Journal of the Royal Microscopical Society

CONTAINING ITS TRANSACTIONS AND PROCEEDINGS

AND

A SUMMARY OF CURRENT RESEARCHES RELATING TO

ZOOLOGY AND BOTANY

(principally Invertebrata and Cryptogamia)

MICROSCOPY, &c.

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Minimis partibus, per totum Naturæ campum, certitudo omnis innititur quas qui fugit pariter Naturam fugit.—*Linnæus.*

FOR THE YEAR

1918

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MICROSCOPY.

A. Instruments, Accessories, etc.*

(1) Stands.

Bausch and Lomb's Metallurgical Microscope, CCM.† — This instrument (fig. 1) which has been made after the design of Dr. Albert Sauveur, of Harvard University, has a foot of horseshoe form. The

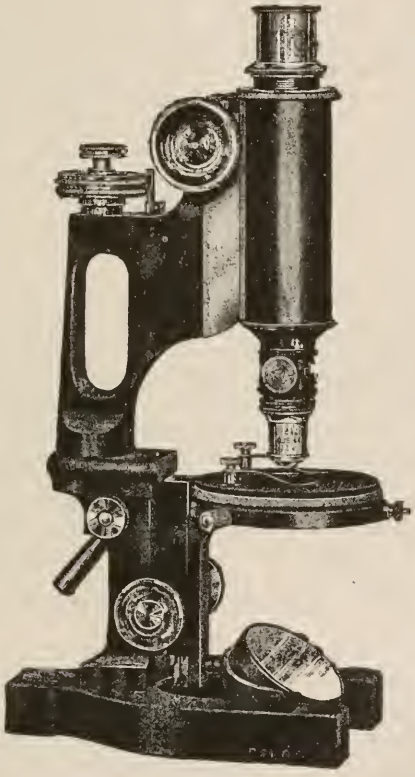

FIG. 1.

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

† Catalogue, Microscopes, Bausch and Lomb Optical Co., New York, pp. 70–1.

pillar, rectangular in section, is provided with inclination joint and clamping lever to secure the instrument in any position by means of vertical and horizontal stops. The arm is of handle type and of enlarged design; it provides ample space for manipulation of object. The body-tube has 39 mm. of outside diameter, and is provided with society screw thread; the oculars are the Bausch and Lomb standard sized eye-pieces. The draw-tube is graduated in single millimetres, with every tenth line numbered. The coarse-adjustment is by standard rack and pinion, and has a stop to prevent pinion from over-riding rack. The fine-adjustment is of lever type, with milled micrometer screw head in two parts for slow and rapid movement, the larger graduated into 100 divisions, each equal to 0·0025 mm. in vertical movement, and provided with hinged indicator, which may be turned back from head; the fine-adjustment ceases to operate when objective touches specimen. The vertical illuminator is a plane glass reflector, readily adjustable, provided with three different sizes in revolving sleeve. The stage is adjustable vertically by standard rack and pinion to increase working distance and allow focusing without displacing vertical illuminator with reference to light; it is circular in shape, 102 mm. in diameter, with distance of 75 mm. from centre to base of arm; it is made of metal with vulcanite top, and is provided with centring screws and spring clips, removable for substitution of attachable mechanical stage. The mirror is plane and concave, 50 mm. in diameter, and adjustable in two planes in a fork mounting.

(3) Illuminating and other Apparatus.

Interference Refractometer.*—R. S. Williams, in pointing out the importance of measuring the refractive index of liquids in estimating their purity, observes that the effect of temperature can be best eliminated by measuring, not the absolute refractive index, but the index relatively to some standard liquid in the same bath. But while a refractometer of the Abbe type, for absolute values, gives results reliable to two units in the fourth decimal place, much greater accuracy can be obtained from an instrument which depends on the interference of light waves. The author finds that with such a refractometer a refractive index may be measured with an accuracy of from 0·00006 to 0·0000003, which is, of course, far beyond the capabilities of a refractometer of the Abbe or Pulfrich type.

Use of the Stereoscope for Examining Superposed Projections.†—H. Hubert believes that his application of the stereoscope to the examination of suspended orthogonal projections is a novelty, and he seems to find it very successful. As an example he quotes the superposition of a topographical surface and an underlying geologic layer. In the ordinary manner superposition of such surfaces would be very confused, while the superposition of three surfaces would be hopeless. But stereoscopic

* Journ. Inst. Brewing, xxiii. (1917) pp. 457–60 (3 figs.).
† Comptes Rendus, clxv. (1917) pp. 1059–60.

fusion of two views renders the superposed projections absolutely independent of each other, because they appear in relief. The transformation of the superposed orthogonal projections into stereoscopic views is very simple, because in constructing each contour curve, it is only necessary to concern oneself with the scale of reduction, and with the position of the centre of the sheet of paper containing the projections. Each contour curve being, in regard to a surface immediately under it, supposed brought near to the observer by a quantity proportional to the graphic equi-distance (a quantity chosen arbitrarily once for all), the scale of reduction is given by the formula α/f, in which α is a variable equal to the distance of the sheet of paper from the observer's eyes, and f a constant equal to the distance between the observer's eyes and the stereoscopic views. As to the centre M of the sheet of paper, it will always lie at that normal to the stereoscopic views which passes through the middle of the line joining the optic centres O, O′ of the two eyes. Consequently its projection in is always in the plane xy passing through the centres S, S′ of the stereoscopic views. Moreover, it is as much to the left (for the right eye) or more to the right (for the left eye) of S and of S′ as the variable α is proportionally smaller. Its position may be obtained either by construction (intersection of D M and of O′ M with xy) or by the calculation $m\mathrm{S}=df/\alpha$, where d is a constant equal to $\frac{\mathrm{O\,O'}}{2}$. The construction of the two views is so much the more simplified, inasmuch as the projection of each curve intended for the left eye is rigorously superposable on the corresponding projection intended for the right eye. The only difference between two corresponding curves is that the projection of the centre M, instead of having the same relation with regard to S and to S′, is placed symmetrically with regard to the middle of the straight line joining these two points. In practice there is advantage in drawing much enlarged the stereoscopic images and in reducing them photographically. There is also a gain in not putting the two corresponding views on the same mount: partly, because each observer can then place them at the most favourable separation, and, partly, because the position of the two views can be also arranged for inversion, which will sometimes be more convenient for the observation of certain details.

