THE ORIGIN OF MONOCYTES IN CERTAIN LYMPH NODES AND THEIR GENETIC RELATION TO OTHER CONNECTIVE TISSUE CELLS

By CLAUDE E. FORKNER,* M.D.

(From The Pathological Institute, Freiburg, i/Br., Germany)

PLATES 12 TO 15

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The purpose of this study was to determine the position of monocytes in certain lymph nodes and, if possible, to ascertain from what cells they arise. The writer (1) has shown in a previous communication that lymph nodes from different parts of the body exhibit marked differences in cytology and that developing monocytes may be found as normal constituents of all the lymph nodes of the rabbit, except the large mass lying in the mesentery of the intestine. At the time of the earlier communication, no reliable method had been found for demonstrating the relationship of monocytes within lymph nodes to other tissue elements. The method used in these observations, which has been fully described in another communication (2), gives true supravital staining of the cells, faithfully preserves this reaction in paraffin sections, and shows their relation to other cellular elements.

The origin of the monocyte has been a much debated question. Various theories to the effect that monocytes arise from myeloblasts, lymphocytes, endothelium, histiocytes, reticulo-endothelial cells, and from undifferentiated cells have been advanced. Each is supported by a certain amount of evidence. However, no clear cut experiments are recorded to show that under normal conditions any of these cells give rise to monocytes. The writer agrees with Maximow (3) that the development of monocytes in the blood-forming tissues—"with the exception perhaps of the red pulp of the spleen"—has never been demonstrated.

* Fellow of The National Research Council, Washington, D. C.

Description of Cells

The protocols to follow will state briefly what kinds of cells have been encountered in various tissues and will describe their morphological relations to each other. It seems expedient, therefore, to describe in some detail the cytological characteristics of the types of cells under discussion with special reference to supravital staining with neutral red and Janus green.

Primitive Undifferentiated Cells (Fig. 10, "c").—These elements can also be called mesenchyme cells. They are undifferentiated and possess multiple potentialities for the formation of various mesenchymal tissues. Entirely unstained by the supravital method, they are pale cells, with a round or oval nucleus and moderate cytoplasm containing no vitally stained granules. They have been described and illustrated by Cunningham, Sabin and Doan (4), by Forkner (1, 5), and others. They may be found in any of the blood-forming organs and probably possess multiple potentialities. In lymph nodes they doubtless represent the stem cells from which lymphocytes, monocytes, macrophages, and fibroblasts are derived.

Lymphocytes.—Maximow (6) believes that lymphocytes are not definitive cells, but are the stem cells from which various other cells develop. In the opinion of others, they represent a definitive cell. They appear as small, intermediate, and large forms which take very little or no neutral red into their cytoplasm when stained supravitally. Their nuclei are usually round, but may be oval, indented, or even horseshoe shaped. The chromatin is characteristically in heavy splotches. These cells usually have in their clear, hyalin cytoplasm a few round, globular, refractive, neutral red bodies. These bodies may at times be numerous and may even be arranged in the bay of the nucleus, in a rosette fashion about the centrosphere. However, the character of the neutral red granules, the character of the mitochondria, the type of motility, and the other general characteristics of the cells do not permit of their confusion with monocytes. Lymphocytes possess abundant mitochondria in their cytoplasm. These structures in themselves are of a different morphology than those in monocytes. They are larger and coarser. The mitochondria are apt to be in clumps near the nucleus, but at times are found scattered through the cytoplasm. The lymphocyte possesses quite a characteristic type of motility. It moves with the nucleus in the forward portion of the cell, as has been shown by Sabin (7).

Monocytes. (Figs. 1, 2, 4, 5, 6, 10).—Other terms which have been applied to these cells are: large mononuclears and transitionals of Ehrlich, endothelial leucocytes, and blood histiocytes. When stained with neutral red and Janus green, the monocytes of the rabbit possess features which permit of their easy differentiation from all other elements in the blood or tissues. They are large in size, as a rule, often exceeding all of the other blood cells in this respect. The

nucleus is often round or indented, more frequently kidney or horseshoe shaped. It possesses a delicate chromatin structure. The cytoplasm is not clear and hyalin, as in lymphocytes, but presents a foggy, or ground glass appearance. Within the cytoplasm are many neutral red bodies, the so-called "segregation apparatus." As Simpson (8) and Sabin (7) have shown, these bodies are, as a rule, arranged in a characteristic pattern, forming a rosette about the centrosphere. It is not uncommon, however, to find a diffuse distribution of these bodies in the cytoplasm. The neutral red bodies in monocytes possess a different shade of color from those in lymphocytes and also a different refractive power. They are, for the most part, non-refractive and are a salmon or brick red color. The mitochondria in monocytes generally appear much smaller than in lymphocytes and, as a rule, are more widely distributed. When a well-marked rosette is present, the mitochondria are frequently more numerous, or at least are better seen, at the periphery of the rosette. These cells have been illustrated by Simpson (8), Sabin (7), Forkner (1), and others.

Pre-Monocytes (Figs. 1, 2, 6, 7, 10).—This is a new term which is proposed for the purpose of designating one of the stages in the development of the monocyte. Prior to the present communication, no one has satisfactorily demonstrated the relations of monocytes to their precursors, under normal conditions, in any of the blood-forming organs. The demonstration in certain lymph nodes of the developmental cycle of the monocyte makes it necessary that a term be used to designate the stage of this cell intermediate between the monoblast and the mature monocyte. The pre-monocyte is characterized by the fact that it is constantly found in all the lymph nodes of rabbits, except in the large mass of lymphoid tissue lying in the mesentery of the intestine (Forkner, 1). It is similar to the adult monocyte of the blood in that it possesses somewhat the same morphological and biological characteristics when seen in supravital preparations. It differs from the adult cell in that the segregation apparatus is less well-developed and that it does not normally, except in rare instances, occur in the blood. The premonocyte is generally somewhat larger than the adult monocyte. Its nucleus is, as a rule, oval and is very faintly stained with the usual histological methods. The chromatin network is less well-developed than in the adult cell. The segregation apparatus consists of from one to several rows of very fine, non-refactive granules which may be arranged around a centrosphere or may be closely packed together in the center of the cell, often obscuring one side of the oval or round nucleus. These neutral red bodies are always in the region of the centrosphere and are never diffusely scattered throughout the cell. The mitochondria are abundant and are identical in morphology with those seen in mature monocytes. Doan and Sabin (9) described the maturation of monocytes in tuberculosis, using the terms monoblast, young monocyte, and monocyte. Their young monocyte corresponds to the pre-monocyte, except that they describe monoblasts and young monocytes in the bone marrow as having deeply blue basophilic cytoplasm as seen in fixed sections. The monoblasts and pre-monocytes which the writer has seen in lymph nodes do not possess a deeply basophilic cytoplasm when stained either with hematoxylin and eosin, or

with Giemsa stains. Their cytoplasm is very lightly stained and presents a very pale appearance

Monoblast (Fig. 10).—This term has been frequently used in the literature to designate the precursor of the monocyte. Bloom (10), however, in a recent paper, has made the statement that monoblasts do not exist. The demonstration in lymph nodes of monocytes in all stages of development allows one to shift the term monoblast from a more or less theoretical name to its proper place as a term designating that particular cell which is derived from a primitive undifferentiated cell and which is the precursor of the pre-monocyte. It is identified in preparations from peripheral lymph nodes by the fact that it is found in close association with pre-monocytes and monocytes in clumps of these cells. It appears to be identical with the pre-monocyte, except that it possesses no demonstrable segregation apparatus, that is, no supravitally stained neutral red bodies. It is easily demonstrable in paraffin sections of supravitally stained lymph nodes (Fig. 10).

