MIGRATION OF LYMPHOCYTES IN PLASMA CULTURES OF HUMAN LYMPH NODES.

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Plates 10 and 11.

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In this study plasma cultures were made from normal and pathological lymph nodes obtained from operations at the Johns Hopkins Hospital. The method was as follows: Pieces were dropped into Locke-Lewis solution, and while in the solution were cut into small fragments 0.5 mm. or less in diameter. About 5 cc. of blood, withdrawn into a small paraffin-lined test-tube, were kept cool in a freezing mixture before centrifugalization. The centrifugalized blood was kept in the original tube surrounded by the freezing mixture and the plasma drawn off as the cultures were made. Cultures were made as rapidly as possible, in the usual manner, the number varying from 20 to 60 in the different series. Sometimes, however, 2 hours elapsed from the time the node was removed until the final cultures were made. The differences in time did not seem to affect the outgrowth from the explant. Both auto- and homoplasma were used, but no differences in the outgrowths were observed.

The lymphocytes were almost always the first cells to migrate out into the plasma clot. In autoplasma migration was observed within 1 to 2 hours, and in homoplasma $1\frac{1}{2}$ to $2\frac{1}{2}$ hours after the cultures were put in the warm box. The number of series of cultures was not sufficient to determine whether the initial latent period varies with the type of plasma and of tissue. The lymphocytes continue to migrate out into the clot for 2 or more days, or until practically all those which are living have left the explant. They migrate out into the plasma clot much more readily than onto the cover-slip over the liquefied areas. Liquefaction of the clot, as a rule, was delayed for

a day or two and varied greatly in extent. In some of the cultures however, the process began almost immediately after the cultures were made and in the course of an hour the explants were more or less surrounded by fluid. In these cultures lymphocytes were seen migrating out into the part of the clot which still remained attached to the explant. Comparatively few lymphocytes, on the other hand, migrated out onto the cover-slip over the liquid area. In some instances one portion of the clot was almost free from lymphocytes while another portion, attached to the explant, was full of them. Lymphocytes which came to the surface of the explant bordering the liquefied area fell to the bottom of this area, as many spherical lymphocytes were seen on the lower surface of the liquid.

The lymphocytes which appeared on the cover-slip over the liquefied area were generally associated with the large wandering cells (Figs. 6, 8, and 9) and with the giant cells. As the last two types of cells migrated out from the explant they occasionally carried with them one or more lymphocytes, the number sometimes being very large, which moved about on the under, free surface of the large cells and tended to remain there, rather than to wander off onto the cover-slip. Small lymphocytes could often be seen creeping around on a large giant cell, or moving back and forth in various directions, reaching the edge of the cell but rarely leaving it to proceed onto the cover-slip; they sometimes came to rest, assuming a rounded form, but still remained attached to the large cell. Apparently they adhered more readily to the flat wandering cells and giant cells than to the cover-slip. In fixed specimens the lymphocytes remained attached to these large cells (Figs. 8 and 9).

Lymphocytes sometimes tended to clump together in clusters of half a dozen or more. This clumping usually took place in the neighborhood of either small wandering cells or some foreign body. It resembled an agglutination without actual fusion of the cells. Only comparatively few of the lymphocytes tended to adhere together in this manner. The same phenomenon was observed in cultures (1) from normal nodes in both autoplasma and homoplasma, (2) from lymphadenitis specimens in autoplasma, (3) from tuberculous nodes in homoplasma, and (4) from metastatic sarcomatous nodes in homoplasma.

Paths of Migration.

In general, the lymphocytes migrated away from the explant and tended to spread out radially from it. When the route pursued by individual lymphocytes was observed in detail, however, it was found that they did not progress in direct radial lines away from the explant but moved in very irregular paths, as shown in Figs. 1 to 5. They sometimes made complete circles within a diameter of 0.033 mm. or less, or came almost to rest and turned and twisted about in a space of 0.006 mm. or less. They often migrated more or less rapidly for a few minutes, then came to rest, assumed a rounded form, and remained stationary for varying periods of time, from a few minutes to an hour or more. When the lymphocytes began to migrate again they did not always continue in the same direction. They crossed one another's paths without apparent deflection, or came into contact with one another without adhering. Contact with other cells or fibrous threads caused more or less deflection from the original course. It was not uncommon to see a lymphocyte come squarely against a strong fibrous thread with force enough to indent deeply the nucleus so as almost to divide it in two, remain there for a few seconds, and then slide off to one side or the other and continue its general course, the nucleus returning to its original form. The nuclei seemed semi-solid and very plastic, and continually changed shape as the cells met one obstruction after another; even when no obstruction was encountered, the nuclei underwent many changes in form as the cells twisted, turned, and elongated during migration. Sometimes the lymphocytes came up towards the cover, or penetrated into the depths of the clot, or migrated between the clot and the cover-slip; but as stated before, they generally migrated in the clot away from the explant. They did not appear to follow the fibrin threads but occupied the spaces between the latter. Migration from the explant continued until very few or only dead ones were left. There were always, of course, many more per cubic millimeter near the explant than towards the periphery of the clot, with occasional exceptions in old cultures.

