THE TRANSFORMATION OF MONOCYTES INTO FIBROBLASTS THROUGH THE ACTION OF ROUS VIRUS.

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PLATES 9 AND 10.

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A few years ago, monocytes in pure culture were found to metamorphose under certain conditions into elongated or stellate cells, and to assume the appearance of fibroblasts from which they became indistinguishable.[1] The processes of the transformed cells were long and sharp, and did not show any of the movements that characterize the pseudopods of monocytes; their nucleus was oval with one or two nucleoli; their cytoplasm contained a number of granules that took up neutral red in the same manner as do the fibroblasts kept in pure culture for over 13 years. As no permanent strain of these cells was obtained, it was not quite certain that the monocytes had metamorphosed into true fibroblasts. But the reality of such a transformation has been demonstrated recently by Fischer,[2] who succeeded in obtaining a permanent strain of fibroblasts from a culture of leucocytes.

In a culture medium, Fischer placed side by side some leucocytes and a fragment of muscle that had been kept in cold storage for a month. A number of tests had previously shown that no fibroblasts grew from the muscle. The leucocytes surrounded the muscle fragment, and every 2 or 3 days the cultures were washed and transferred into a new medium. Generally some fibroblasts could be observed around the muscle after the fourth passage, and their number increased during the following days. Then, the tissues were placed in a medium containing a large amount of embryonic tissue juice. In this manner, a strain of fibroblasts was obtained which remained active *in vitro* permanently.

[1] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 365.

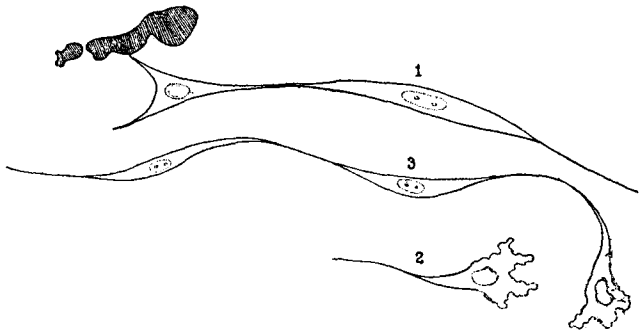
[2] Fischer, A., *Compt. rend. Soc. biol.*, 1925, xcii, 109.

The purpose of the present paper is to describe the conditions that bring about the transformation of the monocytes, to record the attempts to reproduce these conditions, and to give the results of a study of the characteristics of the cells.

Conditions of the Cultures That Bring About the Transformation.

When monocytes are cultivated in a flask instead of in a hanging drop, they never become transformed into fibroblasts. They wander through the medium, invade it progressively, and finally cover the whole area of the coagulum. They scatter themselves, never congregate in a mass, and never form a tissue. Generally, they completely fill the medium of a flask in 3 or 4 weeks. When the coagulum is removed from the flask and cut into fragments which are transferred into another flask, the cells migrate from the old coagulum and invade the new medium, but no fibroblasts ever appear. On the other hand, when leucocytes are cultivated on a cover-glass in a drop of plasma, according to the early technique, they may become transformed into fibroblasts. Although the composition of the medium is the same as in the flasks, the relations of the cells to the coagulum are quite different. The leucocytes are not left undisturbed for weeks, as in the experiments above described. Every few days, the part of the coagulum containing the cells is excised with a cataract knife, washed, and placed in a new medium. When the film that embeds the monocytes is sufficiently thick, when its edges are cut with a very sharp knife, and when they do not fold, the cells easily migrate into the new medium, and no transformation takes place, even when the cultures are kept for 3 months. But if, in the course of the experiments, the edges of the plasma film become slightly folded, or if the coagulum contracts, the cells, instead of migrating into the new medium, may accumulate along the obstacle that prevents their further migration. They congregate in amorphous masses of apparently dead tissue. When this condition arises, the transformation often begins. From the dark mass produced by the accumulation of the monocytes, some elongated and mobile processes start to grow, and fusiform cells appear (Text-fig. 1). At the same time, transition forms are observed. Some ameboid cells show active pseudopods, while one of the processes has become sharp, elongated, and fixed (Text-fig. 2). In some other cells,

two sharp nucleoli appear in the nucleus. There is also a marked tendency to tissue formation. It is well known that monocytes never unite. One of the first signs of the transformation is the union of cells through their processes, and their tendency to form a reticulum. In one culture, a chain of three cells was observed. Two of these cells were typical stellate fibroblasts, while the third one was a monocyte united by a slender process to a neighboring fibroblast (Text-fig. 3). These observations, which were repeated several times during our first study of the phenomenon, showed that the transformation takes place when the cells are packed together through some mechanical



TEXT-FIG. 1. Fusiform cells growing out from clump of dead monocytes.

