PURE CULTURES OF LARGE MONONUCLEAR LEUCOCYTES.

By ALEXIS CARREL, M.D., and ALBERT H. EBELING, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATES 29 TO 37.

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I.

INTRODUCTION.

Pure strains of cells are as necessary in the study of physiological problems as pure cultures of microorganisms in bacteriology. If leucocytes and lymphocytes could be kept for a long time in active condition in media of known composition, many fundamental facts concerning their normal function in the organism and their reactions against bacteria and foreign material would be elucidated. In 1920, several attempts were made in this laboratory to obtain a pure culture of large mononuclear leucocytes. Finally, a strain was isolated from the blood of a hen in January, 1921, by one of us, and maintained in active condition for nearly 3 months. Since then, other strains have been cultivated in the same manner. The purpose of this paper is to describe the characteristics of the pure cultures, the morphological changes of the cells, and their response to certain modifications in the composition of the culture medium.

II.

Technique.

1. Preparation of the Cells.—The blood of an adult chicken which had fasted for 24 hours was taken in cold paraffined tubes through an oiled cannula or syringe and centrifuged at high speed for 10 minutes. After removal of the plasma, a few drops of diluted embryonic tissue juice were placed at the surface of the buffy coat of

leucocytes. 15 minutes later, the coagulum containing the white cells could be removed and placed in a watch-glass with a small amount of Ringer solution. Generally, the film separated into clumps from which almost all the red cells could be washed away. Small fragments were selected and taken from the fluid with the point of a cataract knife.

2. Preparation of the Medium.—Several different media were used. In some experiments, no embryonic tissue juice was used and the medium was composed of plasma alone, or of plasma and Tyrode solution. In other experiments, the medium consisted of two volumes of plasma and one volume of chick embryo juice, or of one volume of plasma, one volume of chick embryo juice, and two volumes of Tyrode solution. Sometimes 20 per cent fibrinogen suspension was substituted for the plasma. In order to prevent the contamination of the white cells by fibroblasts or other cells, the tissue juice was diluted with Tyrode solution and centrifuged for a long time. After the mixture of tissue juice and plasma had been made, the culture medium was examined microscopically and the presence of any contaminating cells could be detected easily. In other cases, the tissue extract, before being used, was kept in cold storage for several weeks until the contaminating cells were dead.

3. Preparation of the Cultures.—The culture medium was placed on a cover-glass of mica, and a small fragment of the leucocyte film was embedded in it before coagulation. A certain relation should exist between the number of white blood corpuscles and the volume of the medium. If the leucocytes were too few in number or if the medium was overcrowded by the cells, the result was negative. When the culture was properly prepared, a great many ameboid cells were seen in the medium after 24 hours. Then the original fragment, which still contained a large number of cells, and the invaded area of the coagulum were divided into several parts and washed in Ringer solution. The section of the coagulum had to be made with greater care than in cultures of fibroblasts. If the knife was not very sharp and the edges of the coagulum were crushed or slightly folded, the cells could not migrate into the new medium. They multiplied in the old coagulum and died. After the first passage, the transfer into a fresh medium was made every 48 hours and even every 3rd, 4th,

or 5th day. The cultures were examined while living, and drawings and photographs were made of the cells. Some of them were stained with neutral red in concentrations varying from 1:10,000 to 1:40,000. A few cultures were fixed in warm Ringer solution containing 4 per cent of formalin, and stained with hematoxylin-eosin, Giemsa's stain, eosin and methylene blue, eosin-azure, and azure.

III.

RESULTS.

The first strain of large mononuclear leucocytes was kept in a condition of active life for almost 3 months. Some other strains were used for shorter periods of time.

1. Migration and Multiplication of the Cells.—After 24 hours, the fragment of coagulum containing the white blood corpuscles was surrounded by a very large number of cells which sometimes invaded the entire area of the medium (Fig. 1). The outer zone was made up of small ameboid cells. In fixed and stained cultures they appeared as polymorphonuclear leucocytes which are pseudo-eosinophilic. The inner zone consisted chiefly of larger ameboid cells. Some of them were very large and more or less elongated and branched, with reticular pseudopods. The others were smaller, more rounded in form, and emitted active filiform or lobar pseudopods. In the preparations stained with eosin-azure, these cells contained a darkly stained nucleus and some showed blue granules in their cytoplasm. They were lymphocytes and large mononuclear leucocytes. Some polymorphonuclear cells were also present in the inner zone. After 24 hours, fragments of coagulum containing the ameboid cells were extirpated from the cultures and placed in a new medium. The cells migrated almost immediately from the old into the new coagulum. They consisted of polymorphonuclear leucocytes, lymphocytes, and large mononuclears, but the number of polymorphonuclear leucocytes had markedly decreased (Fig. 2). If the cells were too closely packed in the old coagulum, their migration into the new medium was slow and death often occurred. After the third or fourth passage, no granular leucocytes remained in the cultures, which were composed merely of lymphocytes and large mononuclear cells. Later, the lymphocytes disappeared also (Fig. 3).

The activity of the cultures was irregular. The migration of the cells (Figs. 4 to 6) from the edges of the old coagulum started from the 1st to the 4th day. When a sharp section of the coagulum was made in the zone of the migrating cells, the medium was invaded sooner. During the first 24 hours, the cells multiplied in the old coagulum and accumulated in large numbers at the periphery where they sometimes formed masses of amorphous material. Later, a few ameboid cells grew from the edges (Figs. 4 to 6) and more or less suddenly toward the 3rd day a large number of them invaded the new medium. In other cases, the invasion of the medium began on the 1st day. The cells were usually disposed in chains which followed almost parallel paths into the new medium on several different planes, but every chain was laterally isolated from the others. When the growing masses were very dense and the cells in contact, the cultures generally died. Often chains of independent cells migrated far into the new medium. This phenomenon occurred generally after 2, 3, or 4 days, according to the technique used, the composition of the medium, and the previous activity of the cells. The multiplication of the cells was slow. However, a few mitotic figures were observed (Figs. 7 and 8). A normal culture was composed of a thick crown of elongated and branched cells, while the central part of the coagulum was practically free of cells. When the old coagulum contracted, or when the crushing or folding of its edges prevented migration in a measure, living cells and apparently dead material were observed in the center of the culture.

The general appearance of the cultures was strikingly different from that of a fibroblast culture. The cells had no tendency to form a tissue (Figs. 2 and 3), but always remained isolated. If they happened to be packed together, they died. When colonies of leucocytes were grown in Gabritschewski dishes, their edges showed no tendency to unite (Fig. 9).

The large mononuclear leucocytes invading the culture medium were almost uniform in shape, elongated or branched, and of irregular form. Their posterior end was generally rounded, while the anterior end emitted very active reticular pseudopods (Figs. 4 to 6). The nucleus was darkly stained by azure while the cytoplasm was clear. In the living cultures stained with neutral red, the cells showed a

segregation apparatus consisting of spherical bodies, often as large and even larger than the nucleus. After each passage, chains of cells of the same type, more or less densely packed, again invaded the medium. But under the influence of many technical conditions, and especially the composition of the culture medium, their rate of proliferation was liable to decrease. The two first strains which were isolated in January, 1921, lost their activity at the beginning of April and died soon afterwards. Another is still active after 3 months of life *in vitro*.

