A New Chamber for Tissue Culture.* (24546)

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Carrell(1) was one of the first to recognize the necessity for apparatus which would permit detailed study of cells grown *in vitro*. Porter(2) developed a flask by flattening 2 sides of a roller tube. Many workers have made use of the hanging drop method. Rose (3) devised a chamber which permits continuous cultivation and observation of cells *in vitro*. Several tissue culture chambers made of acrylic plastic and glass have been described, among others by Christiansen *et al.* (4), Pulvertaft *et al.*(5), and more recently by Toy *et al.*(6). These chambers vary in complexity, are difficult to assemble and to sterilize, are prone to breakage and contamination. The chamber to be described overcomes these difficulties, may be used for observation of cultures at high magnifications by phase microscopy and is suitable for perfusion studies.

Description of chamber. The chamber consists of 5 parts, 2 of metal, 2 of glass and one of rubber. The metal parts may be stainless steel, or brass which is subsequently heavily chrome plated. Aluminum is unsuitable, as binding of threads readily occurs after repeated use. Price considerations make brass the metal of choice. The rubber used is a red silicone rubber "O" ring (American Packing and Gasket Co., Style DC-250, Size 6227-15). Other types of "O" rings made of various kinds of silicone and gum rubber have been found unsuitable. The remaining 2 parts are glass cover-slips of 1 inch diameter and thickness #1 or #2 (Corning Glass Co.). Cover-slips and silicone rubber are the only parts of the chamber which come in contact with the culture or nutrient, and are non-toxic. One metal part, which constitutes the bottom, has 4 holes for insertion of hypodermic needles used in filling and emptying the chamber. A drawing of chamber, with capacity of 0.7 ml, is shown in Fig. 1. It is assembled by placing a cover-slip in the metal bottom, followed by an "O" ring, on top of which is placed another cover-glass and the metal top. The top part is then screwed down with a small stirrup wrench and tightened sufficiently to prevent slippage of the "O" ring when a #22 hypodermic needle is inserted through one of the holes in the base. Degree of tightness is determined by experience of operator and thickness of cover-slip. The chamber may be sterilized in various ways. It may be sterilized by placing the component parts in a 60 mm Petri dish, and autoclaving. This method of sterilization permits the chamber to be used for observation of primary outgrowth from tissue fragments which may be placed on the lower cover glass during assembly. Or, the parts may be sterilized by flaming after dipping in 95% ethanol. Starting with the base, each part is placed after flaming in a sterile 60 mm Petri dish. When the second cover-slip has been placed on the "O" ring, the chamber may be taken out of the dish and the top, sterilized in similar fashion, screwed into place.

Procedure. After assembly, the chamber is easily filled with nutrient or cell suspension. A sterile #22 hypodermic needle is inserted through one of the ports and pushed through the silicone rubber "O" ring by gentle pressure and repeated semi-rotation; this needle serves as air vent. The cell suspension or medium is then introduced into the chamber from a Luer-Lok hypodermic syringe fitted with a #22 needle which is drilled through the "O" ring at the port directly opposite the vent needle. During filling, the chamber is held above the syringe to release entrapped air-bubbles. The vent needle is first withdrawn, then the needle used for filling the chamber. Medium changes are made in a similar manner; repeated changes of nutrient over many

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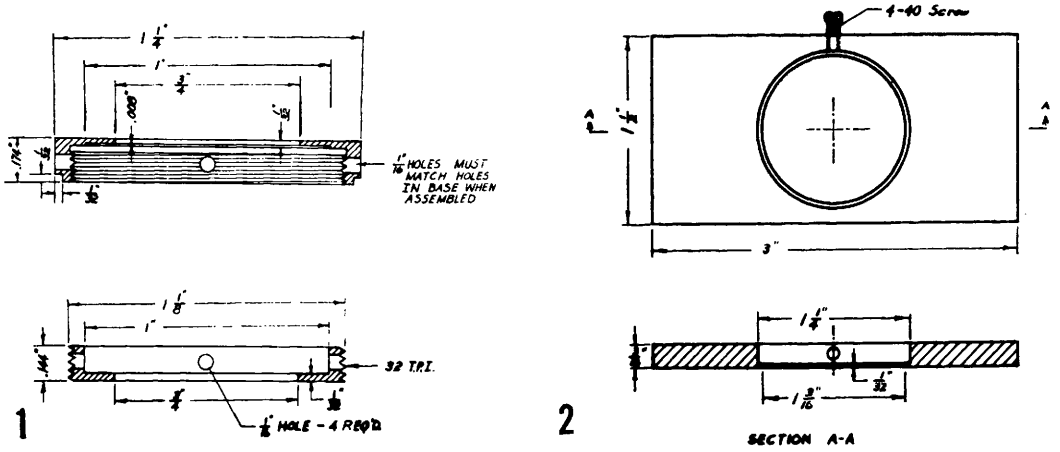

FIG. 1. Drawing showing construction of tissue culture chamber.
FIG. 2. Drawing showing construction of chamber carrier for microscopy.

