the end of one-half hour the vacuum may be broken and air allowed to fill the chamber. This mixture of air and formaldehyd is then exhausted, and the amm ia generator, previously supplied with aqua ammon one half as much as of formalin, is operated in the manner as already described for formaldehyd; the latter having been in a great measure removed with the air, a a small quantity of ammonia will neutralize the residue. Air is again allowed to enter the chamber and the door may now be opened. It is usually found that there is a

slight excess of ammonia, but not enough to cause any annoyance. Neutralization of the formaldehyd occurs very promptly, consequently only the few minutes necessary for operating is consumed by this part of the process. The formalin mixture used consists of 100 parts formalin, 20 parts calcium chloride fully dry, and 10 parts glycerin.

CULTURES.

The cultures employed were in each instance as nearly similar as it was possible to have them. The typhoid and diphtheria were forty-eight hours old bouillon cultures, and the anthrax was of several days growth—old enough so that spores were always present.

Small slips of sterile crash were moistened with the culture, and those to be used in the dry state were allowed to remain in a Petri dish, with the cover slightly raised, at room temperature until the moisture evaporated; those to be used wet were freshly prepared and no time given for evaporation. Each slip was then wrapped in sterile cheese cloth so that it was protected by about ten thicknesses. While the cultures were exposed under a variety of conditions, they were always prepared in the same manner. At the conclusion of each experiment they were removed, the crash slips were placed in sterile bouillon tubes and incubated at 37° C. for seven to twelve days.

It was found that while the controls always grew in twenty-four to forty-eight hours, the growth in many instances in the other tubes seemed to be inhibited so that development did not occur until the sixth or eighth day. In all the experiments with anthrax alone the tubes were incubated ten to twelve days. This particular organism was very virulent, and in cases of doubt as to the growth, mice were inoculated to decide the question. By combining dry heat and formaldehyd, better results odtaineb than with the gas at ordinary temperatures. The degree of heat and the quantity of formalin required for efficient work in a given space were not determined.

In order to decide these points and to complete the work begun two years ago, those experiments were undertaken. It was deemed advisable, first, to determine accurately the effect of dry heat alone upon the organisms selected, which was in reality a question of penetration, because the lowest temperature in any series, 80° C.,would kill diphtheria and typhoid in less than one hour; second, to ascertain the germicidal effect of formaldehyd in large quantity at ordinary temperature, 25°-30° C.; and third, to decide upon the most efficient combination of heat and formaldehyd. All experiments were carefully controlled by 1 to 3 tubes for each germ.

In 26 experiments of cultures buried in hair pillows,or in feather pillows, inside lightly rolled blankets, in cotton mattresses, between mattresses or free in a chamber, with temperature of chamber at 80° C; time, one hour; and vacuum, none, or 15 inches; there were both moist and dried cultures. As was to be expected, typhoid and diphtheria were killed under most conditions, but the degree of heat was not sufficient to destroy the more resistant anthrax spores. The feather pillow, the tightly rolled blanket, and the cotton mattress were not always penetrated. In the third series, the killing of the anthrax in several instances can only be accounted for on the theory that formaldehyd reached it from some source.

The effect of formaldehyd in considerable quantity at room temperature, about 30° C. was not to penetrate the ten thicknesses of cheese cloth,the only protection around the cultures free in the chamber, in sufficient quantity to kill anthrax, and it also failed to reach the typhoid and diphtheria, save under the first condition. Repetition of this set of experiments with identical results prove that when the exposure is limited to one hour, formaldehyd, even in large percentage, at room temperature is practically inert as a disinfectant. The disinfection of bulky articles, such as mattresses and pillows, could not be ac-

complished without an increase in the quantity of formalin or of the degree of heat; but before instituting any change in the methods of procedure, it was decided to attempt the disinfection of lighter articles and those generally recognized as better adapted for treatment with formaldehyd. The cultures were put in the pocket of a coat and the garment so arranged that the gas must penetrate the cloth to reach the germs. The same was done for the cultures placed in the trousers pocket. The letter was sealed and without perforation thrown into the chamber.

