THE FUNDAMENTAL PROPERTIES OF THE FIBROBLAST AND THE MACROPHAGE.

II. THE MACROPHAGE.

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PLATES 8 AND 9.

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The purpose of the experiments described in this article was to elucidate the relations of the macrophage¹ and the monocyte,² and to define these cells by their essential biological properties.

Monocyte and Macrophage as Functional Variations of a Single Type.

The macrophages which migrated from fragments of connective tissue, organs, tumors, etc., of adult animals did not differ markedly from the blood monocytes which had been cultivated \textit{in vitro} for a few days. This fact rendered it probable that both cells merely represent metabolic states of a single type, and that their structure can be modified at will by certain changes in the composition of the medium. Two convergent methods were used. The first one consisted of placing macrophages derived from the subcutaneous connective tissue and monocytes obtained from the blood in media of identical composition, and ascertaining the structural changes occurring after a few days. If monocytes and macrophages merely express different metabolic states of a single cell, they must become identical in appearance when living under the same conditions. By the second method, monocytes and macrophages were studied in media of different composition in

¹ Synonyms: round rhagiocrin cells of Renaut; polyblasts of Maximow; clasmatocytes of Ranvier; endothelial leucocytes of Mallory; adventitial cells of Marchand; pyrrol cells of Goldmann.

² Synonyms: large mononuclear leucocytes of Ehrlich; endothelial leucocytes of Mallory; blood histiocytes of Aschoff.
order to determine whether metabolic changes can modify their cytoplasmic structures.

In a first series of experiments, fragments of leucocytic films and flaps of subcutaneous connective tissue from adult chickens were cultivated in a medium composed of plasma, Tyrode solution, and embryonic juice. After a period varying from 2 hours to a number of days, fragments of the colonies were removed from the flasks, transferred to cover-glasses, and stained with neutral red and Janus green. Camera lucida drawings were made at a magnification of 1600 diameters.

Experiment 1. Monocytes Freshly Removed from the Blood.—Culture 3002-A2, Jan. 14, 1926. Fragments of leucocytic films placed on cover-glasses in a hanging drop of plasma and embryo juice. After 2 hours, preparation stained; camera lucida drawings. A few monocytes are seen among many polymorphonuclear leucocytes. Length of cells, 10 to 15μ; active pseudopods; approximately 10 small neutral red vesicles about 1μ in diameter, containing 1 or 2 small granules; vesicles grouped around the centriole or scattered through the cytoplasm.

Experiment 2. Blood Monocytes after 2, 24, 48, 72, and 96 Hours Cultivation.—Culture 8740-D, Apr. 19. Fragments of leucocytic film cultivated on cover-glasses in equal parts of plasma and embryo juice. After 2 hours, preparation stained; camera lucida drawings. Large number of polymorphonuclear leucocytes (Fig. 1) migrating into the medium among a few red blood corpuscles (Fig. 2); no lymphocytes; some monocytes, all of identical appearance; cells 10 to 15μ long; 8 or 10 neutral red vesicles about 1μ in diameter, radially arranged around the centriole in a few cases; mitochondria as faintly stained dots; no fat globules (Fig. 3). Apr. 20, stained; camera lucida drawings. Active polymorphonuclear leucocytes, unchanged in size or appearance; a few lymphocytes. Without exception, all the monocytes have increased in size, being 30 to 40μ in length; no change in the mode of locomotion and in the general appearance of the cells. The neutral red vesicles are more numerous, from 0.25 to 1.5μ in diameter, and contain granules; no rosette disposition; mitochondria more deeply stained, assuming the shape of rods (Fig. 5). Apr. 21, stained; camera lucida drawings. Active polymorphonuclear leucocytes, unchanged in size or appearance; a few lymphocytes. All the monocytes have increased in size, being 40 to 45μ in length; no change in the mode of locomotion and in the general appearance of the cells; no rosette disposition of the vesicles. They are numerous, 0.5 to 2μ in diameter, and deeply stained. A number of the cells show partially digested polymorphonuclear leucocytes; mitochondria as round and rod-shaped granules, deeply stained (Fig. 6). Apr. 22, stained; camera lucida drawings. Decrease in the number of polymorphonuclear leucocytes. They are unchanged in size or appearance; no lymphocytes. Many of the monocytes are slender, 50 to 75μ in length; no change in the mode of lomomo-
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The vesicles are very numerous, 0.5 to 2μ in diameter, and fill the trophoplasm. In some cases, cells show ingested red blood corpuscles and polymorphonuclear leucocytes; mitochondria as deeply stained granules (Fig. 7). Apr. 23, stained; camera lucida drawings. Very few active polymorphonuclear leucocytes. They are unchanged in size or appearance; no lymphocytes. Most of the monocytes are slender, 25 to 80μ in length. The undulating membrane has disappeared along the sides of the cells. The vesicles are 0.25 to 1μ in diameter, less numerous, and not as deeply stained as in the cells observed after 3 days incubation; no evidence of ingested cells. Mitochondria as deeply stained granules (Fig. 8).

Experiment 3. Blood Monocytes after 2 and 24 Hours Cultivation.—Culture 8741-D, Apr. 20. Fragments of leucocytic film cultivated on cover-glasses in equal parts of plasma and embryo juice. After 2 hours, preparation stained; camera lucida drawings. Normal polymorphonuclear leucocytes; all monocytes identical in appearance; cells 10 to 15μ long; 8 or 10 neutral red vesicles, showing the rosette arrangement in some cases; mitochondria as faintly stained dots; no fat globules; cells identical with those of Fig. 4. Apr. 21, stained; camera lucida drawings. Active polymorphonuclear leucocytes; a few lymphocytes. Every visible monocyte has increased in size, being 30 to 40μ in length; same mode of locomotion and general appearance; rod-shaped mitochondria. The neutral red vesicles are larger and contain dark granules; not grouped around the centriole; cells identical with those of Fig. 5.