Mesenchymal Macrophages.—There is no group of cells which has received more names, nor about which there is more confusing terminology than the cellular elements included under this term. The various terms present in the literature to designate the phagocytic cells under discussion are: macrophage, clasmatocyte, adventitial cell, resting wandering cell, rhagiocrine cell, reticular cell, reticulo-endothelial cell, endothelial leucocyte, pyroll cell, and histiocyte. The writer(11) has recently critically reviewed the literature on this subject and has proposed that we revert to the original descriptive term "macrophage" which was first applied to these cells by Metchnikoff (12,13). It was proposed to limit the term, conforming to our present knowledge, and to include only those cells of mesenchymal origin which possess the property of phagocytosis of débris, or of staining intensely with moderate doses of vital dye in the living state. Where particular groups of macrophages are to be discussed in this paper, they will be designated as: macrophages of the lymphoid tissue, macrophages of the adventitia, macrophages of the common connective tissue, macrophages lining the lymph sinuses, etc. Such cells, supravitally or vitally stained, have been abundantly illustrated in the literature by many investigators (14, 15, 8, 16, 1). They are cells of large size (Figs. 4, 9). The nucleus is usually near the center of the cell, is round or oval, and has a delicate chromatin network. The cytoplasm is abundant and possesses few or many irregularly sized and shaped bodies, scattered throughout without any pattern or definite arrangement. These bodies may represent débris which the cell has phagocytized, or in many instances, they represent vacuoles in which the neutral red or vital dyes have been assembled. The cytoplasmic structures are not only heterogenous in size and shape, but also frequently show various shades of staining. This is in contrast to the uniform homogeneous appearance of the cytoplasmic structures in monocytes. The macrophages lining the sinuses (reticulo-endothelial cells—Fig. 8) are similar in their reactions to dyes and foreign material. The latter cells are fixed, are often flattened or elongated, and can be selectively stained with vital dyes. By appropriate experiments, reported in the protocols of this

paper, it is shown that these cells are among the first to stain with vital trypan blue, whereas the phagocytic macrophages of the reticular syncytium and the free macrophages in the lymphoid tissue may be almost entirely unstained with few doses of this dye. However, this difference is probably dependent on the fact that the former, by virtue of their anatomical position, are more directly exposed to the material passing through the lymph node. It is also significant that the macrophages lining the lymph sinuses (reticulo-endothelial cells) and the phagocytic macrophages of the reticular syncytium are identified with the formation of reticulum fibers, whereas free macrophages are not.

Plasma Cells.—These cells should be described here because they frequently are found in lymph nodes of rabbits and other mammals. So far as the writer is aware, there has been only one description of these cells as they appear in supravital preparations. An analysis of the literature on this point will be considered in the discussion at the end of this paper. On the whole, they are about the size of intermediate lymphocytes, but may be smaller and are often somewhat larger. They possess, as a rule, more cytoplasm in relation to the nucleus than do lymphocytes. The nucleus is generally round or slightly oval, and is often eccentrically placed in a manner which is well-known for these cells. The one special characteristic possessed by these cells, when seen in supravital preparations stained with neutral red and Janus green, is that the cytoplasm has a peculiar and characteristic appearance. It is homogeneous and possesses a faint yellow tinge, almost like that of a nucleated red cell in which the full quota of hemoglobin is not present. Because of this fact, plasma cells in lymph nodes have often been mistaken for developing erythrocytes. Plasma cells in lymph nodes often contain in their cytoplasm, as do lymphocytes, one or a few small, refractive, globular, neutral red bodies. Mitochondria are frequently numerous and of the same type as those described for lymphocytes. In no instance have plasma cells been seen possessing segregation granules resembling those found in monocytes or pre-monocytes. They are easily distinguished from these latter cells.

Materials and Methods

Normal rabbits, guinea pigs, and rats vitally stained with trypan blue have been used in these experiments. The method has been completely described in an accompanying paper (2) which should be consulted for repetition of these experiments. Let it suffice to say here that sections of tissues which have been stained supravitally alone or in combination with vital staining are in many instances more instructive than the living supravitally stained cells. The chief advantage of the method is that the supravital staining is preserved in paraffin sections. A few typical protocols of experiments are given in the following pages.

PROTOCOLS OF EXPERIMENTS

Experiment 1.—Rabbit 2. The animal fresh from stock was etherized. Neutral red, 0.6 per cent, in 0.9 per cent sodium chloride solution, dissolved by warming and then filtered, was injected at body temperature and at 150 mm. of mercury pressure, through a cannula into the exposed left ventricle. A cannula was placed in the right jugular vein, through which the perfusion fluid and blood were allowed to escape. The animal was thus perfused with about 800 cc. of the neutral red solution over a period of 25 minutes. Immediately following, Zenker-formol solution was similarly injected at body temperature through the same cannula, over a period of 20 minutes. Tissue from the various organs was removed, placed in fixing fluid of the same kind, and allowed to remain over night. The method of staining, fixation, dehydration, embedding and counterstaining is described in detail in the accompanying paper (2).

The organs were all well stained except the bone marrow, which contained only a few areas of stained cells in the immediate neighborhood of the nutrient arteries. Much neutral red had accumulated in the stomach and intestines which were dilated and the contents of which were dark red in color.

Peripheral lymph nodes were normal in size and stained a dark red color. On histological examination, all the nodes were essentially alike, except that some contained more monocytes than others. Some of the lymph nodes were heavily laden with monocytes which were situated in the lymphoid tissue in and around the primary follicles. Also in some nodes monocytes were present in large numbers in certain of the secondary follicles, the so-called germinal centers. Most of the germinal centers, however, contained very few or no monocytes. The monocytes, as seen in sections, were often in clumps of from four to twenty or more cells (Figs. 1, 3, 5, 6, 10). These clumps were characteristically a part of the parenchyma of the lymphoid tissue and were not free or separated from the lymphoid cells by any perceptible boundary. Many monocytes were also found diffusely scattered through the cortical tissue, but were almost never present in the medullary cords. These cells were easily recognized by their characteristic appearance. It is interesting to note that they were not in any way related, in their position, to cells lining the sinuses. It is true that occasional monocytes are found in the sinuses (Fig. 5) and in the medullary cords, but this is not at all a common occurence. Their histological appearance cannot be confused with that of the macrophages lining the lymph sinuses (Fig. 8), the cells of the so-called reticulo-endothelium. Monocytes in various stages of development can be easily demonstrated, particularly in areas where monocytic cells are numerous (Fig. 10). The life cycle can be traced from undifferentiated cells through the stages of monoblasts and pre-monocytes to mature monocytes.