2. *Differentiation of the Large Mononuclear Leucocytes.*—In actively growing cultures, the appearance of the cells remained uniform. The strains were composed of ameboid cells, with more or less irregular darkly stained nuclei, and lobar, filiform, or reticular pseudopods (Figs. 5 and 6) which wandered from the edges of the old coagulum into the new medium. However, important morphological changes sometimes occurred. They were observed for the first time in December, 1920. Leucocytes had been taken from the blood of a hen and cultivated in homogenic plasma without the addition of any tissue juice. The coagulum was soon invaded by a large number of ameboid cells, which wandered through it for 2 days. Some of the cells agglutinated in clumps. At the periphery of, or within the clumps, a few cells modified their shape. The pseudopods disappeared and were replaced by sharp processes. On the 3rd day, fusiform and stellate cells resembling fibroblasts were seen in some of the clumps. There was no possible contamination of the culture by fibroblasts from another source because the cells had been taken from the blood and no tissue juice had been added to the plasma composing the medium.

The same phenomenon (Fig. 10 *a* and *b*) was observed subsequently in many cultures. It occurred generally when the migration and the multiplication of the lymphocytes were not very active. The transformation of the cells started in the old coagulum and its origin was multicentral. The cells which showed the first evidence of a change were the elongated lymphocytes with clear cytoplasm and reticular pseudopods. The central end of the cell lost its pseudopods and became a pointed and immobile process, while the peripheral end was still actively ameboid. These transition forms were apparently half fibroblasts and half ameboid cells. Sometimes the nucleus of an

ameboid cell lost its character of being darkly stained by azure, and became pale and oblong with two nucleoli like that of a typical fibroblast. Similar changes occurred in polygonal and branched cells which showed simultaneously fixed processes and reticular pseudopods. At the same time, typical macrophages (Fig. 11*b*) were seen at the periphery of the culture and sometimes in the immediate vicinity of the transition forms and the fibroblasts (Figs. 10*b* and 11*a*). The fibroblasts appeared often near the amorphous masses resulting from the accumulation of lymphocytes on the edges of the old coagulum. Pointed processes seemed to grow from this apparently necrotic material, and after a few hours, fusiform cells with slender processes could be seen. In a few cultures, the fibroblasts migrated from the old coagulum into the new medium where ameboid cells were still wandering (Fig. 10*b*). They were spindle-shaped or stellate cells with an outline not sharply defined, anastomotic processes, pale homogeneous cytoplasm, large oval nuclei, and generally two nucleoli (Fig. 12*a* to *d*). Of four cells united in a chain, two resembled spindle-shaped fibroblasts, the third was half fibroblast and half ameboid, and the fourth was a large mononuclear leucocyte. In this case it seemed that a chain of large mononuclears was being changed into fibroblasts, the mutation beginning in the central part of the coagulum. In other cultures, several fibroblasts were united by long processes in a reticulum.

Cultures which contained large mononuclears, transition forms, and cells resembling fibroblasts were stained with a weak solution of neutral red. The ameboid cells displayed large red granules, which sometimes filled the cytoplasm almost completely. Among the transition cells, some showed a large segregation apparatus and others small red granules. The fibroblastic forms presented very much finer granules, and some were completely free of them. In the latter case, the nucleus was oblong and clear, contained two nucleoli, and looked like the nucleus of a fibroblast. At the same time that their appearance was modified, the behavior of the cells changed. Instead of remaining isolated or forming a chain, they became able to unite sidewise with other cells and showed a tendency to form a tissue, while lymphocytes lived like isolated elements. The cells were acquiring not only a new morphological appearance but also the

property of associating with homologous cells in the manner of fibroblasts.

3. Action of Embryonic Tissue Juice and Serum on the Activity of the Cells.—It seemed probable that lymphocytes and large mononuclear leucocytes would respond to the presence of embryonic tissue juice and serum in the medium as do fibroblasts. But the sensitiveness of their reaction had to be investigated.

(*a*) In the experiments in which the action of embryonic tissue juice was tested, two kinds of media were used. The first was composed of 33.3 per cent normal plasma and 66.6 per cent of a mixture in different concentrations of embryonic tissue juice and Tyrode solution. The second was made of fibrinogen suspension, serum, Tyrode solution, and embryonic tissue juice. Large mononuclears in a condition of low activity at the third or fourth passage were cultivated in media containing 2.5, 33.3, and 66.6 per cent tissue juice. In 2.5 per cent tissue juice, very little or no migration took place and death occurred after one or two passages. On the contrary, the medium containing 33.3 per cent tissue juice was invaded by a large number of lymphocytes. In 66.6 per cent tissue juice, the migration was still greater, but the cells died after a short time, possibly because they were too closely packed. Similar experiments were made with a medium composed of 20 per cent fibrinogen suspension and 10 per cent serum, the presence of serum being necessary to obtain a firm clot which would not dissolve secondarily. The action of 2.5 and 40 per cent embryonic tissue juice was compared. The number of migrating cells was very much larger in the medium containing 40 per cent tissue juice, but they died sooner than in the 2.5 per cent medium. It was evident that the cells were very sensitive to the influence of the embryonic tissue juice, and that its optimum concentration in the medium was about 30 per cent.

(*b*) The action of homologous serum was studied in media composed of 20 per cent fibrinogen suspension, 5 per cent tissue extract, and varied concentrations of Tyrode solution and serum. The cells were obtained from cultures at the third and fourth passages and cultivated in media containing 10 and 50 per cent serum. In the first medium, no migration took place. The cells located in the old coagulum became round, and very few showed active ameboid

movements. They died after the first passage. On the contrary, in the second medium the cells located in the old coagulum retained their activity and a large number migrated into the new medium. After they were transferred to fresh medium, they perpetuated themselves for several generations. Similar results were obtained in media containing 70 and 10 per cent serum. There was no doubt that serum had a marked inhibiting influence on the migration of lymphocytes and large mononuclear leucocytes.

IV.

DISCUSSION.

The cells growing in pure cultures were large mononuclear leucocytes of Ehrlich (endothelial leucocytes, monocytes of Naegeli, blood histiocytes of Aschoff and Kiyono, or hemomacrophages of Metchnikoff). The pseudo-eosinophilic and eosinophilic leucocytes were never observed to be present after five or six passages. The lymphocytes, that is the small and medium sized mononuclear cells of Ehrlich, were seen in large numbers during the 1st week and disappeared afterwards spontaneously. Possibly they were transformed into larger forms, as Maximoff[1] observed in his cultures of lymphoid tissue. Then the cultures were composed exclusively of large mononuclear leucocytes. These cells maintained their activity *in vitro* for nearly 3 months. However, their rate of multiplication was slower than that of fibroblasts. The medium was probably not the optimum one. It may also be supposed that they cannot grow in solid medium in dense masses as fibroblasts do. When they were packed in the coagulum, they usually died after a short time. The actively growing cultures showed a large number of chains of ameboid cells radiating from the original fragment through the medium. But the chains were not in contact laterally. It seemed that the cells created around themselves a field unfavorable to homologous cells. They always remained isolated, while fibroblasts and epithelial cells in pure cultures formed a tissue. The colonies of lymphocytes and fibroblasts when in contact assumed a strikingly different appearance. The edges of fibroblast colonies had a marked tendency to unite.

