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# A Method of Studying Leukocytic Functions In Vivo†

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## Introduction

Passage of the white cells of the blood from the blood vessels into the area of inflammation probably was first described by Dutrochet (1) in 1828 (2,3). In 1843, Addison (4) immersed a frog's foot in hot water and noted that the colorless corpuscles in the irritated web of its foot adhered to the tissue and that the corpuscles went on "congregating in the irritated tissue for an hour or two." In 1846, Waller (5) also described the active nature of leukocytic migration. Addison (6), moreover, in 1849 anticipated Cohnheim's (7) more publicized experiment.

Zimmerman (1852)(9) emphasized that inflammation was a local process characterized by "an abnormal escape of blood corpuscles from the vessels associated with a focal rise in temperature (8)." Virchow (1858) could not decide whether leukocytes were pus cells taken into the blood or, conversely, if pus cells were extravasated leukocytes (10).

Schultze (1865) was able to observe white blood cells kept at body temperature by means of his ingeniously contrived warm stage (11). In his Fig. 13, he depicts a "finely granular cell" in the ameboid form now thought to be characteristic of a lymphocyte. This cell had ingested fine droplets of milk which he had mixed with the human blood cells.

Haeckel (1862) injected indigo into the mollusc, *Tethys,* and was the first to demonstrate that leukocytes were capable of taking up foreign bodies into their own cytoplasm after finding indigo granules within the blood corpuscles (12).

Migration of leukocytes from vessels was further confirmed by F. E. Schultze (13) in 1866, Hering (14) in 1867, and by Cohnheim (7) in his now famous paper of the same year.

Lieberkühn (1870) demonstrated the ameboid motility of leukocytes in salamanders, rabbits, and various other animal species, watching their advancement in small glass tubes (15). He coined the phrase "ameboid motion."

Bizzozero (1871, 1872) observed leukocytes in the larger cells of pus and suspected that the larger cells had devoured the leukocytes (16). Ziegler (1875, 1876) was perhaps the first to advance the concept that mononuclear exudative cells were transformed mononuclear white blood corpuscles (17,18), but, unfortunately, he soon abandoned the idea.

According to Gay, (19) Hayem, Birsch-Hirschfeld, and Koch had previously demonstrated bacteria within the cells of diseased tissues, but the true cytological basis of natural resistance was established by the work of Metchnikoff. Although Metchnikoff gave full credit to the earlier work of other men (Panum, Grawitz, Gaule, and Roser), still, as expressed by Gay (19), "the full exposition and understanding of phagocytosis" was Metchnikoff's own.

Through his work on mesodermic digestion in Medusae, Metchnikoff became convinced that mesoderm retained a primitive digestive function. Although the Coelentera and some Echinoderms possessed an entodermal digestive cavity, he believed that their mesodermal cells retained their powers of intracellular digestion. In 1883, he was struck by the idea that the mobile cells of a transparent starfish larva might serve as defense elements against invaders; whereupon he introduced rosethorns under the skin of a starfish larva *(Bipinnaria asterigera).* As a result the rosethorns were surrounded by the mobile mesodermal elements (20,21).

Soon afterward, Metchnikoff observed spontaneous ingestion and destruction of *Monospora bicuspidata* by mobile cells of the blood of the infested, fresh-water crustacean, *Daphnia magna,* which had made their way into the general body cavity (his Figures 41-43, 1891) (22). Inflammation became to Metchnikoff, in its simplest expression, a reaction of mesodermic cells to an agent extrinsic to an organism. To these mesodermic cells, which digested microbes, he gave the name suggested to him by Claus, "phagocytes," that is, "devouring cells."

The following years saw the application of Metchnikoff's experiments (22,23) to more complex animal forms, with bacteria serving as stimuli to inflammation. In these forms, the mesodermal elements acting as phagocytes were of two general types, macrophages and microphages. The microphages were the polymorphonuclear granulocytes of the blood, and the macrophages were again subdivided into: 1) hematogenous macrophanges, that is, lymphocytes which in turn gave rise to the large mononuclears; and 2) histogenous macrophages, the phagocytic cells of the spleen, peritoneum, bone marrow, sinusoidal lining cells of

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the liver, and, in general, the fixed tissue cells now known as the reticuloendothelial system of cells.