Free macrophages, so-called reticular cells, are likewise numerous in the peripheral lymph nodes, but are not as abundant as monocytes. They are found within the sinuses, in the parenchyma of the lymphoid tissue, and may be scattered through the follicles (Figs. 4, 9), being present at times in the secondary follicles.

These free macrophages vary tremendously in size from that of a large lymphocyte to giant cells with abundant cytoplasm and several nuclei. As a rule the cytoplasmic bodies are scattered throughout the cell without pattern and conform closely to the description previously given. However, certain unmistakable, free macrophages have the cytoplasmic bodies arranged in the form of a very large rosette. This rosette is not composed of the uniform, fine, brick red, non-refractive, neutral red bodies typical of the monocyte, but, on the contrary, the vacuoles are coarse, irregular, of different shades of color, and possess different refractive properties. By diligent search, cells can be found which cannot with certainty be placed in one or the other group. These cells must be considered carefully, for they suggest that under physiological conditions monocytes may be transformed into macrophages. This point will be considered in the discussion to follow.

Plasma cells were found in the lymph nodes of this animal (Rabbit 2). In some areas they were numerous. These areas were chiefly located in the less dense lymphoid tissue and not in the follicles themselves. The plasma cells were easily distinguished from all the other cellular elements.

Mesenteric lymph nodes differ considerably in their cytology from the other lymph nodes, as discussed in detail in an earlier paper (1). The essential differences are: (a) almost complete absence of monocytes in mesenteric lymph nodes, but an abundance in peripheral lymph nodes; (b) presence of more macrophages, chiefly those lining the sinuses, than in peripheral nodes; (c) presence of many more large lymphocytes with abundant basophilic cytoplasm in the mesenteric than in the peripheral lymph nodes.

Spleen.—No developing monocytes were found. Occasional, fully developed monocytes were present. Many free macrophages were present, some of which contained fragmented cellular débris.

Thymus.—An abundance of free macrophages was present, chiefly limited to the cortical zone. No monocytes could be found in either the cortical or medullary zone.

Liver.—No monocytes were present. Many macrophages (Kupffer cells) were in the capillaries. The organ presented an entirely normal appearance.

Omentum.—Paraffin sections demonstrated a few small groups of monocytes, and this possibly represents a place of development for these cells. Their number, however, was insignificant when compared with the number found in the lymph nodes other than the mesenteric mass of lymphoid tissue. Many monocytes appeared to lie on the surface of the serosa, as though they had been present in the peritoneal fluid and had adhered to the omentum. It must be stressed, however, that in supravital films of the omenta of normal rabbits, rats, and guinea pigs, milk spots can be seen composed almost exclusively of monocytes, others containing primarily macrophages, others lymphocytes, and still others primitive undifferentiated cells. Such findings indicate that in the omentum there are present many cells possessing mesenchymal potencies. Many free macrophages were found in the omentum of the animal described in this protocol. Also, many elongated,

fixed macrophages in close association with fibroblasts and connective tissue fibers could be demonstrated.

Bone marrow.—The bone marrow was not well stained in this animal but will be described in some of the protocols which follow.

Many other organs were studied, but their description is not relevant.

Experiment 16.—Rabbit 16. The animal was used in an attempt to inject neutral red more directly into the vessels of the posterior extremities in order to stain supravitally the bone marrow cells. The abdomen was opened under ether anesthesia and a cannula inserted into the abdominal aorta, just above the pelvic brim. Neutral red, 500 cc. of a 0.6 per cent solution in normal saline, was then slowly perfused through the vascular system of the posterior extremities under the same conditions as those in Experiment 1. The same fixing fluid was also used.

After this process the bone marrow was removed intact with blunt forceps. Dehydrating, clearing, and embedding were carried out as in Experiment 1. The marrow was distinctly red, particularly in the neighborhood of the nutrient vessels. Lymph nodes from the popliteal and inguinal groups were also saved.

Microscopically the marrow showed large areas which were ideally stained with neutral red in a manner which renders the differentiation of the cellular types easy. The bone marrow from the long bones of rabbits is very active in blood formation and contains granulocytes, erythrocytes, and megakaryocytes in all stages of development. Macroscopically it is red and highly vascular. It is not within the scope of this paper to describe the histological details of the findings, except to say that monocytes cannot be found in any part of the marrow. Free macrophages are scattered sparingly in the tissue. Macrophages lining the capillaries in the marrow, the so-called reticulo-endothelial cells, are abundant and for the most part are elongated and contain coarse, neutral red bodies in their cytoplasm. They resemble the macrophages lining the lymph sinus in lymph nodes. In no instances can cells be found which can be interpreted as transitional phases between macrophages and monocytes.

It had been demonstrated in the above experiments that all supravitally stainable cells in the body could be easily stained with neutral red and the color preserved in paraffin section. Vital staining with dyes such as trypan blue, Niagara blue, and lithium carmine has long been employed to mark out the cells of the so-called reticulo-endothelial system. It was proposed, therefore, at the suggestion of Professor Aschoff, to use the combined methods of supravital staining with neutral red and vital staining with trypan blue to determine whether or not the developing monocytes really belong to the reticulo-endothelial system, the system of histiocytes, or of mesenchymal macrophages. Paraffin sections of such tissues show that the cytoplasmic structures in the cells which have been previously vitally stained with trypan blue and subsequently counterstained with neutral red present a violet color, whereas those cells which have not been capable of taking up trypan blue and contain only neutral red possess a pure red color, just as though no trypan blue had been introduced. The following experiment was performed.

Experiment 17.—Rabbit 17. A normal rabbit was given a series of five intravenous injectons of a sterile, 1 per cent, aqueous solution of trypan blue. The injections were given every second day. 24 hours after the last injecton of trypan blue, the animal was perfused, through the left heart, with 1000 cc. of 0.5 per cent neutral red, dissolved in normal salt solution. The perfusion and fixation were carried out under the same conditions as in Experiment 1. The general appearance of the tissues changed from a blue to a purple color during the course of the perfusion with neutral red.