TEXT-FIG. 2. Monocyte bearing active pseudopods and a fixed process at the same time.

TEXT-FIG. 3. Two fibroblasts and a transition cell united as a chain.

factors such as contraction of the medium, or tearing of its edges, which prevent their free migration, and determine their accumulation. Around the masses of apparently dead tissues, the transformation may begin. It seems as if the dying monocytes set free a substance which has the power of determining the metamorphosis of the other cells.

Attempt to Reproduce the Conditions Determining the Transformation.

The transformation of the monocytes around the fragments of dead tissue may be attributed to a change in the H ion concentration of the

medium, or to the setting free of some ferment or protein split products by the dying tissues. An attempt was made to reproduce these conditions artificially in several different ways.

Leucocytes were cultivated in media of pH varying from 5.5 to 8.5. When the medium was too acid or too alkaline, the leucocytes did not multiply. But in no case was a transformation observed.

Trypsin and the products of tryptic digestion of muscle were added to the culture. The monocytes did not change into fibroblasts.

Fragments of muscle, brain, spleen, liver, and leucocytic film killed by freezing were cultivated in flasks with living leucocytes. The leucocytes invaded the dead muscles and destroyed them in less than 3 weeks. But they did not become transformed into fibroblasts, although they crowded in immense numbers around the muscle fragments. It appeared that when the cells were free to migrate into the culture medium, the presence of muscle fragments did not determine the transformation. The discrepancies between the results of these experiments and those of Fischer must be attributed to differences in technique. In our experiments, the leucocytes were left undisturbed and could scatter through the entire medium of the flask, while in those of Fischer, the coagulum was excised and transferred to a new medium every few days. When this technique was used by us, the transformation sometimes occurred.

So far, the best method found for determining the fibroblastic transformation of a culture of monocytes is to add some filtered extract of Rous sarcoma to a culture of leucocytes. After a few days, some clumps of dead cells appear in the coagulum. Soon after, the digestion of the fibrin begins. The edges of the digested area are lined with small masses of apparently dead tissue. From these masses grow elongated cells that resemble fibroblasts. Generally they are full of fatty granulations and migrate very slowly into the medium (Fig. 1, *a*). When a fragment of culture is extirpated from the flask and kept in a hanging drop in a medium containing a large amount of tissue juice, the proliferation of these fibroblasts has been observed to continue for a few days. But their growth is far from being active. They resemble closely the fibroblasts from a spontaneous tumor of the chicken, observed some years ago by one of us, which had marked necrotic tendencies and killed the animal by cachexia rather than by metas-

tases.[3] They are far from being normal, as may be seen in the photograph (Fig. 1, *b*), and no permanent strain has been obtained so far.

Morphological Characteristics of the Cells.

The monocytes observed during the first hours of cultivation possessed the appearance that characterizes these cells and manifested the well known reaction to neutral red. The monocytes from a freshly prepared culture of leucocytes in diluted plasma show active pseudopods, and a nucleus that does not generally possess any definite nucleolus (Fig. 2, *a* and *b*). The granules stained by neutral red are small and move swiftly within the cells. They are scattered in the cytoplasm, without forming the rosette represented in the drawings of Sabin.[4] In the cultures that have been kept at a lower temperature, and where the cells are less active, the rosette becomes apparent. The length of these cells varies from about 10μ to 12 or 14μ. On the cinematomicrographic films, the cells are seen to progress rapidly, although they move much slower than the polymorphonuclears. Their mode of locomotion is analogous to that of the octopus. They differ markedly from the lymphocytes, which project short, blunt pseudopods and move slowly, or not at all. This cell is the element described under the names of monocyte, macrophage, endothelial leucocyte, blood histiocyte, or large mononuclear leucocyte, by various authors.