Leber (1888) first described and demonstrated chemotaxis of leukocytes (24). He watched their reaction to an extract of *Staphylococcus aureus* which had been placed in the anterior chambers of the eyes of his experimental animals.

The foundation for our modern concept of lymphocyte function is found in Metchnikoff's paper of 1888 (25). In these experiments, Metchnikoff demonstrated that lymphocytes of the blood of the rabbit and marmot suffering from pulmonary tuberculosis migrated into tuberculous areas and gradually hypertrophied to form cells which he called "large mononuclears." These large mononuclears, in turn, formed macrophages and epithelioid cells. In the same year, Yersin (26) inoculated rabbits with tubercle bacilli intravenously and studied tubercle formation in the liver and spleen from the second through the 41st day. In his animals, he described transformation of lymphocytic function as well as morphology, as the lymphocytes accumulated in the infected area and became the cells known as epithelioid and giant cells.

Ruffer (1890) described this process of lymphocyte change in the response of appendices and tonsils to normal bacterial invaders (27). His description of the process needs no modern revision: "The (lymphocytes') nuclei are surrounded by a small amount of protoplasm of a pale rose color. More careful examination of the lymphoid tissue reveals the presence of other mononucleated lymphocytes differing only slightly at first from the other cells surrounding them. The nucleus of these cells is somewhat paler and its reticulum slightly more apparent, though the protoplasm may not be at all increased in size as yet. Other cells possess a nucleus which is again slightly larger, paler, and more vesicular than the latter, as if the nuclear juice had increased at the expense of the solid part of the nucleus.... Lastly, the nucleus and protoplasm become enormous and the macrophage is formed.... The macrophage is merely a lymphocyte which has assumed a new shape, and... a new function" (p. 491). Excellent figures are appended to this paper.

Metchnikoff's lectures (22) on the comparative pathology of inflammation, delivered at the Pasteur Institute and published in 1892, demonstrated irrefutably the role of the lymphocyte as it hypertrophied to form, first, large mononuclears and then, hematogenous macrophages.

Arnold (1893) introduced various foreign bodies into the mesentery and lymph sac of frogs and followed the gradual transitions of lymph cells to the large connective tissue elements in the ensuing aseptic inflammation (28). Borrel (1894) made similar observations in tubercles in the kidney of the rabbit (29). Kanthack and Hardy (1894-95) traced the origin of their "hyaline cells" (large mononuclear cells) to the lymphocytes of coelomic fluid and established them as morphological units, distinct from the specific granulated cells (30). These investigators observed the same lymphocytic function in blister fluid from the skin of the arm of human volunteers, into which *Bacillus ramosus* had been subsequently inoculated.

Ranvier (1900) studied the functions of clasmatocytes and leukocytes in aspectic inflammation of the peritoneum, mesentery, and appendix in the green frog, salamander, white rat, guinea pig, and rabbit (31). He amply demonstrated that lymphocytes were a source of the defense elements.

Maximow (1902), in an extensive review of his experiments in aseptic inflammation, described: 1) the motility of lymphocytes; 2) migration of lymphocytes from the smaller vessels into the field of inflammation; and 3) their progressive hypertrophy into lymphocytogenous macrophages (his "polyblasts") (32). Maximow inserted a series of celloidin chambers into the connective tissues of rabbits, dogs, and pigeons. More than 20 timed stages, from 19 hours to 65 days of inflammation, were so studied. In 1903, the same investigator (33) extended his studies on new tissue growth in similar chambers, from the fifth hour to the eighth month in the process of scar formation. He demonstrated that macrophages originated from newly emigrated lymphocytes and sessile clasmatocytes.