(5) Microscopical Optics and Manipulation.

Spherometer of Precision.*—The chief feature of this instrument, says J. Guild, is the method employed for detecting the exact contact between the micrometer screw and the surface under test. The micrometer terminates in a small sphere of about 1·5 mm. diameter. A microscope with a suitable illuminating apparatus is mounted above, and the Newton's rings surrounding the point of contact are observed. By watching the behaviour of the rings when the screw is brought up the exact point of contact is determined. The sensitivity is about one-ten-thousandth of a millimetre.

* Trans. Optical Soc., January 10, 1918.

B. Technique.*

(1) Collecting Objects, including Culture Processes.

Method for Discovering Fungi in the Sputum of Bronchitics.† Bazin, after alluding to the difficulty of finding fungi in non-tuberculous sputum, states that if the sputum be cultivated in a suitable medium at 37° C. for forty-eight hours colonies of fungi will be found to have developed. Raulin's fluid as a culture medium does well, but the author prefers glucose-glycerin water. For examining lactophenol was used. By the foregoing procedure he has identified five species of Aspergillus and two of Sterigmotocystis. He adds a note to the effect that iodide of potassium gives remarkable curative results.

Rapid Differentiation of Amœbæ Cysts.‡—D. W. Cutler and R. Williamson, with the intention of facilitating the rapid recognition of intestinal amœbæ, have applied the following method of examination :—A loopful of fæces is emulsified in a drop of 1 in 1000 solution of neutral-red in 0·85 p.c. sodium chloride solution. The preparation is covered with a cover-slip, and examined with $\frac{1}{6}$-inch objective and No. 10 eye-piece. A warm stage is an advantage, but is not essential. In such a preparation the vegetative form of *E. hystolytica* takes up the neutral-red, and the stained amœbæ can be readily seen and recognized. The pink dye is uniformly distributed throughout the endoplasm, while the ectoplasm remains unstained. *Entamœba coli* is not stained, and appears as a light grey body. The method forms an easy and satisfactory means of differentiation between the two micro-organisms. The neutral-red method presents no disadvantage in looking for cysts, which appear as colourless refractile bodies. When difficulty has arisen in deciding to what species of entamœba the cysts belong, the well-known iodine method can be employed. The large amœbæ of the "tetragena" type are usually easily recognized without the neutral-red method. It is the smaller form of *hystolytica*, known as the "minuta" form, which appears only in the fæces in numbers as acute symptoms abate, which offers great difficulty in diagnosis. This type, however, reacts in exactly the same way to the neutral-red as does the "tetragena" type.

Studies in Cattle-plague.§—H. Schein has conducted a series of researches on the etiology and treatment of cattle-plague in Indo-China, and has arrived at the following conclusions with reference to this disease :—1. The goat is a good subject for experimentation. Its

* This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Embedding and Microtomes; (4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, etc.; (6) Miscellaneous.

† C.R. Soc. Biol. Paris, lxxx. (1917) pp. 771-3.

‡ Journ. Path. and Bact., xxi. (1917) pp. 511-3.

§ Ann. Inst. Pasteur, xxxi. (1917) pp. 571-92.

susceptibility to bovine plague is nearly the same as the buffalo, in Indo-China at all events. 2. $\frac{1}{1000}$ c.cm. of virulent blood constitutes a certain lethal dose. 3. $\frac{1}{25000}$ c.cm. of blood is the smallest infective dose. 4. $\frac{1}{10}$ c.cm. of plasma is comparable to the minimal lethal dose. 5. The virus of bovine plague resides in the leucocytes, though it is sometimes found free in the plasma. 6. There are about 25,000 microbes, or groups of microbes, per c.cm. in the whole virulent blood. 7. There are about 10 organisms per c.cm. in the centrifuged citrated plasma. 8. Sensitization of the virus has not given good results in the author's hands. 9. Anti-pest serum does not act upon the virus itself but upon the organism of the experimental animal. 10. "Sero-infection" appears to give good results with the buffalo, provided a sufficient quantity of serum is injected (50 c.cm. per 100 kilos. body-weight for adults, more for young animals), and infecting with the least possible quantity of virus, in order to retard the rapid growth of the parasite.

Vitality of Rinderpest Virus outside the Animal Body.*—A. W. Shilton has carried out a series of elaborate investigations, at Muktasar in Northern India, with reference to the viability of the rinderpest virus outside the animal body under natural conditions, and has come to the following general conclusions :—

1. At the Muktasar Laboratory rinderpest infection was found to persist in certain buildings for forty-eight hours after the removal of sick animals, but not for longer periods of time ; frequently infection was absent after shorter intervals. Ground which was shaded by trees, when contaminated by cattle suffering from rinderpest, was found to be infective to healthy stock eighteen hours after the removal of the sick animals, but not for longer intervals. Ground entirely exposed to direct sunlight did not remain infective beyond eight hours.

2. In the plains, buildings were found to remain infective for twenty hours after the removal of the sick animals, but non-infective after longer intervals. Areas shaded by trees remained infective for twenty-four hours, and those exposed to direct sunlight for six hours after the removal of the sick animals, but not for longer periods of time.

3. The rinderpest virus was found to survive in mixed fæces and urine protected from direct sunlight for periods of fifty-four hours after excretion by sick animals, but when exposed to direct sunlight the virus did not survive for longer periods than eight hours. Saliva and nasal discharge from sick animals did not remain infective beyond forty-four hours.

4. It may be concluded, therefore, that in buildings and on areas infected by the natural discharges of sick animals, the rinderpest virus is unable to survive for more than two or three days, and when air and sunlight are freely admitted its destruction is even more rapid.

5. The carcases of animals which have died from the disease must, however, be regarded as possible sources of infection for some considerable time after death, especially when the air-temperature is low, as it

* Mem. Dept. Agric. India, Veterinary Series, iii. No. 1 (1917).

has been shown that the virus can survive for fifty-one days in blood from a sick animal when this has been freely exposed to the air and allowed to become putrid ; in meat and bones also the virus may persist for many days.