Microscopic examination of the lymph nodes demonstrates clearly that the pre-monocytes and monocytes in the lymph nodes are not stained with trypan blue. On the other hand, many free macrophages (reticular cells), many phagocytic cells of the reticular syncytium, and practically all of the macrophages lining the lymph sinuses (reticulo-endothelial cells) contain an abundance of trypan blue, as well as neutral red. It must be stated that many macrophages in the lymphoid follicles contain very little or no trypan blue. Also, certain cells can be seen to be more abundant than in normal animals, which morphologically represent stages between typical monocytes and typical macrophages and which are, almost without doubt, transition phases in the development of the latter from the former. These cells are likewise intermediate in their reaction to trypan blue, taking only a small amount of this dye. All transition phases can be demonstrated between monocytes containing neutral red but no trypan blue, and macrophages containing an abundance of both colors.

Another experiment was undertaken to confirm these findings. It was proposed to inject much more of the trypan blue to determine whether excessive doses of this dye would alter the above findings.

Experiment 18.—Rabbit 18. This animal was treated with five intravenous injections of 6 cc. each of a 1 per cent aqueous solution of trypan blue, followed by a series of four injections of 8 cc. each of the same solution. These injections were also given on every second day. 24 hours after the last injection, the animal was perfused with 800 cc. of 0.7 per cent neutral red solution and then with fixing fluid, as in the previous experiments.

The results of this experiment are, so far as the point under discussion is concerned, in entire agreement with those of Experiment 17. There are, however, a few additional points which should be mentioned. It will be remembered that in Experiment 17 the macrophages lining the lymph sinuses were strikingly colored with the trypan blue, whereas only a portion of the free macrophages and macrophages of the reticular syncytium were stained. In Experiment 18, practically all of these macrophages contained an abundance of trypan blue,

whereas monocytes lying in close proximity to these cells remained unstained with vital dye, but were richly colored with neutral red (Fig. 9). This fact demonstrates that, even with prolonged administration of large doses of vital dye, the monocytes and pre-monocytes of the lymph nodes remain unstained. In this experiment there were more cells which might be interpreted as intermediate stages from monocytes to macrophages (Fig. 9).

It is interesting also in this experiment that the macrophages of the liver capillaries (reticulo-endothelial or Kupffer cells) were increased considerably in number and for the most part contained a large amount of trypan blue. Also in the sinuses, one sees occasional typical monocytes, often three or more in a clump. Moreover, typical transition forms from monocytes to macrophages can be demonstrated. This finding in the liver will be discussed in another portion of this paper.

DISCUSSION

The literature on the origin of the monocyte shows that no one has as yet proved the derivation of this cell from any fixed cell in the body. Numerous investigations have been reported on the mode of production of these cells under pathologic or tissue culture conditions. These reports are contradictory, excellent workers stating that they arise from endothelium, lymphocytes, myeloblasts, histiocytes, etc., and equally competent investigators denying these statements. The report contained in the present contribution deals with normal animals and demonstrates a site of origin and development of monocytes under physiological conditions. It shows that monocytes are formed in certain specific blood-forming organs and in this respect are analogous to all the other structural elements of the blood. The analogy can be carried even further for, like other white blood cells, they are shown to be derived from primitive, undifferentiated, cellular elements and their various stages of development can be clearly demonstrated.

It is not denied that monocytes may, under abnormal conditions, be abnormally produced in abnormal locations. On the contrary, it has been shown conclusively by numerous investigators that under experimental or pathologic conditions, monocytes may arise in various organs and tissues of the body. This, however, is likewise true of lymphocytes and granulocytes.

The methods here reported for the study of the development of

monocytes in lymph nodes have been equally applicable to the study of these and related cells in all organs of the body. They have demonstrated conclusively that, in the bone marrow of the long bones of rabbits, developing monocytes are not to be found, although the marrow is very active for the production of granulocytes and erythrocytes. This confirms the observations of Sabin and Doan (17). These authors gave the first direct, conclusive evidence that in the rabbit, under physiological conditions, monocytes are not derived from myeloblasts or any other cell in the bone marrow.

Monocytes have been said to arise in considerable numbers in the spleen. Sections of supravitally stained spleens of the animals used in these experiments have failed to show more than occasional monocytes and very rarely pre-monocytes. It is thus apparent that the spleen plays no significant part as an organ in which monocytes are produced.

Maximow (3), Bloom (10), Masugi (18), Rhoads and Parker (19), Witts and Webb (20), and others have maintained that monocytes do not occur in any significant numbers in lymph nodes. All of these investigators employed the supravital technique, together with other methods, for their studies. Maximow (3) and Bloom (10) have used these results as one of their reasons for affirming the original contention of Maximow that monocytes are formed from lymphocytes in the blood stream by individual transformation of the latter cells. On the other hand, Masugi (18) made use of the same evidence to uphold his view and that of Aschoff (21) that monocytes are formed by transformation from histiocytes and reticulo-endothelial cells. It has been possible to utilize the same evidence for the support of each view because a gap has been present in our knowledge of the development of monocytes.

Although Bloom (10) recently has reported that all transition stages between lymphocytes and monocytes may be found in the blood of animals experimentally infected with *B. monocytogenes*, this has not been confirmed by Witts and Webb (20). Furthermore, no cells are shown in Bloom's figures, or described in his paper, which correspond to the early developmental forms of monocytes, as seen in peripheral lymph nodes. It is not intended to imply that the development of monocytes from lymphocytes in the blood stream is impossible, but to indicate that the evidence is insufficient to prove that under physiological conditions this is the mechanism for their formation.

In other experiments of Bloom (22, 23) he cultured lymph from the thoracic duct and after a time recovered monocytes. But one cannot be certain that all the cells in the thoracic duct are lymphocytes. It is not uncommon to find undifferentiated cells which possibly possess multiple potentialities. Even if one assumed that a pure culture of lymphocytes could give rise to a pure culture of monocytes, one would still have to show that this is the mechanism by which monocytes are produced under physiological conditions.

None of my evidence supports the view that monocytes are, under normal conditions, derived from ordinary blood vessel endothelium, macrophages lining the lymph sinuses (reticulo-endothelium), or from free macrophages. Undoubtedly however, desquamated reticulo-endothelial cells do occur under certain conditions in the blood stream, as pointed out by Mallory (24), and many others. Sabin and Doan (25) maintain that under normal conditions there is a practically constant desquamation of endothelial cells into the circulating blood in rabbits and man. It is difficult to conceive of how these cells could give rise to monocytes. Furthermore, no direct evidence for such transformation is recorded in the literature. The reports of Schilling (26) and of others that monocytes have been seen to arise from the Kupffer cells in the liver demand discussion, since it has been shown in this communication (Experiment 18) that monocytes may be found in the liver capillaries in small numbers when animals are vitally stained with trypan blue. In addition, transition forms between monocytes and macrophages and between monocytes and epithelioid cells are demonstrable. It is probably such findings in pathological material that have been responsible for the theory that monocytes arise from Kupffer cells. The evidence, as will be pointed out presently, is decidedly against the theory of the transformation of Kupffer cells into monocytes.