In 1904, Maximow (34), while studying inflammatory connective tissue formation and changes in mast and fat cells of white rats, found that, in as short a period as four hours, the hypertrophy of emigrated lymphocytes resulted in macrophage formation. In the same year, Ziegler (35) studied edema in the skin and subcutaneous tissues of 21 human subjects at autopsy. He traced the gradual transformation of lymphocytes migrated from the blood vessels into large phagocytic cells in areas of edema. Schwartz (1904) introduced both glass and celloidin chambers into the connective tissue of rabbits and, by the second hour, the small and larger lymphocytes were migrating from the vessels in appreciable numbers and, between the 2nd and 10th hours, were transforming into large phagocytes (36).

Helly (1905) injected a large variety of bacteria into the pleural cavities of more than 100 experimental animals and studied the resulting exudates at 24 and 48 hours (37). He observed a continuous series of transition forms between lymphocytes and macrophages. In the same year, Maximow (38) combined celloidin chambers inoculated with various bacteria with the study of aseptic chambers. The septic reactions differed only quantitatively, not qualitatively. He emphasized that the lymphocytogenous origin of the macrophages was found most convincingly in the early (fourth to eighth hour) stages of inflammation.

The thesis of the opposition, mostly Germanic at that time, to this concept of lymphocytic function was that the lymphocytes were incapable of locomotion and, therefore, could not migrate from the vessels into areas of inflammation and form macrophages. Lewis and Webster (39), however, a decade or so later, were to make painstaking demonstrations of lymphocytic locomotion.

Metchnikoff (1905), in his second text, clarified the nomenclature of such cells (23). He stated: "The smaller white corpuscles found in fairly large numbers in the blood and lymph, commonly known as lymphocytes or small lymphocytes, are simply leucocytes with very little protoplasm, which in this state never fulfill phagocytic functions. It is only when it becomes older, when its nucleus, single and rich in chromatin, becomes surrounded by an ample layer of protoplasm, that the lymphocyte becomes capable of ingesting and resorbing foreign bodies. Several authors, with

Ehrlich at their head, still assign to those larger cells the same name — lymphocytes. Others, however, give them the name of large mononuclear cells. Confusion is thus possible, especially as Ehrlich includes under the same term the large mononucleated leucocytes, a very rare form in human blood which is distinguished by the greater staining capacity of its nucleus. To avoid this inconvenience I propose to designate the large lymphocytes by the name of blood macrophages and lymph macrophages (haemomacrophages, lymphomacrophages).... The mesoblastic phagocytes of the vertebrata are divided then, into fixed phagocytes — the macrophages of the spleen, endothelia, connective tissue, neuroglia, and muscle fibers — and free phagocytes.... The fixed macrophages and the free macrophages resemble one another so greatly that it is very often extremely difficult, if not impossible, to differentiate them. For this reason it is often very useful, when the exact origin of a large phagocyte is not known, simply to name it macrophage."

In the decade that followed, there was abundant confirmation of an experimental nature of Metchnikoff's lucid statement. In 1906, Maximow (40) turned to the study of inflammation in cold-blooded animals and chose the axolotl. His Taf. XVII, Fig. 2, and Taf. XVIII, Fig. 10, have been widely published in textbooks of pathology as classic portrayals of lymphocytic migration. In 1907, Zieler (41,42) used a Finsen-Reyn lamp's concentrated beam on the inner surface of a rabbit's ear to study the resulting aseptic inflammation. At 1, 2, 4, 15, and 24-hour stages he noted the migration of small lymphocytes into the area of inflammation and their transformation through cytoplasmic changes into large lymphocytes or polyblasts. In the same year, Renaut (43) completed his extensive work on the potential phagocytes of the connective tissues which he called rhagiocrine cells. He also worked on the source of the potential phagocytes in thoracic duct lymph. He traced many of these phagocytic cells back to lymphocytes.

The material of von Verebely (44) from the Surgical Clinic at Budapest consisted of granulating fatty tissue from patients with primary healed herniotomy incisions, dead two to three weeks later of apoplexy, pneumonia, or pulmonary embolism, as well as diverse patients with laparotomy incisions with primary healing or showing stitch abscesses, and a long series of patients with excised tumors. Von Verebely described the transformation of human lymphocytes into large mononuclear cells, phagocytic for fatty debris in such areas. Because of the surgical nature of his material, quite frequently, he was able to date the exact onset of the initiating stimulus to the fatty tissues. Stages studied were from 24 hours to 5 weeks in duration.