After a short time, the monocytes grow in size. They become from 80 to 140μ in length, and the nucleus is very much larger (Fig. 2, *d*). These giant cells devour with avidity the polymorphonuclears and the red blood corpuscles that are scattered in the coagulum. Soon, the medium contains only wandering monocytes. When stained with neutral red, they show small granules around the nucleus, which may also invade the ends of the cells but are not seen in the pseudopods. Generally the cytoplasm does not contain any unstained and refractile granules. The nucleus does not show any sharp nucleolus. The pseudopods are very active. Sometimes they resemble flagellates.

[3] Carrel, A., *Compt. rend. Soc. biol.*, 1924, xc, 1380.

[4] Sabin, F. R., Doan, C. A., and Cunningham, R. S., *Carnegie Institution of Washington, Pub. No. 361, Contributions to Embryology*, 1925, xvi, 125.

The cells divide freely, and show great phagocytic activity. The fibroblasts that appear in the cultures are of the same size as the giant monocytes. In the camera lucida drawing of a culture of leucocytes from the blood, treated by Rous virus, the fibroblasts are seen as elongated or stellate cells with a large oval nucleus containing one or two nucleoli (Fig. 2, *e*). The pseudopods have become transformed into apparently fixed processes. Small neutral red granules are located in the cytoplasm around the nucleus. At the periphery of the zone they occupy, more or less numerous refractile granules are observed. The red granules are more abundant and more deeply stained than in the fibroblasts described by Evans,[5] and their disposition closely resembles that found in the 13 year old strain of fibroblasts.

To summarize: The first change undergone by the monocytes is a considerable increase in their size. They become about ten times larger and their length equals that of normal fibroblasts. The giant monocytes possess the characteristics of tissue macrophages from which they are indistinguishable. They are the immediate precursors of the fibroblasts, and do not differ essentially from the cells that grow from a fragment of adult connective tissue.

Significance of the Transformation.

It is well known that one of the more important cultural characteristics of monocytes is their inability to form a tissue. When free to move in the medium, they always place themselves at a certain distance from one another. If they are packed together by centrifugation and embedded in a film of plasma, they migrate rapidly from the coagulum. When migration is impossible, they die. Fibroblasts, on the contrary, always live as a tissue. When they are in close contact, they multiply actively without scattering over the coagulum. They remain packed together. The peripheral cells of the growing colony are generally in contact with one another, or are united by their processes. It is important to observe that the transformation of monocytes into fibroblasts generally occurs when, on account of some change in the medium, the life of the monocytes has become impossible.

[5] Evans, H. McL., and Scott, K. J., *Carnegie Institution of Washington, Pub. No. 273, Contributions to Embryology*, 1921, x, 1.

The monocytes transform themselves into cells capable of living under the conditions present in the culture. The metamorphosis of a monocyte into a fibroblast displays the characteristic of an adaptive change which may automatically be produced by substances set free by the monocytes themselves under certain conditions.

The phenomenon that occurs in cultures of monocytes inoculated with Rous virus probably has the same significance. When an area of digestion occurs in the coagulum, masses of necrotic tissue appear along its edges. Around these masses, the fibroblastic forms develop. The monocytes that are highly susceptible to Rous virus become transformed into fibroblasts that are not sensitive to the virus, and that are not even a favorable medium for the growth of the virus. It seems as if there were a tendency for a susceptible cell to transform itself into an immune cell. This phenomenon could be considered as an expression of the general property with which all living organisms or chemical systems are endowed, that of opposing the action of a disturbing factor. But the real significance of these facts cannot be understood until we know whether the transformation of monocytes into fibroblasts is reversible.

SUMMARY.

In normal cultures, the transformation of monocytes into fibroblasts generally occurred when cells became packed together through some mechanical factors that prevented their free migration and determined their accumulation. Various modifications of the medium, the addition of dead tissue, and of trypsin or the products of trypsin digestion, failed to bring about the transformation. The inoculation of cultures of monocytes with filtered extract of Rous sarcoma frequently determined the appearance of fibroblasts. The first change undergone by the monocytes cultivated *in vitro* was a large increase in their size. Later, the giant monocytes became transformed into cells that did not differ essentially from those that grow from a fragment of adult connective tissue.

