Time-Lapse Movie Chamber for Virus and Long-Term Tissue Culture Experiments.* (32202)

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Several types of chambers for time-lapse movie studies of cultured cells are available commercially. These chambers are satisfactory in many respects, since cells can be cultured for limited periods of time within a container of good optical quality, permitting observation even under high magnification phase optics. After several years of experience with various commonly used chambers, it became apparent that they were unsuitable for (a) Long-term cultures requiring many fluid changes, since leaks could develop in the rubber gasket after repeated punctures with hypodermic needles, pH control was difficult, and contamination of the cultures might occur. Some of these obstacles could be avoided by constant perfusion; however, this is a complicated and time-consuming procedure not easily applied to more than a limited number of chambers simultaneously. (b) Studies involving infectious agents in which the often unavoidable gasket leaks and the drop of fluid left after removal of the needle represent a safety risk. Each needle insertion involves a certain chance of breaking the thin glass walls of the chambers.

In order to carry out movie experiments with cultures to be observed over a period of several months, we therefore found it necessary to consider a special approach, particularly when the experiments were concerned with virus. The chamber shown in Fig. 1 and Fig. 2 represents a modification of the Sight chamber (Sight Instruments, Long Beach, Calif.). This chamber as obtained commercially consists of 2 specially molded aluminum plates, between which is placed a 1/8-inch thick silicone gasket with a central circular opening and with cover glasses (43 × 50 mm, #1) on top and bottom. The assembled parts are pressed together with the "Sight chamber press and jig assembly" and aluminum clips are attached to each end of the chamber, holding the aluminum plates in tight position and assuring parallel position of the rectangular cover glasses. For the particular needs described this chamber, without modifications, does not provide obvious advantages over other available chambers; however, it is most useful in the following manner. Rather than seeding cells directly in preassembled chambers, a number of experimental cultures were prepared in large Leighton type tubes (28 × 110 mm, Bellco Glass, Inc., Vineland, N. J.), each tube containing a #1 rectangular cover slip (18 × 75 mm), on top of which a circular 25 mm #1 cover slip was placed. (A slight reduction in the size of the circular cover slip was necessary for its introduction into the large Leighton type tube; two opposite cuts were made, with a diamond point needle and a steel straight edge, removing approximately 3 mm on each side.) Such cultures were observed in order to determine the proper time for their recording with the time-lapse equipment; chamber components were sterilized and kept ready for use. For time-lapse movies, the circular cover slip with the cells growing on the upper side was removed from the Leighton type culture tube with sterile forceps and placed in the Sight chamber inside the circular opening of the silicone gasket on top of the lower cover glass. Two pieces of "O" ring silicone rubber gasket (Bellco Glass, Inc.), 5 mm long or less, were placed on top of the round cover slip close to the Sight silicone gasket; after placement of the upper cover glass and metal plates the chamber was closed in the chamber press and jig assembly. With this procedure the circular cover slip was held tight in place against the lower rectangular cover glass, thus permitting the chamber to be used with either an upright or inverted microscope. For recording under an inverted

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microscope, the two pieces of circular silicone gasket can be omitted.

To inject medium into the chamber initially, and at subsequent feedings, a simple infusion system was designed to avoid repeated puncturing of the gasket. Two hypodermic needles (1 1/4″, #26 gauge) were each fitted with a 3 1/2 cm piece of latex tubing (OD: 5 mm, ID: 3 mm). One of the latex tubes (inlet) was plugged with a glass rod and tightly closed by ligation. The other tube was tightly fitted with a glass tube closed with a serum bottle stopper (plug length 7/32″). The needles were left inserted in the chamber gasket as long as the culture was useful for recording. Culture medium was introduced, as shown in Fig. 1.

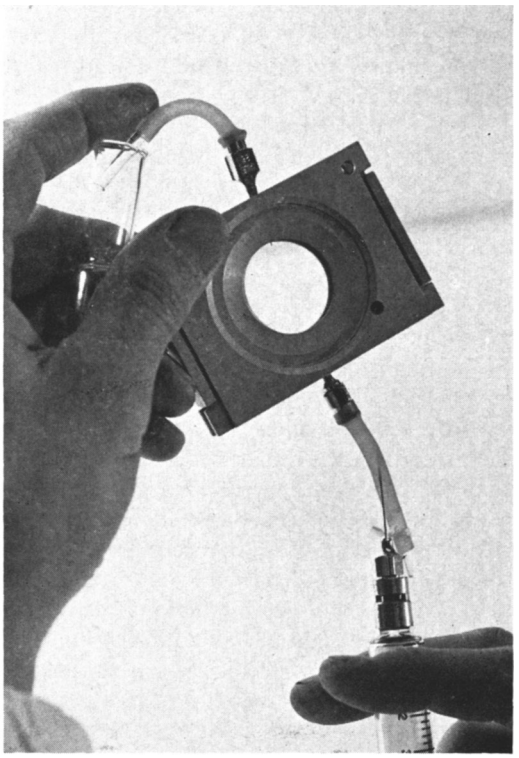

FIG. 1. Procedure for feeding cells in the modified Sight chamber. With rubber cap removed from the outlet tube, medium is injected into alcohol-sterilized area of inlet tube (different place at each feeding). Chamber is held vertically to direct air bubbles toward chamber outlet. Excess fluid is collected in test tube, as shown. At termination of procedure syringe is withdrawn and the puncture area alcohol-sterilized. Glass outlet is flamed and the rubber cap replaced.

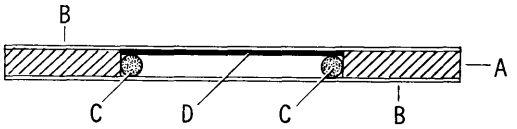

FIG. 2. Relative positions of silicone gaskets, inserted coverslip, and coverglasses. A: Chamber silicone gasket; B: Rectangular chamber coverglasses; C: Two pieces of "0" ring silicone gaskets; D: Circular coverslip with cells growing on lower side. As shown, the chamber is positioned for observation in a regular microscope; when used in connection with an inverted microscope it is turned upside down.

The rectangular cover slip in the large Leighton type tube on which the remaining part of the cell population grew was fixed and stained when the chamber was assembled. This sample facilitated the proper choice of fields for time-lapse recording and served as a record for comparison as the experiment progressed.

The described procedure has been satisfactory in several long-term experiments. As previously mentioned, the technique appears to eliminate most risks involved where infectious agents are used. High contrast movies can be obtained with this chamber through the double-layer cover slips with a 40× objective and long working distance condenser. With the Zeiss photomicroscope movies can be recorded at magnifications up to 145× on 16 mm film.

In virus experiments the chamber and the attached infusion arrangement were autoclaved after use; the serum bottle stopper was removed, and a hypodermic needle was inserted into the inlet tubing to permit expansion during autoclaving. The coverglasses could be removed from the silicone gasket after soaking in xylene for several hours, and the gasket was reusable for the assembly of subsequent chambers.

Summary. A modified time-lapse movie chamber used in connection with coverslip cultures in regular culture containers is particularly suited for long-term culture experiments for work with infectious agents.

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