Parabiotic Chamber for Organ Cultures: Improved Model

MICHAEL G. GABRIDGE

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Received for publication 16 July 1974

A modified parabiotic chamber for organ cultures is described. It consists of a modified organ culture dish, glass cover slip, a cylinder of semipermeable membrane, and a stainless-steel mesh filter support. Silicone and agar seals were used. Diffusion occurred rapidly and with consistent rates at 37 C when a ratio of 5:2 reservoir to inner chamber volume was used. The device is simple to construct and assemble and allows for continuous observation while maintaining a large surface area available for diffusion. The volumes of the reservoir, inner chamber, and agar base can be varied, and the chamber may be used with a wide variety of cell and organ culture systems.

Organ cultures are especially suited to the study of development and cellular interactions. This is because the cells maintain the differentiated state in vitro with nearly normal spatial relationships (2). When organ cultures serve as model systems for the analysis of effects from hormones, drugs, and toxins, it is often desirable to separate the target organ from the source of the soluble agent. This can be done with the proper positioning of a semipermeable membrane, but most of the resulting chambers are designed for surgical implantation (3, 9) and not for in vitro work.

The recently introduced "continuous observation diffusion chamber" (4a) utilizes an upright cylinder of selective membrane material to allow for continual microscopic monitoring of cell and organ cultures. Like most parabiotic chambers (8), however, it cannot accommodate the large reservoir volumes necessary for soluble toxins produced in small quantities while supporting organs or explants at the gas-liquid interface, as required to maintain viability. In our study of host-parasite relationships in tracheal organ cultures, we have developed a device which solves this problem while permitting continuous observation and providing a relatively large and variable ratio of reservoir-reaction chamber volumes. This modified parabiotic chamber was simple to construct and manipulate and has potential for application in a variety of situations where organ (or cell) cultures are employed.

MATERIALS AND METHODS

Chambers. Chambers were constructed from plastic organ culture dishes (60 by 15 mm) (no. 3010, Falcon, Division of Becton-Dickinson, Oxnard, Calif.). The dish was secured in a standard lathe, and a cutting tool was applied to mill off: (i) the lip surrounding the "support shelf," and (ii) the bottom of the reservoir. Figure 1 shows a diagram of a cross section through a standard organ culture dish, with arrows to indicate the edges which were removed. Rough edges were then filed down, and the chambers were washed and dried.

Acid-washed (7) and buffed cover slips (circular, 25 mm) were cemented over the 20-mm hole in the raised shelf of the dish with a silicone sealer (General Electric, Waterford, N.Y.; or Dow Corning, Midland, Mich.). The sealer was allowed to dry for 24 h, and after being tested for leaks chambers were sterilized with 12% ethylene oxide (Oxyfume 12, Linde Division of Union Carbide, New York, N.Y.) for 24 h at 37 C (5). Chambers could also be sterilized with alcohol and/or ultraviolet light. Chambers held in ethylene oxide were filled with sterile distilled water for 6 to 24 h to remove residual gas. Prior to assembly, dishes were routinely exposed to ultraviolet light for 15 min in a laminar flow hood.

Filters. Nuclepore (Nuclepore Corp., Pleasanton, Calif.) membranes with the desired pore size were cut into strips (120 by 11 mm) with a sharp scalpel. Filter supports were constructed of stainless-steel mesh fashioned into a 33-mm diameter cylinder, 10 mm high. The membrane strip was carefully wrapped around the filter support, and the overlapping ends were sealed with silicone cement. When dry, the filters, on their supports, were sterilized by autoclaving.

Assembly. Filters were secured in the dishes with an agar seal of 0.75% Ionagar (Colab Laboratories, Inc., Glenwood, Ill.). The filter and support were first briefly rinsed in sterile water (4 C) to retard diffusion of agar into the filter and were then placed around the raised cover slip in a dish which contained 2.5 ml of molten agar. Once the agar had solidified, medium was added (5.0 ml outside; 2.0 ml inside), the explant was introduced onto the cover slip, and the compound or cells were placed in the outer reservoir. The chamber was then ready for incubation.
The complete chamber thus contained a raised shelf with cover slip surrounded by a cylinder of semipermeable membrane on a mesh support, which was sealed to the dish with agar. A cross section of such a chamber is shown in Fig. 2, and a schematic, dimensional drawing is given in Fig. 3.

