Cytological Preparations from Protein-free Fluids

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During an investigation into the forensic aspects of exfoliative cytology the problem arose of making stained preparations of a few cells in a large volume of protein-free fluid. The method described by Powell (1959), in which the cells are centrifuged on to "evo-stik"-coated rectangular coverslips suffers from two disadvantages. First a special centrifuge head has to be made, and secondly the use of "evo-stik" to promote adhesion precludes the application of alcoholic stains and of the usual methods of dehydration and mounting.

The technique to be described does not restrict the use of any staining methods; it is simple and no modification of the centrifuge head is required. The recovery rate of cells is high and the method may therefore have applications in the examination of body fluids in which only small numbers of cells are present.

The apparatus consists of a 3 in. length of glass tubing of 1 in. diameter. An ordinary $\frac{3}{8}$ in. circular coverslip is placed on a rubber bung of appropriate size which is then used to stopper one end of the glass tube. The cells are fixed by adding an equal volume of 4% formaldehyde to the fluid. This is then poured into the tube, which is centrifuged in an ordinary laboratory centrifuge with a swing-out head. The supernatant fluid is removed and replaced by a similar quantity of physiological saline containing 2 drops of bovine albumin per 10 ml. The mixture is shaken and centrifuged again. The supernatant fluid is pipetted off and the residue allowed to dry on to the coverslip overnight, preferably in a desiccator. The addition of the saline and albumin and the subsequent centrifugation may be omitted if a large number of cells are present, as quite good adhesion is still achieved.

The technique may also be used without preliminary wet fixation, with the advantage that albumin may be added directly to the original fluid. One centrifugation is thus avoided and the recovery rate is higher. It has been claimed by Ferreira and Sabbag (1958) that air drying of smears for 24 hours before fixing has no significant deleterious effects on

FIG. 1.—Centrifugation tube and clip for holding coverslips.

nuclear appearances or cellular morphology and that a correct diagnosis of atypical and malignant cells is still possible. They believe that the results are better after drying for 24 hours than they are after only one hour. Drying may, however, alter the cytoplasmic staining in the Papanicolaou technique and thus interfere with readings of the eosinophilic index.

The staining of circular coverslips in bulk at first presented some difficulty, but this has been overcome by the use of simple holding clips made from stainless steel spring wire of 8 mm. gauge such as is employed in dental practice. The clip is used as a pair of forceps to take the coverslip off the rubber bung, and if always applied in the same way it is obvious throughout the staining process which side of the glass bears the cell smear. Each clip can be labelled individually and any number can be hung side by side in the staining jars on a glass rod passed through the loops.

REFERENCES

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