EXPLANATION OF PLATES.

PLATE 9.

FIG. 1, *a* and *b*. Fibroblasts observed in a culture of monocytes treated with filtered extracts of Rous sarcoma. (*a*) Fibroblasts united by their processes. (*b*) Fibroblasts containing fat granules.

PLATE 10.

FIG. 2, *a* to *e*. Normal monocytes after a few hours cultivation. Camera lucida drawing made 20 minutes after the cells were placed in contact with a 1/50,000 solution of neutral red. × 1055. The stained granules are represented by solid black, and the refractile bodies by black rings. (*a*) Very active monocytes. (*b*) Monocytes in less active condition; rosette disposition of the neutral red granules. (*c*) Red blood corpuscle. (*d*) Giant monocytes after 2 weeks cultivation. (*e*) Fibroblasts resulting from the transformation of the monocytes in a culture treated with filtered extract of Rous sarcoma.

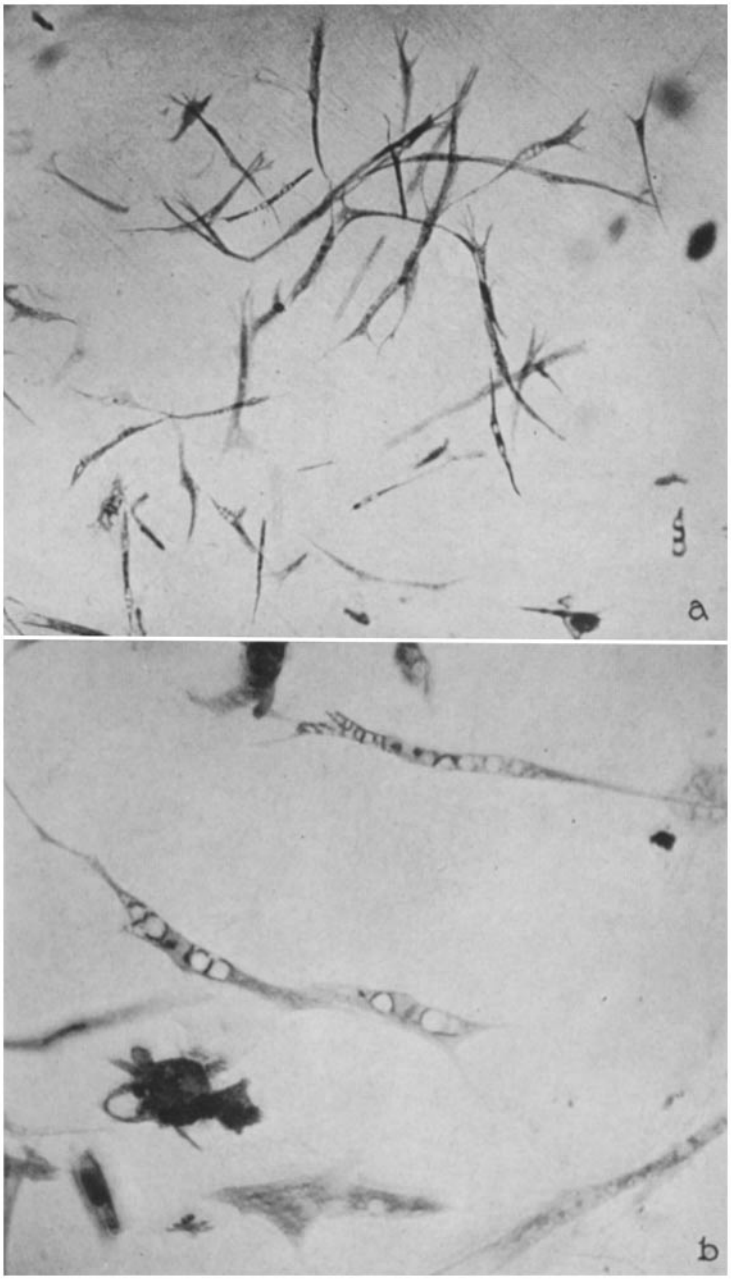


FIG. 1.