TABLE I.

Series.	Distance traversed in successive min.	Rate of travel per min.		
		Maximum.	Minimum.	Average.
		mm.	*mm.*	*mm.*
(17) Carcinoma in homoplasma. 1½ hr. culture; 39°C. (Fig. 4).	(1) 8–4–13–9–8–2–5*	0.0173	0.0026	0.0093
(15) Normal node in homoplasma. 4 hr. culture; 38°C.	(2) 4–11–15–11	0.02	0.0053	0.0136
	(3) 11–13–10	0.0173	0.0133	0.0151
	(4) 9–15–13–4–7–9	0.02	0.0053	0.0113
(8) Metastatic sarcoma in homoplasma. 4 hr. culture.	(5) 18–12–12–18–12–17	0.0243	0.016	0.02
(12) Lymphadenitis specimen in homoplasma. 24 hr. culture; 38.5°C. (Fig. 3.)	(6) 4–15–6–6–7–12–9–8–9–12–17–12–8–9–5–14–21–13	0.028	0.0053	0.0133
	(7) 4–8–10–7–14–8–9–14–12–9–8	0.0186	0.0053	0.0126
	(8) 0–2–3–12–7–6–8–10–21–9	0.028	0.0026	0.0111
	(9) 8–12–8–5–14–9–6–10–13–9–9–8	0.0186	0.0066	0.0125
	(10) 5–7–6–10–5–6–2–9–9–7–11	0 0146	0 0026	0.0093
	(11) 18–15–12–?–?–?–11–15–12–22–7–8	0.0293	0.0093	0.0178
	(12) 8–5–17–5	0.0227	0.0066	0.0111
(16) Normal node in homoplasma. 24 hr. culture; 39–40°C.	(13) 8–7–10–9–8–8–8	0.0133	0.0093	0.0109
	(14) 8–5–9–8–7–6–8–7	0.0120	0.0066	0.0152
	(15) 4–10–5–8–10–13–6–5–8–11	0 0173	0.0053	0.0106
	(16) 17–11–12–6–15–18	0.024	0.008	0.0173
	(17) 7–12–3–8–4–10–3–5–11–8–5–6–15–12–11–8–5–9	0.02	0.004	0.0106
(13) Normal node in homoplasma. 20 hr. culture; 37.5°C. (Fig. 2).	(18) 6–9–6–5–4–7–5–0–0–0–8–6–3–6–7–7–10–3–6–6–2–7–2–8–5–0–0–0–0–2–2–2–1–8–6–7–0–0–0–0–5–5–0–0–0–0–0–0–2–4–4–5–6–0–0–0–0–0–0–0–	0.0133	0.0013	0.0069
(18) Tuberculous node in homoplasma. 48 hr. culture.	(19) 8–21–16–10–15–14–8–12	0.028	0.0106	0.0173

* The figures for the distance traversed are given in millimeters multiplied by 750.

