pretation. However, they do not permit the unreserved conclusion that urogastrone is formed by the small intestine, inasmuch as the effect of control surgical procedures on the excretion of urogastrone is as yet unknown. It should be pointed out that the selection of a proper control experiment is inseparable from the more general question of why urogastrone should be excreted in the fasting urine. Experiments are now in progress which are designed to provide at the same time an answer to this general question as well as controls for the experiments reported here.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A ROLLER BOTTLE TISSUE CULTURE SYSTEM[1]

IN 1936 Gey and Gey[2] described procedures which they employed in maintaining *in vitro* tissue cultures of normal and malignant cells in roller tubes. Cultures are grown in a thin layer of clotted plasma on the walls of ordinary or specially designed glass tubes, and by revolving them horizontally they are alternately in contact with a nutrient medium and with air or any gaseous mixture that may be desired. The rotation gives a constant, slow washing action which seems to promote an adequate exchange of food materials and waste products between the supernatant fluid and the cells. The Gey roller tube method has several advantages over the more usual hanging drop, Maximow slide or Carrel flask techniques, but has certain disadvantages and limitations which we believe to be partially eliminated by the modifications in technique described below.

It is difficult and time-consuming to prepare the sterile human normal and placental sera as described in the original articles.[2,3] For experiments of a few weeks' duration we believe the substitution of the "synthetic medium," described by Baker[4] in 1936, is helpful. In roller tube cultures of various human and animal normal and malignant tissues we have obtained very vigorous and healthy outgrowths, even when this medium has been simplified by omission of hemin, insulin, thyroxine and vitamins A and D. Directions for the preparation of this and other media are given in a recent book by Parker[5] and by Baker and Ebeling[6]. Synthetic media have the great advantage that their components can be varied at will.

The danger of contamination during feeding and transfer of roller tube cultures is a serious one, since contact of the nutrient fluid with the stopper is difficult to avoid. We have found it advantageous to employ an arrangement shown in Fig. 1. A short length of

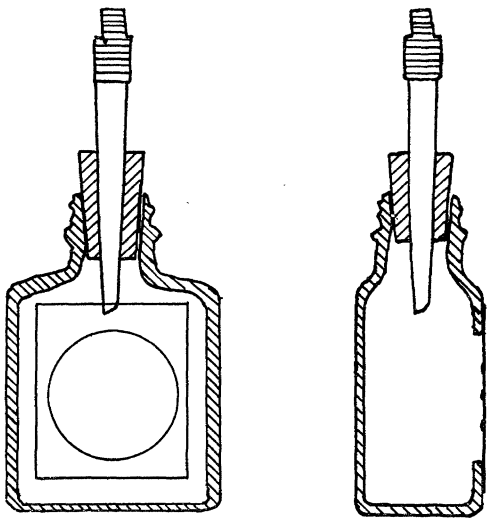

FIG. 1. Cross section views of roller bottle, with stopper and coverslip in place.

Pyrex tubing having a slight constriction at one end is introduced through a hole in a tightly fitting rubber stopper. The two parts are sterilized as a unit by autoclaving in large Pyrex test-tubes. A sterilized, inverted serum vial cap may be used to seal the end of the small tube after flaming it. For ordinary procedures this cap is removed, and the end of the small glass tube flamed. Fluid transfers can then be made by means of sterile syringes and long hypodermic needles.

Observations of the colonies under highest magnification is impossible in ordinary glass roller tubes, due to the thickness and curvature of the walls. Gey[3] in 1933 described the construction of special hexagonal tubes having flat sides of thin glass which were better suited to higher power observations (*i.e.*, 45× objectives). Even with these, however, satisfactory permanent stained specimens would be difficult to prepare and store and would involve sacrifice of the tubes.

[1] Aided by a grant from the National Research Council. From the Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University.

[2] G. O. Gey and M. K. Gey, *Am. Jour. Cancer*, 27: 45, 1936.

[3] G. O. Gey, *Am. Jour. Cancer*, 17: 752, 1933.

[4] L. E. Baker, SCIENCE, n.s., 83: 605, 1936.

[5] R. C. Parker, "Methods of Tissue Culture," Paul B. Hoeber, Inc., 1938.

[6] L. E. Baker and A. H. Ebeling, *Proc. Soc. Exp. Biol. and Med.*, 39: 291, 1938.