Experiment 4. Blood Monocytes Cultivated for 7 Days.—Culture 3020-A2, Jan. 21. Fragments of leucocytic film cultivated in a flask containing 0.5 cc. plasma, 1 cc. Tyrode solution, and 0.5 cc. embryo juice. After coagulation, 0.5 cc. serum and 0.5 cc. embryo juice added to medium. Jan. 22, washed in Tyrode solution, and fresh medium added. Jan. 25, same treatment. Jan. 28, fragments of coagulum transferred to cover-glasses and stained; camera lucida drawings. Length of cells, 30μ; active kinoplasm; trophoplasm filled with large number of neutral red vesicles, deeply stained, resembling berries; diameter of vesicles, 1 to 4μ. Vesicles contained many small granules, irregular in shape, and darkly stained; no rosette arrangement.

Experiment 5. Blood Monocytes Cultivated for 13 Days.—Culture 2954-C, Jan. 7. Fragments of leucocytic film from normal chickens cultivated on cover-glasses in equal parts of plasma and embryo juice; three passages into fresh medium. Jan. 19, stained; camera lucida drawings. Very long and slender monocytes; active kinoplasm; length of cells, 60μ; 15 neutral red vesicles, 0.5 to 2μ in diameter. Some of the vesicles contain 1 to 5 small red granules.

Experiment 6. Tissue Macrophages after 24 Hours Cultivation.—Culture 3136-C5, Feb. 1. A fragment of subcutaneous connective tissue from a normal adult chicken cultivated on cover-glasses in equal parts of plasma and embryo juice; migration of a few cells. Feb. 2, stained; camera lucida drawings. Length of cells, 30μ; active kinoplasm. Trophoplasm contains 8 or 10 neutral red vesicles, 1 to 2μ in diameter, each vesicle having several large red granules, generally agglutinated together; some fat globules between the vesicles.
Experiments 7 and 8. Tissue Macrophages after 24 Hours Cultivation.—Culture 2956-C5, Jan. 14. Fragments of subcutaneous connective tissue from an adult chicken cultivated on cover-glasses in equal parts of plasma and embryo juice. Jan. 15, stained; camera lucida drawings. Long, slender macrophages; very active flagellate pseudopods; length of cells, 60 to 85 μ; about 8 neutral red vesicles, 0.5 to 2 μ in diameter, containing 1 or 2 small neutral red granules.

Experiments 9 and 10. Tissue Macrophages after 24 Hours Cultivation.—Culture 3097-C, Jan. 28. Fragments of subcutaneous connective tissue from an adult chicken cultivated on cover-glasses in equal parts of plasma and embryo juice. Jan. 29, stained; camera lucida drawings. Diameter of cells, 30 μ; approximately 30 moderately stained neutral red vesicles, 0.5 to 3 μ in diameter. Most of the vesicles contain coarse, dark red granules which move more or less actively; many small fat globules between the vesicles. After a few minutes, the fluid in the vacuoles takes up the stain gradually and becomes orange, yellow, or red; vesicles not arranged around the centriole, but distributed through the trophoplasm; active folding and unfolding of the kinoplasm.

Experiments 7 and 8. Tissue Macrophages after 24 Hours Cultivation.—Culture 3777-C, Apr. 22. A fragment of subcutaneous connective tissue from a normal adult chicken cultivated on cover-glasses in equal parts of plasma and embryo juice. Apr. 23, migration of a large number of macrophages; stained; camera lucida drawings. Diameter of cells, 40 μ; active kinoplasm. Trophoplasm contains about 20 neutral red vesicles, 0.25 to 2 μ in diameter, showing rosette disposition; small fat globules around nucleus and vesicles; mitochondria deeply stained, globular, and rod-shaped (Fig. 9).

Experiments 7 and 8. Tissue Macrophages after 24 Hours Cultivation.—Culture 3790-C, Apr. 23. A fragment of subcutaneous connective tissue from a normal adult chicken cultivated in equal parts of plasma and embryo juice. Apr. 24, migration of a moderate number of macrophages; stained; camera lucida drawings. Length of cells, 75 μ; active kinoplasm; trophoplasm filled with neutral red vesicles, 0.25 to 2 μ in diameter; no fat globules; mitochondria deeply stained, globular, and rod-shaped (Fig. 10).

In a second series of experiments, blood monocytes and fragments of subcutaneous connective tissue from adult chickens were cultivated in a control medium composed chiefly of serum, and an experimental medium of Tyrode or Ringer solution. The cover-glass preparations were stained with neutral red or neutral red and Janus green.

Experiment 1. Effect of Tyrode Solution and Serum on Monocytes.—Culture 3386-C, Mar. 2. Fragments of leukocytic film cultivated in flasks in the ordinary medium composed of 0.5 cc. plasma, 0.5 cc. embryo juice, and 1 cc. Tyrode solution. Mar. 3, cultures washed with 2 cc. of Tyrode solution, and patched with 25 cc. plasma and 25 cc. embryo juice. Mar. 4, cultures washed in 2 cc. Tyrode...
solution, which is removed after 10 minutes; Flask 1, 1 cc. Tyrode solution introduced, and Flask 2, 0.5 cc. serum and 0.5 cc. Tyrode solution. Mar. 6, cultures washed in Tyrode solution and fresh fluid introduced into the flasks. Mar. 8, fragments of coagulum transferred to cover-glasses; stained; camera lucida drawings.

1. Monocytes in Tyrode solution. Length of cells, 32 μ; active kinoplasm; trophoplasm filled with faintly stained neutral red vesicles containing no granules; between the vesicles, some fat globules and faintly stained mitochondria. The latter are small dots, hardly visible (Fig. 11).