It was now evident that when anthrax was destroyed the other organisms failed to develop and further work with them was abandoned. At a temperature of 80° C., or more, it was thought that before the formaldehyd entered the chamber the slips of crash must be dried, and in the succeeding experiments the slips were saturated with a bouillon culture of spore-bearing anthrax and placed in the chamber without unnecessary delay. None of the quarantinable diseases is caused by a spore-bearing organism so far as we know, and their infectious principles are not classed as resistant to the disinfectants in common use, but in these experiments disinfection has not been considered successful unless the spores of anthrax were killed.

Although the results obtained with 360 c. c. of formalin were satisfactory it was considered desirable to fix upon the minimum efficient quantity. To that end, experiments were made with 100 c. c. and 200 c. c. of the mixture, and finally with 100 c. c. of formalin, to which was added 400 c. c. of water with calcium chloride and glycerin in the same proportion as for 500 c. c. of formalin. This seemed to indicate that penetration is greater for a small quantity of formaldehyd if a little moisture is present. One chief advantage for formaldehyd is that it is efficient in a dry state, and is, therefore, available for disinfection of articles injured by moisture. For that

reason the water of the formalin should not be increased in actual work. These three series are a proof, if any is necessary, that the germicidal effects in the preceding series were due to the formaldehyd, the heat acting merely as an intensifier.

This cylinder had a capacity of 1,680 liters. The ratio of 1,000 : 1,680 is rather more than 1:2; that 750: 1,680 is something less than 1:2; that of 500: 1,680 is about 1:3 ; while that of 360 : 1,680 is a trifle more than 1:5. Once the capacity of the chamber in which the work is to be performed is known the proper quantity of the mixture required is already determined. Formaldehyd is not recommended even when combined with a high degree of heat, as a disinfecting agent upon which reliance can always be placed for the treatment of articles requiring much penetration, especially when the exposure is limited to one-half hour. In twelve series the quantity of formalin mixture varied from 360 c. c. to 1,000 c. c., or taking the ratio given, from 1:5 to 1:2 mattress and pillows were sterilized ; but in two series in which the proportion of the mixture was as 1:5 the mattresses and pillows were not penetrated. A critical examination of nearly all the published experiments with this agent will reveal instances in which organisms that there was every reason to expect would be killed have survived, and vice versa. It is that occasional unaccountable uncertainty of action that calls forth the warning not to attempt disinfection with formaldehyd in a case in which there is any doubt as to the result.

Care must be taken to arrange the various articles in such a manner that a free circulation is allowed around and among them. If the contents of a trunk were thrown into the chamber in a heap it would not be penetrated. The articles which can be treated successfully will be found in practice to be wearing apparel, books, letters and such other things as do not require deep penetration,

Whenever possible they should be suspended on hooks or lines or so spread that the largest possible surface is exposed. Leather goods, trunks, suit cases, etc., may be subjected to 90° C., dry heat, without injury. The elasticity of rubber is apparently not diminished.

OUTLINE OF METHOD.

1. Heat jacket with steam so that temperature of chamber is 90° C.

2. Pour into formalin generator one-fourth as many c. c.. of formalin mixture as there are liters capacity in chamber, e. g. 625 c. c. for a chamber of 2,500 liters capacity.

3. Obtain a vacuum of 15 inches or one-half an atmosphere.

4. When pressure in generator reaches 40 to 60 pounds open the valve just enough to allow a gradual reduction of pressure by the passage of formaldehyd into the chamber. If this valve is opened too wide the fluid may be driven into the chamber and its contents injured.

5. When it is found that all the gas is driven off, close the valve to the chamber.

6. Maintain temperature of chamber at 90° C., or even higher, by allowing steam to course through jacket.

7. At end of thirty minutes the vacuum may be broken and the mixture of air and formaldehyd exhausted.

8. With a vacuum of 10-15 pounds the ammonia generator may now be operated in the same manner, using one-half as much ammonia water as of the formalin mixture.