TABLE I—*Concluded.*

Series.	Distance traversed in successive min.	Rate of travel per min.		
		Maximum.	Minimum.	Average.
		mm.	*mm.*	*mm.*
(8) Metastatic sarcoma in homoplasma. 48 hr. culture; 38.5–39.5°C.	(20) 8–14–12–12–?–?–0–0–0–0 for 30 min.	0.0186	0.0106	0.0152
	(21) 10–12	0.016	0.0133	0.0146
	(22) 9–8–8–7–10–7	0.0133	0.0093	0.0113
	(23) 18 (Fig. 1)	0.025		
	(24) 12–10–10–6	0.016	0.008	0.0126
	(25) 10–9–6–3	0.0133	0.004	0.009
(12) Lymphadenitis specimen in homoplasma. 44 hr. culture.	(26) 7–15–15–14–11	0.02	0.0093	0.0165
	(27) 13–1–5–11	0.0173	0.0013	0.01
(9) Tuberculous node in autoplasma. 4 day culture (Fig. 5).	(28) 6–6–5–5	0.008	0.0066	0.007
	(29) 5–5–5–4–2–3–5–7	0.0093	0.0026	0.006
	(30) 5–8–2–9–8–11–6	0.0146	0.0026	0.0093
	(31) 5–5–2–6–6–10	0.0133	0.0026	0.0096
(18) Tuberculous node in homoplasma. 7 day culture; 37.5°C.	(32) 10–12–9–6–5–8–3–4–3–8	0.016	0.004	0.0077
	(33) 7–5–6–8–11–11–10–12	0.016	0.0066	0.0116
	(34) 10–13–12	0.0173	0.0133	0.0154
	(35) 5–4	0.0066	0.0053	0.006

Rate of Migration.

The rate of migration was studied by camera lucida drawings made at minute intervals with a magnification of 750 or 1,250 diameters. Most of the lymphocytes migrated out of the field of the camera in a few minutes, so that the period of observation for any one lymphocyte was usually rather short. There was great irregularity in the distance covered during successive intervals. The greatest distance traversed during 1 minute was 0.03 mm., the maximum distances for the different lymphocytes varying from, 0.03 to 0.006 mm. The minimum varied much more; namely, from 0.013 to 0.0013 mm. per minute. This was to be expected, since lymphocytes often slowed down before they came to rest. The average rate varied from 0.02 to 0.006 mm. per minute. Determinations were made in cul-

tures from $1\frac{1}{2}$ hours to 7 days old, but no especial differences were noted in the rate of migration. Measurements were also made of lymphocytes (1) from normal nodes in homoplasma, (2) from lymphadenitis specimens in homoplasma, (3) from tuberculous nodes in auto- and homoplasma, (4) from metastatic sarcomatous nodes in homoplasma, and (5) from a metastatic carcinomatous node in homoplasma, but the rate of migration was essentially the same in all, as shown in Table I. The observations were not extensive enough to draw conclusions as to the meaning of the variations in rate in cultures of different ages or in the different combinations of plasma and explant. We have not been able to discover any differences in lymphocytes from normal and pathological nodes.

Hirschfeld[1] in 1901 observed the ameboid activity of lymphocytes from a case of lymphatic leucemia, using Deetjen's method by placing a drop of leucemic blood on a film of 1 per cent agar which contained 0.6 gm. of sodium chloride, 6 to 8 cc. of a 10 per cent solution of sodium metaphosphate, and 5 cc. of a 10 per cent solution of dipotassium phosphate, per 100 cc.

Structure.

The appearance of the cytoplasm of the living lymphocyte differed from that of the other cells. This was more noticeable in the elongated moving lymphocyte. It is difficult to describe other than that it was darker and less transparent. The cytoplasm seemed to be rather firm in consistency, since it rarely spread out into a very thin layer like that of the wandering cells. The lymphocytes in fresh cultures contained very few granules, and with neutral red only two or three granules at most became red. In older cultures there was a gradual increase in the number of granules which took up the neutral red. They were always small and did not seem to have vacuoles about them. The mitochondria were difficult to see unless colored with Janus green or Janus black No. 2. They were few in number, granular in form, and small. Lymphocytes often contained one or more fat globules (Fig. 1). In the migrating lymphocytes one could usually distinguish a homogeneous ectoplasm and a darker endoplasm which contained the granules and mitochondria.

[1] Hirschfeld, H., *Berl. klin. Woch.*, 1901, xxxviii, 1019.

The nucleus had a peculiar waxy appearance; it appeared to be homogeneous or structureless, and the nucleolus was very difficult to discern.

Shape.