2. Monocytes in serum. Very active multibranched cells, 50 μ in length; kinoplasm packed with smaller neutral red vesicles, deeply stained, resembling berries; practically no fat globules; well stained mitochondria, globular, rod-shaped, and filamentous (Fig. 12).

Experiment 2. Effect of Tyrode Solution on Monocytes.—Culture 3079-A1, Feb. 18. Monocytes which have grown in the ordinary medium since Feb. 16 are washed for 10 minutes with 2 cc. Tyrode solution. After its removal, 1 cc. of Tyrode solution is introduced into the flask. Feb. 21, same treatment. Feb. 24, fragment of coagulum transferred to cover-glasses; stained; camera lucida drawings. Macrophages are of small size with very active kinoplasm; length of cells, 25 μ. The area occupied by the neutral red vesicles and the nucleus is very small. About 15 small neutral red vesicles, 0.25 to 1 μ, are grouped around the centriole. Between them and the centriole is an area lightly stained in red and composed of small granules; rosette arrangement; faintly stained mitochondria as small dots around the nucleus and the neutral red vesicle; no fat.

Experiment 3. Effect of Ringer Solution and Serum on Monocytes.—Culture 3445-C, Mar. 9. Preparation of the cultures in the ordinary medium. Mar. 11, cultures washed in 2 cc. Ringer solution. After the removal of the fluid, 1 cc. Ringer solution is introduced into Flask 1, and 0.5 cc. serum and 0.5 cc. Ringer solution into Flask 2. Mar. 12 and 13, same treatment. Mar. 15, fragments of coagulum transferred to cover-glasses; stained; camera lucida drawings.

1. Monocytes in Ringer solution. Small cells, 25 μ in length; 25 pale vesicles, 0.25 to 1 μ in diameter, and faintly stained mitochondria; large number of small fat globules; active kinoplasm (Fig. 13).

2. Monocytes in serum. Cells active and larger, 50 μ in length; area occupied by the neutral red vesicles, 0.25 to 3 μ in diameter, containing a fluid more deeply stained than in cells cultivated in Ringer solution; few fat globules; large number of mitochondria (Fig. 14).

Experiment 4. Effect of Ringer Solution and Serum on Monocytes.—Culture 3471-C, Mar. 11. Preparation of the cultures by the ordinary method. Mar. 13, cultures washed in 2 cc. Tyrode solution. After removal of the fluid, 1 cc. of Ringer solution is introduced into Flask 1, and 0.5 cc. serum and 0.5 cc. Ringer solution into Flask 2. Mar. 15, cultures washed and fresh medium introduced. Mar. 16, fragments of coagulum transferred to cover-glasses; stained; camera lucida drawings.
1. Monocytes in Ringer solution. Elongated and very active cells, 25μ in length; about 15 faintly stained neutral red vesicles, 0.25 to 1.25μ in diameter, scattered through the trophoplasm, and with no granules; mitochondria as small dots, faintly stained (Fig. 15).

2. Monocytes in serum. Very active cells, with several branches, 40μ in length; deeply stained neutral red vesicles, 0.25 to 1.25μ in diameter, without granules, arranged around centriole; globular and rod-like mitochondria, well stained (Fig. 16).

Experiment 5. Effect of Tyrode Solution and of Blood Serum on Monocytes.—Culture 8496-D, Feb. 1. Fragments of culture of monocytes 3030-A2 are placed in flasks, containing 1.5 cc. plasma and 0.5 cc. embryo juice. After coagulation, Flask 1 washed for 10 minutes in 4.5 cc. of Tyrode solution. After the fluid is removed, 1.5 cc. of Tyrode solution is introduced into the flask. Flask 2 is left undisturbed. Feb. 2, Flask 1 washed with Tyrode solution for 10 minutes. Feb. 3, Flask 1 washed for 5 minutes with Tyrode solution; surface of coagulum of Flask 2 washed for a few seconds in Tyrode solution, and 0.5 cc. serum introduced. In Flask 1, monocytes are elongated, transparent, and in comparatively small numbers. In Flask 2, they are shorter, less transparent, very numerous, and invading the medium actively. Feb. 5, fragments from Flask 2 transferred to cover-glasses; stained; camera lucida drawings. Feb. 6, fragments from Flask 1 transferred to cover-glasses; stained; camera lucida drawings.

1. Monocytes in Tyrode solution. Very active cells, 35μ in length; small segregation apparatus; a few neutral red vesicles, 0.25 to 0.75μ in diameter, faintly stained; no granules; few fat globules.

2. Monocytes in serum. Very active cells, 50μ in length; very large segregation apparatus; many neutral red vesicles, 0.5 to 2μ in diameter, resembling a mass of darkly stained berries; no fat globules.

Experiment 6. Effect of Tyrode Solution and Blood Serum on Monocytes.—Culture 3511-C, Mar. 16. Fragments of leucocytic film cultivated in the ordinary manner. Mar. 18, cultures washed with 2 cc. Tyrode solution for 10 minutes, 1 cc. Tyrode solution introduced into Flask 1, and 1 cc. of 50 per cent serum into Flask 2. Mar. 19, cultures washed with 2 cc. Tyrode solution for 10 minutes, and fresh fluid introduced into each flask. Mar. 20, fragments transferred to cover-glasses; stained; camera lucida drawings.

1. Monocytes in Tyrode solution. Cells, 25μ in diameter; active kinoplasm; faintly stained neutral red vesicles, 0.25 to 0.5μ in diameter; few mitochondria, faintly stained; a few small fat globules.

2. Monocytes in serum. Length of cells, 55μ; neutral red vesicles, 0.25 to 3μ in diameter, more deeply stained; mitochondria well stained; a number of small fat globules scattered about among the vesicles.