9. After the ammonia gas has entered the chamber the vacuum may be at once broken and the chamber opened.

10. Each time, after the formalin generator is operated, it should be thoroughly cleansed with water. If the sirnpy residue is not removed it soon fouls the apparatus and clogs the outlet.

Picro-Carmine and Alum-Carmine as Counter Stains.

B. D. MYERS.

The excellence of picro-carmine was first noticed in staining developing bone which had been decalcified. Picro-fuchsin was being regularly used as a counter stain with hematoxylin. Merely for the experiment picro-carmine was used on one section and left nearly two hours. Much to our surprise and pleasure we found that,instead of our section being ruined, we had secured an excellent differentiation. This was not the first attempt with picro-carmine, but always before,the time had been short, from two to fifteen minutes. The advantage of the stain over picro-fuchsin is noticeable in the superiority of differentiation secured.

The embryonal cartilage cells are better marked by the hematoxylin and picro-carmine, for the alkaline picro-carmine does not fade the hematoxylin as does the acid picro-fuchsin. It is particularly in the zone of calcifying cartilage that this superior differentiation is noticed. The vertically arranged rows of cartilage cells have lost their horizontal septa,but the vertical septa are pronounced and project into the primary marrow cavity as irregular trabeculæ of calcified cartilage. The osteoblasts have enveloped these trabeculæ with a covering of true bone and at the same time the cartilaginous trabeculæ within are being absorbed and true bone substituted.

This true bone, with the picro-carmine, has taken a red which is brilliant in comparison with picro-fuchsin ; and the gradually diminishing and disappearing cartilage which, with picro-fuchsin, has taken a stain not distinguishable from that of the cells of the true bone is,with picro-carmine, beautifully differentiated by a clear pronounced blue,showing the alkalinity of the picro-carmine. This tendency on the part of picro-carmine to bring out the hematoxylin as a blue, while the acid picro-fuchsin

fades it, is very noticeable in the tonsil of dog which was next submitted. In the mucous cells near this gland the nuclei, removed as far as possible from the lumen, are brought out with unequalled clearness. The structure of the blood vessels is also brought out with great distinctness, and the differentiation throughout is very marked.

Quite as striking a contrast between picro-carmine and picro-fuchsin is noticed in a section of the pyloric stomach of a kitten. The stain with picro-carmine is not only more differential,but the unstriped muscle of the stomach and blood vessels is brought out much better by the picro-carmine. During the summer picro-carmine was tried with good results on sections of the fallopian tube of a mare. It has been used with greatest success on tissues which present a mucous surface,and while these successes have been noted, an equal number of failures were encountered, so no claim is made for picro-carmine as a "pan" stain. It seems particularly unsuited for tissues that stain with difficult. Ranvier's picro-carmine was used in most of these experiments, but Bizzozero's was used with equal success. Mayer's recent formula was used in the histological laboratory at Cornell last year with results quite as good as those from Ranvier's.

In the summary, then, we find picro-carmine,in the cases noted, gives, with hematoxylin, a more differential stain than picro-fuchsin, and shows the characteristic alkaline reaction with hematoxylin, bringing out the hematoxylin as a beautiful sharp blue, while the acid picro-fuchsin tends to fade it. Two hours is, in general, the best time for picro-carmine. There is no danger of overstaining. Stohr in his text book of Histology directs that developing bone be stained with hematoxylin and then with picro-carmine.

ALUM CARMINE.—During the summer it was my privilege to prepare some slides of liver of guinea pig to show Anthrax bacilli. I attempted to get a contrast stain and

finally succeeded with alum-carmine. I had tried picro-carmine without success, and had never been able to secure a good stain with picro-carmine on liver. One hour and fifty minutes with alum-carmine gave the best results. The crystal-violet with which the bacilli were stained, and which is washed out much or entirely by the alcohols and clearer, must be sufficiently intense to permit of thorough dehydration and clearing and yet leave a distinct stain. One and one-half minutes will suffice if care is taken not to leave longer than is necessary in alcohol. By this stain the nuclei and the cell body are clearly differentiated and the alum-carmine forms a very good contrast stain with the crystal-violet. The simplicity of the method commends it. With methylene blue a still greater contrast may be secured.

