Conclusion.

In conclusion, said Professor Robertson, it must be apparent to everyone that the problem of preventing mental disorder is not a simple one. The analogous problem is that of the prevention of tuberculosis, with which it has so many points in common, and from the campaign for its prevention much can be learned. The possible causes of mental derangement are legion, and the possible combination of these, in individual cases, beyond counting. It is clear that when sickness of mind threatens or occurs, mental health cannot be regained by any royal road. The claims of the individual sufferer are in the first place paramount and must be studied. Every case of threatened breakdown presents a unique problem. It may have features in common with others; it certainly will present features peculiar to itself. Intensive study, therefore, of individual cases, so as to disclose these causative factors, is the primary duty of the psychiatrist. If that duty be not discharged, treatment will be confined to a few rule-of-thumb precepts, and its results will be disappointing. Secondly, what can be done in a more general way is well illustrated in the case of general paralysis. By disseminating, as widely as possible, information as to its mode of attack and the methods of prevention, a real contribution has been made to the mental hygiene of the community. Encouraged by that success, let us see to it that our knowledge of the origin and development of other disabling conditions is effectively applied wherever it is needed. In the opening up of these preventive channels a great responsibility rests on practising psychiatrists. It is futile to lament that those of unstable equilibrium turn elsewhere for help—to the quack, to the charlatan, to the barren ritual of the latest cult. The blame for this state of affairs must be shared by the psychiatrist. Far too long has he sulked in his cave; now he must come out into the market-place and offer his wares to all who need them. He must not confine his interests to his duties in the mental hospital. He must co-operate with the general practitioner and get into touch with the outer world through the agency of out-patient clinics. An expert body of psychiatrists, trained in every device of medicine and psychology, and diligently applying themselves to the clamant needs of their generation, might avert much needless suffering on the part of those in mental difficulties or about to be overwhelmed. Let none be daunted by the magnitude of the enterprise that is envisaged, but rather find in it an incentive to continued and combined effort, and the hope of a greater reward.

DARK-GROUND ILLUMINATION OF TISSUE CELLS CULTIVATED "IN VITRO."*

BY

T. S. P. STRANGEWAYS, M.A., M.R.C.S., L.R.C.P.,
LECTURER IN SPECIAL PATHOLOGY, CAMBRIDGE UNIVERSITY;
DIRECTOR, CAMBRIDGE RESEARCH HOSPITAL.

AND

R. G. CANTI, M.D.,
LECTURER IN BACTERIOLOGY, ST. BARTHOLOMEW'S HOSPITAL, LONDON.
(From the Laboratories of the Research Hospital, Cambridge.)

THE cultivation of animal tissues *in vitro* is now a recognized method of research. When a fragment of tissue is cultivated upon a cover-slip in suitable medium a zone of cells grows out around the edge of the fragment. Many of these cells become adherent to the surface of the cover-slip and wander over it by amoeboid movement, pushing their way along the plane between the glass and plasma jelly. Such cells are very suitable for microscopical observation, and studies of the cells carried out under direct illumination have already been reported.¹ The thin film of growing cells is peculiarly suitable for observation by dark-ground illumination, and by this method beautiful and instructive pictures of the living vegetative cells can be obtained. The technique for this examination requires considerable experience and care, but presents no great difficulty to workers familiar with critical microscopic technique.

* A demonstration given to the Section of Pathology and Bacteriology of the Annual Meeting at Nottingham on Friday, July 23rd, 1925.

The following description of the apparatus and methods used for the examination and study of tissue cells by dark-ground illumination may be useful to workers not familiar with this method of microscopic examination.

The cultures must be put up on thin glass slides not more than 0.6 mm. in thickness, and the thickness of the whole preparation must not exceed 0.9 mm.

The tissue may be cultivated in plasma or in plasma and embryo extract on a cover-slip sealed over a hollow-ground slide, and just before examination the cover-slip can be removed and transferred to a thin glass slide on which a drop of Tyrode or other suitable saline solution has been placed; the cover-slip is then sealed down with paraffin wax to prevent evaporation.

A better method, however, is to cultivate the tissues directly on the thin glass slide. By this means it is possible to use cultures growing in fluid as well as semi-solid media. Care must be taken not to press upon the cover-slip while handling the cultures as pressure may damage the growing cells. Too violent excursions of the oil-immersion objective will also cause damage.

The microscope we have used is a Zeiss microphotographic stand with mechanical stage and sliding objective carriers. Before any attempt is made to use the dark-ground condenser the objectives must be carefully centred. A triple nosepiece is to be avoided, as the three portions are seldom in exact optical centre and cannot be adjusted. The sliding carriers, on the other hand, are readily adjusted, and with a little experience give no more trouble than a triple nosepiece. Each objective must be centred and kept on its own carrier.