Further observations are necessary to determine the factors influencing the duration of the vitality of the rinderpest virus in dead animals' tissues.

(2) Preparing Objects.

Demonstrating Degeneration of Peripheral Nerves.*—C. Manalong adopted the following technique :—From 2–4 centimetres of the vagus and posterior tibial nerves are laid on a strip of cardboard and treated as follows : 1. Harden in equal parts of Müller's fluid and formaldehyde (10 p.c.) for twenty-four hours. 2. Replace by Müller's fluid for fifteen days. 3. Wash in running water for from twelve to twenty-four hours. 4. Transfer the tissue for fifteen days to the following solution : Müller's fluid 2 c.cm., 2 p.c. osmic acid solution 0·5 c.cm., distilled water 0·5 c.cm. 5. Wash in running water for from twelve to twenty-four hours. 6. Dehydrate in graded alcohols, changing the absolute alcohol twice. 7. Clear in origanum oil, tease under a dissecting microscope and mount in chloroform balsam.

Demonstrating the Cytoplasmic Inclusions of Germ-cells.†—J. B. Gatenby in his work on the snail used the following fixatives : 1. Modification of Flemming's strong formula without acetic acid. 2. Same fluid diluted one-sixth. 3. Champy's fluid. 4. Flemming's strong solution, the acetic acid being replaced by nitric acid. For staining, Ehrlich's hæmatoxylin and eosin, Mayer's acid hæmalum, iron-hæmatoxylin, with Orange G, Bensley's acid fuchsin, methyl-green, and alizarin-toluidin-blue. The following was the best of the fixing and staining methods:—The animals were anæsthetized in chloroform vapour, and after the shells were removed the ovotestis is cut out. This is cut in half longitudinally, and thrown into Flemming without acetic acid, diluted one-sixth with distilled water. Next day they are washed in running water for two hours, then passed through upgraded alcohol to absolute and xylol. Sections, 6μ, are stained in iron-alum-hæmatoxylin by the long method—i.e. ten to twelve hours in iron-alum, ten to fourteen in hæmatoxylin. After differentiation the sections are stained with Orange G or van Gieson. The mitrochondria and nebenkern are intense black and beautifully clear.

(4) Staining and Injecting.

Improvised Staining of Malarial Parasites.‡—G. Senevet used the following improvised eosin-azure stain for malarial blood, in the absence of special stains for this purpose :—

Two solutions are used : 1. Methylen-blue 1 gram, sodium borate

* Philippine Journ. Sci., xii. (1917) p. 171.
† Quart. Journ. Micr. Sci., lxii. (1917) pp. 562–4.
‡ Bull. Soc. Path. Exeta, x. (1917) pp. 540–2.

3 gram, distilled water 100 grams. The solution is left to ripen for eight to fifteen days at a temperature of 37° C. 2. Watery eosin 1 gram, distilled water 100 grams. Stain for two or three hours, for preference, with a weak solution of the stain—one drop of the eosin stain, and one or two drops of the blue stain to 20 c.cm. of neutral distilled water.

Rapid Examination of Malarial Blood.*—E. Roubaud recommends the following rapid method of detecting malarial parasites:—A thick blood-film is quickly dried by heat (45°–55° C.) and then hæmolyzed for five to ten minutes in distilled water. This technique destroys the parasite but leaves the distinctive yellow-brown pigment, which can be shown up more clearly if the film is stained for a few seconds with carbol-violet or thionin. In benign tertian infection the irregular streaks of small pigment-granules of the schizont can be distinguished with ease from the masses of pigment of the female gamete and the dense platelets of the male. The form of the other species of parasite are not so easily demonstrated. For the rapid detection of crescents the author places the dried thick film in a solution of thionin in distilled water, of the strength of 1 to 10,000, for ten to fifteen minutes. The hæmolysis of the film and the staining of the crescents occur simultaneously.

Staining Sporogenous Bacteria.†—L. Tribondeau makes a film from the culture in the usual way, and then fixes it by passing through the flame of a Bunsen six to ten times. When cool the slide is covered with Lugol's solution and then heated to vaporization. Next the film is covered with carbol-crystal violet and then heated to vaporization. After a wash in tap-water, the slide is covered with an aqueous solution of vesuvin (1 to 500). This is allowed to act for one to two minutes. After a wash in tap-water it is dried. The spores stand out a dark violet against the yellow-brown bodies of the bacteria. Instead of the foregoing, carbol-fuchsin and methylen-blue may be used as stain and counterstain.

Staining Young Eels.—A. Gandolfii Hornyold sends the following method, which he hopes may interest readers of the Journal. He writes from the Marine Biological Laboratory, Mallorca:—"In small eels the ovaries and the testicles have exactly the same appearance, namely, that of a very fine transparent band or ribbon, and it is only when the eels are about 24–26 c.cm. long that it is possible to distinguish the two sexes with a pocket-lens. Walter, in his monograph, 'Der Tenpaal,' advises to examine a fragment of the organ under the microscope with a low-power × 50. If no eggs can be distinguished, the eel is most probably a male. The following method gives good results: I pour a little alcohol (90 p.c.) on the organs, and it isthen quite easy to detach a fragment with a fine forceps, as coagulation renders

* Bull. Soc. Path. Exot., x. (1917) pp. 702–3.
† C.R. Soc. Biol. Paris, lxxx. (1917) pp. 880–1.

them white and more visible. I then put it on a slide in water to which is added a small drop of Loeffler's methylen-blue. I watch the staining under the microscope with a low power (I use Zeiss AA, with eye-piece No. 2 or 4). Staining takes place rapidly, and it is much more easy to distinguish the eggs than in an unstained fragment. One can afterwards mount in glycerin or in Apathy's gum-syrup. By this method I have been able to distinguish the sexes in eels 23·5 c.cm. long. I have tried vital staining on elvers. Neutral broth gives very good results, and does not appear to harm them. These stained elvers are very pretty in aquaria."