Fusulina Cylindrica Shell Structure.

ALVA J. SMITH.

The living Fusulina cylindrica was a member of the animal sub-kingdom Protozoa, class Rhizopoda, order Foraminifera, family Nummulitidæ. The shape of the shell of the young is a spheroid, but changes during the growth of the animal to an ovaloid, which resembles a grain of wheat in both form and size. This shell is composed of longitudinal chambers arranged spirally around a central spherical chamber, making about ten complete whorls in the adult shell.

The average length of the Fusulina is about 6 mm. and the thickness 2.5 mm. The spheroidal nucleus or central chamber is about 1-10 mm. in diameter and is provided with many circular openings, through which the animal protrudes its thread-like pseudopodia, and is connected by a small open entrance to the second chamber. The second chamber is about 3-100 mm. in width, while its length embraces slightly over one-half of the nucleal chamber.

Each succeeding chamber extends a little beyond its predecessor. This lapping of the chambers at the ends causes the increase in the longitudinal dimensions of the shell as it grows by the addition of chamber after chamber. The size of the chambers and the thickness and strength of their walls increase from the center out. An open passage bearing a resemblance to the siphuncle in cephalopods lies as a trough along the ventral side of the chambers and cuts away the lower half of the septa where it passes through them.

The name "involute sinus" has been proposed by Professor Williston for this trough-like passage. The width of the openings in the septa increases from about 1-25 mm. at the nucleus to 1 mm. in the outer whorls. The septa are also punctured by many minute circular openings (foramina) which were once occupied by the pseudopodia of the animal, and later served as ways for the protoplasm of the animal to commuicate from chamber to chamber. The outer walls of the chambers possesses very few if any foramina or other openings. They are slightly more convex than the general curve of the whorl, and extend in graceful double curves from the girdle to either end, giving a corrugated appearance to the outer surface of the shell. The living Fusulina was evidently one composite body, occupying all the chambers of the entire shell at the same time, with a common vitality ; a continual circulation of protoplasm taking place from chamber to chamber through the minute foramina and the siphuncle-like openings in the septa. The first chamber occupied by the young Fusulina is nearly spherical. A spherical first chamber is found in a great number of Foraminifera whose later forms bear no resemblance to a sphere, the form of the succeeding chambers and the final shape of the adult shell depending upon the order in which the multiplication of chambers takes place and their manner of attachment to the parent mass.

In the Fusulina the animal occupied the central spherical shell for a time; then a portion of its ameboid contents spread out through an opening in the shell, forming a belt about 3-100 mm. wide on the outside, its length embracing slightly over one-half the perimeter of the shell. This strip of living matter soon secreted a calcareous covering, which is the second chamber of the shell. The third chamber is formed by a similar process along the sides of the one already formed. A continual repetition of this process completes the shell as we now find it fossilized in our limestone.

Dana says "the cells of rhizopods are each occupied by a separate animal." While it is possible for this to be the case with some species of rhizopods, it is impossible with the Fusulina, for an independent animal occupying the central cells would have access to neither food nor oxygen, after being enclosed by the outer portions of the shell. The possession of the trough-like siphuncle indicates the flowing of matter from chamber to chamber along this course, as also do the thickened ends and rounded corners of the septa where cut by this trough. We know that an irritation of the bodies of conchiferous animals produces an increase in the calcareous secretions at the point irritated. Then the increased thickness and rounded corners of the septa where cut by the stolon passage may point to an irritation of the Fusulina at these points, which could only come by a flowing of the protoplasm through the involute sinus. Only by a system of circulation through the openings in the septa can an ameboid animal secure the essential food and oxygen to maintain life while inhabiting the recesses of a chambered shell like the Fusulina cylindrica.—*Kans. Acad. Sci.*

Moore's Bacteriology.—"It would be difficult to find anywhere in the world, in the same number of pages, as many important and useful suggestions."—A Critic.

BIOLOGICAL NOTES.