FIG. 1.—Diagrammatic drawing of lantern. A. Metal shield bent at right angles holding a pointlite lamp; the whole slides on B, a steel rod to which it is fixed with a thumbscrew C. This arrangement allows the lamp to be moved as required for centring with the lens D. The lens carrier slides in a sleeve, E, fixed to the front of the body of the box F. The top and back of the box are open and the bottom is made of a cast brass plate, G, to which is fixed the steel rod which carries the lamp-holder. This plate is pivoted at its centre and rides on a similar cast brass plate, movement laterally being obtained by the thumbscrew H. The lower plate is in turn pivoted at its forward end to a bar, J, fixed to the table, so that the whole lantern can be tilted up or down by the thumbscrew K.

The following instructions for centring the objective may be useful to those not accustomed to critical work.

1. Swing out the lower substage diaphragm, and the Abbé condenser, leaving the upper substage diaphragm in position; close this as far as possible.
2. Place the 2/3 inch objective with its sliding carrier in position and, using diffuse light, focus on to the aperture of the diaphragm.
3. Centre the 2/3 inch objective to the aperture of the diaphragm, using the key.
4. Replace the Abbé condenser, and place a centring slide on the microscope stage.
5. Focus on to the cross-line of the centring slide with the 2/3 inch objective and place the cross-line exactly in the centre of the field.
6. Remove the 2/3 inch objective and replace by the 1/8 inch, and later by the 1/12 inch, each on its own carrier. Using the key, centre these lenses to the cross-line.

All the lenses are now centred to the aperture of the upper substage diaphragm and hence to each other.

The source of light used for illuminating the object is a pointolite of 100-c.p. This is fixed in a special lantern constructed of metal plate, made by Maflin. In the front is a Zeiss aplanatic condensing lens (Model Ic.) with iris diaphragm. The lamp is capable of being centred to this lens, and the whole lantern with the lamp can be tilted either vertically or horizontally as one unit. Fig. 1 will make the construction of this lantern clear.

The examination of the cultures is made in an incubator regulated by a Hearson thermostat and containing the microscope (Fig. 2).

FIG. 2.—Incubator arranged for use.

The incubator consists essentially of a wooden box 20 inches long by 9 inches wide by 12 inches high.

The microscope is placed inside close to the right-hand end in order that a camera lucida attachment with drawing-board may be employed. The tube of the microscope projects through a hole 2 inches in diameter in the top of the box. This hole has its centre $3\frac{1}{2}$ inches from the front and 3 inches from the right-hand side. To the left of this opening a square is cut out of the top of the box and fitted with a sheet of glass through which a view may be obtained of the interior. This square is fitted with a hinged wooden door. In the front of the box opposite the mirror of the microscope is a window, fitted with glass, through which the light is admitted to the microscope from the lantern.

The back of the box is divided vertically into three equal portions, the left and right being closed with a door, hinged at the bottom, giving access to the heating elements and the microscope respectively. The middle partition is open, and is fitted with a velvet sleeve through which the microscope is manipulated.

The heating elements consist of two 16-c.p. carbon filament lamps run from the mains, one of which is kept permanently alight and the other is connected in series with a Hearson's capsule thermostat regulated at 38°C . The connexions required are shown in Fig. 3.

FIG. 3.—Heating elements showing connexions.

The capsule is protected from the direct radiation of the lamps by means of a small cardboard tray suspended beneath it.

The mirror in the model of the microscope employed is ordinarily attached to the substage condenser, so that it moves with it. When a focused source of light is used this form of apparatus is inadmissible, and the mirror is

therefore removed from the microscope and attached to the floor of the box on a sliding base. The microscope is placed so that it stands vertically over the mirror, and is kept in position by a horseshoe-shaped plate of lead 1 inch thick, cast so that it fits the base of the microscope stand (Fig. 4).

This arrangement keeps the microscope rigidly in position during the various manipulations required, and at the same time allows the whole instrument to be moved at will and its position adjusted as required.

FIG. 4.—Mirror and lead plate to fit base of microscope stand.

The dark-ground condenser is made by Swift with numerical aperture 0.95. The objectives used are $\frac{1}{8}$ inch oil immersion by Swift with numerical aperture 0.95, or a $\frac{1}{12}$ inch objective by Zeiss suitably stopped. The selection of the objectives is of considerable importance. Many of the stops supplied to bring down numerical aperture of the ordinary $\frac{1}{12}$ inch do not fit closely to the back of the lens, and thus perfect definition cannot be obtained. The authors advise an objective of the correct numerical aperture. The usual eyepieces are used, and also an X10 Telalugic by Swift.