Experiment 7. Effect of Tyrode Solution and Serum on Monocytes.—Culture 3020-A, Jan. 21. Fragments of leucocytic film cultivated in the ordinary manner. After coagulation, 1 cc. Tyrode solution introduced into Flask 1, and 0.5 cc. serum and 0.5 cc. embryo juice into Flask 2. Jan. 22, cultures washed with 2 cc. Tyrode
solution for 10 minutes, and after removal of the fluid, patched with 1 cc. of 0.5 cc.
plasma and 0.5 cc. embryo juice. After coagulation, fresh fluid added to each
flask. Jan. 23, same treatment. Jan. 27, fragments from Flask 1 transferred to
cover-glasses; stained; camera lucida drawings; Flask 2, ordinary treatment.
Jan. 28, fragments from Flask 2 transferred to cover-glasses; stained; camera lucida
drawings.

1. Monocytes in Tyrode solution. Length of cells, 30μ; small vesicular ap-
paratus; about 20 neutral red vesicles, 0.25 to 1.5μ in diameter, faintly stained;
no granules in the vesicles; about 15 fat globules.

2. Monocytes in serum and embryo juice. Length of cells, 40μ; about 40
neutral red vesicles, 0.25 to 2.5μ in diameter, many of them containing red granules.

Experiment 8. Effect of Tyrode Solution on Tissue Macrophages.—Culture
3841-C, Apr. 29. Fragments of subcutaneous connective tissue from an adult
chicken cultivated on cover-glasses in equal parts of plasma and embryo juice.
Apr. 30, extensive migration of macrophages into medium; one preparation
stained; camera lucida drawings. Diameter of cells, 45μ; many neutral red
vesicles, 0.25 to 3.5μ in diameter; rosette disposition; no fat globules; numerous
mitochondria, well stained granules and short rods grouped around nucleus and vesicular area (Fig. 17).

Other preparations transferred to a flask containing 0.5 cc. plasma, 1 cc. Tyrode
solution, and 0.5 cc. embryo juice. After coagulation, culture washed in 2 cc.
Tyrode solution for 10 minutes; fluid removed, and 1 cc. Tyrode solution intro-
duced into the flask. May 1, a few active cells migrating into the fresh medium;
washed in 2 cc. Tyrode solution, and fresh Tyrode solution introduced. May 3,
same treatment. May 5, fragments of coagulum transferred to cover-glasses;
stained; camera lucida drawings. Very active cells, 25μ in diameter; neutral red
vesicles, 0.25 to 1.5μ in diameter; rosette disposition; no fat globules; mitochondria
well stained granules with a few rods grouped around nucleus and vesicular area
(Fig. 18).

The results of these two series of experiments may be summarized
as follows:

In the first series, blood monocytes and tissue macrophages were
cultivated in identical media for various periods of time. The
monocytes, examined 2 hours after the preparation of the cultures
(Figs. 3 and 4), were 10 to 15 μ in length. Their undulating mem-
brane moved unceasingly and showed its folds as flagellate pseudopods.
The neutral red vesicles, 6 to 10 in number, were radially arranged
around the centriole, or scattered irregularly through the tropho-
plasm. The mitochondria were small and round (Figs. 3 and 4).

After 24 hours (Fig. 5), the size of the cells increased, their length
reaching 30 to 40 μ. The neutral red vesicles were augmented in number and size, and filled with orange-red fluid containing small or large dark granules. No fat globules were seen. The mitochondria assumed the appearance of rods or short filaments. Every monocyte present in the preparation was transformed in this manner. The change became more marked after 48 hours (Fig. 6). The trophoplasm was filled with debris of polymorphonuclear leucocytes. After 72 hours (Fig. 7), the size of the cells reached 75 μ. The segregation apparatus and the mitochondria showed a corresponding increase. The undulating membrane was very active. The polymorphonuclear leucocytes were normal (Fig. 1). However, a number of them and also some red blood corpuscles had been phagocyted and were being digested by the monocytes. All the monocytes present in the preparation had become large cells, indistinguishable from tissue macrophages. After 96 hours cultivation (Fig. 8) the polymorphonuclear leucocytes, lymphocytes, and red corpuscles had practically disappeared from the preparation, and the segregation apparatus of the monocytes had slightly decreased in size. The undulating membrane moved only at the ends of the cells.

The tissue macrophages were always larger than the monocytes just obtained from the blood. Their size varied from 30 to 85 μ. The undulating membrane and its flagellate-like folds moved in the same manner as those of the monocytes. The number of the neutral red vesicles varied from 15 to 30 or more. They were often grouped around the centriole in a typical rosette. Their diameter varied from 1 to 2 μ. Generally, they contained 1 or 2 small red granules. A few fat globules were seen between the vesicles. The mitochondria were located around the nucleus and the mass of the vesicles, and assumed the shape of dots and rods (Figs. 9 and 10).

As these results clearly show, blood monocytes increase in size when cultivated in plasma and embryo juice in the presence of polymorphonuclear leucocytes, and in 24 hours they reach the dimensions of the tissue macrophages. At the same time that the size and proliferating activity of the cells are augmented, the segregation apparatus becomes larger, dark stained granules appear in the vesicles, and the mitochondria assume the shape of rods and short filaments. The rosette arrangement of the neutral red vesicles around the centriole
is present in the tissue macrophages, as well as in the monocytes. However, it is not a constant characteristic of either of these cells. There is no difference between the mitochondria of monocytes and of macrophages, after the monocytes have been cultivated in plasma and embryo juice. As all the large mononuclear cells present in the medium exhibit a similar appearance and size, they certainly derive from the monocytes observed in the fresh cultures. It is obvious that, when cultivated under the same conditions, the blood monocytes become indistinguishable from the macrophages of adult connective tissue (Figs. 7 to 10).