L. H. PAMMEL.

Tsetse Fly Disease.—Plimmer and Bradford (Veterinarian 72: 648) give a preliminary note on the morphology and distribution of the organism found in the Tsetse fly disease. The organism is called by them Trypanosoma brucii, and according to Buetschli belongs to the order Flagellata and to the sub-group Monadina. According to these investigators the reproduction is by division which is of two kinds, longitudinal and transverse, and conjugation. This consists essentially of the fusion of micro-nuclei of the congugating organs. Amœboid forms are found with and without flagella but always possessing a macro- and micro-nucleus. The spleen is the organ in which these forms occur in greatest abundance. The whole spleen is filled in every part with plasmodia. The enlargement of the spleen is caused by masses of plasmodia.

Mallein.—John A. W. Dollar summarizes as follows concerning the report of the Glanders Committee appointed by the Board of Agriculture of England. (Veterinarian 72: 657). The evidence before the committee in regard to the use of mallein is somewhat conflicting. It appears certain that judged by the reaction of the injection of mallein a considerable number of cases of disease are detected which would not have been diagnosed as glanders by the ordinary clinical signs. Mallein is therefore a very sensitive diagnostic reagent.

Milk of Tuberculous Animals.—The Seventh International Congress of Veterinary Surgeons make the following recommendations with regard to the utilization of milk of tuberculous animals. The milk of tuberculous animals should not be used for human food if the animals are emaciated or affected with tuberculosis of the udder. In accordance with the mode of procedure in Denmark and Sweden, the emaciated tuberculous dairy animals should be immediately removed from the farms and slaughtered, compensation given to owners. In regard to flesh they recommend that all organs affected with tuberculosis, together with

their appendages should be detected and removed. In the case of local tuberculosis where the centers are limited to the internal viscera, and are healed, the meat may be disposed of in a raw state to be used as food.

Texas Cattle Fever.—In a recent number of the New York Medical Journal, Dr. Theobald Smith discusses the Aetiology of Texas Cattle fever with special reference to recent hypotheses concerning the transmission of malaria. The paper gives a brief history of the subject from the time that he began his work and reviews the current theories regarding the transmission of malaria. The summary is, indeed, a most excellent one. Since the aetiology of the American disease has been cleared up the same malady has been found to exist in Finland, Roumania, Italy, Australia, South Africa, and German East Africa, and he thinks it will be found in other similarly situated countries whenever a migration of cattle has taken place, which will tend to mingle immune and susceptible animals. "The recent investigations of Ross, confirmed and materially extended by Koch and his colleagues, showing that in the Proteosoma infection of certain birds the blood parasite completes its cycle of development in one and the same insect by reappearing finally in the salivary glands, so that the insect becomes infectious a certain number of days after drawing infected blood, introduces a most interesting modification of the course pursued by the blood parasite in Texas fever."

Rusts.—Mr. M. A. Carleton of the Division of Vegetable Physiology and Pathology has recently published the results of his work on the Cereal Rusts of the United States. (Bull. U. S. Dept. of Agr. Div. Veg. Phys. and Path. 16.) This work is largely a physiological investigation, and from his studies Mr. Carleton says: "At least six and probably seven distinct rusts affect the cereals of the United States, as follows: Orange leaf rust of wheat (Puccinia rubigo-vera tritici), orange leaf rust of rye (P. rubigo-vera secalis), crown rust of oats (P. coronata Corda), black stem rust of wheat and barley (P. graminis tritici Eriks. and Henn.), black stem rust of oats (P. graminis avenæ Eriks. and Henn.), and maize rust (P. sorghi Schw.).

Bartholomew has published (Trans. Kans. Acad. Sci. 16:168) a valuable list of the Kansas Uredineæ. He has brought together a comprehensive list of the diseases as they occur in that state. Kansas has had several valuable collectors, Hitchcock, Kellerman, Carleton, Swingle and Norton, all of whom have added largely to the known species of the Uredineæ of that state. Bartholomew cites some of the literature of the species and gives a complete list of the host plants, making this, indeed a very valuable contribution.

MICROSCOPICAL APPARATUS.