Therefore, we have constructed flat roller bottles, as shown in the figure, which appear to offer many advantages. A hole 33 mm in diameter is drilled in the wall of an ordinary commercial flat-walled, two-ounce bottle. Then a 43 by 50 mm No. 1 or No. 2 cover glass is cemented over this opening with a suitable plastic cement which should be non-toxic and insoluble in water after dry heat sterilization, but soluble in alcohol as is "Gelva" (kindly supplied by the Shawinigan Resins Corporation), or soluble in acetone as is "Glyptal number 1202" (made by the General Electric Company). These cements seem somewhat soluble in water if heated for only three or four hours at 160 degrees Centigrade and hence should be left overnight at this temperature. Since 80 per cent. alcohol is used in many staining procedures and easily dissolves the "Gelva," this cement is recommended for use when the cover slip will be removed. "Glyptal" may prove better for cementing cover slips which are to be left on and used several times before renewal. In practice the bottle is washed and dried; a thin layer of cement is applied with a medicine dropper around the edges of the hole and on the adjacent flat outside wall of the bottle; then a cover slip is placed on the cement and manipulated to remove air bubbles. A fairly loose non-absorbent cotton plug is inserted in the neck of the bottle which is placed, cover slip side up, in a wire basket or rack, ready for dry heat sterilization.

To plant the cultures, bottles are lined up on a sterile rack or towel, cover slip side down, the cotton plugs are removed, and the necks flamed gently. Then, in each bottle, from two to four drops of sterile heparinized plasma are placed directly on the inside surface of the cover slip and distributed evenly, after which the excess is drawn off, leaving a very thin film containing about one drop of plasma. It is unnecessary to deposit any plasma on the other walls of the bottle unless tissue is to be planted there as well. Next, from one to twenty or more fragments of finely chopped tissue in Baker's medium or saline are drawn into a pipette, concentrated in the tip, and allowed to fall on the cover slip. The excess fluid is now drawn off and a sterile stopper unit is placed in the flamed neck. After allowing the clot to harden slightly, fluids may be added through the small Pyrex tube by means of a hypodermic needle and syringe. (A total of about 5 cc is satisfactory.) Usually no embryo extract or other clotting factors will be required. Finally, the tip of the small Pyrex tube is flamed and covered with a sterile inverted serum vial cap. The culture is now ready for incubation.

Cultures seem to grow better if they are rotated from six to fifteen revolutions per hour, thus giving an aerating and washing action to the supernatant fluid as Gey[2] describes for his roller tube method. However, they may be grown in these roller bottles without motion, as in Carrel flasks.

We recommend the use of a small synchronous-type electric motor such as the Telechron C-2 or C-4,[7] which will be found to have ample torque to rotate a shaft carrying a balanced roller bottle box capable of holding twenty-four bottles at a speed of fifteen revolutions per hour. Using a stock motor with a shaft speed of one r.p.m., only one small four-to-one reduction gear is required. Elaborate bearings are unnecessary, and the whole apparatus will occupy a space of less than $3 \times 2 \times 2$ feet inside an incubator.

In staining cultures, the preliminary fixation and washing are best done with the cover slip still on the bottle. Then, after allowing the bottle to remain in contact with the proper solvent for a few hours, the cover slip and preparation may be removed *in toto* for subsequent staining and mounting, leaving the roller bottle ready for re-use. Since the colonies grow in a very thin plasma coagulum and tend to form extensive sheets, often only one or a few cells thick, exceptionally good cytoplasmic and nuclear detail may be observed without any intermediate transfer or sectioning procedures, and the usual difficulties encountered in staining cultures in thick clots are eliminated.

Numerous possible uses for the apparatus suggest themselves. The bottles are well adapted to the taking of frequent photomicrographs or cinematographs. The injection of test solutions, viruses or bacteria can be timed accurately, and since a very thin plasma clot may be used, penetration to the cells is very rapid. By the use of two holes and cover slips in opposite sides of the bottle, treated and untreated tissue could be nourished by the same nutrient solution, or the growth and action of viruses or bacteria on immune and non-immune tissues could be studied readily in the same container. Cultures of different endocrines could be handled the same way and their interactions on one another or their response to hormones determined.

A similar technique using thin organic films in place of cover slips has been developed in collaboration with the Haskins Laboratories[8] for use in radiation experiments.

The method obviously has advantages in studies in which optimal cultural conditions should be combined with microscopic studies of living and stained tissue, and hence appears to be a useful improvement in technique.

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[7] C–2M for larger installations.
[8] Haskins Laboratories, 480 Lexington Ave., New York, N. Y.