AN IMPROVED TECHNIC FOR MASSIVE TISSUE CULTURE

GEORGE O. GEY

Surgical Pathological Laboratory Johns Hopkins Hospital and University

Although there are a number of more or less standardized methods for the plating and maintenance of tissue cultures, for some specific purposes these methods and the technic used cannot well be adapted. In order to eliminate the cumbersome procedures of the hanging-drop methods of culture that have been devised, and to eliminate some of the difficulties of the flask methods, a special culture tube has been designed, as described in the present article.[1]

The essential principle of this method consists in the utilization of the entire inner surface of the culture tube for the growth of the tissue cells implanted in it. In addition, it is possible to bring about a sort of washing action on the growing tissue cells by revolving the tube slowly at a controlled speed, thus allowing the supernatant fluid to bathe them constantly. The revolving action of the tube is brought about by a special device. The necessary slow movement has been found in preliminary experiments to be from one-half to one hour per turn, or just enough to prevent a violent action of the supernatant fluid which would wash the growing cells free from the inner wall of the tube. By a simple adjusting mechanism the necessary slow movement can be varied and regulated to any desired speed.

The revolving action of the tube not only permits the supernatant or washing fluid, containing the necessary food substances, to come into contact with the growing tissue cells at definitely controlled intervals, but at the same time allows a definite period of exposure to any gaseous mixture desired during the gas phase of the turn, when the cells are covered by a minimum amount of fluid. The gaseous mixture can be used as one means of accurately controlling the pH of the medium. In previous culture methods those cells which came free of the medium due to liquefaction were ordinarily lost or recovered with difficulty unless patching of the

[1] The author wishes to acknowledge the previous description of a "roll-culture" method by Hans Löwenstädt (Arch. f. exper. Zellforsch. 1: 251, 1925), who contributed it as a method originally suggested by A. Carrel (Berl. klin. Wchnschr. 50: 1097, 1913). During the time the method described in the present paper was being developed, and up until it was first submitted for publication, the author was unaware of a previous description of a somewhat similar principle of cell culture.

medium was practised (as has frequently been done in order to maintain the unity of the culture where the plate or flask method is used). Oftentimes these cells are the very ones whose continued cultivation is desired. With the roller tube method these cells can be allowed to re-implant themselves in another portion of the tube shortly after they have become free; or they can be collected and recultured in another tube, since fairly large quantities of cell suspension can readily be obtained.

In the cultivation of such types of tissue, for example, as the more rapidly growing tumors, the fibroblastic stroma actively invades the solid medium of the culture, while (in most media) the more malignant cells come free and are to be found in the liquefaction droplet. The cells from the stroma can be maintained indefinitely, as is well known, in suitable media. The more active tumor cells, on the contrary, usually liquefy most types of media, come free very soon after the primary culture is made, and lie dormant or die in the stagnant liquefaction droplet. In the roller tube method of culture a means is provided whereby such actively fibrinolytic cells are allowed to come free from a large number of cultured fragments contained in the same tube while the cells of the fibroblastic stroma are actively invading the culture medium. From such tubes the free cells can easily be concentrated either by a sedimentation or slow centrifugation method and thus be selectively separated from the stroma cells which have invaded the medium. Such a separation is most common in the primary and secondary cultures. Further transfer through a series of culture tubes by this method makes possible a still more effective separation of the two types of cells and thus permits a gradual change to pure cultures of the cells desired by a method chosen to take advantage of this physiological difference in their behavior. In addition, it is possible gradually to change the composition of the medium from one which at first is most favorable for one type of cell to one which is most favorable for another type. During all of this process no stagnation of the medium occurs, as there is constantly supplied a fluid medium which rapidly dilutes any toxic products. In the flask method Carrel has demonstrated the need for a supernatant fluid but no provision is made for the constant washing action and the re-implantation effect which are so advantageous for permanent strains of tissue, and which are obtained by the roller tube method.