The resting lymphocytes (Figs. 1, 6, and 7), and those that had fallen to the bottom of the liquefied area, were usually more or less spherical and presented the characteristic picture of a thin shell of cytoplasm enclosing a comparatively large nucleus eccentrically placed. The migrating or moving lymphocytes, on the other hand, had a peculiar, elongated shape, with the nucleus always at or very near the anterior or forward-moving end (Figs. 1 to 5 and 8 to 10). The cells seemed to possess a distinct polarity. The degree of elongation varied during successive minutes, but the nucleus was always near the anterior end with a small amount of ameboid cytoplasm in front which was continually changing shape and was sometimes so small in extent as to be scarcely visible. This anterior cytoplasm was homogeneous and free from granules. There was usually very little cytoplasm at the side of the nucleus; the bulk of it, in these migrating cells, lay behind the nucleus and was more or less finger-shaped, consisting of a thin ectoplasm enclosing an endoplasm which contained mitrochondria and granules. The nuclear region was often the broadest part of the cell, but the nucleus itself was very plastic and was continually changing shape, although it seemed to be more solid than the cytoplasm.

When the lymphocytes came to rest and assumed a rounded form, the large cytoplasmic tail formed the thicker part of the cytoplasmic rim and forced the nucleus into a slightly eccentric position; when the lymphocytes began to move and stretched out, this thickened part of the cytoplasmic rim formed the elongated tail. The resting as well as the migrating cells possessed a distinct polarity.

We were not able to distinguish a centriole or a central area as in the mesenchyme cells of chick embryos, so that it was impossible to determine whether polarity had any relation to a centriole. The living lymphocytes in the plasma clot were remarkably constant in size, shape, and structure, and showed no tendency toward transition to other types of cells. They were easily recognized, either at rest

or during migration, and their general appearance was so distinctive that they were never confused with the other types. Occasionally a large mononuclear cell was seen which resembled a lymphocyte in every particular except size. The general appearance of the cytoplasm and nucleus was similar. They migrated with the nuclear end first and were then elongated, but assumed a more or less rounded form when at rest (Fig. 4).

In fixed and stained preparations the lymphocytes found on the surface of the large wandering cells and giant cells, and more rarely on the cover-slip, showed a greater diversity of form than the living ones in the plasma clot (Figs. 8 to 10). This was probably due to the peculiarities of the environment during life, where they had a more or less flat surface to which they could adhere and upon which they could spread out.

Neither mitosis nor amitosis was observed in lymphocytes. All the lymphocytes studied were mononuclear.

SUMMARY.

1. Lymphocytes were usually the first cells to migrate out into the plasmic clot from explanted pieces of lymph nodes.

2. Their paths of migration were irregular but in general they proceeded away from the explant.

3. The lymphocytes migrated at rates varying from 0.03 to 0.0013 mm. per minute. The rate of any one varied from minute to minute, and they often came to rest for varying lengths of time.

4. The migrating lymphocytes were very much elongated, with the nucleus always near the anterior end. The elongated tail contained the endoplasm with a few granular mitochondria and usually a few granules which took up neutral red.

5. The lymphocytes in cultures made from normal and pathological lymph nodes in auto- and homoplasma showed no differences.

EXPLANATION OF PLATES.

PLATE 10.

FIG. 1. 48 hour culture from a metastatic sarcomatous node in homoplasma. A migrating lymphocyte (No. 23, Table I) at ½ minute intervals. The change from the migrating form to the resting form and back to the migrating is shown. Distance traversed during the minute 0.025 mm. × 1,250.

FIG. 2. 20 hour culture from a normal node in homoplasma. A migrating lymphocyte (No. 18, Table I) drawn at 1 minute intervals for 1 hour. The broken arrow points to the explant. × 750.

FIG. 3. 24 hour culture from a lymphadenitis specimen in homoplasma. Migrating lymphocytes in the same field drawn at 1 minute intervals. × 750.

FIG. 4. 1½ hour culture from a metastatic carcinomatous node in homoplasma. A migrating lymphocyte (No. 1, Table I) and a large lymphocyte drawn at 1 minute intervals. × 750.

FIG. 5. 4 day culture from tuberculous node in autoplasma. Four migrating lymphocytes in the same field (Nos. 28, 29, 30, and 31, Table I) drawn at 1 minute intervals. × 750.

PLATE 11.