[1] Maximoff, A., *Compt. rend. Soc. biol.*, 1917, lxxx, 225.

In some cultures, the fibroblasts of a colony seemed to be attracted by the fibroblasts of the neighboring colony and a group of several colonies soon formed a continuous layer of connective tissue (Fig. 13 *a*). On the contrary, the peripheral edges of the leucocyte colonies never coalesced, even when growing in close contact (Fig. 13 *b*).

The large lymphocytes were more sensitive to all influences than the fibroblasts. The slightest error in technique brought about the death of the cultures. This peculiarity increased the difficulties of the experiments very much, because marked differences of growth were observed which were due merely to technical errors. However, the cells responded readily to physical and chemical modifications of the composition of the medium by changes in the rate of migration and in their morphology. As cells cultivated *in vitro* respond to an antigen by the production of an antibody,[2] the use of pure strains of macrophages may, therefore, become of interest in the study of immunity.

The large mononuclears became transformed into cells which assumed the appearance of fibroblasts. These cells were spindle-shaped or stellate, with long and slender processes uniting sometimes in a reticulum with those of other cells. When stained vitally with neutral red, they could be distinguished from the large mononuclears present in the same culture by the neutral red reaction of Evans.[3] The nucleus was similar to that of the fibroblasts. They had also acquired a tendency to tissue formation which lymphocytes do not possess. Besides the typical cells, there were many transition forms characterized by a nucleus less darkly stained and more oval, by the progressive substitution of sharp processes for the pseudopods, and by a decrease in the size of the segregation apparatus. The macrophages had evidently differentiated into fibroblasts. It is probable that Maximoff observed the beginning of a similar transformation in his cultures of lymphoid tissue.[1] He mentions that the large lymphocytes cultivated in hypotonic plasma and bone marrow extract underwent a modification. Their cytoplasm was larger and less basophilic, while the nucleus became paler.

[2] Carrel, A., and Ingebrigtsen, R., *J. Exp. Med.*, 1912, xv, 287.

[3] Evans, H. M., and Scott, K. J., *Carnegie Institution of Washington, Pub. No. 273, Contributions to Embryology*, 1921, x, 3.

The differentiation of large mononuclears into fibroblasts is merely the confirmation of previous observations. Long ago, Renaut[4] found that young connective tissue formed inside uterine cysts, completely lined with epithelial cells, and thought it derived from ameboid cells which had migrated through the epithelium. He considered that fixed connective tissue cells, endothelial cells, clasmatocytes, chromoblasts, vacuolar cells, and osteoblasts originated from an indifferent lymph cell. The transformation *in vitro* of connective tissue macrophages into fibroblasts came as a partial confirmation of this view. In 1912, pure cultures of ameboid cells from connective tissue became transformed into fibroblasts.[5] Cultures of peritoneal macrophages were observed by Maximoff[6] to undergo differentiation and to yield colonies of fibroblasts. Maximoff[6,7] never observed the transformation of polyblasts into fibroblasts, probably for technical reasons. Foot,[8] in cultivating white blood corpuscles of chicks, saw no real fibroblasts in the preparations. But in cultures of leucemic blood, Awrorow and Timofejewskij[9] found that the lymphocytes, in multiplying and undergoing differentiation, were converted into various forms—macrophages, giant cells, and even spindle-shaped and stellate connective tissue cells.

It is well known that the genetic relationship between the types of connective tissue cells is very close. Sabin[10] has shown lately that clasmatocytes and monocytes, that is large mononuclear leucocytes, are identical in origin. Their differentiation into fibroblasts is, therefore, a phenomenon which could easily be expected and was already considered as certain. Policard and Desplas[11] consider that fibroblasts derive from lymphocytes in granulating wounds. According to Dubreuil,[12] the large mononuclear leucocytes wander into and become fixed in the connective tissue, grow larger, and transform

[4] Renaut, J., Traité d'histologie pratique, Paris, 1893, i, pt. 2, 968.

[5] Carrel, A., *J. Exp. Med.*, 1912, xvi, 165.

[6] Maximoff, A., *Arch. Russ. Anat., Hist. et Embryol.*, 1916, i, 105.

[7] Maximoff, A., *Compt. rend. Soc. biol.*, 1917, lxxx, 237.

[8] Foot, N. C., *J. Exp. Med.*, 1913, xvii, 43.

[9] Awrorow, P. P., and Timofejewskij, A. D., *Virchows Arch. path. Anat.*, 1914, ccxvi, 184.

[10] Sabin, F. R., *Bull. Johns Hopkins Hosp.*, 1921, xxxii, 314.

[11] Policard, A., and Desplas, B., *Compt. rend. Soc. biol.*, 1917, lxxx, 745.

[12] Dubreuil, G., *Arch. anat. micr.*, 1913–14, xv, 53.

themselves into clasmatocytes or rhagiocrins. Then their segregation apparatus decreases, and they become fixed connective tissue cells. The differentiation *in vitro* of lymphocytes into large mononuclears observed by Maximoff,[7] and of the large mononuclears into fibroblasts recorded by us, is a confirmation of the ideas of Renaut and his school,[4,12] who consider the lymphocyte as the origin of all connective tissue cells. The transformation of large mononuclears into fibroblasts *in vitro* has more significance than the mere final demonstration of a phenomenon which was practically known. Thus, it shows that cell differentiation may occur *in vitro* under conditions which can easily be controlled. There is, therefore, a basis for the hope that the immediate cause of the change will be discovered.

The pure cultures of large lymphocytes were found to respond more readily than fibroblasts to the presence of embryonic tissue juice or serum in the medium. Lymphocytes and polymorphonuclear leucocytes displayed a similar sensitiveness to the same factors. This means that leucocytes are submitted to the influence of the two classes of substances which we found to be instrumental in increasing and inhibiting the rate of cell multiplication. The activating substances,[13] as is well known, are contained in embryonic tissues and also in muscle, epithelial cells, and white blood corpuscles of adult animals, in the aqueous extracts of these tissues, and probably in their secretions. Their presence in a culture medium greatly increases the activity of fibroblasts,[14] epithelial cells, and also leucocytes. If leucocytes could be activated *in vivo* by tissue juices as much as *in vitro*, important consequences should follow. The humors in which tissue juices or cell secretions are set free would acquire the power of increasing the activity of leucocytes, lymphocytes, and fibroblasts. Aqueous extract of inflamed connective tissue, and peritoneal exudates, containing white blood corpuscles, have been found to possess such a power.[15] The death of groups of cells within a tissue would also set free their juices and stimulate the activity of leucocytes and lymphocytes. Should white cells secrete more freely or undergo leucolysis, the substances which were set free would stimulate the

[13] Carrel, A., *J. Exp. Med.*, 1913, xvii, 14.

