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A Study of Certain General Conditions Leading to the Differentiation of Cells.

MONTROSE T. BURROWS.

From Private Research Laboratory, Pasadena, California.

In previous articles[1, 2] I have given evidence to show that heart muscle cells may show a variety of reactions and changes in the tissue culture. It was shown further that each of these various forms of reactions and changes is a response of the cell to a given environment.

Under certain conditions these cells may stretch out from the fragment as an otherwise inactive syncytium. Again they are seen in other cultures migrating out as large mononuclear cells. Under other conditions they separate, flatten on the surface of the medium, grow actively, divide by mitoses and resemble actively growing mesenchyme or sarcoma cells. In other cultures they migrate from the fragment as single spindle-shape, mesenchyme-like cells and they have been seen to differentiate into rhythmically contracting cells. These latter cells have been removed from the environment suitable for such activity to one suitable for growth and division, and have changed from contracting cells to cells which flatten on the surface of the medium, grow actively and divide by mitosis.

A careful study of the condition suitable for these various activities has shown that the growing cells are cells flattened against a film of material which flows out from the fragment over the surface of the medium. The syncytia which stretch out from the fragments and the migrating spindle shaped heart muscle cells are cells which migrate directly into the clotted plasmatic medium from fragments which do not disintegrate and throw out a surface film. The

[1] Burrows, M. T., *Am. J. Physiol.*, 1917, xlv, 556.

[2] Burrows, M. T., and Johnston, C. S., *Archiv. Int. Med.*, 1925, xxxvi, 293.

large mononuclear cells are cells migrating into a clot of plasma which fails to coagulate to fibrin and serum but which is undergoing slow dissolution. The contracting cells are cells which are either stretched through the serum of the coagulated plasma clot between well formed fibrin fibrils and a surface film of material which has spread out from the fragment, or cells which are stretched through the serum between the fragment itself and the ends of fibrin fibrils.

Contraction in these cells does not begin at once but always late in the life of the culture, when the plasma clot in the neighborhood of the fragment has become transformed into dense fibrin fibrils and serum.

Cells placed in the fibrin of such cultures become immediately inactive. Cells placed in the serum round off, become inactive and show the sharp conture of no or very little interchange with the medium. In the surface film alone do these cells flatten out, show a low surface tension and evidence of activity. In the contracting cells we could believe, therefore, that activity was located only at the one end in contact with the surface film or the fragment. They are polarized cells. Contraction, we could believe, is the result of this polarization of the muscle cells.

The next question is whether this polarization is peculiar for inducing rhythmical contractions in cells or is it necessary for function in all cells? It had been suggested to me by several biologists that I would probably find that simple mesenchyme and other cells will contract rhythmically if polarized in a similar manner in the culture.

After applying this condition of polarization of the functioning heart muscle cell to the body as a whole we appreciated that probably all functioning cells of the body are polarized in a similar manner. The gland cells are stretched between a basment membrane, a rich net of capillaries, nerves, lymphatics, etc., and the open gland duct. The nerve fibers are stretched between the brain and an end organ. It is the nerve fibers which exhibit pain and not the brain itself.

It seemed possible, therefore, that the polarization noted in the contracting heart muscle cell is peculiar to all functioning cells and that the type of function is determined by the cell itself, differentiated from the egg as it is at a much earlier time. The fact that cancer may arise from any tissue suggests again that the conditions leading to a change from a state of function to a state of growth may be similar in all tissues and that the type of function may be a fixed property of the cell itself.

It became of interest to study the reaction of striated skeletal muscle, smooth muscle, simple mesenchyme and various forms of epithelial cells in the cultures. After preparing more than 30000 cultures of these tissues, I observed that each type under certain conditions can grow, can show migration without growth and also at times some evidence of that form of differentiation peculiar to them in the mature organism. Three isolated contracting skeletal muscle cells and 2 isolated contracting smooth muscle cells were observed. In each instance these contracting cells were polarized in a manner quite identical to the isolated contracting heart muscle cells. The skeletal muscle cells had a rhythm identical to the heart muscle cells. They contracted at a rate of about 90 per minute. The smooth cells contracted but they showed the slow rhythm peculiar to these cells in the body. A few polarized mesenchyme cells were also observed but they showed no evidence of contraction. Single polarized epithelial cells were never seen. These cells dissolve the fibrin clot very soon.[3] Their only evidence of differentiation is the formation of tubes or alveoli. Membranes of epithelial cells are formed readily in the cultures and they are invariably stretched between ends of fibrin fibrils and the fragment through a liquid cavity. Evidence of rhythmical contraction was never observed in such membranes. Nerves frequently become stretched also between the fragment and the fibrin through a serum cavity but they never show any evidence of contraction.

The snappy rhythm of the contracting heart is evidently a peculiarity of striated muscle cells in general in the body. The same conditions which produce this rhythm in these cells induces the characteristically slow rhythm in the smooth muscle cells and causes no contraction whatsoever in the simple mesenchyme cells and other cells.

Some experimental evidence has thus been obtained for the first time which indicates that the peculiarities of the function of the various cells of the body are a property of these cells differentiated from the egg at an earlier time. The change of the cell from its early growing embryonic phase to the differentiated and functioning adult phase and vice versa is due on the other hand to common general changes in the organization of the whole. Further evidence is also given to show that function instead of growth in these cells is due to external influences limiting activity to one end of these cells or, in other words, it is due to a polarization of the cell.

[3] Burrows, M. T., *J. Cancer Res.*, 1927, xi, 72.