On the other hand, one does find evidence for the hypothesis that monocytes may be transformed into free macrophages, and this probably represents one of the modes of formation of the latter cells. Such proof is recorded in the protocols of this paper. It is consistent with Simpson's (8) production of macrophage showers in the circulating blood. These showers were generally preceded by a marked monocytosis and the presence of cells which probably represented transition phases between the two types of cells. A typical protocol shows that the shower phenomenon first occurred after twenty-six injections over a period of 2 months. Masugi (18) has recently contested the findings of Simpson (8), although he seems not to have repeated the experiments. He explains the macrophage showers of Simpson as due to aspirated pericardial fluid.

Tissue cultures of the buffy coat of the blood (27, 28) have likewise shown that monocytes readily may be converted into macrophages. Carrel and Ebeling (29) compared the results of tissue cultures of monocytes from the blood and macrophages from the subcutaneous tissues of chickens. They found a transformation of monocytes into macrophages.

From the experiments recorded in this communication and from the experiments of many workers in the past, it appears to be proved that macrophages in lymph nodes may arise directly from the undifferentiated cells of the reticular syncytium, or that they may arise from monocytes which, in their turn, are derived from the same mother cells.

A schematic outline of the development of monocytes and macrophages in lymph nodes is presented in the accompanying diagram.

Diagram Representing Normal Development of Monocytes and Macrophages in Lymph Nodes

Common Endothelial Cell

(a) Lines ordinary blood vessels.
(b) Does not produce reticulum.
(c) Does not stain with vital or supravital dyes.
(d) Does not phagocytize débris.
(e) May desquamate into blood as transient inhabitant.

Fixed Macrophage

(Phagocytic cell of reticular syncytium)

(a) Produces reticulum fibers.
(b) Stains with supravital and vital dyes.
(c) Phagocytic for débris.
(d) Cytoplasmic structures have no characteristic pattern.

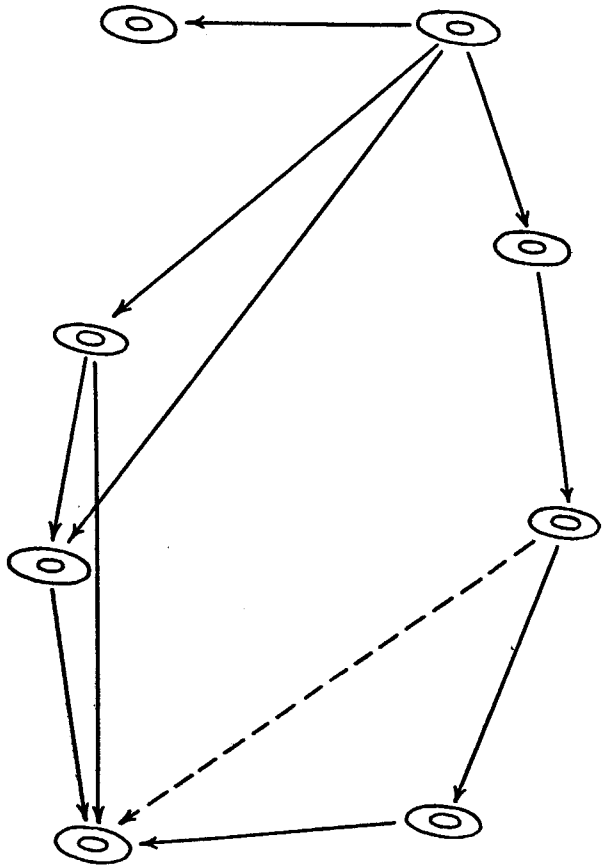
Macrophage Lining Sinus

(Reticulo-endothelial cell)

(a) Produces reticulum fibers.
(b) Stains with vital and supravital dyes.
(c) Phagocytic for débris.
(d) Cytoplasmic structures have no characteristic pattern, size, or shape.
(e) May become detached to form free macrophage.

Free Macrophage

(a) Does not produce reticulum fibers.
(b) Markedly phagocytic for débris.
(c) Stains with vital and supravital dyes.
(d) No pattern to cytoplasmic structures.
(e) Is a wandering cell.
(f) Occurs in blood as transient inhabitant.



Primitive Cell (Undifferentiated mesenchyme cell)

(Undifferentiated, non-phagocytic cell of reticular syncytium)

(a) May produce reticulum fibers.
(b) Contains no supravitally or vitally stainable granules.
(c) Possesses multiple potentialities.

Monoblast

(a) May or may not be identified with production of reticulum fibers.
(b) Somewhat more differentiated than the primitive cell.
(c) Found in association with pre-monocytes and monocytes.
(d) Contains no vitally or supravitally stainable granules.
(e) Not found in blood.

Pre-Monocyte

(a) Does not form reticulum fibers.
(b) Somewhat more differentiated than monoblast.
(c) Found in association with monoblasts and monocytes.
(d) Contains the beginning of a rosette of neutral red bodies.
(e) Does not stain with vital dyes.
(f) Rarely found in normal blood.
(g) May be experimentally produced in blood.

Monocyte

(a) A fully differentiated cell.
(b) Contains many neutral red bodies, usually in form of a rosette.
(c) Is smaller than pre-monocyte.
(d) Is a normal constituent of the blood.

All the cells mentioned in the diagram may be seen in peripheral lymph nodes when stained and sectioned according to the writer's methods (2). They may originate in other manners besides those there given, but the diagram indicates one common method of their origin under physiological conditions. The peripheral lymph nodes are the only organs of rabbits in which one can constantly find monocytes in all stages of development.

McJunkin (30) has thought that monocytes are derived from what he calls "reticular lymph vessel endothelium" in lymph nodes. His methods, in my hands, have proved unsuccessful for the demonstration of monocytes, supravitally stained in paraffin sections of lymph nodes. On the other hand, many free macrophages and macrophages lining the lymph sinuses can be stained and demonstrated by his methods. Possibly McJunkin has confused these elements with monocytes.

What is the relation of monocytes to the reticulo-endothelial system? The experiments (Nos. 17 and 18) recorded in the protocols have demonstrated that monocytes and pre-monocytes, in the lymph nodes, remain unstained when the animals are repeatedly injected over a long period of time with trypan blue. These facts are in agreement with the general opinion that monocytes do not stain with vital dyes. There is one exception, however, which must be mentioned here. Doan, Sabin, and Forkner (31) have shown that some monocytes, epithelioid cells, and epithelioid giant cells may be stained vitally when directly exposed to trypan blue over a long period of time. This result might have been predicted, since Kiyono (14), Evans and Scott (15), and others, have demonstrated that in connective tissues even fibrocytes may become vitally stained after prolonged treatment with trypan blue or Niagara blue. The only conclusion which one safely can make with regard to the relation of monocytes to the reticulo-endothelial system is that they do not stain vitally by the usual methods which are used to demonstrate this system.