In the second series of experiments, macrophages originating from monocytes and tissue macrophages were placed in nutrient and non-nutrient media, in order to ascertain whether their morphology could be modified by a nutritional change. It is known that serum is a nutrient medium for the monocytes, whereas they starve in Tyrode or Ringer solution. The control monocytes, which were kept in serum, continued to resemble tissue macrophages (Figs. 12, 14, and 16). The segregation apparatus was well developed and the vesicles often looked like red berries and contained some granules. The mitochondria appeared as short filaments, deeply stained with Janus green. The cells remained large and very active. Their length varied from 40 to 70 µ. The experimental monocytes were deprived of food in flasks containing only Tyrode or Ringer solution as a fluid medium (Figs. 11, 13, and 15). Their size decreased, and the nucleus also became smaller. At the same time, the segregation apparatus lost its importance, the granules disappeared from the vesicles which were filled with pale fluid, and the mitochondria became shorter, less numerous, and faintly stained. Some fat globules appeared between the vesicles. It was evident that in the cells subjected to starvation, the neutral red vesicles and the mitochondria became profoundly modified. Similar phenomena were observed in tissue macrophages cultivated in a medium composed chiefly of Tyrode solution (Figs. 17 and 18). They reverted to a smaller type, markedly resembling monocytes.

Monocytes and tissue macrophages do not differ essentially from one another. They represent functional states of a single type, which readily responds to a change in nutrition by modifying its protoplasmic
organs. Cell morphology is determined by, and varies according to, the chemical composition of the pericellular fluid.

Mode of Locomotion of the Macrophage and Structure of Its Kinoplasm.

The mode of locomotion of the macrophage was studied on cinematographic films of cultures of blood, connective tissue, and Rous sarcoma. Both direct light and dark-field illumination were used. The photographs were taken at a speed of 60 exposures per minute and at a magnification of 400 diameters. As the films were projected at a speed of 10 or 11 exposures per second, the rate of motion of the cells was increased approximately ten times. It was thus possible to observe simultaneously on the films the progression of the cells and the movements of their organs. The small monocytes observed in freshly prepared cultures moved more slowly than the polymorphonuclear leucocytes, and more rapidly than the lymphocytes. The mode of progression of the three types of cells differed widely. The lymphocytes sent forth short, blunt pseudopods, and advanced like small worms. The polymorphonuclear leucocytes projected forward long arms, into which the rods streamed. Often they dragged a thin tail behind them. While the polymorphonuclear leucocytes moved like amebae, the monocytes assumed an appearance more nearly comparable to that of an octopus. Around their body and processes, thread-like pseudopods moved unceasingly. They could be distinguished at first sight from the polymorphonuclear leucocytes and lymphocytes by their peculiar way of carrying themselves. After the monocytes had assumed the appearance of macrophages, their mode of locomotion was not modified. The movements of both monocytes and macrophages growing from adult connective tissue were identical, and neither cell could be distinguished from the other.

The study of macrophages photographed by direct light and by dark-field illumination led to a clear conception of their structure. These cells are composed, as is well known, of a central body or trophoplasm, and a marginal portion or kinoplasm. The trophoplasm contains the nucleus, the mitochondria, and the neutral red vesicles. Although the cell body is plastic, and the nucleus as well as the cyto-

3 The cultures were filmed by Mr. Heinz Rosenberger.
plasmic organs glide nimbly into the advancing process during the progression of the cell, it is relatively immobile when compared to the kinoplasm. The kinoplasm is the peripheral and thin cytoplasm from which thread-like pseudopods seem to extend themselves. These filiform appendages move with great activity. When the flagellated pseudopods are examined on films taken with dark-field illumination, they appear to be the folds of an extremely thin membrane which projects itself from the kinoplasm and is a part of it. This membrane undulates incessantly, like a delicate silk veil when blown by the wind. Under direct light, the membrane becomes invisible. Its folding and outer edges only are then seen, to be mistaken for thread-like pseudopods. When the macrophage stops progressing and assumes a circular shape, it appears surrounded by the membrane which completely unfolds itself and moves like the waves of the sea on a sandy shore. Under dark-field illumination, the presence of the membrane is detected because its surface as well as its folds become lighted. When, in the course of a slow undulation, the convex surface of a fold is illuminated from the side, the membrane is seen as an extremely thin structure covering part or all of the periphery of the cell, and molding itself upon any impinging bodies, fibrin, bacteria, and other cells. When a lymphocyte approaches a macrophage, it is rapidly enveloped by the foldings and appears to glide into them toward the body of the cell, where it is digested. The incessant motion of the undulating membrane is probably the cause of the inability of the macrophages to unite and form a tissue as fibroblasts do. Possibly it is an essential function of the cytoplasm, and its suppression, when cells are crowded, may bring about death.

Macrophages obtained from the subcutaneous connective tissue of adult chickens were studied by the same method, and found to move exactly as do monocytes. Normal blood monocytes, large monocytes growing in flasks, sarcoma cells, and tissue macrophages possess an undulating membrane. It is a characteristic common to the four groups of cells.

