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BRIEF COMMUNICATION

A Device for Preparing Cell Spreads

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Studies on blood cells or whole cells derived from solid organs which involve the use of techniques such as immunofluorescence, autoradiography and quantitative histochemistry or combinations of these (e.g. Balfour, Cooper and Alpen, 1965), require preparations occupying a small area in which the cells are in fairly close apposition to each other and are sufficiently flattened to display their cytoplasmic and nuclear structure.

Organ imprints produce well flattened preparations suitable for these methods, but the cells are not a representative sample of the whole population and when using immunofluorescence, proteins present in the interstitial fluid may obscure cytoplasmic staining. It is therefore necessary to make suspensions of washed cells derived from an organ or a representative sample of it.

Bots, Went and Schaberg (1963) studying cerebrospinal fluid, devised a method in which cell suspensions were placed in a narrow vertical container and allowed to sediment on to a slide. A ring of filter paper surrounding the lower opening of the container provided the capillary tension necessary to extract the fluid and cause the cells to flatten on the slide. The time required to prepare a cell spread by this method was 30 minutes and the delay could be a distinct disadvantage in experiments involving radioactive isotopes. The instrument described here is based on a similar principle but uses centrifugal force to speed up the sedimentation of the cells; by this means it is possible to prepare six slides in 15 minutes.

The apparatus consists of a hexagonal Perspex block mounted on the spindle of an electric motor and containing six holes each with an inlet on the upper surface and an outlet in the centre of one face. The cell suspensions are placed in the holes and centrifuged; the cells are driven out on to slides, one of which is clamped over each outlet and the fluid is absorbed by a ring of filter paper interposed between the slide and the block.

The Perspex block (1) is a regular hexagon measuring 10·2 cm (4 in.) across flats and 2·5 cm (1 in.) thick, each face measuring $5{\cdot}8 \times 2{\cdot}5$ cm ($2\frac{5}{16} \times 1$ in.). The centre of the block is drilled to fit the vertical spindle of a motor (2). A horizontal hole 0·65 cm ($\frac{1}{4}$ in.) in diameter and 2·5 cm (1 in.) deep is drilled in the centre of and normal to each face. This opens into a similar vertical hole drilled into the upper surface of the block at a distance of 2·5 cm (1 in.) from the face. Each face is relieved, leaving a raised portion 0·5 mm (0·020 in.) in height and 1·5 cm (0·625 in.) in diameter around each hole. The inner surface of the hole is reamed and the raised area is polished to produce a flat surface with a sharp edge at the outlet of the hole.

At each corner of the block, metal lugs (3) are attached, radially to the upper and lower surfaces and made to project 1·5 cm ($\frac{5}{8}$ in.) beyond the edge of the block. Each pair of lugs accommodates a hinge-pin (4) for the attachment of a metal pressure plate (5)

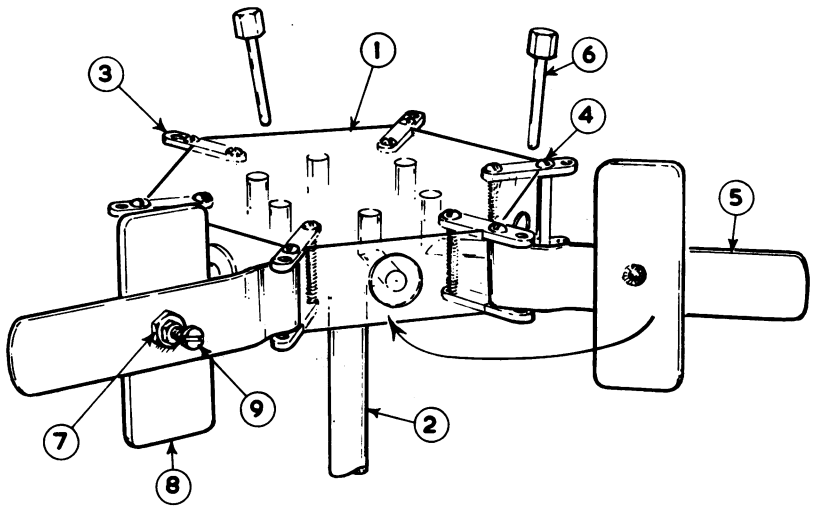

FIG. 1.

8·5 × 1·9 cm ($3\frac{1}{2} \times \frac{5}{8}$ in.) which is bent over at one end to form the hinge. The plates can be locked between the next pair of lugs by a cotter pin (6). A 6 B.A. hank bush (7) is rivetted to each plate on the outer surface (when in the locked position) in line with the hole. A metal backing plate (8) 6·6 cm × 2·5 cm × 4·5 mm ($2\frac{5}{8} \times 1 \times \frac{3}{16}$ in.) is fastened to the inner surface of the pressure plate by a screw (9) threaded through the hank bush. The backing plate has universal movement on the end of the screw, the adjustment of which controls the pressure on the slide. When the apparatus is loaded the ring of filter paper is aligned over the outlet of the hole with the slide between it and the backing plate.

It is important that the pressure on all six slides should be equal and of the order of 4 kg. This may be checked as follows: clamp six microscope slides in position, without filter paper discs; attach a spring balance to one of the adjusting screws, pull back and note the reading when the slide begins to move. Repeat this process with the remaining pressure plates and adjust the screws if necessary. Insufficient pressure allows the majority of the cells to be pulled into the filter paper with the fluid and too great a pressure prevents the preparation drying out in 15 minutes. A steel punch 0·56 cm ($\frac{1}{4}$ in.) external diameter is required for the preparation of the filter paper discs.

TECHNIQUE

Preparation of suitable cell suspensions

Leucocytes can be separated from whole blood by any of the accepted methods or they can be used after being in culture for several days. In both cases the cells are washed and resuspended in Hanks solution containing 5 per cent BSA. The optimum cell concentration is about 14,000/mm^3. Peritoneal macrophage suspensions can be treated in the same way.

To obtain suspensions of cells from solid organs such as lymph nodes, spleen or thymus, the tissue is cut into small pieces which are forced through a Borel sieve or similar device into Hanks solution as before. After centrifugation and resuspension the concentration is adjusted in the same manner as for peripheral leucocytes. If there are many large cells in the preparation, as for instance in cultures of transformed lymphocytes or in the case of

spleen and lymph-node cells growing in millipore chambers, the concentration may have to be correspondingly reduced. However, it is important to keep the density as high as possible, since the cells require the support of their neighbours in order to counteract the unequal pull exerted by the filter paper on the liquid. This pull is liable to damage the cells, particularly the nucleus. If a thin preparation is required the cells can be protected from damage by coating the slide with the following mixture; non-fluorescent gelatin N.280 (The Gelatine and Glue Research Association, London) 10 g, egg white 10 g and glycerol 10 g in 100 ml saline. About 0·3 ml of this mixture is sufficient to cover one slide, which should be allowed to dry in a 37° oven until it is tacky.

Loading

A Whatman MM3 filter paper 1½ in. diameter, pierced in the centre with the punch is held against the block so that its hole is exactly centred over the outlet. The slide is then placed outside the filter paper, with its long axis vertical, and both are clamped in position by locking the pressure plate, 0·1 ml of the cell suspension is then placed in each hole via the inlet.

Centrifugation

The speed is adjusted to 400 rev/min and the suspensions are centrifuged for 15 minutes. The slides are unclamped and should appear dry at this stage, since retention of moisture causes the cells to round up again. The spreads can be fixed and stained at once or stored in boxes with silica-gel for 1–2 months.

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