An Easily Made Tissue Culture Perfusion Chamber

By D. A. T. DICK

(From the Department of Human Anatomy, Oxford)

With one plate (fig. 3)

SUMMARY

A perfusion chamber is described with which a tissue culture can be irrigated successively with different immersion media. Only 1–2 ml. of medium are required for each change, which can be accomplished within 1 minute. Simplicity of design permits speed and economy in manufacture. Perfusion by suction instead of by positive pressure has eliminated certain drawbacks of previous designs. Mixing effects in the chamber have been studied. Any component of an immersion medium can be reduced to 0.5% of its initial concentration by the use of only 2 c.c. of fresh medium. The chamber has been used in the techniques of immersion refractometry and interferometry of living cells.

INTRODUCTION

The number of perfusion chambers which have been designed for the growth and study of tissue cultures since the method was originated by Harrison in 1907 is now very considerable. Initially the purpose of irrigating the explant with fresh medium was to improve the supply of nutritive material and the removal of metabolites, and the early designs of Burrows (1912) and Romeis (1912) were directed solely to that end. Carrell (1931), De Haan (1938), and Lindbergh (1939) introduced numerous ingenious improvements with basically the same purpose. Schade (1933), who was interested primarily in the physico-chemical aspects of tissue culture, provided means for the control not merely of the liquid but also of the gaseous phase of the environment. With this increased interest in the environment of the culture as a study in itself and not merely as an improvement in culture technique, De Haan (1938) and Schade (1933) introduced the first chambers which allowed microscopic observation of the culture during the course of perfusion, although under very unfavourable optical conditions. More recently Hu and others (1951), Christiansen and others (1953), and Schwöbel (1954) have produced designs which provide vastly improved facilities for observation, in particular by phase-contrast microscopy.

The optical requirements may be summarized as follows:

1. The optical path through all points of the chamber must be constant. This means that the chamber must have plane-parallel walls of uniform thickness and refractive index. In addition the immersion medium must be optically homogeneous, i.e. there must be no concentration gradients. Such gradients are particularly likely to occur when the medium is changed and it is essential to avoid this by thorough irrigation. These conditions are especially...
important in interference microscopy, which is much more easily disturbed by gradients in optical path in the medium, slide, or cover glass than is phase-contrast.

(2) The total thickness should generally not exceed 1 mm. in order to allow focusing of the microscope condenser. This can be increased if a condenser with a long working-distance is available.

(3) The chamber should be capable of being used with a coverslip of standard thickness to meet the optical requirements of the objective.

**NEW REQUIREMENTS**

The advent of new techniques which are being applied to cells in tissue culture has given rise to some entirely new requirements in a perfusion chamber. The present chamber was designed for the study of tissue cultures by the technique of immersion refractometry (Barer, Ross, and Tkaczyk, 1953; Barer and Joseph, 1954). The new requirements will also apply, however, to much projected biochemical and pharmacological investigation, in which the effect of various compounds on the morphological characters of cells in tissue culture will be tested. Briefly, it is necessary to effect repeated complete changes in the medium in which the culture is immersed, (a) in a minimum time, and (b) with the use of as small a quantity of fresh medium as possible. This latter requirement may be dictated either by the scarcity or by the expense of some constituent of the medium, e.g. by the expense of the bovine plasma albumin employed in refractometry.

For this purpose none of the perfusion chambers already referred to are suitable. In every case a large reservoir is connected to the culture chamber by a system of tubes and control taps. To change the irrigating medium the reservoir must be emptied and refilled and the new medium run in until the chamber is thoroughly flushed out. As the volume of the connecting tubes and control taps creates a considerable 'dead space' which must be flushed out and refilled in addition to the chamber itself, large demands are made both on time and on the supply of medium if a complete change of medium is to be ensured.