Staining Tubercle Bacilli.*—L. Tribondeau adopts the following procedure: The dried films of sputum are flooded with carbol-fuchsin, and heated to vaporization thrice. The stain is then thrown off and the film treated with nitric acid (acid 1, water 2) until it is yellow. It is then washed in running water, followed by alcohol (90 to 100 p.c.), until the film is of a pink colour. After a rapid wash in tap-water, it is treated with a saturated aqueous solution of picric acid (1 vol. plus alcohol 90–100 p.c. 1 vol.), or with a solution of methylen-blue (medicinal M.B. gr. 0·50, distilled water 150 c.cm.). These counter-stains are allowed to act for five to ten seconds. The slide is then quickly washed and dried. The picric acid treatment is preferable if tubercle bacilli only are sought for; the methylen-blue if the cell-elements, etc., are to be shown.

(5) Mounting, including Slides, Preservative Fluids, etc.

Preservation of Fermentation Organisms in Nutrient Media.†—A. Klocker makes an important communication on this subject. Hansen's conclusion that a 10 p.c. solution of cane-sugar forms an excellent medium is confirmed, but beer-wort is also very good. The Pasteur flask is undoubtedly the best form of vessel for prolonged preservation. The present observations were made, during a period of more than thirty years, on 820 cultures of yeasts and moulds. These included Saccharomycetes, Schizosaccharomycetes, Torulæ, Mycoderma, Endomyces, Monilia, Chalara, *Oidium*, and *Mucor*. For the most part the nutrient medium employed was a 10 p.c. solution of cane-sugar, in which 461 cultures were grown, but 290 cultures were made on beer-wort and 69 on other media. Of the 461 cultures on cane-sugar solution (231 of these being Saccharomyces) 403 survived, whilst 58 perished. In the case of the 290 cultures grown on beer-wort (190 Saccharomyces) 268 survived and 22 perished. Thus it must be concluded that fermentation organisms can be kept alive for upwards of thirty years. The exceptions to this rule are:—(1) The asporogenic varieties of Saccharomyces; (2) *Saccharomycodes Ludwigii*; (3) Schizosaccharomyces; and (4) *Aspergillus glaucus*. Of the first only 44 p.c.

* C.R. Soc. Biol. Paris, lxxx. (1917) pp. 780–2.

† C.R. des Travaux du Laboratoire de Carlsberg, ii. pt. 6 (1917). See also Nature, Dec. 13 (1917) pp. 289–90.

survived on cane-sugar and 21 p.c. on beer-wort; of the second only one in nine survived on cane-sugar for more than 7·5 years, but all five cultures on beer-wort survived for twenty-five years. Only two ont of five cultures of Schizosaccharomyces on cane-sugar survived, but ten out of eleven of those on beer-wort were living. Of six cultures of *A. glaucus* only one survived, and two of the remaining five perished in less than two years.

(6) Miscellaneous.

Rapid Method for obtaining Hæmolytic Serum.* — A. Sézary recommends the following rapid method :—Into the peritoneal sac of a rabbit a single injection of washed sheep's-corpuscles is made. The quantity of red corpuscles is that which is found in 35 c.cm. of defibrinated blood. The animal is bled eight days afterwards. The hæmolytic serum is just as powerful as if the animal were injected four or five times.

Detection of Bile-pigments in Blood-serum.†—A. Fouchet obtains blood by venous puncture (5 c.cm.). The serum is obtained by centrifuging or by leaving it to exude. Five drops are placed on some white surface, and then a similar quantity of the following reagent is added : Trichloracetic acid, 5 grm. ; water, 20 c.cm. ; perchloride of iron, officinal, 2 c,cm. The two are thoroughly mixed with a glass rod. In less than twenty minutes a colour is obtained ; this is greenish-blue and stable. If there be $\frac{1}{20000}$ of biliration the colour comes out at once.

* C.R. Soc. Biol. Paris, lxxx. (1917) pp. 797–8.
† C.R. Soc. Biol. Paris, lxxx. (1917) pp. 826–8.

Metallography, etc.

Heterogeneity of Steels.*—By treating polished sections of steel with a cupric reagent and then removing the deposited copper by solution in ammonia, G. Charpy and S. Bonnerot find that pearlite appears white and ferrite dark, i.e. the appearance is the reverse of that obtained by etching with ordinary reagents, such as nitric or picric acid. The parts which are darkened are those which receive the first deposit of copper, even in samples which appear to have received an almost uniform coating of copper. The selective action of the cupric reagent is determined mostly by the phosphorous and other elements in the ferrite, and not by the carbon, as with acid reagents. The dendritic structure of cast-steel is shown very clearly by this method of etching, the dendrites appearing white and the remainder of the mass, which has solidified later and contains the greater part of the foreign elements, dark ; and the authors have used it to trace the progressive deformation of the dendrites during rolling of the steel. The dimensions of the dendrites and their variation with conditions of cooling were also studied. Variations in size of ingot have little effect on the size of the fine dendrites formed by contact with the walls of the ingot-mould ; those which form in the centre of the ingot increase considerably in size with increase in size of ingot. In ingots of relatively pure steel and of medium size, variations of 1 to 10 in the relative size of the dendrites in the peripheral and central portions were observed.

Tungsten-Molybdenum Alloys.†—Alloys of tungsten and molybdenum were prepared by Z. Jeffries by mixing the powdered metals (obtained by reduction of the oxides in hydrogen) in the requisite amounts, pressing into briquettes and sintering in hydrogen at 1300° C., and then heating just below the fusion temperature (previously determined approximately) for twelve minutes. After such treatment the alloys were completely crystalline, and could be rolled or drawn. The melting-points were determined by a novel electrical method, depending on the number of watts consumed in melting a wire in an atmosphere of hydrogen, the melting-points and fusion-wattage of the pure metals being known. The melting-point curve shows that the alloys form a continuous series of solid solutions. This was confirmed by microscopical examination of the structure of the alloys. When properly prepared, all consisted of homogeneous polygonal crystals. The alloys were etched with a boiling solution of hydrogen peroxide. Examination of etching-pits in the pure metals showed that they both crystallized in the isometric system, the crystal units being cubes.

* Comptes Rendus, clxv. No. 17 (1917) pp. 536–40 (6 figs.).
† Trans. Amer. Inst. Mining Engineers, No. 115 (1916) pp. 1225–36 (11 figs.).