The question of the relationships between lymphocytes, monocytes and plasma cells in the rat has been discussed by Bloom (32). He has concluded that plasma cells often possess rosettes of neutral red bodies and that all transitions exist between plasma cells and monocytes in lymph nodes. The present work on rabbits does not support the conclusions of Bloom.

The question naturally arises: How do monocytes reach the blood stream, a point which has already been discussed by the writer (1). They probably gain admission to the capillaries by means of their own motility in much the same manner as do the granular leucocytes. It is true that in the peripheral lymph nodes developing monocytes are frequently found in close proximity to the capillary vessels (Fig. 10). It is also probable that many lymphocytes which develop in the spleen and lymph nodes wander into the blood stream.

SUMMARY

1. The theories for the origin of monocytes from myeloblasts, lymphocytes, endothelium, macrophages, and primitive cells are reviewed and considered.

2. Monocytes in all stages of development have been demonstrated to be present constantly in large numbers in all the lymph nodes of the body, except in the large mesenteric group.

3. The relations of these cells to undifferentiated cells, lymphocytes, macrophages, plasma cells, and endothelium are described.

4. The origin of adult monocytes from primitive undifferentiated cells through the stages of monoblasts and pre-monocytes is described and illustrated.

5. The demonstration in certain lymph nodes of innumerable monocytes in all stages of development permits of a shifting of the term "monoblast" from a more or less theoretical name to its proper place as a term designating that particular cell which is derived from a primitive undifferentiated cell and which is the immediate precursor of the pre-monocyte.

6. The term "pre-monocyte" is proposed to designate the intermediate stage between the monoblast and the mature monocyte.

7. Evidence is advanced to show that monocytes are an independent strain of cells, but that under physiological conditions they may be transformed into macrophages, this representing at least one way in which the latter cells normally are produced.

8. In no organs or tissues other than in certain specific lymph nodes, chiefly the peripheral group, can one constantly find monocytes in all stages of development.

9. Developing monocytes occasionally may be found in small num-

bers in the spleen, mesenteric lymph nodes, Peyer's patches, subcutaneous connective tissues, lungs, and omenta of normal rabbits, but their presence is by no means constant and their numbers are insignificant in comparison with those found in the peripheral lymph nodes.

10. Monocytes and pre-monocytes do not stain by the common methods used for the demonstration of the reticulo-endothelial system and therefore must be considered for the present as independent of this system, except in so far as monocytes may be transformed into macrophages.

11. Plasma cells, stained with the supravital technique, as seen in lymph nodes, are described. No basis has been found for the theory that plasma cells and monocytes are closely related structural elements.

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EXPLANATION OF PLATES

PLATE 12

FIG. 1. Peripheral lymph node of a normal rabbit. × 1000. Paraffin section. Stained supravitally with neutral red. Here is an area of developing monocytes in the less dense lymphoid tissue at the periphery of a primary follicle. This is not an isolated or single group of monocytes. The picture may be duplicated many times in almost any section of the peripheral nodes, when properly stained. All stages are present, from monoblasts to mature monocytes with well-formed rosettes.

FIG. 2. Peripheral lymph node of a rabbit. ×820. Paraffin section. Stained supravitally with neutral red. Counterstained lightly with Goodpasture's acid polychrome methylene blue. This figure shows developing monocytes within a primary follicle of the lymph node. The size of the cells can be compared with that of the lymphocytes at the left of the figure. The delicate, faint outlines of the nuclei can be clearly seen.

PLATE 13

FIG. 3. A follicle in a peripheral lymph node of a normal rabbit; a low power view of the same area shown in Fig. 2. × 280. Paraffin section. Supravitally

stained with neutral red. Lightly counterstained with Goodpasture's acid polychrome methylene blue. Not all follicles contain monocytes. Many contain more monocytes than this and some may be almost entirely composed of such cells. It will be seen that they are independent of reticulo-endothelium and of common blood vessel endothelium. They develop from the undifferentiated parenchymal cells of the stroma.

FIG. 4. Peripheral lymph node of normal rabbit. ×1200. Paraffin section. Stained supravitally with neutral red and counterstained by a modification of Foot and Menard's rapid silver method. The upper part of the photograph represents the peripheral portion of a primary follicle. In the center, at the periphery of the follicle, two monocytes with well-developed rosettes can be seen, "a." To the right is a free macrophage showing the character of the cytoplasmic structures in these cells, "b."

FIG. 5. Peripheral lymph node of a normal rabbit. ×1200. Paraffin section. Staining as in Fig. 4. Here monocytes and pre-monocytes are shown in a peripheral sinus. They rarely appear in the sinuses. They are shown here as free cells which have probably made their way into the sinus by means of their own motility.

FIG. 6. A group of monocytes, pre-monocytes, and monoblasts in a peripheral lymph node of a normal rabbit. ×1200. Staining as in Fig. 4.

PLATE 14

FIG. 7. Peripheral lymph node of a normal rabbit. × 1200. Stained as in Fig. 4. Here is shown a group of early pre-monocytes and monoblasts. The former have small rosettes not fully developed, whereas the latter are entirely similar, except for the absence of the neutral red bodies.

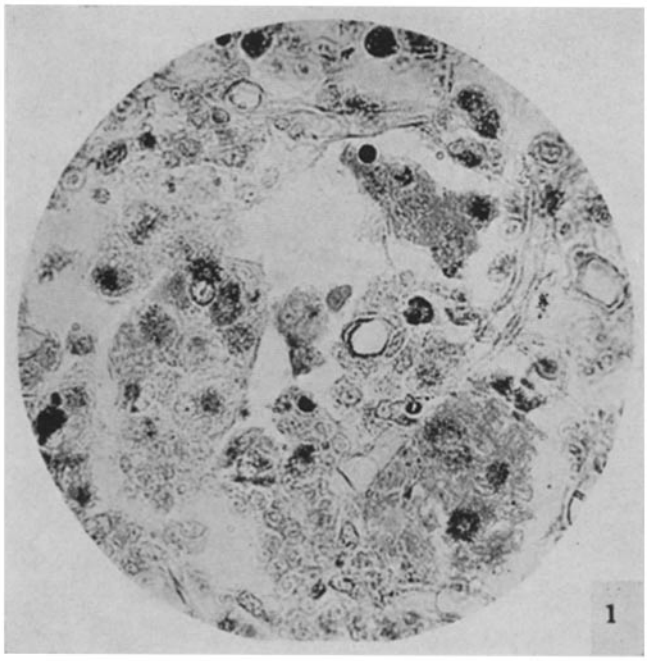
FIG. 8. An area of lymphatic tissue between follicles of lymphoid tissue of a normal rabbit. × 1200. Stained as in Fig. 4. The macrophages of the sinuses (reticulo-endothelial cells) are shown. These cells contain reticulum fibers and also irregular masses of neutral red. Monocytes cannot be found developing from these cells. Compare the monocytes of Fig. 7 with the reticulo-endothelial cells of Fig. 8.

