J. clin. Path. (1958), **11**, 455.

TECHNICAL METHODS

A Glass Tissue Culture Chamber for Use in Time-lapse Cinematography

F. L. CONSTABLE AND MARGARET A. J. MOFFAT

From the Department of Bacteriology, University of Edinburgh

(RECEIVED FOR PUBLICATION MARCH 3, 1958)

Numerous tissue culture chambers have been devised with the object of providing the optical requirements necessary for microscopic observation of the culture, and some of these incorporate a means of perfusion to facilitate changing medium, or to introduce various agents during the period of observation (Carrel, 1931; de Haan, 1931, 1938; Lindbergh, 1939; Parker, 1950). A few have been designed specifically for use in microcinematography in conjunction with phase-contrast or interference microscopy in which the optical requirements are exacting and impose strict limitations upon the design (Hu, Holmes, Pomerat, Livingood, and McConnell, 1951; Pomerat, 1951; Christiansen, Danes, Allen, and Leinfelder, 1953; Schwöbel, 1954; Dick, 1955; Richter and Woodward, 1955).

Requirements

The chamber herein described was developed to meet the following general requirements:

(1) The purpose of the chamber is to permit time-lapse colour cinematography with interference microscopy of the growth of normal or virus-infected tissue culture cells.

(2) It is essential that the walls of the chamber be optically flat and parallel and of uniform refractive index. The thickness of the chamber must not exceed 1 mm. and the medium must be homogeneous.

(3) The culture must be maintained for at least three days, and this, in view of the small dimensions of the chamber, necessitated by optical considerations, implies the use of perfusion, which need not be continuous.

In addition the following were considered to be desirable: (*a*) The material to be glass which combines relatively easy working with ease of sterilization; (*b*) the method of perfusion to be as simple as possible, avoiding large reservoirs, complicated tubing and valves; (*c*) the adhesive for sealing the chamber to be more durable than wax, to provide a perfect seal, to be non-toxic and to withstand sterilization at 160° C.; and (*d*) the construction to be within the capabilities of a small laboratory workshop and the cost to be as low as possible.

Construction

The body of the chamber is made from a thin glass microscope slide. 3 in. × 1 in. × 1 mm., a hole approximately ½ in. in diameter being cut in the centre, with a No. 1 coverglass, 0.18 mm. in thickness, above and below to form the walls.

At each side of the perforation a short groove is cut in the glass, parallel to the long axis of the slide. These should be no deeper than is necessary to accommodate a stainless steel hypodermic needle No. 20, which should not project above the surface of the glass within the area of the coverglass seating.

At this stage all components of the chamber are cleaned to the requisite standard for tissue culture.

To assemble the chamber one coverglass is cemented over the hole in the slide on the surface on which there are no grooves; two hypodermic needles are bent into the shape of a crank and are cemented

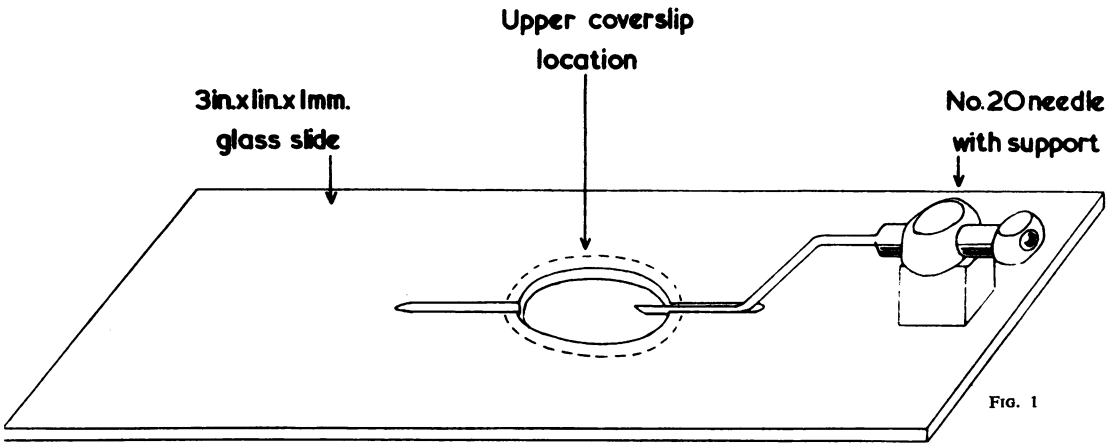

FIG. 1

into the grooves so that the points project slightly into the chamber. Additional support is given in the form of small wooden blocks under the needle fitting to facilitate the attachment of syringes or tubing. Finally, a coverglass is cemented in place to close the chamber, the only access to which is now through the needles (Fig. 1).

A satisfactory cement was found to be " araldite " which is a two-component epoxy resin, obtainable from Aero Research Limited, Duxford, Cambridge. At room temperature this adhesive takes at least 12 hours to set, but by raising the temperature this period can be reduced to as little as 30 minutes.