Method for Distinguishing Sulphides from Oxides in Steel.*— It is shown by G. F. Comstock that light grey inclusions seen in polished unetched sections of steel do not always indicate manganese sulphide, as commonly believed. Iron oxide occasionally presents a similar appearance. In cases of doubt the nature of the inclusions can be definitely determined by the use of boiling alkaline-sodium picrate, the reagent used to darken cementite. This reagent attacks sulphide inclusions, changing the colour from light grey to black, while oxide inclusions are quite unattacked. Numerous photomicrographs are given illustrating the use of the reagent. It is made by dissolving 25 grm. sodium hydroxide in 60–70 c.cm. water, adding 2 grm. picric acid, and heating till the acid is dissolved, when the volume is made up to 100 c.cm. with water. To etch, the solution is brought to boiling, the specimen immersed, and boiling continued for ten minutes. Then the specimen is removed, washed, and dried.

Annealing of Bronze.† — An elaborate study has been made by C. H. Mathewson and P. Davidson of the combined effects of time and temperature of annealing and of cold-working with regard to the production of uniform structures and growth of recrystallized grain in bronzes containing 4 to 8 p.c. tin. The time necessary for the removal of the cored structure of the solid solution by diffusion is the same whether the alloys have or have not been cold-worked after casting. A given grain-size produced by annealing after deformation corresponds to the same degree of diffusion whatever the combination of time and temperature used in the annealing. Alloys which have been rendered homogeneous by a preliminary annealing develop a coarser grain on subsequent cold-working and annealing than alloys which are similarly treated but receive no preliminary annealing before cold-working. Numerous photomicrographs are shown in support of these conclusions.

An Unusual Feature in the Microstructure of Wrought-Iron.‡—A peculiar structure sometimes shown by wrought-irons high in phosphorus is described by H. S. Rawdon. The ferrite crystals present a mottled appearance after prolonged etching with an acid reagent such as 5 p.c. nitric acid in alcohol, which extends in streaks over the surface of the specimen. This intra-crystalline etch-pattern is quite distinct from the etching-pits which result from prolonged etching of ordinary wrought-iron. It is considered to be due to the non-uniform distribution of dissolved phosphorus in the ferrite, the area constituting the mottled etch-pattern being relatively high in phosphorus. By using Stead's cupric chloride reagent the patterns can be more strikingly developed. The fractures in some wrought-iron articles which had failed in use, and which showed these features in their structure, ran parallel to the

* Trans. Amer. Inst. Mining Engineers, No. 120 (1916) pp. 2103–10 (17 figs.).
† Int. Zeitschr. Metallographie, viii. (1916) pp. 181–218 (28 figs.).
‡ Engineering, cv. (1918) pp. 77–9 (18 figs.).

mottled bands, and are considered to have been caused by them owing to the brittle character of ferrite rich in phosphorus. Attention is drawn to the slowness of diffusion of phosphorus in ferrite, which accounts for the persistence of the non-uniform structure during the heating and rolling operations undergone by the wrought-iron articles before reaching the finished condition.

MICROSCOPY.

A. Instruments, Accessories, etc.*

(3) Illuminating and other Apparatus.

Microscope Illumination.†—N. J. Clark has patented an apparatus for illuminating microscope specimens by means of a low-voltage electric lamp which is supported immediately below or within the substage condenser and is adjustable for focussing. As shown (fig. 1), the lamp B and a reflector A are carried by an arm C, clamped to a vertical rod E by a

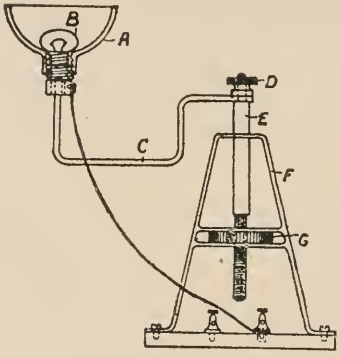

Fig. 1.

nut D, the rod E being mounted on a stand F so that it may be rotated or may be raised or lowered by means of a nut G. The illumination of the specimen may be regulated by means of an adjustable resistance in the lamp circuit. The lamp may be used with a dark-ground condenser to obtain oblique illumination or dark-ground effects.

(4) Photomicrography.

Spirochæta icterohæmorrhagica.‡—A. C. Coles has succeeded in photographing the minute spirals, 10–12 to each 5 μ, in spirochætes obtained from the common rat in England. They are diagnostic of the newly-created genus, the *Leptospira* of Noguchi.

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

† English Mechanic, Feb. 1, 1918, p. 16.

‡ Lancet, March 30, 1918, pp. 468–9 (6 figs.).

Photomicrographs in Colour.*—H. R. Eggleston describes the process of making lantern-slides representing photomicrographs of stained sections. The process is as follows:—Lantern-plates are sensitized by bathing for five minutes in a $2\frac{1}{2}$ p.c. solution of ammonium bichromate containing 5 c.cm. of ammonia to the litre, the temperature of the bath being not above 60° F. The plates are then rinsed for two or three seconds in clean water, drained and dried as uniformly as possible, being kept in the dark while drying. The sensitized plates are then exposed through the glass under the negative to the light of an arc lamp, the average exposure being about three minutes at 18 inches distance. The exposed plates are then developed by rocking in trays of water at about 120° F. until all soluble gelatin is removed. The plates are then rinsed in cold water, fixed in hypo, and washed free of the hypo. They are then ready for staining. The staining is done with a 1 p.c. solution of dye containing 1 p.c. of acetic acid, the dye being selected to simulate most closely the original stain of the section. When sections stained with two different colours are being photographed, negatives are made through suitable colour-filters, and are then dyed in the two stains and placed face to face, so that a two-colour slide is obtained. Suppose a section is stained red and green Two negatives are made on panchromatic plates—one with a red filter, which will cause the green to appear as clear spaces in the negative and will not record the red, and the other with a green filter, which will record the red and not the green. The slides made from bichromated gelatin are stained—that from the red negative with the original green stain, and that from the green negative with the original red stain. The filters used are Wratten M filters. The choice of the filter is decided by visual trial. Thus photographing a section stained with hæmatoxylin and eosin the A (red) filter shows no trace of the eosin and gives a good strong negative of the hæmatoxylin. The B and C filters are used together for the other negative, giving a blue-green colour and recording the eosin and hæmatoxylin both fully, and from these two negatives positives are made and stained with a blue and a red dye.