**Characteristics of the Colonies of Macrophages.**

When fragments of a leucocytic film are placed in a medium composed of 0.5 cc. plasma, 1 cc. Tyrode solution, and 0.5 cc. embryonic
extract, ameboid cells migrate in large numbers into the coagulum. The polymorphonuclear leucocytes, which move rapidly, occupy the outer zone of the invaded area, while the inner zone consists chiefly of the slower monocytes which later multiply and scatter everywhere. Eventually, small islands composed of monocytes appear at a distance from the main colonies. The monocytes rapidly phagocyte the polymorphonuclear leucocytes and in 3 or 4 weeks spread on the entire medium in a more or less regular formation. They do not build up a tissue, as fibroblasts do. Sometimes they unite as chains. They never come into contact at their sides. Instead of remaining packed together, they disperse through the whole coagulum. They may congregate around some foreign bodies, such as fragments of muscle or protein precipitate, which they devour. But as soon as the food is exhausted, they scatter again. When the original fragments of the leucocytic film have been removed, the coagulum appears as homogeneous. As long as the cells remain normal, the flask seems to be empty. When the segregation apparatus develops abnormally, the colonies may look like whitish spots on the transparent background. If death occurs, the colonies become opaque. Digestion of the coagulum does not take place even after the death of the cells. As a rule, digestion of the coagulum down to the glass is a characteristic of the malignant transformation of the cells after inoculation with Rous virus. The clot seems to be moth-eaten, and on the edges of the digested areas small masses of necrotic tissue may be seen. Simultaneously, some cells assume the appearance of fibroblasts. The transformation of the macrophages into fibroblasts never occurs spontaneously when the cells are allowed to migrate freely, as they do in a normal culture and when no sarcoma virus is present. If macrophages crowd together through the action of some mechanical factor, or following inoculation with Rous virus, they die or become transformed into fibroblasts.

Colonies of tissue macrophages were obtained from the subcutaneous connective tissue of adult chickens, and from other normal or pathological tissues. Their appearance and behavior have always proved to be identical with those of the monocytes.
Rate of Growth of the Colonies.

In a medium composed chiefly of blood serum, the rate of multiplication and migration is such that monocytes originating from fragments of a film about 3 by 2 mm. entirely cover the coagulum of a flask 5 cm. in diameter in approximately 3 weeks. The rate of growth of the colony may be appraised in terms of the time the cells take to invade the culture medium and the number of cells contained in the unit of surface. However, no method has yet been developed by which the rate of multiplication may be accurately measured. Only considerable differences can be appreciated. There is no difficulty in detecting the variations in the rate of multiplication of macrophages or monocytes in media widely different in nature. When colonies of monocytes are cultivated in a hanging drop and transferred to a new medium every 2 or 3 days, the size of the colonies generally doubles in 4 or 5 days. The velocity of the multiplication of the cells is probably less great than that of epithelial cells and fibroblasts.

Food Requirements of Macrophages. Their Susceptibility to Arsenous Oxide.

Monocytes and macrophages do not feed on egg albumin, nucleic acid, amino acids, crystallized egg albumin, egg yolk, or pure embryo juice. They have the property of living and multiplying in blood serum and differ from fibroblasts and epithelial cells, which die in blood serum and multiply indefinitely in embryo juice. Although they may proliferate for several weeks when cultivated in plasma or serum, it is far from certain that they reach their optimum metabolic activity in this medium. In fresh blood cultures, monocytes almost immediately phagocyte polymorphonuclear leucocytes and red corpuscles, and their length increases from 10 or 15 μ to 70 or 80 μ. When the food is exhausted, they generally revert to a smaller type, even in the presence of blood serum. The increase in size of the cells during the first days of cultivation is probably due to the abundant food supplied by polymorphonuclear leucocytes, lymphocytes, and red corpuscles. Monocytes and macrophages also absorb with avidity minute fragments of dead muscle, or particles of precipitated
protein. Fragments of muscle, killed by heating or freezing and placed in the immediate vicinity of a colony of monocytes, are soon invaded by migrating cells which creep along them and begin their work of erosion. The muscle fibers assume the appearance of worm-eaten logs. They finally disintegrate under the repeated attacks of the monocytes, which migrate through the medium heavily charged with debris. As long as the cells congregate around the muscle fibers, they remain very large. After the supply of food is exhausted, they become smaller. A similar phenomenon takes place when drops of a protein precipitate are deposited near a group of monocytes. It seems as if discrete particles of proteins are better food than proteins in solution. However, when the fluid medium contains a minute amount of proteose, the rate of proliferation is also markedly augmented. If a larger concentration of proteose is used, the monocytes accumulate many fat globules and neutral red vacuoles, and die after a short time. It is significant that a concentration of proteose, which greatly increases the rate of growth of the fibroblasts, brings about the death of the macrophages.

Monocytes are endowed with a peculiar resistance to the action of arsenous oxide. When they are cultivated for 24 hours in a medium containing arsenous oxide at a concentration of 1/180,000, they die in large numbers. However, some of the cells resist the poison and multiply actively. A concentration of 1/200,000 is much less injurious. The cells proliferate in spite of having been treated by arsenous oxide, and after a few days the cultures assume a normal appearance. While macrophages resist arsenous oxide at a concentration of 1/200,000, fibroblasts are killed when their medium contains arsenous oxide at a concentration of 1/800,000. If fragments of Rous sarcoma are placed in a medium containing 1/400,000 arsenous oxide, the fibroblasts and the degenerating sarcoma cells rapidly disappear and only normal macrophages survive. Tissue macrophages possess approximately the same food requirements and the same susceptibility to arsenous oxide as blood monocytes.

DISCUSSION.

The essential properties of the blood monocyte and the tissue macrophage appear to be identical, as shown by the appearance of the
colonies, their action on the medium, the mode of locomotion and the structure of the cells, their rate of growth, their food requirements, and their susceptibility to certain toxic substances. The cells differ slightly in the size and development of their segregation apparatus and mitochondria (Figs. 3 to 10). The radial arrangement of the neutral red vacuoles around the centriole, or rosette disposition, considered by Sabin, Doan, and Cunningham as being characteristic of the monocyte (Figs. 3 and 16), is frequently observed also in the tissue macrophage (Figs. 9, 17, and 18). In normal macrophages, as well as in monocytes, mitochondria are always present (Figs. 12 and 17). Monocytes and macrophages cultivated in media of identical composition become identical in structure. There is no difference between a monocyte that has been kept for a few days in plasma and embryo juice, and a tissue macrophage (Figs. 5, 7, and 10). Both cells have approximately the same size; their segregation apparatus is well developed; the neutral red vesicles may be arranged in a rosette around the centriole or scattered through the cytoplasm; and the mitochondria appear as short filaments. The disposition of the neutral red vesicles around the centriole, their size and number, the condition of the mitochondria, and the presence of the fat globules, are determined by the composition of the culture medium and not by the origin of the cells. A monocyte supplied with an abundance of food in a solid medium becomes a macrophage (Fig. 7). A macrophage is merely a monocyte in a more active metabolic condition.

