antigen is an important contributory factor in the production of pneumococcus antisera.*

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## A Simplified Method for Quantitative Tissue Culture in vitro.

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*Objects of Method:* (1) to be able to observe the growth of tissue using high power microscopic objectives, as in the original cover slip method of Maximow; (2) to secure quantitative growth as has been made possible to a certain extent by the Carrel flask;* and (3) at the same time to retain the advantages of the cleverly devised Borel flask in which the bottom is detachable, so that the tissue can be fixed and stained without distortion, and thus retained as a permanent record of the tissue grown. The present method is a simplified adaptation and combination of all 3 of these techniques.

The simple apparatus consists of but 3 parts: (1) A glass ring 3 inches in diameter, 5-6 mm. in height, with parallel, flat, ground surfaces, 2-3 mm. in thickness. These are made from heavy pyrex tubing. (2) Two thin sheets of mica 3¼ inches in diameter. (3) An ordinary 4 inch petri dish.

*Method: Absolute* cleanliness of the glassware is essential We sterilize the glass ring within a petri dish, and sterilize the mica sheets separately in another petri dish for convenience in handling them. Working preferably in a sterile bacteriologic transfer room, to one edge of the sterilized glass ring is applied a rim of vaseline (1% paraffin) by means of a sterile wide mouthed pipette. One of the sterile mica sheets is placed upon it and pressed down. This is then turned upside down within the petri dish where it forms a chamber, the cover being the bottom, and the ring its wall. This is now ready to receive any medium, solid or liquid, and the tissue for culture. As the entire chamber is readily accessible by simply removing the cover of the petri dish, the fragments of tissue can be arranged

* We beg to express our gratitude to Dr. Wm. H. Park for his interest in this work and to Miss Lillian Gross for her technical assistance.

* Since presenting this paper we have learned that a somewhat similar, but more elaborate apparatus with a metal ring has been used by Carrel.

and grouped as desired. The area is large enough so that 50 or more such bits of tissue can be planted within the chamber, thus offering an opportunity for quantitative work, or for comparative studies by growing different tissues under identical conditions.

After the media and tissue have been added, the chamber is sealed

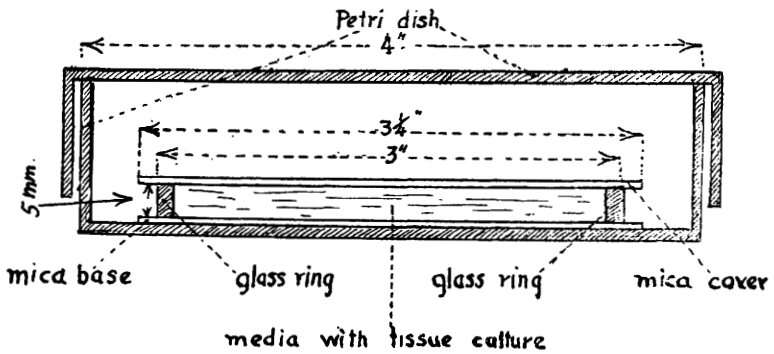


FIG. 1.

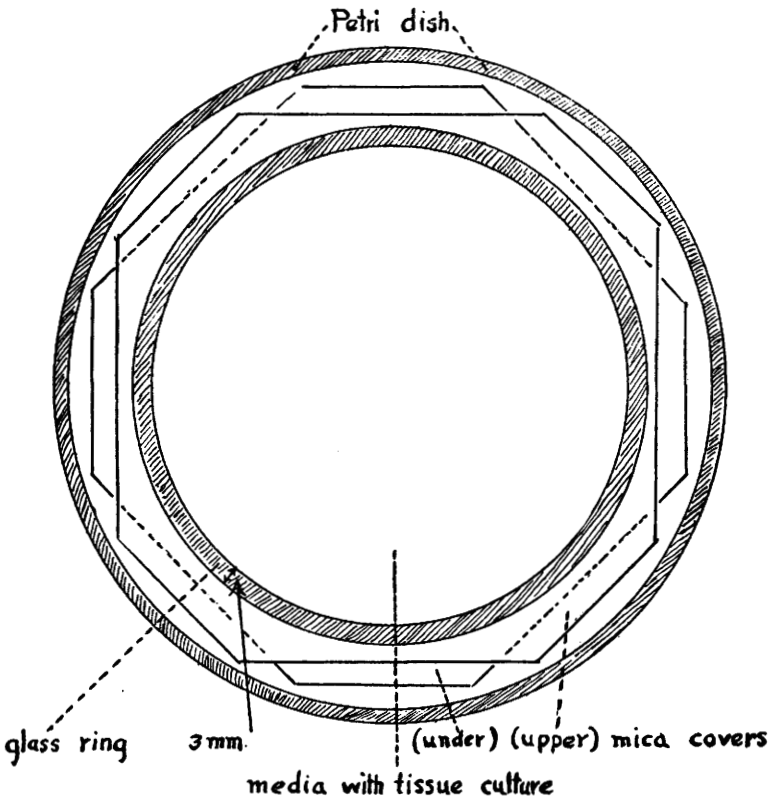


FIG. 2.

by the other mica sheet in the same manner as the first one. This culture chamber is kept in the petri dish for convenience in handling.

To examine the tissue the chamber is removed from the petri dish, inverted, placed under the microscope, and the growth observed under oil immersion. No special handling is required. To remove the products of metabolism for study, to wash tissue, or to renew its food supply, the mica cover is either partially elevated on one side or wholly removed, after placing the petri dish on a slight slant. The fluid, totalling 1-3 cc. is removed by a sterile, capillary pipette, the surface washed off by running in Tyrode's solution and removing it in the same way by pipetting. Pieces of dead tissue or living tissue for transfer can be removed with needle and lancet. Fresh food is then added and the chamber again closed by a fresh, sterile mica cover. When the culture is ready for disposal after it has grown sufficiently or has been used for inoculation, etc., the remaining material can be fixed and stained. It is only necessary to remove the bottom mica from the chamber, wash off the excess media and drop the mica into fixing fluid. The size of the mica sheet, 3¼ inches square, permits cutting into several smaller pieces and preparing various comparatively stained and fixed specimens for study, all from the same culture and grown under identical conditions. In this way, too, a permanent record is available for reference.

The chief value of the method appears to be in the opportunity afforded for quantitative studies of the products of cell metabolism. It can be applied to much larger volumes by simply increasing the size of the chamber. It offers a quantitative method of analytical approach to the whole field of cell physiology. The apparatus is easily cleaned and readily handled. It is applicable to either solid or liquid media.

We have successfully used the method for experimentally growing numerous types of tissue and for the multiplication of vaccine virus as an approach to some of the larger problems concerned with filtrable virus disease. We present the technique with the hope that it may save others many hours of discouraging labor, and thus contribute toward the ultimate solution of some of these as yet unsolved fundamental biologic problems.