In Fig. 1 two types of roller tube are shown. A round type is used for the maintenance of permanent strains of cells, where constant microscopic observation is not necessarily important. With the round tube a large quantity of tissue can be kept under continuous cultivation and under fairly close observation by an extremely simple culture technic. Repeated sub-culture, on slides

for microscopic study, or in other tubes for further culture, necessitates only the removal of a suspension of the cells that have been washed or wiped free from the walls of the tube. These cells are either concentrated by allowing the tube to stand on end for sedimentation or freely washed off by direct centrifugalization of the tube. Such suspensions of cells can also be removed and centrifuged in separate tubes designed for the purpose. The available inner surface of a tube with a wall 100 mm. in length and 15 mm. in diameter will permit the cultivation of as much tissue as can be grown suitably on from 50 to 100 large slides of the Maximow type. The comparative labor involved in the two methods in order to maintain the same amount of tissue is readily appreciated.

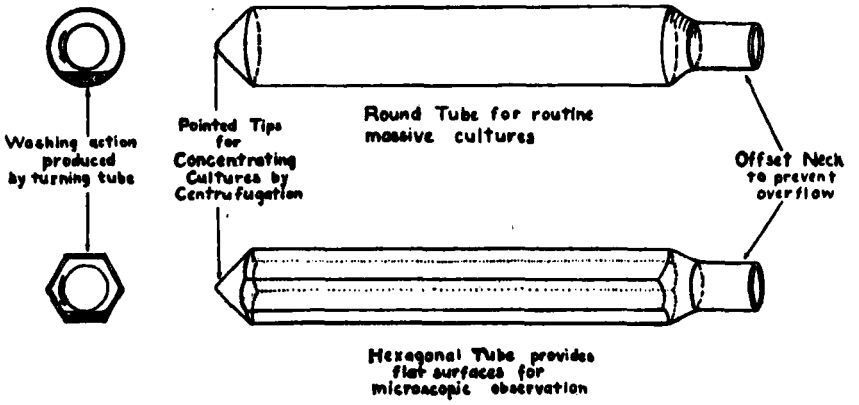

FIG. 1. ROLLER CULTURE TUBE FOR HORIZONTAL OPERATION

For occasional microscopic observation during continued cultivation the hexagonal tube shown in Fig. 1 has been designed. It supplies a more suitable flat surface for microscopic observation and at the same time retains all of the desirable features of the round type of tube, which permits only a fair estimation of the condition of the cells on microscopic examination.

To date several types of cells have been maintained by the roller tube method of culture. In one instance a single fragment of a strain of sarcoma cells (WRS 338) was inoculated into a roller tube and within a few weeks a sufficient number of cells were reimplanted in other areas of the tube to cover its inner surface almost completely. The frequency with which the washing fluid should be removed depends, of course, upon both an estimation of the activity of the cells cultured and the extent of spread brought about by re-implantation. An exchange of from 1 to 3 c.c. of fluid every two to three days has been found sufficient to maintain the strain of cells referred to even at a time when the cells covered the greater portion of the inner wall.

A chamber, illustrated in Fig. 2, has been designed, containing

some 50 or more thin-walled brass tubes mounted in a cylinder about 6 inches in diameter and 12 inches long. This cylinder will hold over one hundred roller tubes and when mounted in a gas tight chamber, which also contains the driving mechanism, occupies a space only 8 x 10 x 15 inches, thus making it suitable for insertion into almost any incubator. The gas-tight chamber with self-contained driving mechanism was devised in order to permit the use of cotton plugs in the roller tubes as an aseptic seal, a carbon dioxide gas mixture being supplied from an external source and made to pulsate by a simple gas-delivery apparatus. Any tube desired can be removed from this chamber by shutting off the main gas supply and removing the small slip of glass, sealed on and