The polymorphism of the cell was further ascertained by experiments in which macrophages derived from monocytes or obtained from adult connective tissue were caused to revert to a less active metabolic state. When blood macrophages (Figs. 12, 14, and 16) were cultivated in Tyrode or Ringer solution, the starved cells became smaller (Figs. 11, 13, and 15), the rod-like mitochondria assumed the appearance of dots, the neutral red vesicles decreased in number and diameter, and their segregation granules disappeared. A similar phenomenon was observed when tissue macrophages (Fig. 17) from the subcutaneous connective tissue of adult chickens were cultivated in nutrient and

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non-nutrient media. The starved tissue macrophages grew smaller (Fig. 18), lost part of their segregation apparatus, reduced their mitochondria, and became almost similar to monocytes freshly removed from the blood. It is obvious that macrophages can revert to a form closely resembling monocytes, when they are deprived of food. These facts completed the demonstration that monocytes and macrophages are the expression of various metabolic states of a single type. Monocytes, macrophages, and fibroblasts are closely related forms. While the transformation of a monocyte into a macrophage is easily brought about by an abundant food supply, that of a macrophage into a fibroblast requires the presence of substances of unknown nature, probably set free by dead cells.5

Between monocytes and macrophages, the main difference is one of size (Figs. 3 and 10). In both cells, the mode of locomotion and the movement of the cytoplasmic organs are identical. Both cells are surrounded by an undulating membrane which moves unceasingly (Figs. 6 and 9). The external boundary of this appendage is irregular and invisible with direct light. This characteristic distinguishes the macrophage from the fibroblast which is limited by a sharp line of geometric design. The surface of the fibroblast is rigid, and the motion of the protoplasm takes place only at the end of the processes. When a macrophage begins to lose its activity, its sides become limited by a sharp, regular line, while the undulating membrane remains present at both ends (Fig. 8). The structural characteristics of fibroblasts and macrophages which are clearly noted in cinematographic films explain the differences observed in the constitution of the colonies. The fibroblasts that move only through their end processes can come into close contact at their sides. They form circular colonies where each component cell flows radially toward the periphery. On the contrary, macrophages are incapable of remaining packed together because they are surrounded by a membrane which moves continuously. They progress far more rapidly than the fibroblasts, and scatter through the coagulum without forming any dense colonies.

Both macrophages and fibroblasts possess a segregation apparatus which increases in size when the cells receive an abundant food supply.

(Figs. 3, 6, and 7). At the same time, the mitochondria augment in length and number. The segregation apparatus of the macrophage is far larger than that of the fibroblast on account of the greater metabolic activity of the former cell. When macrophages are fed on certain split products of proteins, or on fragments of dead muscle, they multiply more actively and develop more numerous and larger neutral red vesicles which generally contain small granules. If they are starved in Tyrode or Ringer solution, the neutral red vesicles lose their granules, decrease in size and number, and the cells themselves become smaller. In the macrophage, as in the fibroblast, the development of the segregation apparatus and of the mitochondria depends on the composition of the pericellular fluid.

The ability of the macrophage to multiply when fed upon blood serum or particles of proteins, and the fact that it dies in pure embryo juice, sharply distinguish this cell from the fibroblast. The fibroblast stops multiplying in blood serum and does not phagocyte other cells and fragments of proteins. It may feed upon substances set free in the medium by leucocytes and thyroid gland, and proliferate indefinitely in plasma and embryonic juice. A similar phenomenon occurs with proteose which, at a certain concentration, promotes the growth of fibroblasts and kills the macrophages. A third property distinguishing the macrophage from the fibroblast is a different susceptibility of the cells to arsenous oxide. The fibroblast dies in a concentration of arsenous oxide which is completely innocuous for the macrophage. The causes and the mechanism of the contrasting characteristics of the two types of connective tissue cells are still unknown.

It is certain that the innate physiological properties of the macrophages determine their behavior within the organism. The knowledge of these fundamental characteristics necessarily throws some light on the mechanism of the phenomena in which the cells participate. Macrophages are rarely present in the blood, and monocytes are not

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8 Carrel, A., unpublished experiments.
found in the subcutaneous connective tissue. In the blood, as well as in the tissues, the structure of the mononuclear cell depends upon the composition of its medium. It is probable that the nutrition of spherical monocytes in suspension in a fluid is not as active as when the cells, stretching themselves on the fibrin framework of a coagulum, greatly increase their surface. The only response that the large mononuclear can make to the physicochemical conditions of the circulating blood is probably to assume a monocytic form. On the other hand, when monocytes migrate from the capillaries into the tissues, they automatically become transformed into macrophages, as they do in a solid medium containing an abundant food supply.