The apparatus is assembled as follows. The pointolite lamp is first adjusted in the lantern so that the centre of the lens is in alignment with the point of light of the lamp. To ensure this the light is focused through the lens on to a strip of white paper about 4 feet away, and the bright image of the glowing tungsten ball of the lamp is thus readily obtained. The lamp is now moved vertically and horizontally as required until the image of the tungsten ball is clearly seen surrounded on all sides by an equal halo of coloured light. It will be found that as the lamp is moved the halo moves to one or the other side of the circle of light, and a truly centred beam is only obtained when the coloured halo is seen encircling the light evenly. The lamp is now fixed in front of the microscope incubator and focused on the mirror at such a distance that the size of the tungsten ball on the mirror is not larger than 10 mm. in diameter. This is ensured by pasting upon the concave surface of the mirror a piece of paper with a scale marked upon it as shown in Fig. 4. The mirror is now turned round so that the plain surface receives the light. The microscope, with all the lenses removed, is now adjusted so that the light passes directly upwards through the draw-tube. This is a detail of great importance and requires practice. The iris diaphragm of the substage is placed in position. The beam of light is now thrown upon the ceiling through the draw-tube, and by alternately opening and closing the iris diaphragm on the substage and that in front of the condensing lens of the lantern it is possible to judge if the beam of light from the lantern is passing centrally through the draw-tube; the two circles of light, which may be caused to increase or decrease in size by opening one or other of the iris diaphragms, must coincide exactly and the circle of illumination must show relatively sharp edges, blurring on one side or the other indicating that the beam of light is not central. When the light has once been centred, the lantern, the microscope, and the mirror must on no account be moved again or shaken. If accidental movement occurs during subsequent manipulations the whole process of centring must be repeated. A piece of white paper is now inserted on the top of the substage about $\frac{3}{4}$ inch below the opening in the microscope stage. The light is carefully focused on this sheet of paper by moving the condensing lens on the lantern in or out, care being taken

that the lantern is not moved while adjusting the lens. The image of the tungsten ball on the paper should not exceed 10 mm. in diameter. The paper is now removed and the iris diaphragm of the substage swung out and the dark-ground condenser gently placed into position on the substage. Great care must be taken not to touch the mirror or to move the microscope while this manipulation is being carried out. The condenser is then racked up to the level of the stage. The 2/3 inch objective, which has already been centred, is put in position and a No. 1 eyepiece inserted into the draw-tube. The surface of the condenser is brought into focus and the rings engraved on the surface are carefully centred by means of the centring screws of the carrier of the condenser. The apparatus is now ready for use.

To examine the cultures, switch on the heating bulbs of the microscope incubator and allow the temperature to become steady at 38° C., if this has not already been done. Open fully the iris diaphragm of the lantern and note again that the dark-ground condenser is centred correctly. Rack down the condenser about 1/4 inch. Take the culture to be examined and on the under side of the glass slide place a large drop of cedarwood oil. Place the slide in position on the stage of the microscope and rack up the condenser until the oil on the slide touches the top of the condenser. Air bubbles must be avoided. Focus the central fragment of tissue in the culture and clamp slide to the stage. Rack up the condenser; a dark circle gradually appears in the centre of the field, which becomes smaller as the condenser is raised, and suddenly a very bright image of the tungsten ball is seen focused on the fragment. This bright image should be exactly in the centre of the field. When the light is seen truly centred through the fragment the 2/3 inch objective is removed carefully and replaced either with the 1/8 inch or the 1/12 inch oil immersion as required. The iris diaphragm of the lantern is closed to an opening of about 1/8 inch in diameter and the oiled objective brought down and focused on the central fragment. The condenser is now racked slowly up, and if the centring is true a small area of light will be seen in the centre of the field. If the centring is not quite true this area of light will appear to one side of the field, and a slight adjustment of the objective with the key of the carrier is permissible in order to bring it to the true centre. If, however, the area of light is quite outside the field then the apparatus must be again set from the beginning. The worker is warned that it requires considerable practice to obtain this true centring, but unless the necessary skill is acquired good results cannot be expected. When the light is correctly centred the culture is moved by the mechanical stage so that cells at the edge of the central fragment which are in single layer are brought into the field. To obtain good definition the objective and condenser are carefully focused and the light of the lantern is adjusted by the iris diaphragm of the lens. A mercury green line filter placed in front of the lantern renders the light approximately monochromatic and increases the sharpness of the image; in addition, light of this colour is useful in preventing eye-strain.