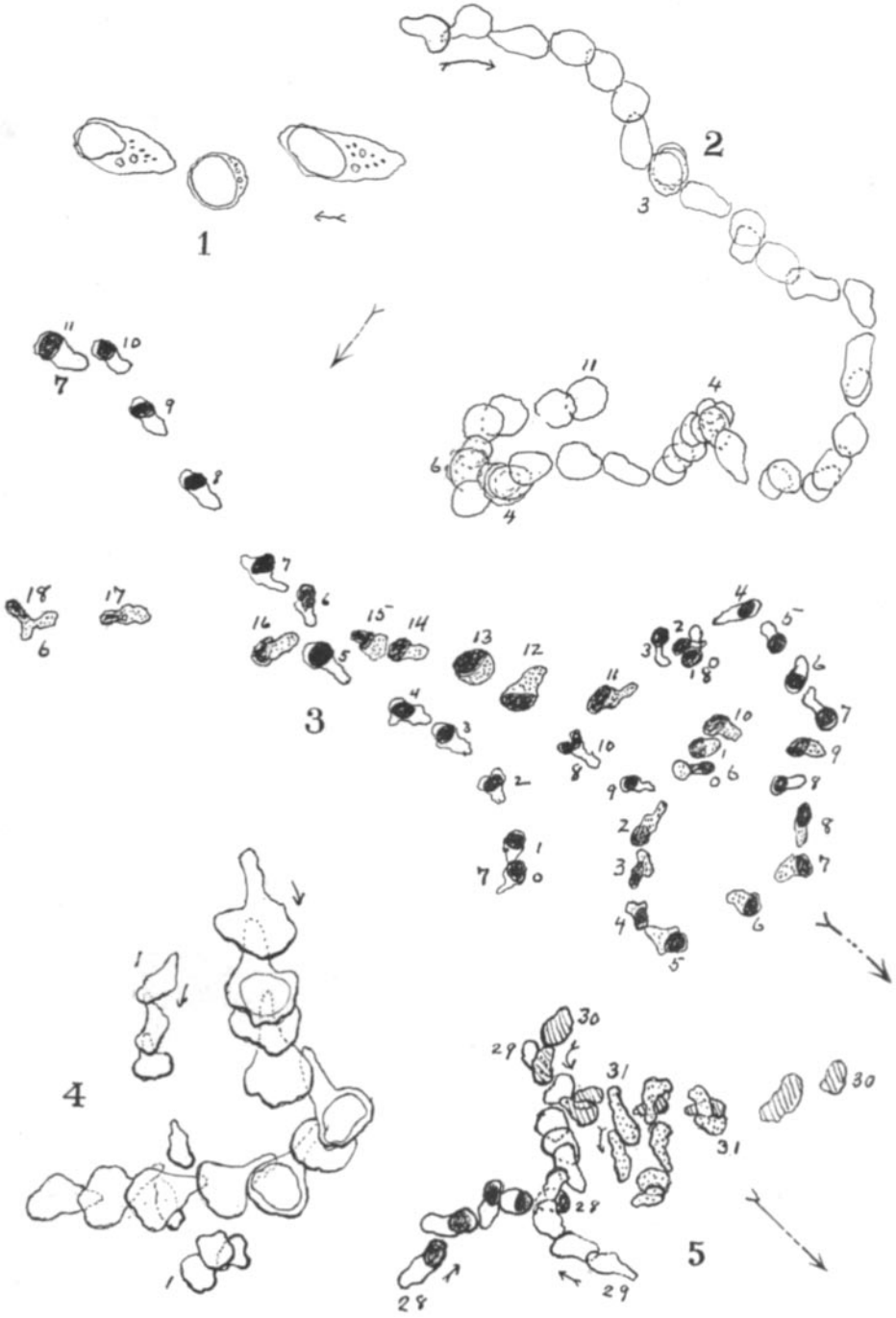
FIG. 6. 7 day culture from a normal node in autoplasma. Four resting lymphocytes and two large wandering cells on the cover-slip. Neutral red and Janus green were added at the time of observation. The lymphocytes showed neutral red granules and green mitochondrial granules. The large wandering cells had a deep red center (dark granular in the figure) near the nucleus, surrounded by an extensive area of fat globules. Mitochondria were abundant about the fat area and extended into the clear ectoplasm. × 750.

FIG. 7. 96 hour culture from a metastatic sarcomatous node in homoplasma. Two resting lymphocytes showing neutral red granules. Neutral red was added at the time of observation. × 1,250.