(Carrel and Ebeling: Transformation of monocytes.)

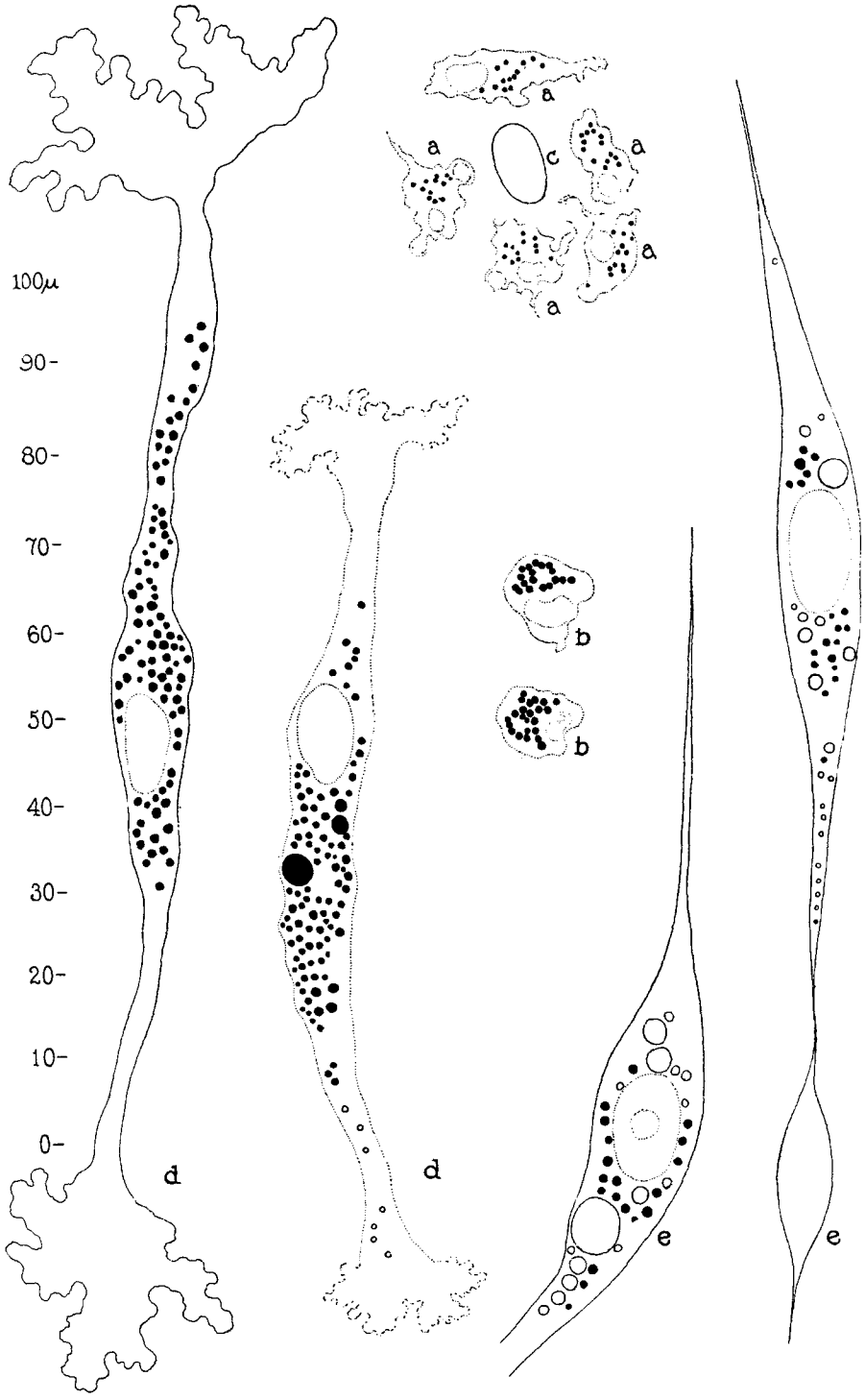


FIG. 2.

(Carrel and Ebeling: Transformation of monocytes.)