48 hours improves the quality of the finished product. After washing, the unwanted parts of the agar are cut out and discarded and the remaining specimen flooded with freshly boiled (gas-free) distilled water. This prevents liberation of dissolved air during drying. Where tap water has a high mineral content it may be necessary to equilibrate the specimen for some hours against distilled water.

With freshly boiled water almost to the brim of the petri dish, the specimen is floated gently loose from the glass with a clean, grease-free scalpel and with slight rocking of the petri dish. The latter is then tilted and the specimen floated on to a grease-free glass plate such as a lantern slide, using the fingers as a guide. It is essential that the slide is absolutely clean and grease-free (flaming it over a bunsen burner for about 30 seconds gives the best results). A little experience and care is required in dealing with sheets of agar larger than about 2 in. square; using plenty of water for support is the secret.

When the agar is in position on a lantern slide it is trimmed and all bubbles trapped between agar and glass are removed with excess boiled water. Excess water is drawn off by tilting slightly and applying blotting paper to the agar edges. The upper agar surface must not be blotted. The preparation is then put in a horizontal position to dry in an incubator or oven at 55 to 60°C. Any tendency for the agar to creep out of position in the first few minutes of drying is due to excess water and/or tilting of the specimen. The process of drying takes 1 to 3 hours depending on the size of the specimen and after this period it is transparent and is firmly adherent to the glass. Staining with protein stains such as amido (naphthalene) black can then be done with little risk of detaching the agar. Sometimes, although not experienced personally, a preparation fails to take the stain, and Norris (1960) finds that a previous rapid wash with water removes the difficulty. Should the agar become partially or completely detached it may often be replaced on the slide; on re-drying it will be found adherent once more.

After staining and drying the preparations have similar properties to photographic plates. They may be stored between paper sheets; contact prints, enlargements, or lantern slides may be made for direct projection. The latter are often of excellent quality and superior to photographs of the same material. Fig. 1 shows a contact print (A), and enlargements $\times 3$ (B), and $\times 24$ (C) of the same material. Note that the definition of lines at the highest magnification is still good and the picture is devoid of 'graininess' which is usually apparent in prints from photographic negatives at this degree of enlargement.

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A micro method for agar-gel precipitin reactions

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The technique described here was devised with the object of conserving scarce reagents (especially sera) used in agar-gel precipitin tests. Although it is possible to use for this purpose a 'miniature Ouchterlony plate', in which small holes are cut in agar coated on to a microscope slide, in our experience it is difficult both to cut the holes cleanly and to ensure precision in their spacing. In these circumstances repeatability of results is often unsatisfactory. In the method to be described an agar layer on a microscope slide is used; but, instead of introducing the reagents into holes cut in this agar, they are placed in holes drilled in a perspex block applied to its surface. In effect this provides a series of hollow cylinders resting on the agar, into which the reagents are free to diffuse. The capacity of the holes can be controlled accurately by selection of an appropriate thickness of perspex and a suitable diameter of drill. Similarly a high degree of precision can be obtained in positioning the holes.

PERSPEX BLOCKS

Figs. 1 and 2 show the apparatus assembled. The thickness of the perspex and diameter and spacing of the holes will depend on individual requirements, but for general purposes we have found that perspex 3 mm. thick and holes made with a number 35 drill are satisfactory. The capacity of these holes is 0.02 ml. The spacing of the

FIG. 1. Diagram of the apparatus.

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FIG. 2. *The apparatus assembled.*

holes in the preparation shown in Fig. 2 is 6 mm. between centres.

For quick and accurate preparation of the perspex blocks some form of template is desirable. The one we use is made from sheet brass 3 mm. thick through which holes have been drilled at various spacings. This template is clamped to a piece of perspex and holes drilled through the appropriate section. The perspex is then separated from the template and cut with a hacksaw to a suitable size (about 1 inch square).