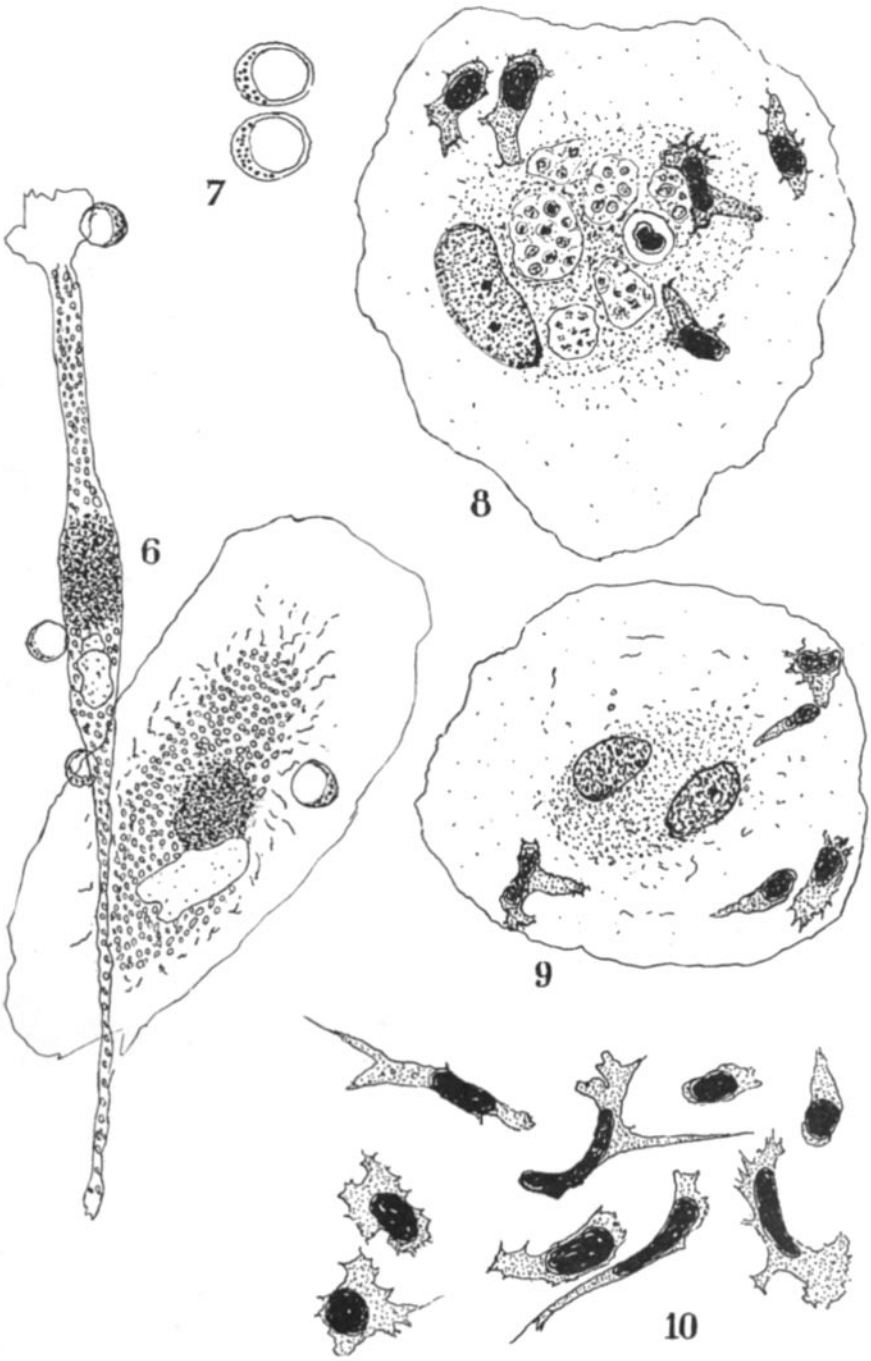
FIG. 8. Fixed and stained specimen from a 48 hour culture of a tuberculous node in homoplasma. Five migrating lymphocytes are shown on the surface of a large mononuclear wandering cell which is spread out on the surface of the cover-slip. The wandering cell contains dead lymphocytes in various stages of digestion. × 1,250.

FIG. 9. Five migrating lymphocytes on the surface of another large wandering cell from the same culture as Fig. 8. × 1,100.

FIG. 10. Nine migrating lymphocytes from the surface of other wandering cells from the same culture as Fig. 8. × 1,250.



(Lewis and Webster: Plasma cultures of human lymph nodes.)



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