It should be noted here that the dark-ground method of illumination shows up one thing only, and that is the interface between two surfaces of different refractive index. When each of the substances on either side of the interface is clear then the interface is shown as a white line with blackness on either side of it. If, however, one of these substances should contain, for example, particles of a different refractive index, then this substance will appear granular or cloudy, according to the size of the particles. A bright light will show up as a cloud the presence of particles which, owing to their fineness, a less bright light would leave as a complete blackness.

Cultures of tissue cells of about eighteen to twenty-four hours' incubation which are growing well should be selected for examination. In such cultures the outlines, internal structures, and complex internal movements of the vegetative cells are clearly shown. The vegetative cells when growing upon the cover-slip usually have a feather-like outline. The cytoplasm is clear and in it the various cell structures are seen embedded; the nucleus appears as a clear oval or rounded body with a sharp outline and contains

one or two distinct, slightly opaque bodies—the nucleoli. In the cytoplasm the most prominent objects are small globules of fat which are present in varying numbers in practically all cells cultivated *in vitro*. These fat globules are seen as very bright refractile spheres. The fat globules vary much in size and become larger and more numerous as the culture grows older. A varying number of fine somewhat refractile granules are also seen; these are quite small and not so bright as the small fat globules. The mitochondria are clearly seen in the cytoplasm as fine threads which vary greatly in length. The centrosphere shows as an obscure area on one side of the nucleus. The cell undergoing mitosis is not well shown by dark-ground illumination, as such cells become spherical in shape and thus are not so suitable for critical study as the flattened vegetative cell. The fat globules, however, and some of the small granules and some of the mitochondria can usually be made out lying round the clear spindle-shaped area of protoplasm, in which can be seen indications of the chromosomes, especially when the cell is at metaphase.

The structures described above can be well seen in cultures examined under dark-ground illumination at room temperature without the use of a microscope incubator, but the fascinating movements of the cell and its structures do not take place under these conditions.

REFERENCE.

¹ Strangeways, T. S. P.: Observations on the Changes seen in Living Cells during Growth and Division. *Proc. Roy. Soc., B*, xciv, p. 137.

Memoranda:

MEDICAL, SURGICAL, OBSTETRICAL.

A METHOD OF REDUCING CERTAIN DISLOCATIONS OF THE SHOULDER.

I WAS recently called to a colliery ambulance room to see an old man—an underground haulier. He was sitting on a bench with the right arm hanging down by his side, and he was apparently in great pain.

Half an hour before the patient had been pushed by his horse and had fallen against the side, receiving a sharp blow on the upper and back part of the right shoulder-joint. He had "felt his arm go out" and had "felt sick." He had a very obvious subcoracoid dislocation of his right shoulder-joint. He had never before had a dislocation.

Stripping him of his shirt, I tried Miller's method of reduction with countertraction by means of a towel round the chest for some time, but without success. I then carried out Kocher's manoeuvres, but these also failed to effect reduction. I resorted, in turn, to hyperabduction with traction, to leverage with the patient's arm round my waist, and to the heel in axilla method. These were all unavailing. I was about to administer an anaesthetic when it occurred to me to try a simple manipulation of the head of the humerus, which I could feel medial to the biceps and coraco-brachialis muscles. This was immediately successful—despite the fact that the patient was not faint, nor were the muscles relaxed. Further, the successful method caused the patient no pain at all. The plan was as follows (for right shoulder):

1. With the patient recumbent, the right arm is grasped at the elbow and lifted forward and upward until the patient's arm is almost extended above his head. This places the arm in position for movement 2, and relaxes the biceps, coraco-brachialis, and deltoid muscles.

2. With the patient's arm still raised, pressure is applied to the elbow as if trying to make the head of the humerus touch the table. The line of force is thus vertical, from the elbow through the shaft and head of the humerus. At the same time the operator's left fingers are spread out on the back of the patient's right shoulder, while the thumb in front presses the head of the humerus downwards and so helps it to travel back under the muscles whence it came. By this manoeuvre the head of the bone passes back round the neck of the scapula to a position below the glenoid cavity. The dislocation is thus converted into a subglenoid one.

3. The patient's arm is now brought down parallel with the trunk.

4. Finally, with the operator's clenched left fist in the axilla to act as a fulcrum, the patient's elbow is now approximated to the side—when the head of the bone will re-enter the capsule, and will be felt—and heard—to slip over the labrum glenoidale into position.

After-Treatment.—In this it is important that the elbow on the injured side be well supported, thus preventing the head of the humerus from pressing on the damaged lower part of the capsule.

Pontycymmer, Glam.

VERNON NEWTON, M.R.C.S.Eng.