(5) Microscopical Optics and Manipulation.

Dividing-engine for Ruling Diffraction Gratings.†—"Nature" records that part 1 of vol. xxx. of the Proceedings of the Royal Society of Victoria contains a description of a new dividing-engine for ruling diffraction gratings by J. H. Grayson, of the University of Melbourne. The design and construction of this machine have occupied Grayson, whose skill in work of this type is well known, for seven years, and the completion of the task places spectroscopists under a great debt of gratitude to him. His paper contains a detailed description of the machine, and gives full particulars of the methods used for grinding and testing the screw. The machine is set up in a room of its own in the basement of the University, and is driven by a $\frac{1}{40}$ h.-p. hot-air engine placed in an adjoining room. Ruling-diamonds are broken stones, in which the fracture along a cleavage-plane intersects an outer

* Trans. Amer. Micr. Soc., xxxvi. (1917) pp. 279–81.
† Nature, March 21, 1918, p. 51.

crystalline face and gives a good knife-edge. Grayson finds the stones from the diamondiferous drift of New South Wales best for this purpose, and when ruling properly such a diamond makes no noise. The photographs which accompany the paper show that the rulings are extremely regular, and warrant the hope that gratings ruled on the machine will give exceptionally clear spectra. The verdict of spectroscopists on the gratings will be awaited with considerable interest. In the meantime all will congratulate Grayson on the completion of his work, and the University of Melbourne on the public-spirited way in which it has provided facilities for that work.

Dispersion and other Optical Properties of Carborundum.*—H. E. Merwin's investigations on carborundum show that for wave-lengths ranging from 755 $\mu\mu$ to 416 $\mu\mu$, w ranges from 2·616 and ϵ from 2·654 to 2·812. Microscopical study of several samples of granular carborundum revealed no definite variations in the refractive index for red light, even in grains of different colour.

Measurement of Magnifying Power.†—W. M. Bale describes a simple method of measuring the magnifying power of a microscopical combination. His procedure is :—(A) Measure with the camera lucida the exact diameter (at 10 inches) of the magnified field ; (B) measure with the stage micrometer the actual diameter of the field. Then A divided by B is the magnifying power. At the same time there are sundry precautions to be taken to ensure success, especially if the investigation does not concern the centre of the field. These difficulties the author fully deals with.

(6) Miscellaneous.

Balsam Problem.‡—At a Meeting of the Optical Society on April 11 J. W. French stated that for cementing optical parts together Canada balsam is almost invariably employed. Although on starting or starring of the balsam-layer actual separation of the parts or deformation of the optical surfaces frequently occurs, there is no appreciably better substance known. Optical parts may be combined with an air-space between the surfaces by optical contact, with or without scaled edges, by optical welding or by cementing. The disadvantages of the various methods were enumerated, the loss of light at transmission surfaces being particularly discussed. A considerable number of balsam specimens, of ages varying up to ten years, had been opened, and photomicrographs of the balsam-layer were exhibited. In all cases there were fluid layers between the harder balsam and the glass surface, and the photographs demonstrated particularly the smallness of the adhesion to the glass. Specimens artificially produced were also exhibited. In many cases the age of the specimen was shown to be deducible from the configuration. So-called granulation of balsam was stated to be due to the action of moisture on the balsam surface. No trace of crystallization of glass-

* Journ. Washington Acad. Sci., vii. No. 14 (1917) pp. 445–7.
† Journ. Quekett Micr. Club, xiii. (1917) pp. 1–8 (1 fig.).
‡ Nature, April 18, 1918, p. 139.

quality balsam was found in any of the experiments, but a number of the photographed specimens showed definite right-angled fractures occasionally observed in torn gelatin films.

British Resources of Sands and Rocks used in Glass Manufacture, with Notes on certain Refractory Materials.*—The above is the title of a valuable Supplementary Memoir, by P. G. H. Boswell, with contributions by W. B. Wright, H. F. Harwood, and A. A. Eldridge. The title gives a clear guide to the contents, seven chapters of which deal exhaustively with the raw materials suitable for glass manufacture found in the British islands; there is also one chapter devoted to American grade glass-sands. The book includes elaborate tables of mechanical and chemical analyses, and some of the plates are microphotographs of British and American glass-sands.

Petrographic Microscope.†—F. E. Wright's contribution on this subject is an interesting exposition of the possibilities of petrographic examination. He does not deal much with constructional principles, but limits himself to a description of the results which can be obtained with the view of advocating a wider use of the instrument.

B. Technique.‡

(1) Collecting Objects, including Culture Processes.

Medium for Cultivating Bacillus tetani.§—W. J. Tulloch has discovered the following selective medium for the enrichment of *B. tetani* against other organisms accompanying it. The preparation of the medium is as follows:—Take 1 lb. of chopped meat, add 1 litre of water, boil 30 minutes, cool to 45° C., adjust reaction of fluid so that it is slightly alkaline to litmus. Trypsinize as for Douglas's broth; incubate in open vessel for five days at 37° C. Filter products of putrefaction through paper, add sodium formate 1 p.c. of total, adjust reaction of fluid to neutral point for phenolphthalein. Fluid is then filtered through a Berkefeld and Doulton filter in series, stored under paraffin in sterile flask mounted with a hooded delivery pipette, so that medium may be distributed into tubes. Before use each tube of 10 c.cm. is enriched by addition of $\frac{1}{8}$ part of fresh rabbit kidney, removed (after killing animal) by sterile operation. Author usually employs tubes containing 5 c.cm. of medium and adds $\frac{1}{16}$ part of kidney to each. To ensure sterility, 5, 1, 0·5, 0·1, and 0·01 c.cm. are inoculated into meat tubes which are incubated anaerobically for fourteen days and should show no evidence of growth.

* Longman, Green and Co., London, 1917, 92 pp. (7 pls. and maps).

† Trans. Optical Soc. Amer., i. No. 1, Jan. 1917, pp. 15–21 (1 pl.).