**Assays.** Glucose was measured as total hexose by the Dubois method (4), whereas bovine serum albumin was measured as protein by the method of Lowry et al. (6).

**RESULTS**

The described chambers were relatively simple to construct, and the dish with the cover slip in place could be washed and used indefinitely. The cover slip could be cemented either to the upper or lower surface of the raised shelf in the floor of the dish. The latter arrangement had the added benefit of providing a narrow ridge around the cover slip area, which kept small explants from sliding off the shelf during manipulations. The silicone and agar provided leak-proof seals, and, if the membranes were handled carefully and inspected prior to use, leakage was not a significant problem.

Diffusion rates across the Nucleopore mem-

![Diagrammatic cross section through a standard organ culture dish; arrows indicate edges to be removed.](image)

![Diagrammatic cross section through completed parabiotic chamber. (a) Modified organ culture dish; (b) layer of agar to seal the membrane to dish; (c) cylinder of semipermeable membrane and filter support; (d) shelf from original dish; (e) circular, glass cover slip cemented over central hole in dish with silicone cement.](image)

![Schematic, dimensional drawing of completed parabiotic chamber. See Fig. 2 for legend.](image)

branes were quite high (Fig. 4). Molecules the size of glucose (molecular weight, 180) diffused very rapidly and reached equilibration (99% theoretical maximum) by 24 h at 37 C when using 5.0 ml of glucose solution in the reservoir and 2.0 ml of distilled water in the inner chamber. Larger molecules, such as bovine serum albumin (molecular weight, approximately 60,000), took approximately 96 h to reach equilibrium (Fig. 4).

Results between experiments were quite consistent and reliable. Membranes of several pore sizes were evaluated using different chambers in multiple diffusion runs (Table 1), and standard deviations were less than 8% of the means when pore sizes of 0.05, 0.10, and 0.20 μm were used.

![Concentration of hexose (starred circles) and protein (closed circles) in the inner chamber after various times of diffusion through 0.10-μm Nuclepore membrane. At time 0, outer chamber (reservoir) contained 5.0 ml of 880 μg of glucose per ml and 780 μg of bovine serum albumin per ml, and inner chamber contained 2.0 ml of distilled water. Static incubation was at 37 C.](image)

**TABLE 1. Results of repeated diffusion runs (48 h at 37 C) using bovine serum albumin with membranes of various pore sizes**

| Pore size (μm) | No. of expts. | Protein in inner chamber (μg/ml) |
|---------------|--------------|---------------------------------|
|               | Range        | Mean               | SD*   |
| 0.05          | 371–435      | 395                | 23.5  |
| 0.10          | 430–495      | 465                | 27.4  |
| 0.20          | 345–450      | 401                | 34.2  |

* Outer reservoir contained 5.0 ml of bovine serum albumin (1.102 μg/ml), for a theoretical maximum value of 787 μg/ml for the inner and outer chambers at complete equilibration at infinite time.

**SD. Standard deviation.**
Median values were within 3.5 μg/ml or <1% of the means, indicating an unskewed distribution of results.

The procedures used in constructing and assembling the chambers did not impair growth of tracheal organ cultures (Fig. 5). Rings of hamster trachea which were maintained in modified mycoplasma medium (1) consistently sustained viability, as measured by percentage of the epithelium remaining intact and the vigor of ciliary beating. Levels were equal to, or greater than, controls maintained in petri dishes.

**DISCUSSION**

We found the parabiotic chamber described here to be a significant improvement over other chambers. Most other chambers fail to allow for continuous observation and relatively large reservoir volumes or are difficult to construct and manipulate. The current chamber eliminates these difficulties and minimizes leakage and breakage by utilizing modified plastic dishes with silicone and agar seals.

