A SUBSTITUTE METHOD FOR CULTIVATING FRAGMENTS OF NORMAL AND TUMOROUS TISSUES

Translation No. 1460

JULY 1965

U. S. ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

A SIMPLE METHOD FOR CULTIVATING FRAGMENTS OF NORMAL AND TUMOROUS TISSUES

[Following is the translation of an article by S. G. Drozdov, Institute of Poliomyelitis and Viral Encephalitis, AMN, USSR, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) 1963, 8(5) pages 630--631. It was submitted on 9 Jan 63. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The methods of culturing the most diverse types of cells which have been widely used in recent years have guaranteed the tremendous success of experimental and practical virology (and many other divisions of biology). However for a number of problems it is important to study the influence of a biological or chemical agent not on the cell (or cellular population) but on an organized tissue which has preserved cell differentiation and its own functional status.

The quest for simple methods for the prolonged cultivation of tissue fragments has been carried out since the time of the first works by Karrelya. The main mission consists of supporting the explant on the border between the liquid and gaseous medium of the culture, guaranteeing a specific constancy in the composition of these media, and creating a good exchange between them and the fragments of tissue. A satisfactory solution to this problem was proposed by Trowell (5, 6), who used small platforms made out of metallic mesh (stainless steel, tantalum) which fixed the explant in the gaseous medium in contact with the upper layers of the liquid nutrient medium. However Trowell's method is complex and this makes its wide application difficult. In the Wistar Institute (USA) a simpler and more convenient method is used for the cultivation of fragments of tissues and organs which may be used for studying problems of virology, oncology, pharmacology, radiology, etc. This method was developed by Jensen and Castellano /2/ for the cultivation of tumorous explants, and then by Jensen and others for the cultivation of fragments of organs and tissues of animals and man. The application of this method increases the possibilities of experimental biological research, therefore we consider it expedient to describe it based on the materials of practical work and on the basis of several literary sources. *

* We will take this chance to express thanks to the coworkers at the Wistar Institute (Carp, Jensen, Gwatkin) who acquainted us with the described method and rendered great help in its practical assimilation.

Apparatus for cultivation. The cultivation of tissue fragments is carried out in Petri dishes with a diameter of 60 mm. A special platform is placed on the bottom of the dish. It is made from a close-woven- mesh of stainless steel 20x20 mm in size and at a height of 5 mm. From the two sides of the edge on which it is resting there are arched notches for the passage of air when the dish is being filled with medium. The construction

of the platform can be seen in the drawing. The close-woven mesh guarantees a capillarity which promotes wetting of the upper part of the platform. A paper backing the same size as the platform is placed on the latter and the tissue fragments are laid on it. For this backing a special coarsely porous thin paper or thin porous filter paper is required. The paper backing which is wetted by the medium guarantees the uniform nourishment of the explant, and also serves as the base on which the growth of young cells is disseminated.

Nutrient medium. The most generally used is the Eagle nutrient medium in Earle's salt solution /1/ with the addition of 5--10% serum of a horse or calf and antibiotics (penicillin and streptomycin based on 100 units/ml). The optimum pH is 7.0; the usual frequency of replacing the medium is two times a week.

Preparation of tissues. The fragments of tumors or normal tissues are cut with a sharp scapel usually in a large sterile Petri dish. If the organ or tissue was taken under unsterile conditions it is washed in Hanks salt solution with an increased dose of antibiotics (up to 500--1000 units/ml) and dissected into fragments also in this solution which is changed several times. The optimum size for fragments is 2x2x2 mm. With larger sizes during the process of cultivation central necroses may be formed, since the diffusion of nutrient substances is difficult. Two or three fragments of tissue are placed on the supporting paper which has been wetted with several drops of nutrient medium (in a small sterile Petri dish). It is expedient to locate the fragments on a diagonal to the slip of paper at distances of 5--7 mm from each other. Then they are straightened out on the paper (if this is required) and it is transferred by means of a scapel to the metal platform in the Petri dish. Under visual control 12--15 ml of nutrient medium is added. The level should be such that the paper is well wetted but would not be emersed if the dish was tilted lightly.

If the effect of carcinolytic or pharmacological substances are being tested they may be added directly to the medium in the necessary concentration. When testing the effect of viruses on tissue the fragments are infected before they are transposed to the dish. This makes it possible to create a higher infecting dose which is necessary in a number of virological experiments. Prior to transfer to the platform the infected fragments are maintained on the paper for 1--1½ hours at 36° for a more complete adsorbtion of the virus. Then they are washed in 2--3 changes of Hank's solution for removing the nonadsorbed virus. The paper is transferred to the platform in the Petri dish and the medium poured in.

Cultivation is usually conducted at 36--37° in incubators which are equipped with a channel of air with 5% CO_2. This makes it possible to maintain a constant pH in the nutrient medium in the nonhermetic Petri dishes.

Secondary cultures. A normally developing explant produces the growth of young cells which infiltrate the supporting paper. Part of them fall to

the bottom of the dish, forming small colonies and then a monolayer. A cover glass can be laid on the bottom of the dish and the cellular colonies on it may be investigated in a native and a stained form. The cells which have formed a monolayer may be removed with trypsin and subinoculated by the usual methods. The prolonged cultivation of fragments makes it possible to obtain several series of subcultures by the successive transfer of the platform with the fragments to new dishes after a certain period of time. These possibilities were successfully used by Koprowski and coworkers /3/ for studying chronic viral infection of human tissue.

Control of the growth and multiplication of the cells. For a control of the viability of the cells in the explant during various periods of cultivation the following methods were used: Microscopic investigation of fragments stained with trypan blue; histological study of stained sections of the fragment; inoculation of the explant in susceptible animals (when cultivating tumorous tissues); studying the capability for growth of the dispersed cells of the fragment in a subculture; determining the level of cellular protein /4/.

Results. The transplanted tumors of white mice, S37, S180 and Ca755 /2/ were cultivated by the described method and the possibility was shown of maintaining the viability of explants up to 90 days (for Ca755). Jensen, Gwatkin and Biggers successfully cultivated ovaries, Fallopian tubes, salivary glands, and the foreward lobe of the hypophisis of white mice. Here they observed not only the preservation of the viability of the cells, but also the functional activity of the organs. Koprowski and coworkers /3/ cultivated the mucous membrane from the cheek of a man for more than 30 days. Carp used a culture of fragments from the intestine of a human embryo for the multiplication and study of the poliomyelitis virus.

Literature

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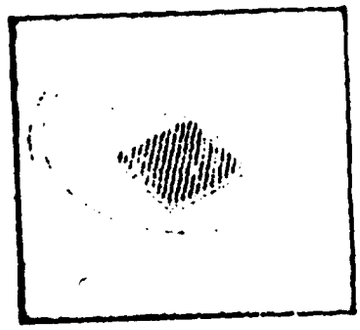

Counting platform made of stainless steel and placed in a Petri dish with a diameter of 60 mm.