[14] Carrel, A., *J. Exp. Med.*, 1913, xviii, 287. Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 317.

[15] Carrel, A., unpublished experiments.

activity and multiplication of the macrophages and other cells. By this mechanism, the destruction of a number of leucocytes within the organism should automatically be followed by an increase in activity and by the multiplication of the remaining cells.

It is known that blood serum possesses the power of inhibiting the multiplication of homologous fibroblasts,[16] and that leucocytes respond still more readily than fibroblasts to the retarding influence of serum *in vitro*. Very likely, serum possesses a similar action *in vivo* because the number of leucocytes would increase indefinitely if serum were not endowed with this property. As a consequence of the sensitiveness of leucocytes to serum, these cells must be more active in fluids containing serum proteins under a lower concentration. The relative activity and migration of leucocytes in interstitial lymph, inflammatory exudates, and blood may depend in some measure on this property. It is also probable that the increase of the growth-inhibiting action of serum in the course of life determines a decrease in the activity of the white cells and modifies their secretions. Possibly this is one of the mechanisms by which the profound changes brought about by age in blood serum may be related to certain diseases of the period of senescence, such as cancer. On the other hand, it must be remembered that within the organism the leucocytes are placed in a very complex medium, and that their response to the action of serum and tissue juices may be modified and even prevented by other factors.

V.

CONCLUSIONS.

1. Pure strains of mononuclear leucocytes were isolated from the blood of adult chickens and kept in active condition for nearly 3 months.

2. The cultures were composed of large mononuclear leucocytes which migrated and proliferated *in vitro* at a slower rate than fibroblasts. The cells had no tendency to form a tissue, as do fibroblasts and epithelial cells. They were much less resistant than fibroblasts.

3. Differentiation of the large mononuclears into cells assuming the appearance of fibroblasts took place under certain conditions.

4. The activity of the large mononuclears was increased by embryonic tissue juice and inhibited by homologous serum.

[16] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 599.

EXPLANATION OF PLATES.

PLATE 29.

FIG. 1. 24 hour culture of leucocytes taken from the blood. In the lower left corner is seen part of the original mass of cells. The coagulum is invaded by a large number of cells. The inner zpne is composed chiefly of lymphocytes and mononuclear leucocytes and the outer zone of polymorphonuclear leucocytes. The intermediate zone which appears as a darker ring is occupied by large mononuclear ameboid cells. × 62.

PLATE 30.

FIG. 2. Second passage. The culture is still composed of mononuclear and polymorphonuclear leucocytes. The inner zone contains irregularly shaped, more or less elongated, ameboid cells, and the outer zone polymorphonuclear leucocytes, the number of which has decreased considerably. × 133.

PLATE 31.

FIG. 3. Twelfth passage. Pure strain of large mononuclear leucocytes. The polymorphonuclear leucocytes have completely disappeared. × 240.

PLATE 32.

FIGS. 4 to 6. Large mononuclear cells, with active pseudopods. × 1,000.

FIGS. 7 and 8. Mitotic figures in a pure culture of large mononuclears. × 1,000.

PLATE 33.

FIG. 9. Colonies of leucocytes in a Gabritschewski dish, showing their lack of coalescence. × 2.5.

PLATE 34.

FIG. 10. Culture 26330, eighth passage. (*a*) Pure strain of mononuclear leucocytes undergoing differentiation. There are a number of mitoses scattered throughout the preparation. × 100. (*b*) Higher magnification of the same culture. × 250.

PLATE 35.

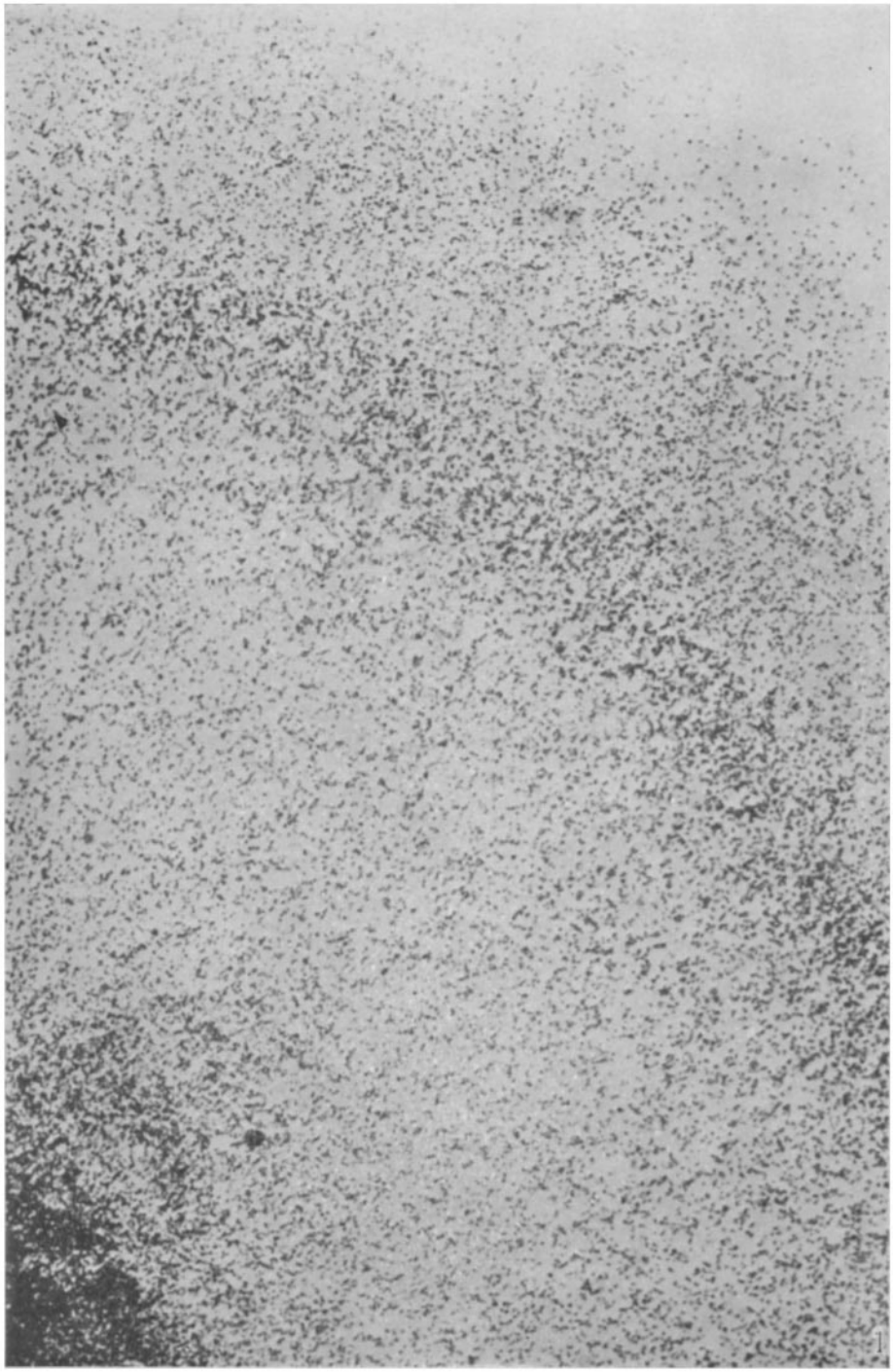
FIG. 11. Culture 26330, eighth passage. Higher magnification of two neighboring cells from the culture shown in Fig. 10. (*a*) Large mononuclear assuming the appearance of a fibroblast. × 1,000. (*b*) Large mononuclear, actively ameboid. × 1,000.