‡ This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Embedding and Microtomes; (4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, etc.; (6) Miscellaneous.

§ Lancet, April 20, 1918, p. 578.

Cultivating the Parasite of Epizootic Lymphangitis.*—A. Boquet and L. Nègre first obtained cultures of *Cryptococcus farcinosus* in horse-dung agar, but after two or three passages the organism was transferred to Sabourand's medium, potato and carrot. After a time the coccus develops a mycelium which forms spores and chlamydospores. The cultures were successfully inoculated on horses.

(2) Preparing Objects.

Modification of Bouin's Fluid.†—A. C. Hollande, after praising the eminent qualities of Bouin's picro-aceto-formalin mixture, states that it may even be improved by the addition of neutral acetate of copper. He gives the following formula : Picric acid, 4 grm. ; neutral acetate of copper, 2·5 grm. ; 40 p.c. formalin, 10 c.cm. ; glacial acetic acid, 1·5 c.cm. ; distilled water, 100 c.cm. The acetate of copper is dissolved in 100 c.cm. of distilled water ; then are added 4 grm. of picric acid. After the picric acid is dissolved the fluid is filtered, and to the filtrate are added 10 c.cm. of the 40 p.c. formalin and the acetic acid. The pieces to be fixed are immersed in the fixative for three days. They are next washed several times in distilled water during twenty-four hours. They are afterwards passed through upgraded alcohols. This is important, as direct passage to strong alcohol gives rise to a precipitate. The author gives a list of eight fluids through which the fixed tissue should be passed.

Short Method of Preparing Histological Material.‡—L. W. Strong publishes a modification of the usual routine by which the time is reduced to three days, with considerable saving in labour and reagents : 1. Fix in 10 p.c. formalin in 80 p.c. alcohol overnight. 2. 95 p.c. alcohol, eight to ten hours. 3. Acetone, from one-half to two hours. 4. Chloroform-paraffin, overnight in warm place. 5. Paraffin, four hours ; 48° C. m.p., two hours ; 52° C. m.p., two hours. 6. Embed.

(3) Cutting, including Embedding and Microtomes.

Electrifying the Microtome.§—H. E. Metcalf derived his inspiration from an electric sewing-machine. The device consists of a small motor with a cork friction-wheel, and mounted on a base, so that when put underneath a sewing-machine fly-wheel a strong spring in the base will press the cork friction-wheel against the fly-wheel of the sewing-machine. The motor was turned on its side with its base bolted to a block, and the block bolted to the table. The microtome-wheel was then backed up on to the cork friction-wheel of the motor until the requisite tension was secured, and then it was bolted to the table. Thus both motor and microtome are both rigidly fastened down.

Variations in speed are obtained by means of the foot-controller which accompanies the motor. Although this controller allows only six

* Comptes Rendus, clxvi. (1918) pp. 308–11.
† C.R. Soc. Biol. Paris, lxxxi. (1918) pp. 17–20.
‡ Trans. Amer. Micr. Soc., xxxvi. (1917) pp. 280–1.
§ Trans. Amer. Micr. Soc., xxxvi. (1917) pp. 267–9 (1 fig.).

variations in speed, it is regulated satisfactorily. While cutting serial sections the assistant never has to touch the fly-wheel with his hands, as he is able merely by using the foot-control to move the paraffin-block a fraction of an inch at a time, as well as being able to cut sections one by one if necessary. In this manner colloidin sections have been cut with a slanting knife. Much time and expense are saved by this device.

(4) Staining and Injecting.

Simple Method for Double-staining Sporulating Bacteria.*—C. Botelho recommends the following procedure :—Dissolve light green 4 grm. and acid-fuchsin 2 grm. in a solution of glacial acetic acid and distilled water, 50 c.cm. of each. The material, with a drop of water, is placed on a slide and fixed by heat. The film is then covered with the stain and heated to vaporization. This procedure is repeated three or four times. When cool wash with distilled water. Dry and examine. Spores are stained red, the bacilli green.

New Method of Staining the Tubercle Bacillus.†—C. Cépède recommends the following procedure :—The sections or smears are first treated with carbol-fuchsin, heated for five minutes. They are then immersed in the following solution for two to three minutes :—Methylen-blue in excess ; lactic acid, 40 c.cm. ; distilled water, 160 c.cm., 1 part ; alcohol 95 p.c., 4 parts. The preparation is then washed in tap-water, and if any red remain the blue solution must be reapplied. Then dry and examine. In urine the technique is slightly different. Before colouring with fuchsin the preparation is treated with a soda solution to which 5 p.c. alcohol has been added. This removes the fat from the smegma bacillus, and prevents confusion.

(5) Mounting, including Slides, Preservative Fluids, etc.

New Counting Chamber.‡ — J. W. Cropper read a note on a "New Counting Chamber for the Enumeration of Protozoa and other Organisms" (from the Marcus Beck Laboratories, Royal Society of Medicine). The chamber was designed on the principle of the hæmocytometer, but with a considerably larger area—namely, 5 × 5 mm.—of the platform ruled in squares, the latter also being increased in size. For various practical reasons the depth of the chamber was retained as $\frac{1}{10}$ mm. as in the older chambers. Thus the organisms or cells in a comparatively large volume—namely, 2·5 c.mm.—could be counted, and in cases where the number present was scanty it was possible to count a sufficient number of organisms to minimize the statistical errors which were inseparable from a small count. The size of the smallest squares had been so arranged that they occupied the central half of the diameter of the field of the microscope, using a $\frac{1}{6}$-in. objective and a × 7 eye-piece, this permitting a rapid count being made. In cases where the organisms or cells could be easily recognized with a low-power magnifica-

* C.R. Soc. Biol. Paris, lxxxi. (1918) pp. 183-4.
† Comptes Rendus, clxvi. (1918) pp. 357-9.
‡ Lancet, March 2, p. 337.

tion, it had been found preferable to employ an extemporized device, consisting of a large-sized microscope-slide on which columns $\frac{1}{2}$ mm. in width had been ruled. On this a ring of paraffin-wax, applied while molten by means of a turn-table, was placed. A definite volume—say, 10, 20, or more c.mm.—of the suspension of organisms to be counted was expelled from a graduated capillary pipette on to the ruled slide, and a cover-slip was allowed to fall upon it. The organisms in the *whole of the drop* were easily and rapidly counted by working along the columns from end to end. The chamber and ruled slides could be obtained from Messrs. H. F. Angus and Co., Wigmore Street, W.