New 1-12 inch Immersion Objective.—Messrs. R. and J. Beck have a new 1-12 inch immersion objective with a numerical aperture of 1.4 and an aplanatic cone of 1.35 N. A. It is perfectly achromatic, and the makers modestly and rightly claim no more than this. It is exceptionally free from color, the definition is excellent, and the increased quantity of light passed is most noticeable. It bears comparatively high eye-piecing well. The working distance is of coarse rather less than in objectives of lower aperture. The price brings it within the reach of all workers requiring an objective of this description, being $40 or $48 with correction collar.

New Immersion Condenser.—Messrs. Beck have out a new immersion condenser, with a numerical aperture of 1.36 to 1.4, and an aplanatic cone of 1.3 N. A. The combination consists of four systems of lenses, the front of which is a hemisphere with three combinations behind, and constructed on the principle of an oil-immersion objective. Testing this condenser in connection with the objective 1.4 N. A. above described is one much pleased with its performance. The working distance is 0.6 inches. By an ingenious arrangement the optical part of the condenser can be reversed in the mount so that it may be used with microscopes fitted with under-stage fitments instead of the usual focussing substage. The top lens is also removable. The price of the optical part is $13; of the mount, with iris

diaphragm and carrier for stop, $5 ; the stops and colored glasses in brass box, $3. Total, $21.

MICROSCOPICAL MANIPULATION.

Preparing Nutrient Agar-Agar.—Dr. T. Yokote, in the Centralblatt fur Bakteriologie, describes a method for preparing nutrient agar-agar for culture purposes, that can be carried out without special apparatus, or loss of time and material as follows :

Fat sinew-free beef, 500 grams, cut into small pieces, is shaken with a liter of distilled water, in a flask. After a thorough agitation the flask is put on the sand-bath and heated, at first gently, but with gradually increasing intensity until a lively boil is reached. After boiling for one hour and a half, the flask is removed and the liquid filtered while hot, through paper. To the filtrate add 15 gm. of agar in shreds, return to the sand-bath and boil for about one hour. Remove and add 10 gm. pepton and 5 gm. common salt, agitate until dissolved and then neutralize the liquid with a concentrated solution of sodium carbonate, or potash lye. The soda should be added until a piece of litmus paper plunged into the broth shows a weak but distinct alkaline reaction. Let cool down to the neighborhood of 50 degs. C. (122 degs. to 125 degs. F)., add the white of 2 eggs, and agitate thoroughly (on the thoroughness of the agitation depends the rapidity of the subsequent filtration). Return the vessel to the sand bath and heat until the temperature of the sand in the neighborhood of the flask reaches 110 degs. C. (230 degs. F.), and mantained at this point for from 1 to 2 hours. (The regular maintenance of the temperature at this point has also considerable influence on the character of the product). During the boiling the water lost by evaporation must be restored by frequent additions of boiling water. After boiling the requisite length of time filter through a moistened pleated filter This operation, when everything goes right, ought not to take more than 5 minutes, but it is frequently very slow taking sometimes as much as 6 hours.

A New Method of Demonstrating the Presence of Malarial Organisms in the Blood.—Prepare blood films in the usual way and let dry in the air ; then fix for a few minutes in absolute alcohol. After drying, the fixed blood films are exposed to the vapors of iodine for from ten to fifteen minutes. To this end some metallic iodine is placed in a small glass dish provided with a well-fitting cover,and the specimens, blood-side down upon little tripods of glass or a similar contrivance, so as not to come in direct contact with the iodine. When the specimens present a well-marked yellow color tbey are removed, carefully dusted off with a camel's hair brush and mounted in a drop of syrup of levulose. The color of the red blood corpuscles is now very like that of the fresh blood, somewhat intensified, and the malarial organisms appear as in fresh specimens. If the finger has been carefully cleansed and clean glasses have been used, no foreign material will be present to interfere with the examination. Unfortunately the color of the red corpuscles fades after twelve to twenty-four hours, so that the preparations cannot be preserved. For teaching purposes the method will be found very convenient at times when fresh specimens of malarial blood cannot be readily procured.—Md. Med. Journal.

MICROSCOPICAL SOCIETIES.

