PURE CULTURES OF CELLS.*

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The cells surrounding a fragment of tissue growing *in vitro* nearly always belong to several different kinds of tissues. It is important to isolate and cultivate a pure strain of cells of a determined type. I have attempted, therefore, to develop a technique by which pure cultures of cells might be obtained.

In its essentials the technique consists in isolating the cells of the selected type and in propagating them by repeated passages from medium to medium.

During the first few days of the growth of a culture of tissues, it was generally impossible to isolate cells belonging to a definite type. When the culture had undergone several passages, however, the cells grew more actively and spread over a larger area of the medium. Often a large group of cells belonging morphologically to one type could be seen growing in a thin layer. By careful microscopical examination it could be ascertained that no cells of a different type were present.

Then the part of the plasmatic jelly which contained the selected cells was isolated by cutting it away from the remaining part of the culture with a sharp cataract knife. The little film containing the cells was washed for one or two minutes in Ringer's solution and was then placed in a new medium. The new medium was composed of two parts of hypotonic plasma and one part of embryonic extract. A second microscopical examination showed that all the cells placed in this medium belonged to the type selected.

After the cells had been allowed to multiply for two or three days they underwent a second passage. They were allowed to grow again and subsequently underwent several passages. Generally after a few passages the cells grew together, forming a dense tissue.

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The washings and passages were repeated as often as was necessary, and in this way it was possible to study the morphological changes in cells belonging to a known strain.

The experiments were carried out with ameboid and round connective tissue cells of the chick. The primary cultures were obtained from cultures of connective tissue, which were more than two months old. The old cultures were selected because it seemed that these cells had adapted themselves to their new form of life and that they could stand the washings and the passages better than the cells contained in the younger cultures. All the experiments in which the cells were taken from cultures more than sixty days old were successful. Two experiments only will be described.

PURE CULTURE OF AMEBOID CELLS.

Experiment 1. Primary Culture.—On March 20, 1912, a fragment of plasma at the peripheral part of a culture of heart sixty-three days old was resected. The fragment contained large ameboid cells only. Each cell could be seen individually. No round cells or spindle cells were present. The fragment was washed and put in a new medium. On March 21 no ameboid cells had passed into the new medium, but the number of cells contained in the old fragment of plasma had markedly increased.

First Passage.—On March 21 the culture was washed in Ringer's solution and put in a new medium. On March 22 the cells contained in the old plasma had increased so much that a real tissue was formed, but no ameboid cells were in the new plasma.

Second Passage.—On March 25 the area covered by the cells had become very large. The culture was therefore divided into two fragments (culture 1196–1 and culture 1196–2).

1. *Culture 1196–1.*—On March 26 the original fragment of plasma was surrounded by a large number of active ameboid cells. On March 28 the area covered by the cells had more than doubled. It was divided into two pieces and washed.

Third Passage.—One of the pieces was put in new plasma on March 28. On March 30 the culture had increased greatly in size and was again divided into two parts and washed.

Fourth Passage.—One of the pieces was put in new plasma on March 30, and grew abundantly.

On April 3, 6, 9, 12, 13, and 15, the culture underwent its fifth, sixth, seventh, eighth, ninth, and tenth passages. During that period, the growth, which was very luxuriant, was composed only of ameboid cells, and these were similar to those of the original culture.

After April 18 there was a very abundant growth of ameboid cells. On April 22 the culture was divided into two parts, and then underwent its twelfth

passage. The cells grew rapidly and on April 25 a thirteenth passage was made. On April 26 the cultures were composed of a mass of dense tissue, from the peripheral part of which radiated many ameboid cells.

2. *Culture 1196–2.*—On March 26 large ameboid cells, disposed in short columns, grew in the new medium. On March 28 the old plasma was surrounded by an immense number of cells covering a large area. The culture was divided into two parts and washed.

Third Passage.—On March 28 one of the parts was put in a new medium, and a very large number of ameboid cells, the pseudopodia of which were very short, grew in the new plasma. On April 1 the culture was divided into two parts.

Fourth Passage.—On April 1 one of the parts was put on a piece of silk veil and in a new medium. On April 2 the ameboid cells had covered a large area of the veil which was then divided into two parts.

Fifth Passage.—On April 2 one of the parts was put in new plasma, and on April 4 thick columns of ameboid cells had grown into the new medium from the periphery of the silk veil.

On April 5, 6, 9, and 12, the culture underwent its sixth, seventh, eighth, and ninth passages. The plasma contained in and around the silk network became denser, and the growth of the cells slower. After the tenth passage on April 15 chains of elongated cells appeared in the new plasma. No ameboid cells were observed.

On April 17, 20, 23, and 26, the culture underwent its eleventh, twelfth, thirteenth, and fourteenth passages. The growth was very slow and was composed only of polygonal and fusiform cells. No ameboid or round cells could be seen.

PURE CULTURE OF ROUND CELLS.

Experiment II. Primary Culture.—On April 1, 1912, a culture of connective tissue that had grown very actively for seventy-two days was selected. The central part was composed of a dense tissue surrounded by elongated cells. In the peripheral part of the plasma many round cells were scattered. With a cataract knife, the central portion was removed, leaving the crown of round cells. Microscopical examination showed that no spindle cells had been left. On April 3 the number of the round cells had increased enormously, but no fusiform cells had appeared.

First Passage.—On April 4 the culture was washed in Ringer's solution and put in a new medium. On April 5 the cells multiplied very rapidly; on April 6 a few spindle cells appeared; and on April 7 a large number of fusiform cells were scattered through the new medium.

On April 8 the culture underwent its second passage, and on April 9 the fusiform cells had increased greatly in number. The culture was divided into two parts on April 10.

After the third passage, on April 10, the new tissue increased in density.

On April 13, 16, 18, 22, and 24, the cultures underwent their fourth, fifth, sixth, seventh, and eighth passages. They were composed of a dense mass of tissue from which radiated a crown of elongated cells. There were no round or ameboid cells.

SUMMARY.

In experiment I a group of ameboid cells was isolated from a culture of cardiac muscle sixty-three days old, and cultivated in plasma. After several passages, they formed a dense tissue from which ameboid cells radiated. The culture was divided into two parts. The part cultivated in plasma alone kept its morphological characters and continued to produce ameboid cells. The part cultivated upon silk in plasma became modified; the cells lost their ameboid characters, and were transformed into large elongated cells which were united in chains, or interlaced to form a network.

In experiment II the round cells taken from a culture of connective tissue seventy-four days old multiplied rapidly. They transformed themselves into elongated cells and produced, after a few passages, a mass of dense connective tissue. From the tissue a large number of elongated cells were constantly growing.

In both experiments the tissues originated from the ameboid or round cells extirpated from cultures that were sixty-three and seventy-four days old respectively. These cultures were still growing actively thirty and forty days later; that is, more than one hundred days after the extirpation of the original fragments from the organism.[1]

These experiments show that from old cultures it is possible to isolate and propagate cells that belong to a definite type. A tissue, formed by a pure strain of cells, can be obtained in this way, and this new method may be of value in cytological investigations.

[1] The cultures were still living actively on June 2, 1912.