AGAR-COATED SLIDES

In order to ensure a water-tight seal between the perspex and the agar it is essential that the surface of the latter should be absolutely flat. For the preparation of single slides we use a perspex trough 2 mm. deep. The slide (1 mm. thick) is placed in the trough on a level surface and molten agar poured over it until no meniscus is visible at the sides of the trough. When the agar has set the coated slide is removed by cutting round its edges with a scalpel.

Larger numbers of slides may be prepared in a similar manner in larger trays. In order to ensure that the slides are level a base layer of agar should first be poured into the tray and allowed to set. The slides are then placed on this layer and further agar poured in in sufficient quantity to cover the slides to a depth of 1 mm. It is important that the slides should be placed well away from the sides of the tray so that the meniscus formed when the agar is poured in does not interfere with the flatness of the agar coating on the slides. The slides are separated from the surrounding agar with a knife as before. It is simplified if a firm agar (3%) is used for the base layer. For the coating layer we use 1.5% ion agar in 0.85% NaCl; 0.02% sodium azide is added to ensure sterility.

APPLICATION OF BLOCKS TO AGAR AND FILLING THE HOLES

The greatest source of difficulty with this technique is the provision of a watertight seal between perspex and agar. To ensure this the perspex should be pressed firmly on to the agar and slid gently over its surface to allow air bubbles to escape through the holes. The surface of the agar must not be visibly moist.

When filling the holes with reagents air bubbles must be excluded. If care is not taken these are liable to form at the bottom of the holes during filling and will interfere with diffusion into the agar. The holes should be filled from the bottom by means of a Pasteur pipette drawn out into a fine capillary. The preparation is stored in a moist chamber while precipitation lines are forming.

STAINING AND MOUNTING THE PREPARATION

When the precipitation lines have developed sufficiently the perspex block is lifted gently off the agar. Faint outlines of the holes are visible on the surface of the agar and these should be marked with a drop of Indian ink. The slide is dried by placing it face downwards on a piece of filter paper and leaving at 37°C. overnight. It is then washed in running tap water for 48 hours before staining.

A suitable general purpose stain is 0.5% azocarmine. Thirty minutes' exposure is generally adequate, although staining of faint lines may take longer. After staining, the preparation is rinsed in tap water and differentiated in 2% acetic acid until no more stain can be removed. The slide is then rinsed again in water, dried, and mounted under a coverslip. Fig. 3 shows examples of the finished preparations.

FIG. 3. Examples of finished preparations, stained with 0.5% azocarmine.

SUMMARY

A micro agar-gel precipitation technique is described in which the reagents are applied to the agar by means of a block of perspex through which holes have been drilled. The method allows a high degree of precision in placing the reagents on the agar with consequent good repeatability of results.

CORRECTIONS

We very much regret that Figs. 2, 4, and 7 of the paper 'Sarcoma of breast, with particular reference to its origin from fibroadenoma' by R. C. Curran and O. G. Dodge (*J. clin. Path.*, 15, 1-16) have been printed upside down. In the legends accordingly please read 'right' for 'left' and vice versa. Also Figs. 12 and 13 refer to Case 37, not to Case 36 as printed.

Professor N. H. Martin asks that the last phrase of the last sentence of the first paragraph of the Discussion in his paper 'Serum sialic acid levels in health and disease' (*J. clin. Path.*, 15, 71) 'whereas an increase in the α fraction would have little effect on this ratio', be deleted.

A method for obtaining concentrates of eosinophils from blood

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In a previous publication (Spriggs and Alexander, 1960) we described an albumin gradient method for separating the different white cells of blood. This was applied to the isolation of tumour cells but it was also noted that very pure suspensions of neutrophil polymorphonuclears could be obtained by the same method. It has now been found that the eosinophil leucocytes can be collected by the albumin method in a separate layer, and if the blood sample comes from a patient with eosinophilia it is sometimes possible to pipette these cells off in a high degree of purity.

The albumin gradient method need not be described again, but it should be noted that all equipment *must be siliconed*. The cells form a series of layers according to their specific gravity, as shown in Fig. 1. The platelets

FIG. 1.

are on top, with most of the leucocytes below them, and any residual red cells not removed by the preliminary sedimentation are lower still. When eosinophils are

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