Manchester Society.—We have received from the Manchester Microscopical Society their Annual Report and Transactions for the year 1898. Most of the papers are well illustrated with excellent plates. Mr. A. T. Gillanders on "Scale Insects," Mr. W. H. Pepworth on Myxomycetes," Mr. W. Moss on "The Genitalia of the British Hyalinia," Mr. Chas. Bailey on "Maize," Mr. Frank Paulden on "Peripatus leuckarti," an Australasian form, and Mr. William Blackburn on "Myriothela phrygia." The annual address by the President, Prof. Weiss, of Owen's College, is printed in full, the subject being "Life." Besides the usual field-work the Society has a sub-section for practical work in mounting and technique. It possesses a

library, instruments, and a cabinet of micro- and lantern slides; has recently extended its usefulness by organizing lectures with demonstrations for the benefit of outside societies and institutions. Eighteen such lectures have been given in the Manchester district during the past twelve months and the scheme has been eminently successful. The report and transactions can be obtained from the Hon. Secretary, Mr. E. C. Stump, 16 Herbert Street, Moss Side, Manchester, post free for one shilling and eightpence.

NEW PUBLICATIONS.

New Catalogues.—Send to Mr. J. H. Steward, of 406 and 407, Strand, and to Messrs. A. Clarkson & Co., of Holborn Circus. The former contains several microscopes of excellent design, and a full list of accessories, but is cumbered, as is too often the case, with types of microscopes of antiquated patterns. Messrs. Clarkson's catalogue is almost entirely devoted to second-hand instruments, all offered at moderate prices, and nearly all are of good and recent models.

The Fixation, Staining and Structure of Protoplasm, a Critical Consideration of the Theory and Technique of Modern Cell study. By Dr. Alfred Fischer (Leipsic), royal octavo, 362 pages, 1 double plate and 21 figures in text. Published by G. Fischer, Jena, 1899. The history of the closing cycle of botanical activity will undoubtedly show that one of its most noticable and unusual features has been the enormous amount of energy devoted to the study of the structure of protoplasm. Research in this phase of biological science may be said to have had its origin chiefly in an effort to determine the mechanism of the nucleus as a vehicle of heredity, and it has been directed for the greater part to the morphology of the chromatin in mitotic division, and to the behavior of the 'attractive and directive bodies,' with some effort to take into consideration the structure of protoplasm and the general organization of the cell. The results of these investigations have filled a great amount of space in all classes of botanical Journals,

beside the special periodicals devoted to the subject, and have covered an untold area of the costliest plates. The early specialization of a large number of the younger workers in this line has led to the publication of many articles on the subject utterly devoid of literary form, filled with local and personal terms, uselessly recounting technique, and giving the most merciless repetition of details of observation with no attempt to summarize the results, or give the general significance of the phenomena described: making the preparation of such a work as the book under discussion doubly necessary.

Then again the time seems at hand when the cytologist may be fairly asked to interpret to his botanical brethren the vast amount of detail accumulated in the last decade by his method of research. So far as the general discussions of recent date may be taken as a reply to this pertinent inquiry, the summary of well-grounded facts and established theory shows a very small residum of actual progress. Thus one of the most prominent cytologists in America has taken occasion to say, in a recent review of knowledge of the cell, "And yet if we take account of the actual knowledge gained, we cannot repress a certain sense of disappointment, partly that microscopical research should have fallen so far short of giving the insight for which he had hoped, but still more because of the failure of the best observers to reach any unanimity in the interpretation of what is actually visible under the microscope. * * * I would like at the outset to express the opinion that, if we except certain highly specialized structures, the hope of finding in visible protoplasmic structure any approach to an understanding of its physiological activity is growing more, instead of less remote, and is giving way to a conviction that the way of progress lies rather in an appeal to to the ultra-microscopical protoplasmic organization, and to the chemical processes through which this is expressed." (E. B. Wilson, in Science, p. 34, July 15, 1899).

The chief value of the book consists in its collection of methods used, and facilitates the ready selection of those which give promise of results in new methods of attack.

INDEX.