TECHNIQUE FOR CULTIVATING A LARGE QUANTITY OF TISSUE.*

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In a previous article,[1] a technique for cultivating large quantities of tissue was briefly described, but the results given by this method were inconstant, and by it the growth could not be watched under the microscope. Moreover, the fluids which exuded from the culture fell into and mixed with the water covering the bottom of the box, aeration was difficult, and bacterial infection often occurred. In order that we might study the functions of growing tissues, it was necessary to improve the method. The procedure which we now employ in the preparation of the tissues, the medium, and the cultures, and the method of examining the cultures will be described in this article.

1. *Preparation of the Tissues.*—By means of a sharp instrument, the tissues are divided into very small fragments in Ringer's solution, care being taken not to crush the tissues. The tissues of chicken fetuses can be easily reduced without crushing to fragments measuring about 0.5 or 0.1 of a millimeter in diameter. More friable tissues, dog's thyroid, for example, cannot be cut so finely. The fragments are suspended in Ringer's solution, and are then taken up in a large pipette. Each drop of the fluid contains approximately the same number of fragments. By depositing the same quantity of fluid on each plate, we obtain cultures which contain practically the same quantity of tissue.

2. *Preparation of the Medium.*—The best culture medium is plasma diluted with about two fifths of its volume of distilled water. Serum mixed with one fifth of its volume of a 2 per cent. agar

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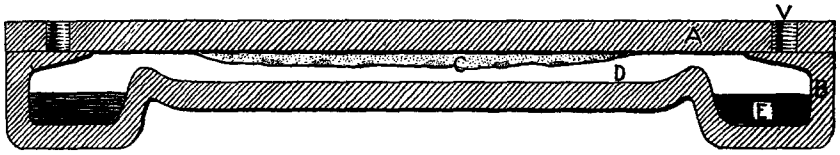
[1] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 387.

solution can also be used, but the growth is less abundant than in plasma. When plasma is employed in the preparation of cultures which have been previously washed in Ringer's solution, it coagulates very slowly. Complications, such as concentration of the medium by evaporation, and bacterial infection, may then occur. In order to accelerate the coagulation of the plasma in these cases, muscle, or embryonal extracts, or serum is added.

3. *Preparation of the Cultures.*—The cultures are prepared in Gabritschewski boxes or in larger glass boxes. A Gabritschewski box consists of a flat, circular glass dish covered by a circular glass plate (text-figure 1). The dish measures 110 millimeters in diam-



TEXT-FIG. 1. Cross-section of a Gabritschewski box. A represents the cover; B, the box; C, the culture; D, the lower inner surface; E, the groove filled with water; V, the hole.

eter, and is 12 millimeters in height. Around the periphery of the dish, at the bottom, runs a groove which contains water, while the fluids exuded from the culture are retained in the central depression which measures 65 millimeters in diameter. The cover is sealed to the circumference of the dish by vaselin. At the ends of the same diameter and near the edge of the cover are two holes. When the cover is in place, the atmosphere of the box is isolated from the external atmosphere unless the cover is rotated so as to bring its two holes in apposition with two similar holes in the upper edge of the dish. The culture medium and the fragments of tissue are distributed evenly over the lower surface of the plate, and may cover an area 65 millimeters in diameter. After the medium hardens, the tissues adhere to the plate and this can be turned over and sealed to the dish.

The larger boxes consist of deep circular dishes covered by glass plates. These boxes, as well as the Gabritschewski boxes, are sterilized by dry heat. The bottom of each dish is covered with water.

A large Petri dish, fixed on a stand, receives the fluid which exudes from the culture. The cover is sealed to the dish with vaselin. The culture on the under surface of the plate may cover an area about 150 millimeters in diameter.

When the culture is to be washed, the tissues are not placed directly on the covers, but are first deposited on a silk veil which is stretched upon a rectangular glass frame having an area of about 14,000 square millimeters.

A culture on a silk veil is prepared in the following way: The glass frame on which the silk is stretched is placed on the glass cover and is fixed to it by paraffin. Then the silk is moistened with Ringer's solution, or with plasma diluted with distilled water. The tissues in Ringer's solution are then deposited on the cover by means of a large pipette, and are so spread that the fragments are evenly distributed and are separated by free spaces. When the fragments are crowded in a small area, they do not grow abundantly and their life is short. Then a quantity of plasma is added that is about three times as large as that of the suspension of tissue. If necessary, a few drops of serum or of muscle extract are also added. During the coagulation of the medium, the plate is protected from atmospheric dust. As soon as the medium has become sufficiently hard, the plate is placed on the box, which is closed hermetically by vaselin.

When, after a few days, the culture has to be washed, the cover is placed above a jar filled with cold Ringer's solution. The frame is detached with a knife and deposited in the fluid. After one or two hours, it is removed from the Ringer's solution, placed on another cover, fixed with paraffin, and covered by a new medium. By this procedure, rejuvenation of large cultures can be brought about,[2] but bacterial infection frequently occurs.

4. *Examination of the Cultures.*—The boxes are incubated at a temperature adapted to the nature of the tissues, generally 38° or 39° C. The tissues grow as extensively as in small cultures in a hanging drop. The growth can be watched without the help of a microscope. After one or two days, the outline of the fragments

[2] Carrel, *Jour. Am. Med. Assn.*, 1911, lvii, 1611.

becomes less sharp, and is surrounded by a narrow opalin area. The size of the fragment increases. When the tissues are growing rapidly, a little fluid exudes from the medium and collects on the under surface of the tissue. Simultaneously the tension of the atmosphere in the box diminishes. If there is bacterial infection, the colonies are easily recognized by their opacity and by their sharp outline.

The cultures in the Gabritschewski boxes may be examined microscopically, although a high power cannot be used on account of the thickness of the cover. If minute morphological examination of the tissues is required, the cultures are washed for one hour in Ringer's solution at 0° C. and are then fixed in isotonic sodium chlorid solution containing 2 per cent. formalin. They are stained afterwards without being detached from the glass plate. If the medium is very thick, the culture, after having been fixed, is removed from the plate and divided into fragments which are embedded in paraffin and cut in serial sections.

When the cultures are to be inoculated into animals, the fragments of tissue and the surrounding cells are removed, washed in Ringer's solution, cut into small pieces, and injected under the skin.

For the study of the substances that develop in the medium during the life of the tissues, the culture is cut into small fragments, which, together with the exuded fluid, are taken up in a large pipette, placed in a centrifuge tube, and centrifugalized. The substances produced by the tissues may be studied in the supernatant fluid.

This technique for cultivating a large quantity of tissue is still far from perfect, and will be modified in the future. Nevertheless, by means of it, new problems may be studied.[3]

[3] Carrel and Ingebrigtsen, *Jour. Exper. Med.*, 1912, xv, 287.