CONTRIBUTIONS TO THE BIOLOGY OF TISSUE CELLS.

I. THE RELATION OF CELL CROWDING TO TISSUE GROWTH IN VITRO.

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PLATE 37.

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INTRODUCTION.

When small bits of tissue are cultivated in vitro, do they persist and the cells multiply indefinitely? As a first step toward answering this question the attempt was made to determine whether it was possible to obtain growth and multiplication from one single cell with the ultimate development of cell colonies comparable to those formed in the cultivation of bacteria.

It was necessary to develop a method for isolating living cells from tissues in vivo or in vitro. Under both conditions the cells are strongly adherent to one another by protoplasmatic connections. These anastomoses between the cells in the organism vary with the different tissues and are very strong in tissues which have a vital significance. In culture, the cells are, in addition, connected by means of fibrin threads in the clot. It is impossible to separate out an individual cell from an ordinary culture with a knife or other instrument; the injury is too great. To overcome the difficulties, a method for isolating cells from tissue cultures was worked out by Rous. He digested the tissue culture by means of trypsin and finally obtained a suspension of tissue cells. A different technique has been used in the present study.

Method 1.

Ordinary absorbent cotton is finely cut between two fingers, by means of a small pair of scissors, to a fine “powder” and sterilized

by dry heat. Cultures are then made in the following way. A small drop of fresh concentrated embryonic tissue juice is placed on a sterile cover-glass; a small amount of the finely cut cotton is stirred into the drop with the point of a needle or cataract knife in order to spread out the drop. The small short cotton threads will then form a rather compact meshwork in the drop. A small piece of tissue is cut out from another culture, placed in the meshwork of cotton, and the drop stirred a little. The cotton threads seem to have the ability to gather around the piece of tissue and establish a good contact to the supporting apparatus is established. The cover-glass is placed on the slide, sealed with paraffin, and brought into the incubator. After 24 hours in the incubator a large mass of cells may be observed growing on the cotton threads. At the start, a single layer of cells covers the cotton threads; later on they fill up the spaces between the threads (Fig. 1).

Connective tissue cells show a typical spindle shape, particularly those in the closest contact with the cotton threads, but toward the middle of the fluid medium spherical forms may be observed. When the time comes to transfer these cells as isolated individuals to a new medium, the cover-glass is unsealed and the excess fluid which contains the decomposing products formed during the growth is disposed of by aspiration with a fine pipette, and a little Locke’s fluid containing 1/8 per cent gelatin is carefully added to preserve the fragile cells from injury. The cells having been washed once this way, a new drop of Locke’s fluid is added and the drop containing the cotton threads, with the adherent cells, is carefully aspirated and expelled on a cover-glass on which is a drop of fresh plasma. The cells are stirred with a needle and a drop of fresh embryonic tissue juice is added and stirred in. After a short interval the plasma coagulates upon the preparation, fixing the cells in place.

RESULTS.

When examined under the microscope the cells will be seen to have assumed a spherical form, and if the cotton culture from which they were transferred is also examined, it is apparent that all the cells which eluded the pipette have become spherical. This same phenomenon
has also been already described by Rous and Uhlenhuth. The picture now presented looks very much like a mass of leucocytes.

Upon closer observation of the individual fibroblasts it is possible to perceive an active ameboid movement. Pseudopodia of rounded outline are put out and retracted, and active currents pass through the granulated protoplasm. Broad tongue-like pseudopodia can be seen without any granules into which, after a time, the granules suddenly flow. These granules may have a remarkable rate of speed.

As a rule, after a few hours, the cells will have assumed a typical spindle shape characteristic of fibroblasts. When this change has taken place all movement appears to cease. In this condition the cells remain apparently unchanged in outline but the protoplasm fills up gradually with vacuoles and fat granules, death takes place, and the protoplasm decays and dissipates.

It has not been possible to observe the division and proliferation of an isolated cell, though hundreds of cells have been studied with this object in view. Growth by proliferation has been observed only when a number of cells were in close contact in a culture.

These observations lead to the question of whether intercellular contact is essential to the cells for their growth and multiplication, and what significance the contact has in the growth and multiplication of the cells.

Before discussing the problem, it will be well to describe other methods by which the attempt was made to determine whether isolated cells are able to multiply. For the results of the experiments just mentioned may have been due to a technical error. Anyway, it has to be taken into consideration that not impossibly the manipulations may injure the cells, despite the signs which seem to indicate perfect vitality.