PLATE 36.

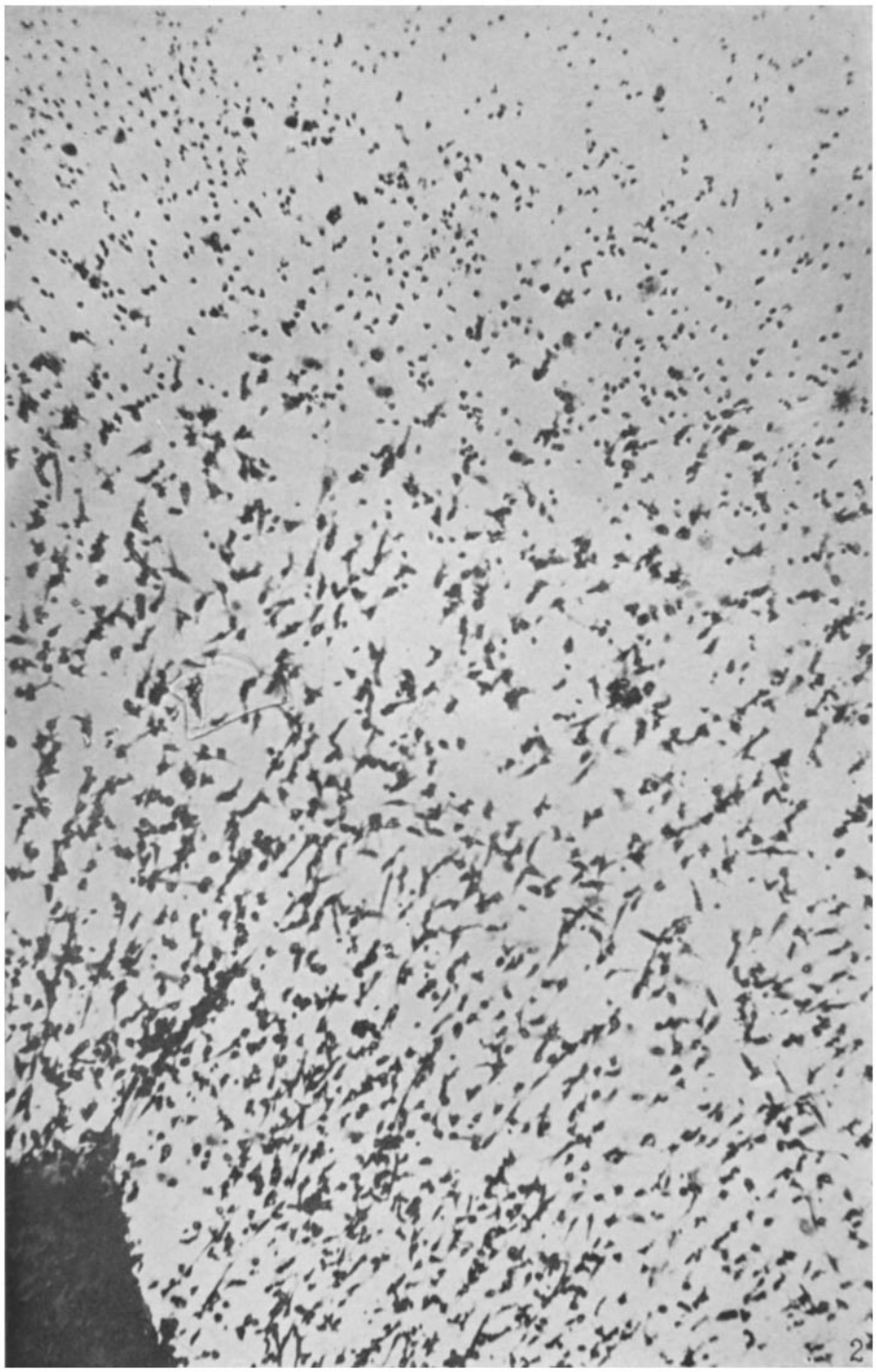
FIG. 12. (*a* to *d*) Large mononuclear leucocytes transformed into spindle-shaped and stellate forms. × 1,000.

PLATE 37.

FIG. 13. Semidiagrammatic drawing of colonies of (*a*) fibroblasts and (*b*) leucocytes. The colonies of fibroblasts have united at their edges.



(Carrel and Ebeling: Large mononuclear leucocytes.)