The abnormal multiplication of monocytes and macrophages in certain diseases is probably due to an increase in their food supply. The formation of an exudate richer in proteins than normal lymph, the death of leucocytes, lymphocytes, or red corpuscles, the presence of blood cells in the tissues, the destruction of groups of cells by toxins, bacteria, and parasites, certain split products of proteins, etc., may give to monocytes and macrophages the food required for their proliferation and bring about monocytosis, as well as local accumulation of macrophages in the tissues. Like amebae, macrophages utilize for their nutrition the nitrogenous particles that they phagocyte. The various substances taken up by the cells are elaborated in their rhagiocrin apparatus. The neutral red vesicles increase in size and segregate a number of granules, which disappear if the colonies are starved. The substances manufactured by the rhagiocrin organs are probably used by the cell itself, or set free in the medium. Some of the products of the macrophages consist of substances which promote the multiplication of epithelial cells and fibroblasts and are analogous to those present in embryo juice. Monocytes may secrete these substances in the culture medium and stimulate the growth of colonies of fibroblasts. They transform proteins from serum and from the nitrogenous material of living or dead cells into food which they may bring to fixed tissue cells. The nature of their secretions depends

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10 Renaut, J., Arch. anat. micr., 1906-07, ix, 495.
possibly upon the composition of the substances they ingest. It is probable that the products of macrophages fed upon muscle fibers or bacteria are not identical. Antibodies may be a mere by-product of the nutrition of the macrophage. However, it is not known in what measure the cells use bacterial proteins for their own nutrition or even whether they use them at all. They can simultaneously secrete natural hemolysin and growth-promoting substances for epithelial cells and fibroblasts, oppose a foreign invader, and stimulate the tissues of the organism. The secretion of growth-promoting substances may explain the resumption of the activity of epithelial cells and fibroblasts in cicatrization, regeneration, sclerosis of organs, and tumor growth. However, proliferation of resting fibroblasts and epithelial cells can also be determined by certain split products of the protein molecule. The proliferation of cells within the inhibiting humors of the adult organism may be due to the production of proteoses by degenerating material as well as to the setting free in the tissues of proteins of the embryonic type. The secretion of ferments is not observed to take place in pure cultures of macrophages. The macrocytase of Metchnikoff remains inside of the cells or, if it is set free, the conditions of the medium do not allow any manifestation of its activity. When macrophages are transformed into malignant cells by Rous virus, they digest the fibrin of the medium and give to the coagulum its characteristic appearance. Proteolysis only occurs around sick or dead macrophages.

There is no doubt that the function of the macrophages is far more important than was thought by Metchnikoff. In some cases, they are the defenders of the organism against infections, but they may also be the direct source of the invasion of the tissues by a disease such as Rous sarcoma. The destruction by phagocytosis of cells and bacteria, the secretion of antibodies, the reproduction of Rous virus, and the genesis of sarcoma cells, are phenomena of secondary importance,—a pathological adaptation of general physiological properties. The main function of the macrophages is related to the nutrition of the

properties of fibroblast and macrophage. II

Their dual and opposite rôles in the war on, and the propagation of, disease are accessory phenomena.

This study of the biological characteristics of the fibroblast and the macrophage is still very incomplete. However, the methods by which this important aspect of cytology may profitably be investigated have been built up and can be widely used. They will give rise to a thorough knowledge of the fundamental properties of both types of connective tissue cells, and ultimately reveal the mechanism of the normal and pathological phenomena in which these cells are involved.

CONCLUSIONS.

1. Monocytes and tissue macrophages become identical in appearance when they live under identical conditions. Macrophages cultivated in nutrient and non-nutrient media acquire different structures. Monocytes and tissue macrophages are mere functional variations of a single type.

2. The structure of the segregation apparatus and of the mitochondria of monocytes and macrophages depends on the composition of the pericellular fluid and on the metabolic state of the cells.

3. The monocyte and the macrophage are endowed with a number of physiological properties which become apparent when they grow in pure cultures.

4. The knowledge of these fundamental characteristics explains the behavior of the cells within the organism.

EXPLANATION OF PLATES.

The yellow tone in the original drawings is omitted in reproduction.

PLATE 8.

Fig. 1. Culture 8740-D7. Polymorphonuclear leucocyte after 2 hours cultivation in plasma and embryo juice.

Fig. 2. Culture 8740-D7. Red blood corpuscle.

Fig. 3. Culture 8740-D7. Blood monocyte after 2 hours cultivation in plasma and embryo juice.

Fig. 4. Culture 8741-D1. Blood monocyte after 2 hours cultivation in plasma and embryo juice.

Fig. 5. Culture 8740-D6. Blood monocyte after 24 hours cultivation in plasma and embryo juice.
Fig. 6. Culture 8740-D14. Blood monocyte after 48 hours cultivation in plasma and embryo juice.

Fig. 7. Culture 8740-D15. Blood monocyte after 72 hours cultivation in plasma and embryo juice.

Fig. 8. Culture 8740-D19. Blood monocyte after 96 hours cultivation in plasma and embryo juice.

Fig. 9. Culture 3777-C. Tissue macrophage after 24 hours cultivation in plasma and embryo juice.

Fig. 10. Culture 3790-C. Tissue macrophage after 24 hours cultivation in plasma and embryo juice.

Plate 9.

Fig. 11. Culture 3386-C1. Blood monocyte cultivated in Tyrode solution for 4 days.

Fig. 12. Culture 3386-C2. Blood monocyte cultivated in serum for 4 days.

Fig. 13. Culture 3445-C1. Blood monocyte cultivated in Ringer solution for 4 days.

Fig. 14. Culture 3445-C2. Blood monocyte cultivated in serum for 4 days.

Fig. 15. Culture 3471-C1. Blood monocyte cultivated in Ringer solution for 3 days.

Fig. 16. Culture 3471-C2. Blood monocyte cultivated in serum for 3 days.

Fig. 17. Culture 3841-C1. Macrophage from subcutaneous tissue of an adult chicken after 24 hours cultivation in plasma and embryo juice.

Fig. 18. Culture 3841-C2. Macrophage from subcutaneous tissue of an adult chicken after 5 days cultivation in Tyrode solution.
(Carrel and Ebeling: Properties of fibroblast and macrophage. II.)
(Carrel and Ebeling: Properties of fibroblast and macrophage. I.)