Metallography, etc.

Comparison Screen for Brass.*—A simple method for determining approximately the grain-size of annealed brass is described and illustrated by O. W. Ellis. A glass screen is employed carrying prints on transfer-paper of photomicrographs of standard samples which have been subjected to different but known heat-treatments, arranged in the form of a ring. The image of the structure of the sample under examination is projected on to the centre of the screen, and direct comparison is then readily made with the standard samples.

Effect of Great Hydrostatic Pressure on Metals.†—Z. Jeffries has found that cylinders of pure aluminium and of an aluminium-copper alloy (88 p.c. aluminium), after subjecting to hydrostatic pressure up to 12,400 kilograms per square centimetre at temperatures of 25° and 40°C., are practically unchanged in dimensions, or in regard to hardness, strength, and microstructure. Both the metals tested had fine-grained structures, and owing to the haphazard orientation of the crystals it is considered that the resistance to deformation would be equal in all directions, and hence no permanent deformation would result from great hydrostatic pressure. A single crystal or a piece of metal composed of a few larger grains, on the other hand, would show different degrees of resistance to deformation in different directions, and might suffer permanent deformation under great hydrostatic pressures.

Etching with Chromic Acid and Hydrogen Peroxide.‡ — This mixture is stated by S. W. Miller to be a useful etching reagent for all bronzes and brasses, and also for silver. The reagent is made by adding a few drops of hydrogen peroxide to a very dilute solution of chromic acid. Addition of hydrogen peroxide causes strong effervescence, and turns the solution a very dark brown colour. If the chromic acid solution is too strong large gas-bubbles are evolved which prevent uniform etching. The specimen is immersed in the effervescent solution for a few seconds and then washed immediately in running water. Photo-

* Journ. Inst. Metals, xviii. (1917, 2) pp. 171–2 (1 fig.).
† Journ. Inst. Metals, xviii. (1917, 2) pp. 243–7.
‡ Journ. Inst. Metals, xviii. (1917, 2) pp. 253–5 (2 figs.).

micrographs of a brass and a bronze are given illustrating the effects of the reagent. The reagent is recommended particularly for the etching of brass welds.

Heat-treatment of Grey Cast Iron.*—The behaviour of cast iron under the influence of heat has been studied by J. E. Hurst in connexion with the cracking of Diesel engine piston-heads, which during working attain a temperature of at least 900° C. Chemical analyses of different parts of cracked piston-rods show that the combined carbon is gradually all converted into the graphitic form in the portion exposed to high temperature. Microscopical examination showed an increase in the dentritic structure and in the number of graphite plates in the portion subjected to the influence of heat; while the extreme edge of the piston is directly in contact with the flame the extent of the dentrictic structure is less, the graphite is more finely divided, and numerous small holes appear. Experiments with samples of a high phosphorus grey cast iron showed that under certain conditions—annealing at high temperatures (above 900° C.)—a portion of the free carbon is reabsorbed. The structure at the extreme edge of the piston-head is connected with this phenomenon. Fracture ensues from the internal strain caused by the slight volume-changes which accompany these changes in the condition of the carbon, as well as from the weakening of the iron caused by the separation of carbon.

Uniformity of a Cast of Acid Open-hearth Steel.†—The results of an investigation to determine the general quality and uniformity of a cast of steel from a 40-ton acid open-hearth furnace are given by T. D. Morgans and F. Rogers. The steel was made for the manufacture of H.E. shell, and contained approximately 0·5 p.c. carbon. Chemical analyses, tensile and hardness tests, and microscopical examinations were made on the top, middle, and bottom billets of each of twenty-one ingots. The manganese-content showed a slight decline from the first to the last ingot poured. Beyond this and the usual slightly higher percentage of elements found in the upper parts of the ingots, all properties approached a high degree of uniformity. The structure consisted of a ferrite network, whose meshes were filled with generally lamellar pearlite. Towards the edge of the billets the network size was smaller, and was largest towards the centre. No variation in structure which would prove detrimental to the steel in use was found.

Microstructure of Commercially Pure Iron between Ar_3 and Ar_2.‡ The very pure iron (99·84 p.c. iron) known as "Armco" iron, which is made in the ordinary basic open-hearth furnace, is peculiar in showing brittleness when subjected to mechanical treatment between 900° and 800° C.; at any other temperature outside this range the material is remarkably ductile and malleable. The cause of this unusual red-shortness has been investigated by W. J. Brooke and F. F. Hunting by

* Journ. Iron and Steel Inst., xcvi. (1917, 2) pp. 121–8 (8 figs.).
† Journ. Iron and Steel Inst., xcvi. (1917, 2) pp. 209–18 (21 figs.).
‡ Journ. Iron and Steel Inst., xcvi. (1917, 2) pp. 233–9 (14 figs.).

heating samples above 1000° C., allowing to cool slowly, and quenching individual samples at various temperatures. Photomicrographs of all the quenched samples are given. The microstructures of all samples quenched above 900° or below 800 C° were as normally obtained with pure iron, but those quenched between these temperature-limits showed a peculiar "eutectoid" constituent, with double boundaries at the junctions of many of the crystal boundaries. The composite character of the constituent was always characteristic, the central structure being more or less pearlitic, surrounded by a ferrite zone which connected up in a definite manner with the adjacent crystal grains. The composition of the "eutectoid" has not been determined by the authors, but a combination of sulphide, phosphide, and carbide is suggested. Increasing the oxygen-content of the iron was not found to increase the amount of the eutectoid. From the coincidence of the temperatures of appearance and disappearance of the constituent with those of the brittle range, it is considered very probable that the constituent is the cause of the red-shortness shown by "Armco" iron.

FAY, HENRY—**Microscopic Examination of Steel.**
[A guide to others engaged in the inspection of steel.]
Wiley & Sons and Chapman & Hall: iv and 18 pp. (55 photos.).