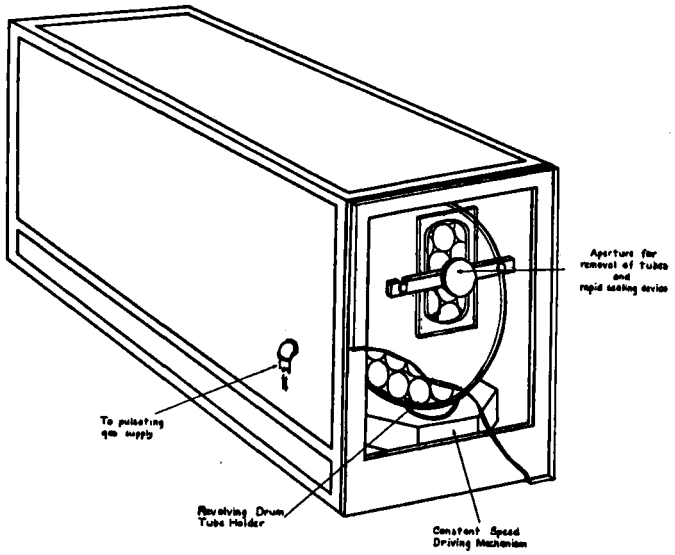

FIG. 2. GAS-TIGHT CHAMBER FOR ROLLER TUBE CULTURES

clamped over an aperture just large enough to permit the removal of the tube when the drum is rotated.

Where no chamber is needed, the roller tubes can be sealed in a number of ways to prevent loss of gas or moisture from the contents. The use of the gas chamber eliminates any undesirable effects of paraffin-impregnated corks, paraffined paper sealing caps, or rubber stoppers, such as are customarily used as hermetic seals. Where a constant gas mixture is not available, alveolar air can be expelled into the chamber and thus used as the equilibrating gas mixture. The author has used alveolar air for the equilibration of stored biologic fluids and for the equilibrium of tissue cultures at different times, for a period of over nine years. Only a little practice is necessary in expelling alveolar air so as to obtain a fairly constant percentage mixture of carbon dioxide. The

equilibrating carbon dioxide mixture allows the use of unmodified body fluids or an almost exact duplicate of their inorganic composition and allows especially the use of a diluting saline fluid containing the normal concentration of sodium bicarbonate.

In addition to the strain of sarcoma cells referred to above, which has been kept under constant cultivation by the roller tube method, a culture of human bronchiogenic carcinoma has also been maintained for several months with the use only of the pleural fluid from the same host. In this instance a non-activating medium was used which simulated the normal body fluids, permitting constant washing of the cells without making it necessary to disturb them in any way, as is so frequently necessary when such an attempt is made by other methods. In attempts to cultivate similar and other carcinomata from human sources, whatever feeble growth occurs, even in what seems the most activating media, the usual hanging drop method causes so great a loss during transfer from one medium to another that eventually the entire tissue becomes so diminished in size that further culture is impossible. The washing action, in a way, simulates the flow of the body fluids. Here again the simplicity and the effectiveness of this method can be readily appreciated.

The method described above has many advantages. It permits the maintenance of permanent strains of many types of cells in laboratories where sufficient technical help is not available to maintain the large numbers of cultures necessary when other methods of tissue culture now in use are employed. It eliminates to a great extent the cumbersome and detailed transfer technic which is involved when the cover slip method is used. Inasmuch as the entire medium is available for the growth of the cells contained in it, this simple roller tube method is markedly economical, and the amount of medium used becomes almost directly proportional to the amount needed for the cultures it contains. In this laboratory the slide technic has been developed to a very high degree for the cultivation of the cells of malignant human tumors. In most instances, however, the best growth can be obtained only in those cultures where a large excess of medium has been used, which actually constitutes a loss, as it is not primarily used for tissue growth. The improved technic eliminates to a great extent this loss and permits the growth of larger quantities of tissue when comparative amounts of supporting medium are used.

Note: The practical and more technical details of this method were made possible through the cooperation of the Department of Embryology of the Carnegie Institute of Washington, in whose laboratories most of the apparatus was designed and built.