The ratio of reservoir to reaction chamber volume used was 5:2 ml or 2.5×. These levels can be altered according to the size of the dish and the depth of fluid tolerated by the explant. The tracheal rings used here are approximately 1 mm in thickness, and the level of fluid over the cover slip is about the same.

Diffusion rates vary according to the size of the molecules, the pores of the membrane, temperature, and mixing. Molecules the size of glucose and albumin diffused across membranes with 0.05- to 0.20-μm pore size rapidly (<96 h to equilibrium) at 37°C. The trachea organ culture system is not amenable to agitation, but it would be possible to promote diffusion in other situations by gently mixing the contents of the reservoirs to promote exposure of the agent to the membrane. This could be done with minimal trauma to the tissue by using a rocker platform or by circulating the reservoir contents with gentle flow in a peristaltic pump. The diffusion rate is also related to the thickness of the membrane, and Nuclepore membranes proved optimal in this regard. The pores are straight and not convoluted as in cellulose ester filters, and the membranes are extremely thin (approximately 10 μm) and can withstand autoclaving.

The surface area available for diffusion is relatively large in this system due to the use of a cylinder of membrane material. Under the conditions described (2.5 ml of agar, 5-ml reservoir, 2-ml reaction chamber volume), there was approximately 8 to 9 cm² of membrane surface exposed on both sides to fluids. This value could be increased or decreased by varying the level of the agar on the bottom of the dish or the depth of the fluids in the reservoir and inner chamber.

The upright, cylindrical membrane also permitted continuous microscopic monitoring of the explant. The dish was simply removed from the incubator and placed on the stage of an upright or inverted microscope. Because of the short working distance of high power objectives, one is limited to magnifications such as those obtained with a 10× objective and 15× eyepieces. However, higher power objectives can be used on an inverted microscope if the standard stage is replaced by a thinner sheet of Plexiglas containing an opening for travel of the objective.

The parabiotic chamber described here represents a significant modification of the previously available chambers with continuous observation capabilities (4a, 8). It should have potential application in several areas, including pharmacology, endocrinology, and host-parasite interactions.

**ACKNOWLEDGMENTS**

The expert technical assistance of Cynthia J. Chelcun is gratefully appreciated. Samples of semipermeable membranes were generously supplied by the Nuclepore Corp., Pleasanton, Calif.

This investigation was supported by contract DADA
17-73-C-3016 from the U.S. Army Medical Research and Development Command.

LITERATURE CITED
1. Collier, A. M., W. A. Clyde, and F. W. Denny. 1969. Biologic effects of *Mycoplasma pneumoniae* and other Mycoplasmas from man on hamster tracheal organ cultures. Proc. Soc. Exp. Biol. Med. 132:1153-1158.
2. Dawe, C. J. 1963. Symposium on organ culture: studies of development, function, and disease. Nat. Cancer Inst. Monogr. 11:1-252.
3. Dent, P. B., D. Y. Perry, M. D. Copper, and R. Good. 1968. Nonspecific stimulation of antibody production in surgically bursectomized chickens by bursa-containing diffusion chambers. J. Immunol. 101:799-805.
4. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
4a. Gabridge, M. G. 1974. Continuous observation diffusion chamber for cell and organ cultures. In *In Vitro* 9:455-457.
5. Kelsey, J. C. 1967. Use of gaseous antimicrobial agents with special reference to ethylene oxide. J. Appl. Bacteriol. 30:92-100.
6. Lowry, O. H., N. J. Roseborough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
7. Paul, J. 1961. Cell and tissue culture. The Williams & Wilkins Co., Baltimore.
8. Powell, K. R., H. J. Huth, and J. D. Cherry. 1973. Simple parabiotic chamber for the study of microorganisms in organ culture. Appl. Microbiol. 25:491-492.
9. Shelton, E. 1966. Differentiation of mouse thymus cultured in diffusion chambers. Amer. J. Anat. 119:341-358.