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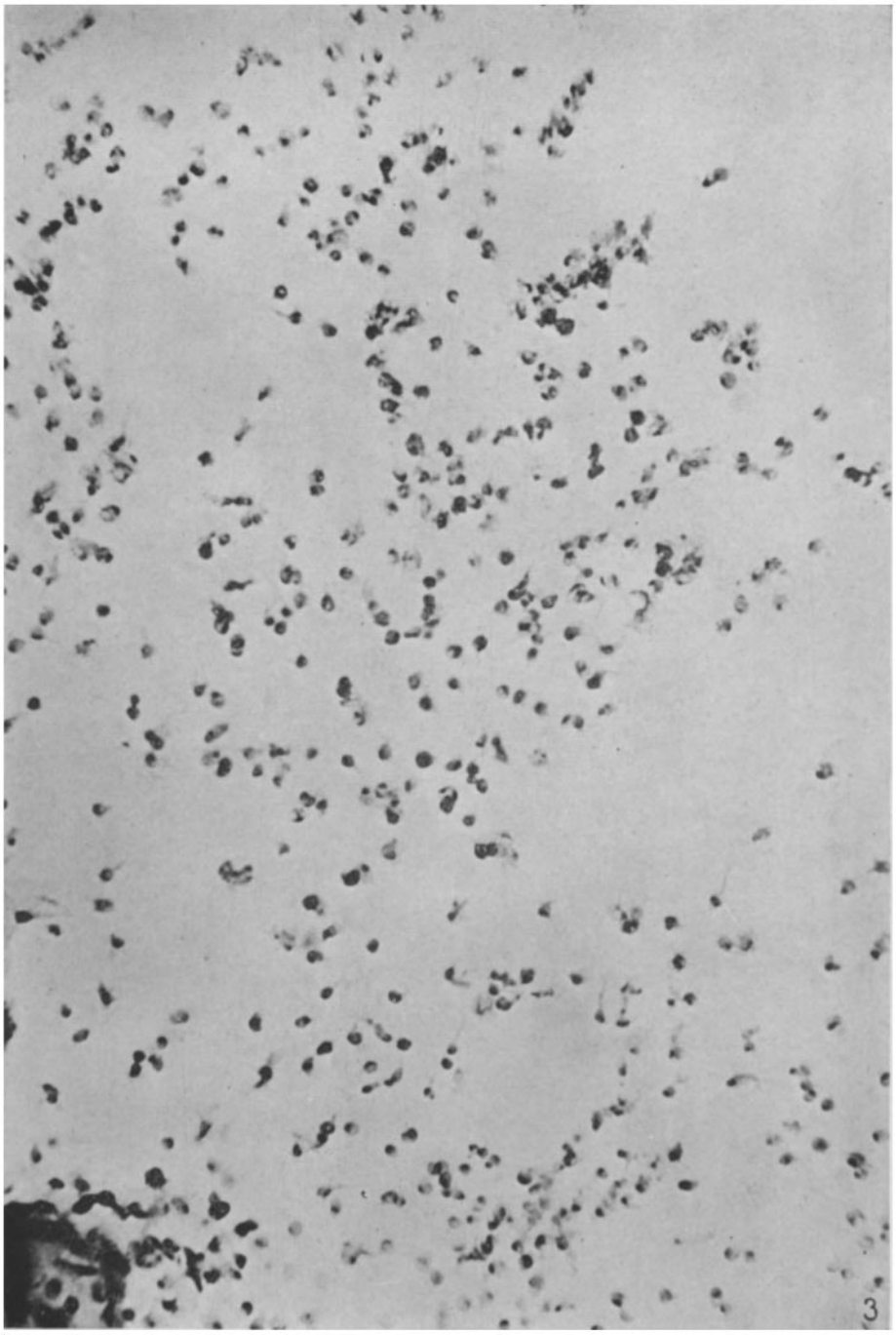

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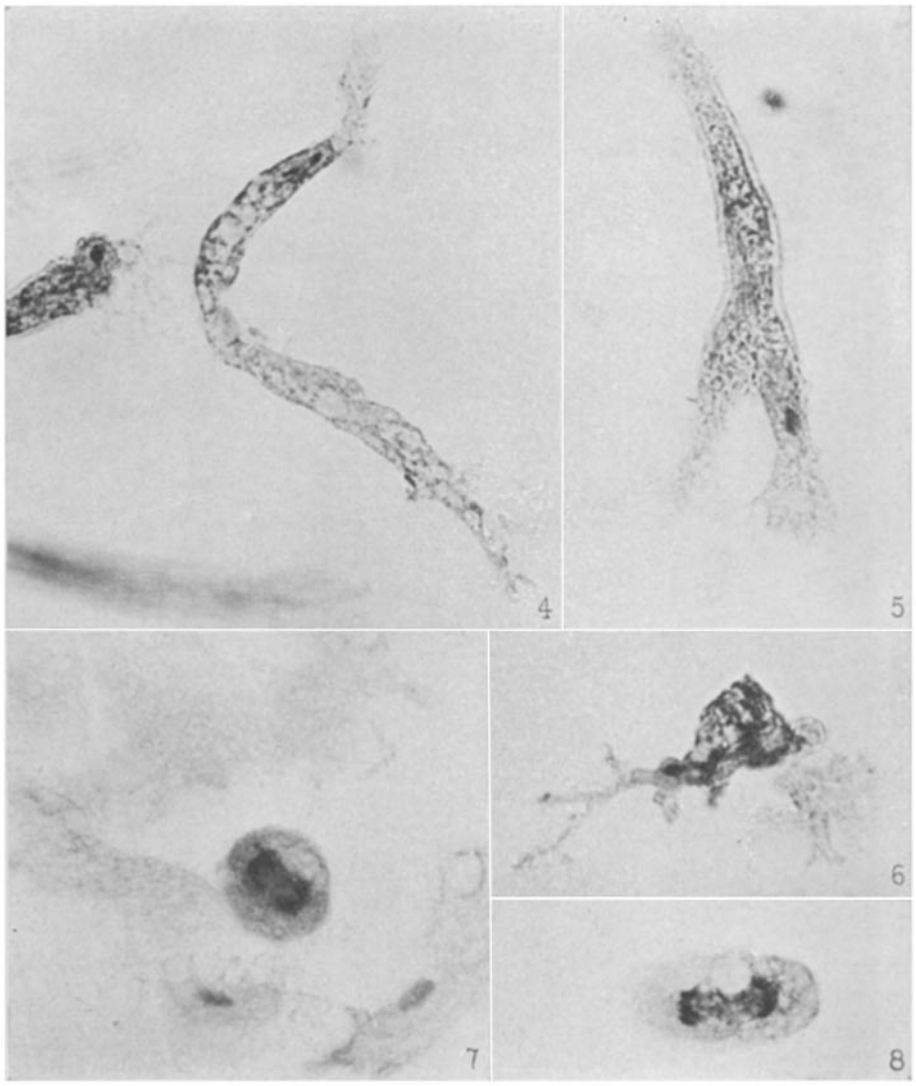

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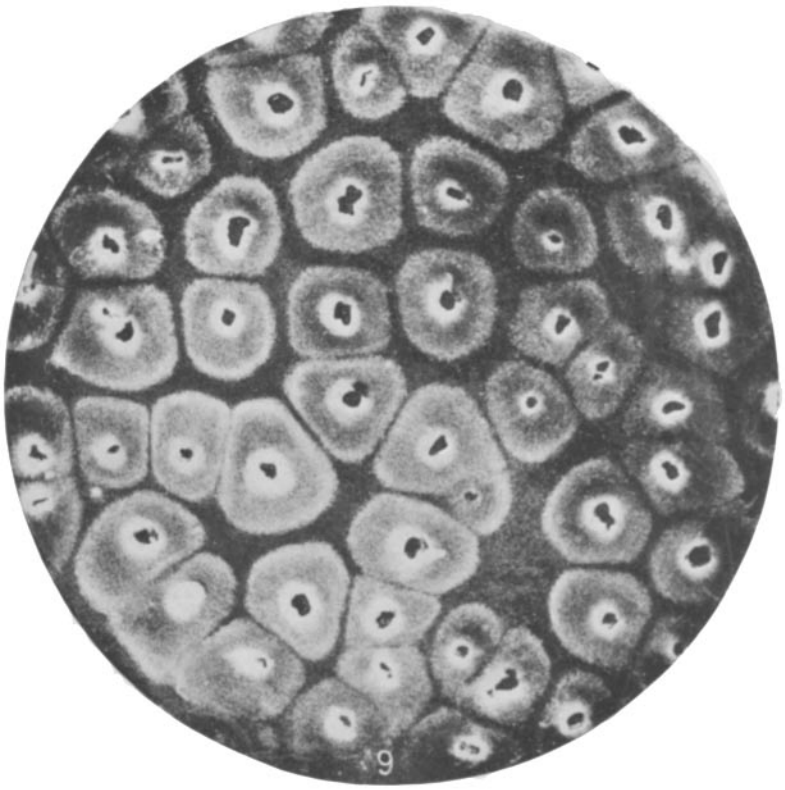