When ordinary cultures were cut in two halves and transferred to a fresh medium, it was frequently noted that the cells began to grow out first from the center of the cut edge of the culture, that is to say from the point at which the cells were most crowded, and only a little later on from the periphery. This has also been noted by Carrel. From the observation it might be deduced that the central part of the

3 Carrel, A., personal communication.
culture has a greater ability to proliferate than the peripheral part, where the cells are more scattered. Some experiments were undertaken to see how minute the fragments of a piece of tissue from a culture could be and retain the ability to multiply when transferred to a favorable culture medium. It was found that if the transplanted piece consisted of a few scattered cells no growth took place, and the cells took on the aspects of degeneration at a time when the control culture seemed to be in perfect condition. By contrast, when the small clumps of cells had been taken from the central portion of the culture where it was dense, growth was extensive. The tissue used in these experiments was derived from a 4 month old strain of fibroblasts. The small fragments were obtained by clean cuts with a sharp cataract knife. After a little practise fragments could be cut so small that they just could be distinguished with the naked eye.

Here it could be objected that the poor growth of the fragment containing only scattered cells was a result of retraction of the fibrin clot. In the attempt to maintain uniform conditions during the separation of the small fragment from the mother culture, another technique was devised.

Method 2.

A culture of fibroblasts (the 10 year old strain of The Rockefeller Institute) was permitted to grow for about 20 hours, and then the cover-glass was unsealed and an incision was made in the periphery of the new growth with an ordinary sharp cataract knife, in order to separate from the mother culture a piece of tissue at the periphery where the cells were rather scattered. The cover-glass was replaced on the slide, it was sealed with paraffin, and an outline drawing was made of the tissue—the big piece as well as the small one. The culture was brought back to the incubator for another 30 hours; at the end of the second incubation a new drawing was made and the area measured. It was observed that the mother culture had greatly increased in size, but the fragment, separated from it by means of the wound in the clot, consisted of scattered cells and had rather decreased in size. Fig. 2 and Text-fig. 1 illustrate this experiment. It can be seen from Fig. 2 that a peninsula had grown on the surface

of the cover-glass from the central part of the main tissue, but that the separated small piece had not increased in size, much less encroached on the open space.

DISCUSSION.

We are not dealing with the growth of independent cells in the ordinary tissue culture but with a partial organism—a piece of tissue, obeying the laws of regeneration.

Text-Fig. 1. A, outline drawing of the culture shown in Fig. 2 after incision. The limits of the mother culture, the separated culture, and the wound are to be seen. B, outline drawing of the same culture after 24 hours incubation. The mother culture has almost doubled in size since the wound was made but the separated fragment has ceased to grow.

The embryonic tissue juices contain substances which promote growth in vitro for an indefinite length of time. Extracts of tissues from the adult organism also possess growth-promoting substances but not of the same grade as the embryonic tissue juices. Carrel has shown that certain tissues give an extract with a greater activating power than others. Extracts of leucocytes or spleen possess nearly as much activating substance as embryonic tissue juice. The

mechanism of growth and multiplication of tissue cells is a profound and complex phenomenon. Besides the growth-promoting factors found in the tissue juices, there may possibly be something, produced in the body of the cell or certain cells only, which initiates cell division and is transported directly from one living cell to another.

The cytotropic character of tissue cells has already been noted by several investigators. Roux was the first to describe it. Recently Rous has noticed the striking ability of isolated tissue cells to reunite. He never saw any division or multiplication of isolated single cells. Also Burrows mentions the important relation of cells in tissue cultures to each other. He expresses himself as follows: "The tissue culture cannot be compared in detail with the bacterial culture. The tissue cells planted in plasma do not grow at the expense of the plasma. Single cells may show movement in this medium but they do not grow. . . . Growth takes place only about fragments of tissue. . . . . The nutrient material for the growth of these cells comes from the cells disintegrating within the fragment."

SUMMARY.

1. A method has been described by which it is possible to scatter isolated tissue cells in a suitable medium.

2. Experiments were undertaken to determine whether minute fragments of fibroblastic culture are able to persist and multiply when transferred into a new culture medium.

3. Cell divisions were not observed in isolated cells under the conditions of the experiment. Growth took place only when the tissue cells were numerous and close to one another.

EXPLANATION OF PLATE 37.

FIG. 1. Fibroblasts growing in a meshwork of cotton threads in a drop of embryonic tissue juice.

FIG. 2. A culture of fibroblasts at the end of 48 hours incubation. After 24 hours incubation the culture chamber had been opened and an incision made in the clot whereby a small part of the edge of the culture was separated from the mother portion. The culture was resealed and incubated again, stained, and photographed. It can be seen that whereas a peninsula of cells has grown into the wound from the mother culture, the separated tissue has failed to increase.

Roux, W., Arch. Entwicklungsmechn. Organ., 1894, i, 161; 1896, iii, 127.