There are three further incidental disadvantages from which previous designs suffer. Though they are almost invariably entitled 'simple' culture chambers, for the most part complicated precision work in either glass, acrylic plastic (Perspex), or even stainless steel is involved in making them. This must make their construction slow and expensive. Secondly, those designs which employ coverslips sealed on to the upper and lower surfaces of the chamber are very liable to leak, since positive pressure is applied to introduce the fresh medium. Great care has to be taken to make the seals strong enough to withstand this hydrostatic pressure, which, although it is small, exerts a considerable thrust on the coverslips owing to their large area. Thirdly, no attention has apparently been directed to the problem of ensuring that the new medium shall sweep out the old with a minimum of
mixing and eddying. These processes must increase the volume needed to ensure complete replacement.

**Design and Construction**

The new culture chamber is essentially a modification and simplification of that designed by Christiansen and others (1953). It is constructed from acrylic plastic (Perspex) sheet, \( \frac{1}{25} \) inch (1 mm.) thick. This is readily obtainable and can be easily worked by a competent carpenter or mechanic without special tools. A high degree of precision is quite unnecessary.

A line drawing, plan, and elevation of the chamber are shown in fig. 1. Provided that the sawing and drilling are done fairly slowly, overheating is not a problem. The two grooves on the under surface on each side of the
control hole are produced by a fine circular saw. At the extreme ends of the grooves holes are drilled through the sheet. The reservoir and nozzle are turned from plastic rod and are attached to the upper surface over the corresponding holes by moistening the opposing surfaces with chloroform. While the chamber has been designed for convenience to the dimensions 3 inches by 1½ inches, it may be reduced without modification to 3 inches by 1 inch size, if for instance it is necessary to fit a warm stage apparatus.

**ASSEMBLY AND OPERATION**

To set up the chamber, a 2½ inches by ½ inch coverslip is sealed over the lower surface with paraffin wax, covering the central hole and the whole of both grooves leading into it. The coverslip, 7/8 inch by 7/8 inch, carrying the culture to be examined in a hanging drop, is sealed on to the upper surface over the central hole. No special strength is required in these wax seals. The irrigating medium is run into the reservoir with a Pasteur pipette. A piece of rubber tubing attached to a ground-glass tap leading to a suction apparatus is placed on the nozzle and the medium is sucked into the chamber. The formation of air-bubbles may be avoided by tilting slightly as the fluid runs in. Care must be taken not to empty the reservoir completely with risk of sucking air into the chamber. A simple syphon arrangement as illustrated (fig. 2) or a filter pump may be used to provide suction.

To change the medium in the chamber it is only necessary to suck out the remainder of the old medium from the reservoir with a Pasteur pipette, refill with new medium, and suck it through.

The optical properties of the chamber are excellent, provided that care is taken to seal the coverslips flat on to the surfaces of the chamber.
THE CHAMBER IN USE

It is seen that, apart from its simplicity, the chief innovation in this design is the substitution of negative for positive pressure to effect flow. The medium is sucked into the chamber rather than introduced by gravitational or other force. This has made it possible to eliminate almost entirely the ‘dead space’ between the reservoir and the chamber. Since the volume of medium needed for complete replacement is thus reduced, the size of the reservoir can also be reduced. This modification has also entirely eliminated the risk of leakage at the paraffin-wax seals, since the suction tends to draw the coverslips against the chamber instead of the reverse.

The time required to replace completely the medium in the chamber depends, first, on the rate of flow of medium, which is directly proportional to the degree of suction applied and inversely proportional to the viscosity of the medium. Secondly, it depends on the amount of mixing which takes place, and this will be discussed below. Even with media of relatively high viscosity, such as concentrated protein or acacia gum solutions, complete replacement can normally be brought about within one minute.