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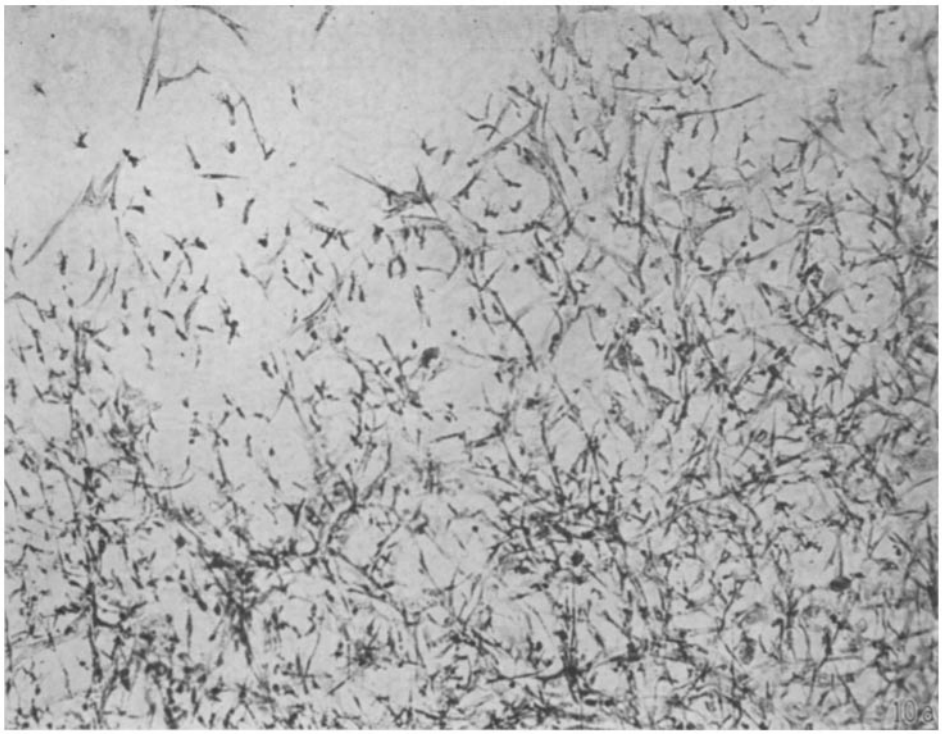

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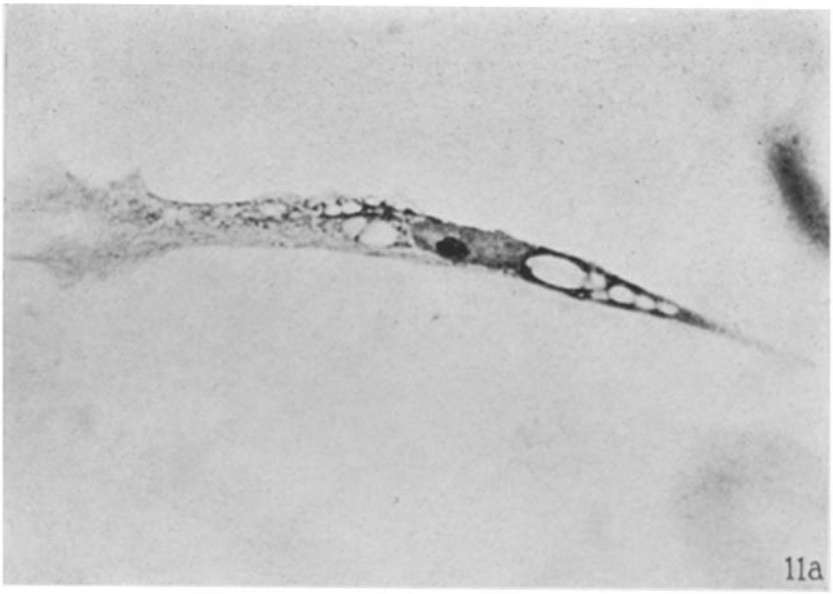