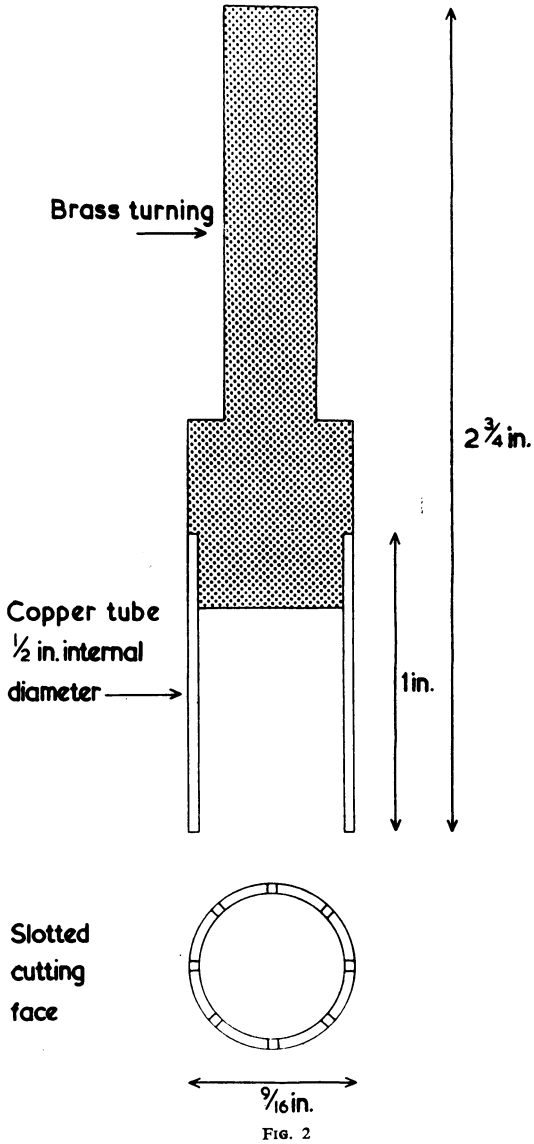

FIG. 2

Cutting the Glass

Holes can readily be cut in glass slides if a power-driven vertical bench drill is available. An effective cutter was made by turning a brass rod to the dimensions shown in Fig. 2, and soldering to it a length of copper tubing. If a lathe is not available, short lengths of tubing of suitable diameter soldered together in telescope fashion will suffice. Slotting the face of the cutting edge with a fine hacksaw increases the efficiency. In operation the tool should be fed with coarse carborundum paste and the drill speed should be fairly low. To prevent cracking, a perfectly flat support must be provided for the slide, and drill pressure carefully regulated, especially towards the end of cutting. A carborundum wheel in a dental drill serves to cut the needle grooves. The operation should take less than five minutes.

Sterilization

The complete chamber is sterilized in the hot air oven at 160° C. for one and a half hours when the cement sets extremely hard and may become black.

The Chamber in Use

Preparation of Cultures.—After sterilization the chamber is filled with a suspension of cells at a density of approximately 100,000 cells/ml. in an appropriate medium. This is injected from a syringe directly attached to one of the needle fittings and by tilting the slide all air can be excluded. After removal of the syringe it is necessary to seal the needle ends, for which small plugs of sterile cotton-wool are satisfactory, to prevent blockage by dried medium. A short period of incubation at 37° C. is required to allow the cells to settle on the upper coverglass, after which the chamber is turned over and is ready for observation in a microscope incubator.

Changing Medium and/or Perfusing Chamber.—This may be accomplished in one of two ways, depending on whether or not the slide can be moved from the microscope stage.

If the slide can be moved the fluid is changed by means of a syringe, as in the initial filling. If, however, it is to remain *in situ*, a method of perfusing the chamber must be provided. For this purpose a reservoir is placed inside the incubator, fluid being drawn into the chamber under negative pressure applied by an externally situated syringe, connexions being made by polythene tubing and needle adaptors.

The syringe may be operated by hand, or for prolonged irrigation, either continuous or intermittent, by a suitably geared motor. To introduce fresh medium or agents such as viruses, it is only necessary to replace the reservoir and this operation can be carried out during filming.

Although the volume of the fluid in the chamber is small it should support cell growth without renewal for as long as 48 hours, and in our experience only one or two manipulations, either to introduce virus or

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.

We are indebted to Mr. T. C. Dodds, Mr. G. A. Wilson, and Miss Sheila Heath for technical assistance.

One of us (M. M.) was in receipt of an Edinburgh University Graduate Research Scholarship.

References

Carrel, A. (1931). *C.R. Soc. Biol. (Paris)*, **106**, 7.
Christiansen, G. S., Danes, B., Allen, L., and Leinfelder, P. J. (1953). *Exp. Cell Res.*, **5**, 10.
Dick, D. A. T. (1955). *Quart. J. micr. Sci.*, **96**, 363.
Haan, J. de (1931). *Arch. exp. Zellforsch.*, **11**, 365.
—— (1938). *Acta neerl. Morph.*, **1**, 12.
Hu, F. N., Holmes, S. G., Pomerat, C. M., Livingood, C. S., and McConnell, K. P. (1951). *Tex. Rep. Biol. Med.*, **9**, 739.
Lindbergh, C. A. (1939). *J. exp. Med.*, **70**, 231.
Parker, R. C. (1950). *Methods of Tissue Culture*, 2nd ed. Hoebner, New York.
Pomerat, C. M. (1951). *Methods in Medical Research*, Vol. 4. Year Book Publishers, Chicago.
Richter, K. M., and Woodward, N. W., Jnr. (1955). *Exp. Cell Res.*, **9**, 585.
Schwöbel, W. (1954). Ibid., **6**, 79.

Preparation of Stained Films of Sickle Cells

H. L. ENGLISH
From Winwick Hospital, Warrington

(RECEIVED FOR PUBLICATION SEPTEMBER 23, 1957)

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."