Effects of mixing

The effects of mixing and eddying in the chamber have been studied. In experiments with solutions coloured with methylene blue it was found that solutions of relatively high viscosity (e.g. containing more than 20% of acacia gum) produced the most satisfactory clearance of the old medium. When viscous solutions were interchanged or when a viscous solution replaced one of low viscosity, mixing was negligible and the fresh viscous medium entered the chamber as a separate wave, effecting complete and instantaneous replacement with less than 0.5 ml. of medium. (The volume of the reservoir is 1.2 ml. and that of the chamber 0.13 ml.) With non-viscous solutions (e.g. salines) there was considerable mixing of old and new media. Several designs with two channels leading from the reservoir and entering the chamber at different angles were tried. None of these, however, was found significantly more satisfactory than that with single straight entrance and exit channels. More efficient clearance was produced in this case by creating an intermittent rather than a continuous flow of the fresh medium. When a concentrated aqueous solution of methylene blue was washed out of the chamber with water, it was found that 2 ml. of water were sufficient to reduce the concentration of dye in the chamber (estimated colorimetrically) to between 0.4% and 0.6% of the initial value. Clearance was least satisfactory when a viscous solution was replaced by a non-viscous one. In this case a layer of dense viscous solution persisted on the floor of the chamber. If, however, the chamber was slightly tilted during the change, the viscous layer tended to gravitate towards the exit channel and clearance to less than 0.4% of initial concentration was achieved with 2 ml. of medium. Here also intermittent flow was more efficient in effecting clearance than continuous flow.
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Experience with the chamber

The culture chamber has been used in refractometric measurements on chick heart fibroblasts and snail amoebocytes. It is, of course, a condition of its use that the culture or tissue to be examined should adhere firmly to the upper coverslip, whether by natural migration on to it or by attachment by means of a plasma clot. Fig. 3 illustrates an amoebocyte of Helix aspersa which has undergone seven successive changes of acacia gum solutions while in the culture chamber. There is no sign of damage, in marked contrast to the appearance produced after a small air-bubble has been in contact with the cell for approximately 15 seconds. This observation must call in question the validity of the interferometric cell-thickness determinations made by Mellors and others (1953), in which the cell was examined when suspended in an air-bubble. Such a cell must have been so grossly damaged as to bear little resemblance to the normal.

The Departmental workshop has so far made one dozen of these culture chambers and they appear to offer no great difficulty in manufacture.

Use under sterile conditions

While the chamber was designed for experiments in which the culture is required to survive only for a few hours and sterility is therefore not essential, it may be easily adapted for use under sterile conditions. The chamber may be sterilized by ultra-violet irradiation (Carlson, Holländer, and Gaulden, 1947) and the reservoir protected from infection between changes of medium by a sterile glass cap. The coverslips may be easily attached so as to preserve the aseptic condition of the chamber. Then, provided that the irrigating fluid is sterile and is introduced into the reservoir with a sterile pipette, complete asepsis is maintained. There is no need to sterilize the suction apparatus.

I am greatly indebted to Dr. R. Barer for his continuous advice and encouragement in the preparation of this paper. Mr. A. B. Alder, F.R.C.S.,

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**Fig. 3 (plate).** Series of photographs showing effect of changing the immersion medium on snail amoebocytes. All photographs were taken with a Winkel-Zeiss semi-apochromatic phase-contrast objective ×40, N.A. 0.75. The heavy absorption of light in the phase plate emphasizes the halo around refractile details.

A, amoebocyte in 0.9% saline.

B, same amoebocyte in 18% neutralized acacia gum solution of the same tonicity as 0.9% saline. The cytoplasm and immersion medium have the same refractive index. The consequent absence of contrast between them makes the cytoplasm ‘disappear’.

C, same amoebocyte in 25% neutralized acacia gum solution. The cytoplasm has a lower refractive index than the immersion medium and therefore appears relatively bright (‘reversed contrast’).

D, same amoebocyte returned to saline. Note that active amoeboid movements have continued. The dark lines at the periphery of the cytoplasm are apparently thickened protoplasmic folds produced during movement.

E, same amoebocyte in saline after four further changes of immersion medium (different concentrations of acacia gum). The cell is still motile.

F, same amoebocyte in saline after an air-bubble 1 mm. in diameter had rested on it for approximately 15 seconds.
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made some helpful suggestions. My thanks are due to Mr. P. J. Peade for his skilled execution of the design. This work was carried out while on leave of absence from the Department of Anatomy, University of Glasgow, for which I must thank Prof. G. M. Wyburn.

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