fresh medium, were found to be necessary during the three-day period of filming.

Discussion

The chamber as constructed and used was found to fulfil all of the requirements. Some difficulty was experienced, however, in carrying out the static perfusion technique, but by exercising patience and by excluding all air bubbles this method of changing the medium is practicable. In addition to using this system to perfuse nutrient fluid for cell growth it may be used as a means of adding any desired infective or chemical agent. As pointed out by Dick (1955), an important advantage of the negative pressure technique is the virtual elimination of the large "dead" space inherent in certain other systems.

The needles are the only metallic components of the chamber, but being of stainless steel are not toxic to the tissue culture. The cement also is not toxic, and if used with discretion need scarcely come into contact with the medium. The heat of sterilization sets the cement so hard that it is impossible to detach the coverslips and although the needles can be recovered the slide must be rejected. A high melting point wax was used at first for one or both coverslips to permit repeated use of the slide, but the glass cutting proved so easy and quick that it was decided to regard the chambers as expendable rather than forgo the advantages of a permanent seal.

Soda glass coverslips, if adequately cleaned and sterilized, were found to support a monolayer of HeLa cells and were optically adequate for interference microscopy. The fluid layer, being thin and homogeneous, does not interfere with colour interpretation and light absorption is minimal.

Summary

An easily made glass tissue culture chamber is described which fulfils the requirements necessary for the observation of normal or infected tissue culture cells by time-lapse colour cinematography. A method of perfusion under negative pressure is incorporated in the design.

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Preparation of Stained Films of Sickle Cells

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It is known that red cells from a patient showing sickle-cell anaemia or sickle-cell trait contain an abnormal haemoglobin (haemoglobin S). When these cells are incubated under conditions of reduced oxygen tension, such as by sealing a wet film between two slides, sickling occurs, the process taking from 15 minutes to several hours. The cells may show irreversible sickling, but usually revert to their normal shape on the admission of air. If, therefore, the slides are separated to prepare a blood film, which is fixed and stained in the usual way, the cells will be found to present a normal appearance. Attempts to fix the cells before separating the slides is likewise unsuccessful, since no cells remain after staining.

In view of the comparative rarity of the condition in the United Kingdom, a stained preparation would be useful for demonstration purposes, and it has been found possible to do this by the following method.

Method

A drop of oxalated blood was placed on a slide and covered with a second slide. The cells were observed under the microscope until sickling occurred. Two tablets of paraformaldehyde on a piece of wire gauze were placed on a tripod and heated with a Bunsen burner until a concentrated stream of formaldehyde was being evolved. The two slides were then rapidly separated to produce blood films and held face downwards in the formaldehyde. Fixation was immediate and drying was rapid. They were then stained with Leishman's stain.

I wish to thank Mr. F. W. Eels for his assistance in finding this method.

The next number of the *Journal* will contain the papers read at a Symposium on the Pathology of the Cell organized by the Association of Clinical Pathologists in honour of the centenary of the publication of Virchow's "Cellular Pathology."