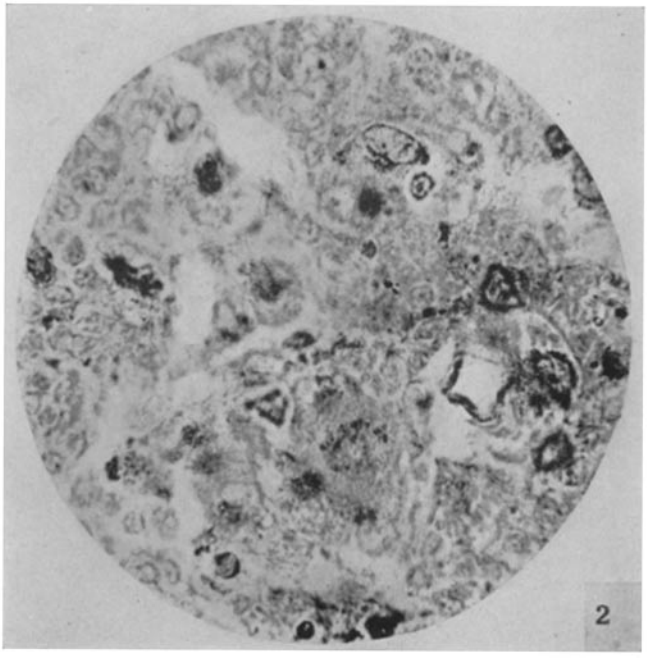
PLATE 15

FIG. 9. Peripheral lymph node of rabbit treated with 9 doses of trypan blue (Experiment 18). Subsequently stained supravitally with neutral red. Paraffin section × 820. Here can be demonstrated intermediate stages in the transformation of monocytes into free macrophages. "a" points toward a cell which has a large rosette of neutral red bodies. In addition, a few globules of trypan blue have been taken into the cell. The granules of the rosette are larger than those usually seen in monocytes. It is a transition form but still possesses most of the characteristics of a monocyte. The cell to the right of the arrow leading from small letter "a" represents another step in the transition. It possesses an abundance of both neutral red and trypan blue. The cell indicated by "b" is a genuine macrophage which has probably developed from a primitive cell directly without first having

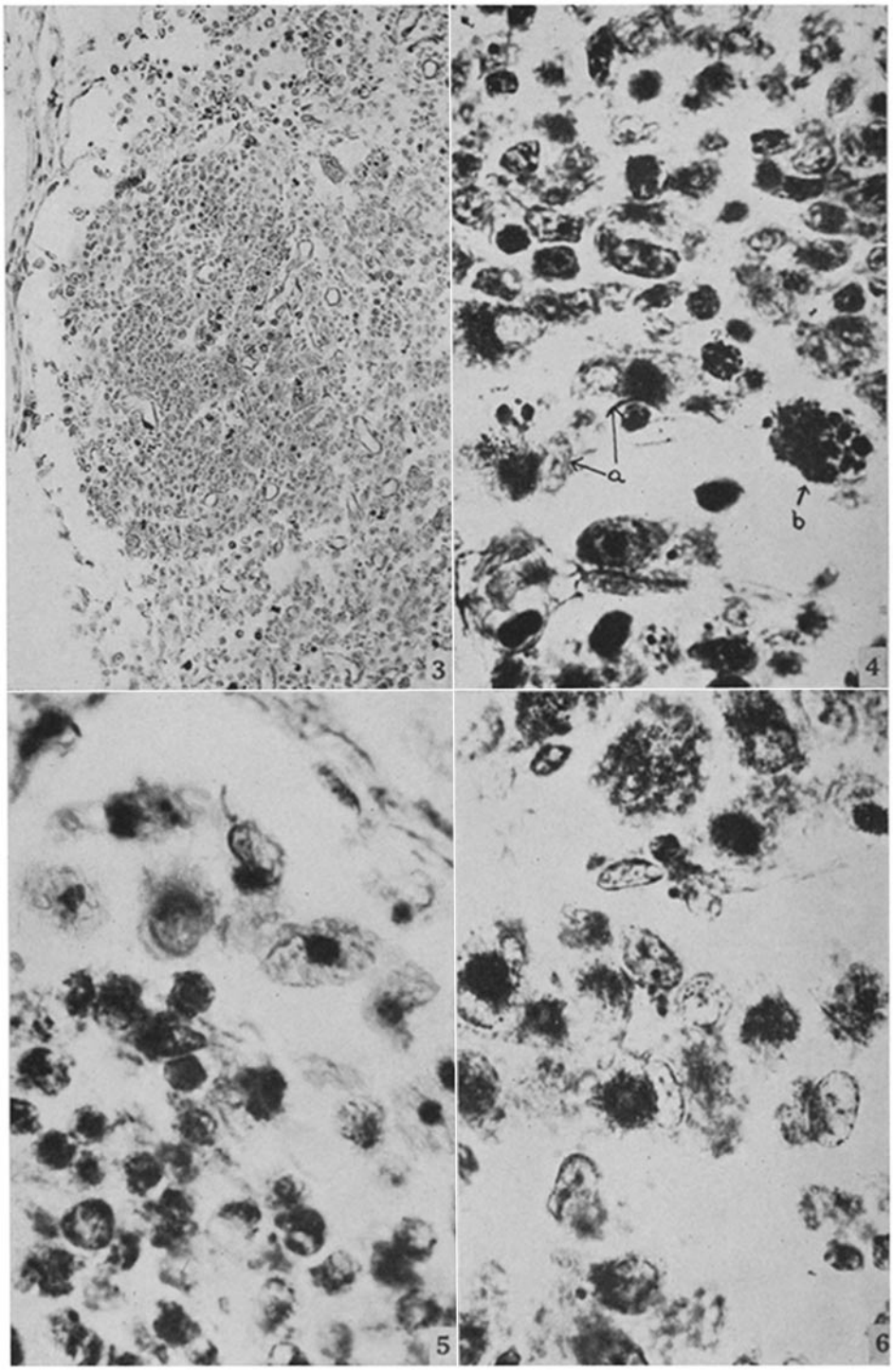
been a monocyte or pre-monocyte. It contains both dyes in abundance. "c" indicates a fully developed monocyte. It contains much neutral red, but no trypan blue. "d" indicates a developing monocyte, a pre-monocyte, with a characteristic rosette containing only neutral red bodies.