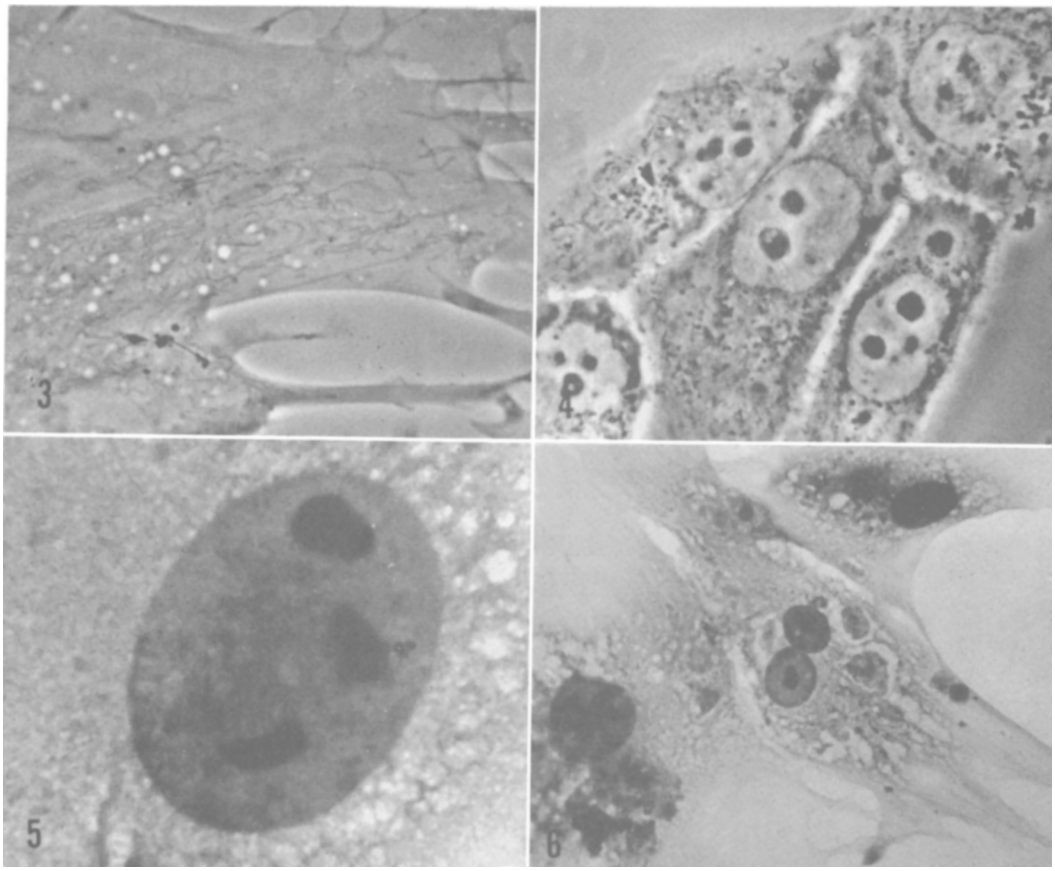

FIG. 3. Phase photomicrograph of part of a cell derived from plaque, a precursor lesion of bovine ocular squamous carcinoma, showing mitochondria. × 1200.
FIG. 4. Phase photomicrograph of human amnion cells. × 1200.
FIG. 5. Photomicrograph of nucleus of a cell derived from plaque, a precursor lesion of bovine ocular squamous carcinoma, after staining and mounting of chamber culture. × 1200.
FIG. 6. Photomicrograph showing inclusion-like bodies in cells derived from plaque. × 270.

days are possible without contamination. For culture identification and for protection of cover-slips the chamber is incubated in a 60 mm Petri dish, with the culture cover-slip at the bottom. Sub-cultures of cells growing in the chamber are made by trypsinization. Examples of cells examined by phase microscopy in the chamber are shown in Figs. 3 and 4; similar cells, fixed and stained, are shown in Figs. 5 and 6. For perfusion experiments, it is essential that the bottle used for collection of effluent be effectively plugged against aerial contaminants. For microscopy, the chamber is placed in a small rectangular carrier which will fit the slide clips of any microscope stage (Fig. 2). The carrier is provided with a locking screw which is useful in perfusion experiments where the position of the observed area must not change. Cell staining may be carried out either *in situ* or after removal of the cover slip on which the cells are growing, which is the method of choice.

Discussion. A tissue culture chamber is described which is simple in construction and use. It consists of 5 parts, 3 of these are standard items readily available. The parts of the chamber in contact with cells or nutrient are non-toxic. The apparatus is suitable for examination of cultivated cells at high magnifications by phase or bright-field microscopy. Cultures can be maintained for long periods of time in the chamber and subcultures are readily made. The chamber has proved valuable in tissue culture studies of material derived from cases of human leukemia, mouse leukemia, mammary carcinoma of mice, and bovine ocular squamous cell carcinoma and its benign precursor lesions.

Summary. A simple tissue culture chamber is described which permits phase microscopy at high magnifications and easy staining for permanent record. The chamber is also suited to perfusion studies, permits observation of cells over extended periods, and is useful for time lapse photography.

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Survival Time of Endotoxin Treated Rats as Response Metameter for Corticotropin and Hydrocortisone Bioassays.* (24547)

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The protective effect (survival) of adrenal cortical steroids to lethal doses of typhoid endotoxin in adrenalectomized rats(1) has been suggested as the basis of assay method for adrenal cortical steroids(2), but no detailed study of the method has been published. A protective effect has been shown in normal rats, mice, and rabbits against a variety of endotoxin preparations(3,4,5) by such materials as cortisone, hydrocortisone, corticosterone, adrenal cortical extract, and corticotropin. Desoxycorticosterone acetate was without effect. Halberg, Spink, and Bittner (6) showed that cortisone, hydrocortisone, 9-α-flurohydrocortisone, and their acetates, and to some extent aldosterone, prevented hypothermia which precedes death and prolonged survival time of adrenalectomized mice treated with Brucella somatic antigen (endotoxin). We investigated the effect of hydrocortisone on hypothermia and survival time of adrenalectomized rats given a lethal dose

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