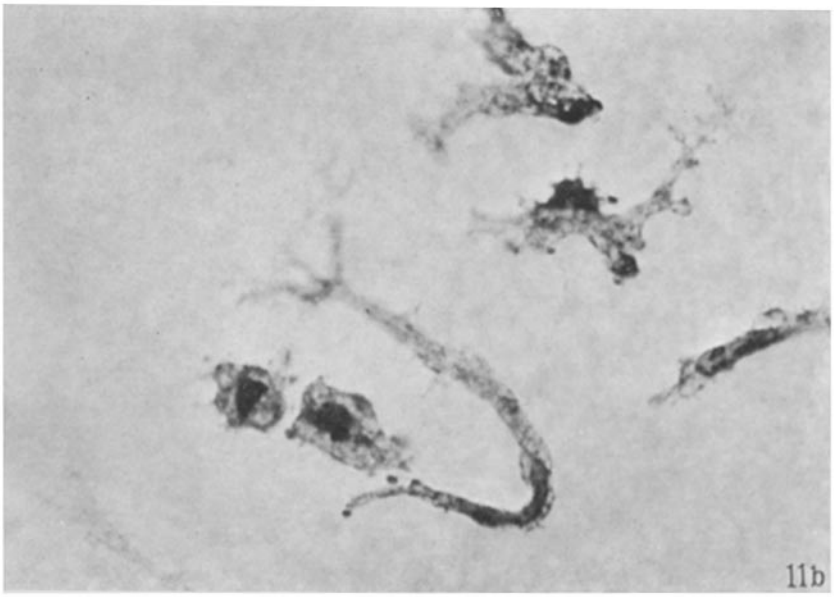

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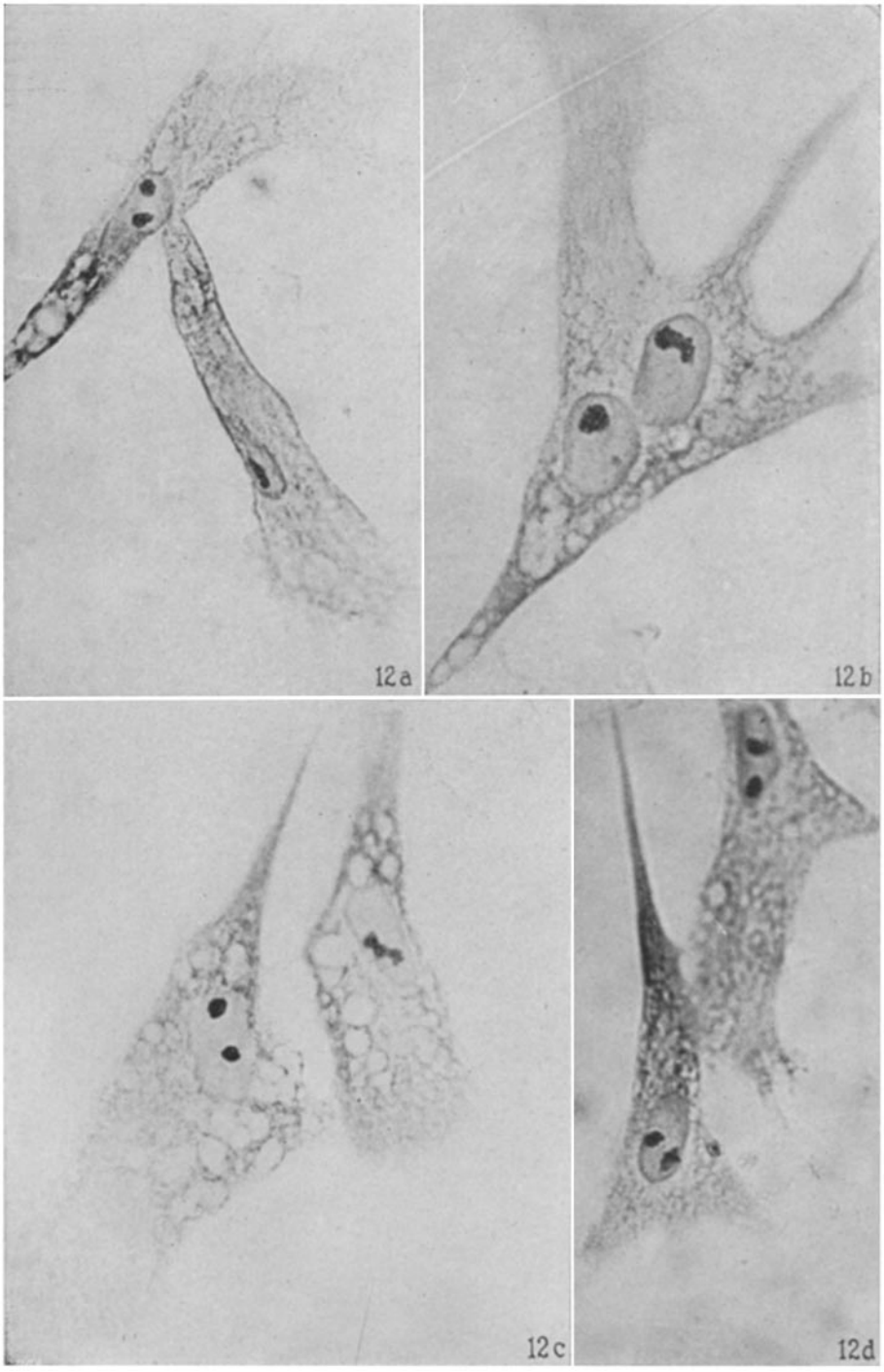

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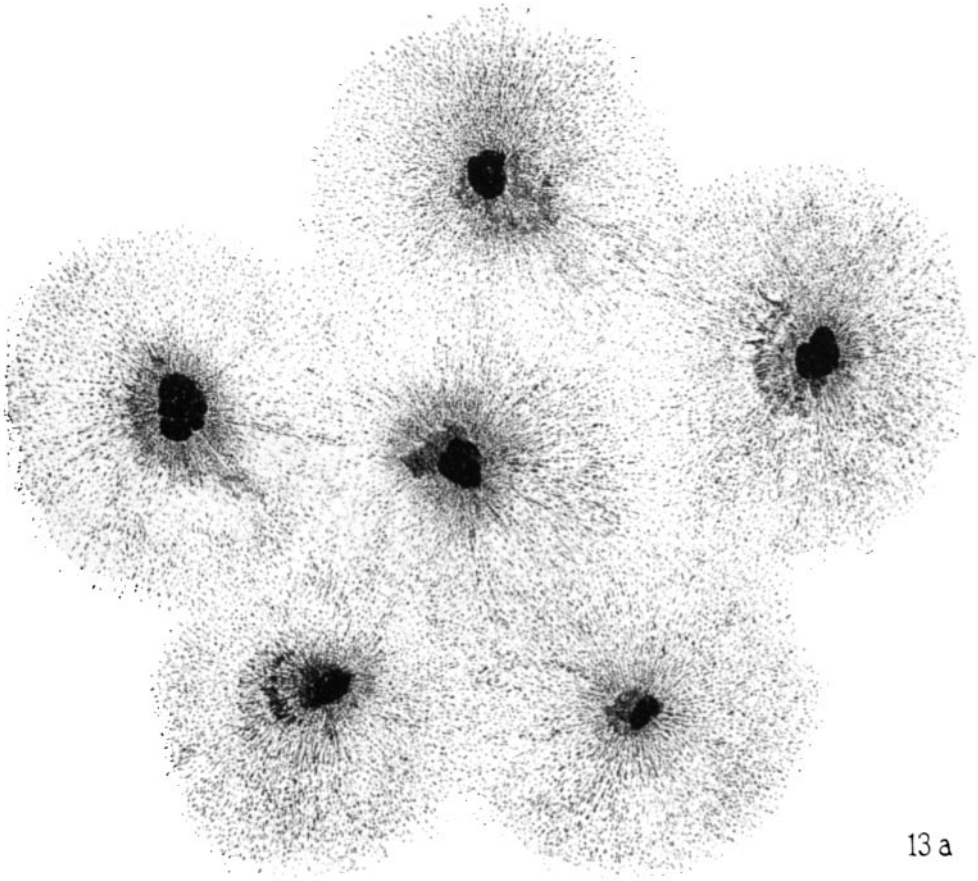
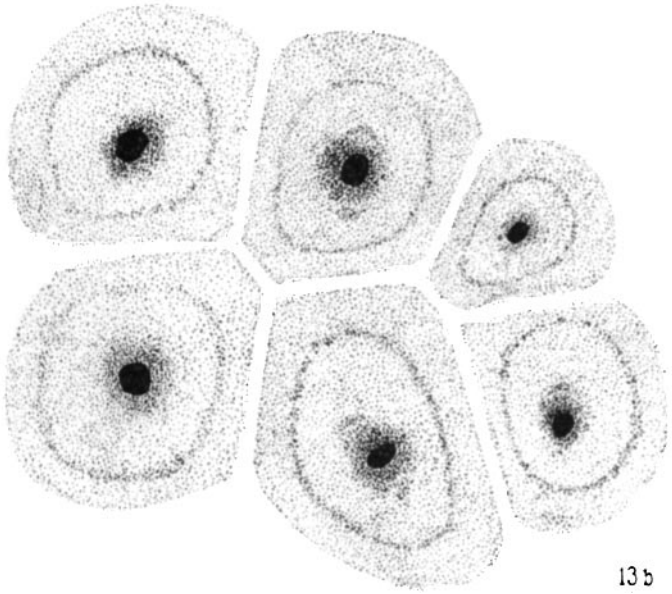


13 a



13 b

(Carrel and Ebeling: Large mononuclear leucocytes.)