FIG. 10. An area of developing monocytes closely adjacent to a capillary blood vessel in a peripheral lymph node. Paraffin section × 820. Stained as in Fig. 1. The small letters "a" point to cells which are regarded as monoblasts. They do not possess rosettes and yet are somewhat more differentiated than the primitive cells "c." The letter "b" indicates a pre-monocyte and "d" a well-developed monocyte. At the upper right hand corner a small capillary blood vessel is seen.





(Forkner: Origin of monocytes in lymph nodes)



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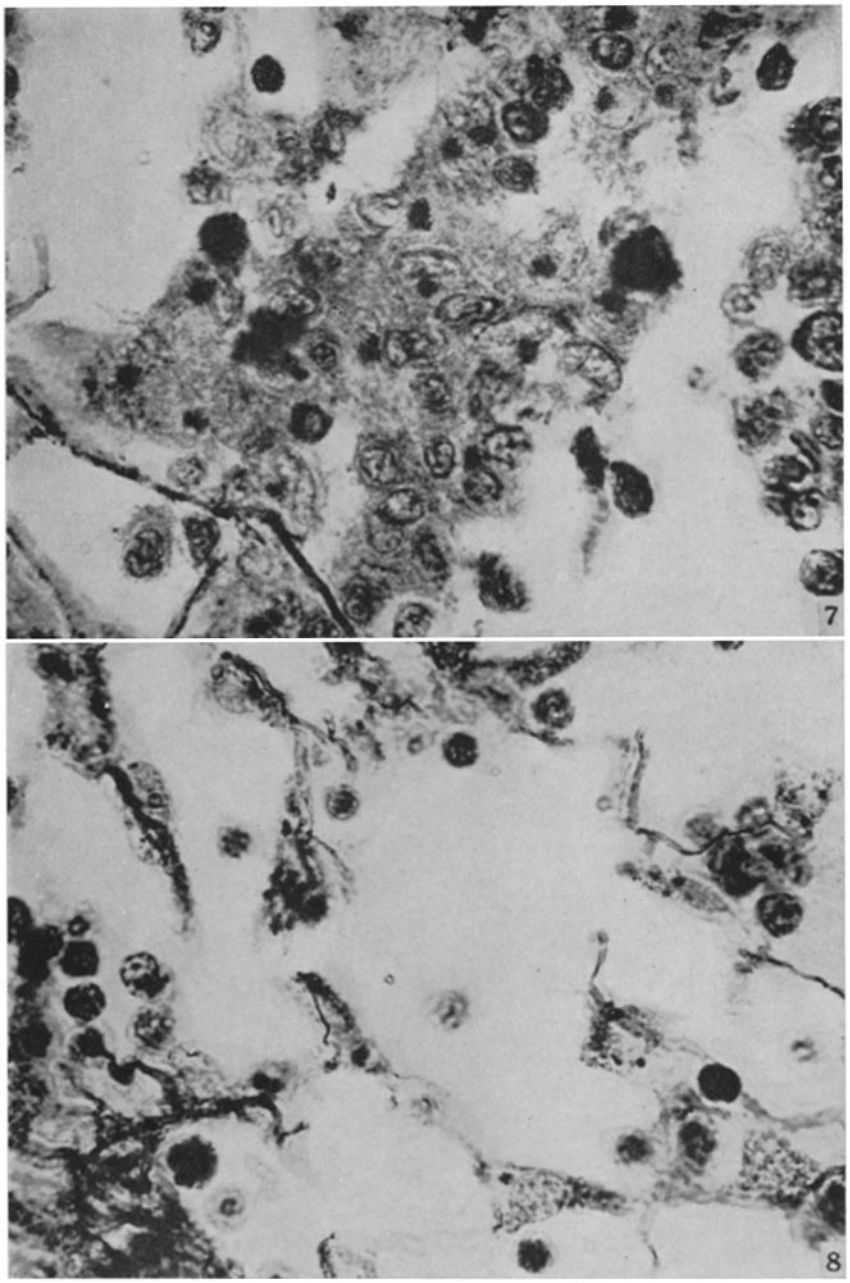

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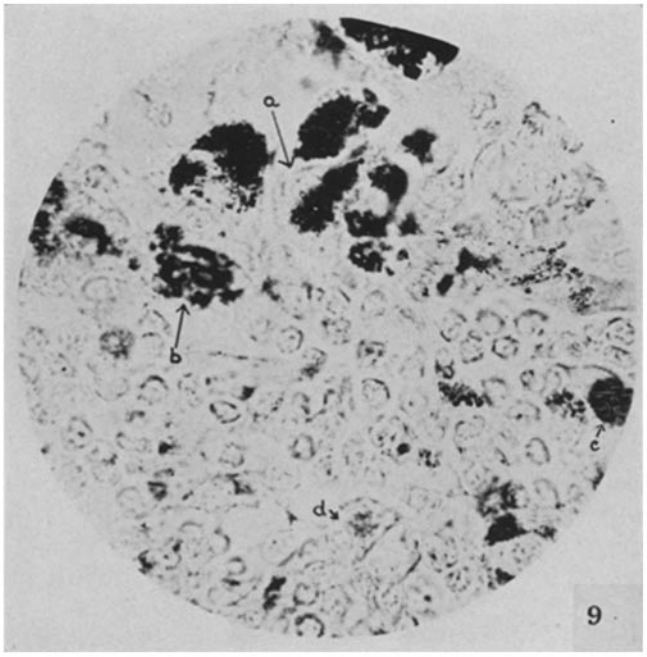

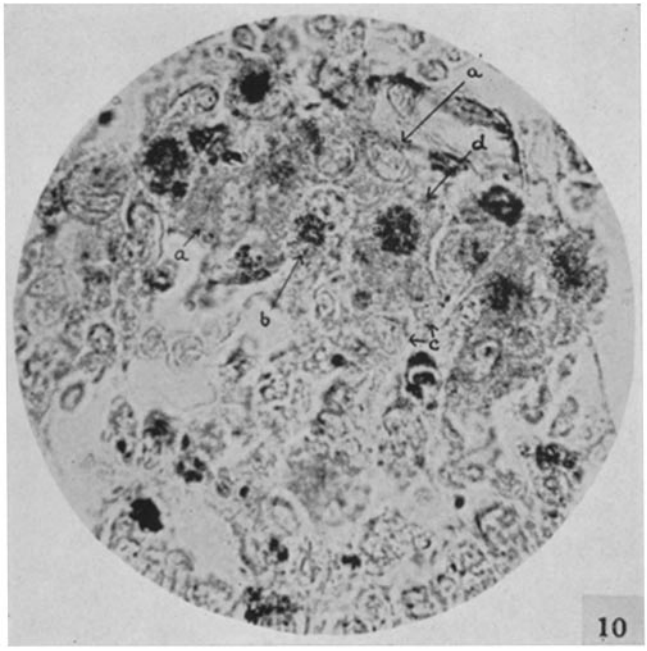

(Forkner: Origin of monocytes in lymph nodes)