In 1909, Fischer (45) introduced elder pith saturated with turpentine into the subcutaneous tissues of mice and rats and studied the sites of inflammation at 0.75, 1.25, 2, 3, 5, and 18-hour stages. He, too, emphasized the necessity of early stage observation to catch the lymphocytes in process of migration from the smaller vessels.

In the following year, Babkina (46) supplied a partial solution to the puzzling problem of the apparent sluggishness of lymphocytic acquisition of phagocytic powers in the lymphatic tissues themselves. She embedded celloidin tubules in the spleen, lymph nodes, and marrow of rabbits and observed that the local lymphocytes remained inert for a long time but that, still later, they hypertrophied and were transformed into phagocytic polyblasts (macrophages). She explained this sluggishness by pointing out that such tissues already contain large numbers of cells which were immediately phagocytic, namely reticulum cells, and that lymphocytic transformation into phagocytes in such areas is therefore seldom required.

In 1911, Homen (47) studied tubercle formation within sciatic nerves of rabbits and guinea pigs. He inoculated virulent human type tubercle bacilli into these structures and studied the reaction in serial stages from the sixth hour through the fifteenth month. The origin of epithelioid cells from polyblasts and, ultimately, from lymphocytes, as well as from fibroblasts and adventitial cells, was apparent to him. In the same year, von Fieandt (48) injected virulent human tubercle bacilli into the internal carotid arteries of dogs and observed the transformation of lymphocytes into macrophages in the resulting tubercles in the meninges and cerebrum within 12 to 14 hours.

Also in 1911, Wallgren (49) published careful descriptions of experimental hepatic tuberculosis in rabbits, after injecting the organisms into cecal veins. In two to four days, the predominant lymphocytes showed numerous transitional stages to polyblasts. In his Fig. 1, Taf. XIII, eight stages in the development of the lymphocyte to epithelioid cell are depicted. Here, for the first time, changes in the cell center, centrioles, and attraction sphere were delineated, as progressive changes in lymphocytic function took place.

In 1912, Downey and Weidenreich (50), studying the reaction of the lymphocytes of the appendix of the rabbit to the influx of intestinal bacterial flora, confirmed Ruffer's (27) findings with respect to the phagocytic transformation of the lymphocytes. In 1913, Dubreuil (51) made an intensive study of lymphocytic functions. He employed an unusual method in proving the derivation of the mononuclears from the lymphocytes in that he correlated concomitant granular mitochondrial changes as the lymphocytes changed function. He also employed postvital staining with neutral red to show the same functional relationships of the lymphocyte.

Aschoff (52), in his extensive contributions to our knowledge of a general defense system of cells, excluded the lymphocytes from his scheme of a reticuloendothelial system, because, under the conditions in which his experiments were performed, he was uncertain as to whether lymphocytes were phagocytic for vital dyes. Even while Aschoff was performing his work, Tschaschin (53,54) was already demonstrating the small lymphocytes phagocytosed vital dyes until their cytoplasmic content was indistinguishable from ordinary macrophages. Downey (55,56) soon after demonstrated that the lymphocytes within the blood itself were capable of phagocytosing colloidal dyes, if only the dyes were first made available to these cells by the simple expedient of double ligature of a vessel with study of the leukocytes in the interposed segment.

Policard and Deplas (1917) described the cellular constituents of normal and infected human granulation tissue (57). These workers made counts of the cell types found at the surface, at an intermediate level, and at the depths of the granulations, at timed intervals from the 12th to the 25th day. They described the manner

in which lymphocytes migrated from the smaller vessels, transformed into the macrophage, and finally settled down as fibroblasts in the ultimate scar.

The reports of Bergel (1920) dealt with the lipolytic enzymes of the lymphocyte (58). In one series of experiments, he described the breakdown of the fatty constituents of the leprosy and tubercle bacilli within the cytoplasm of lymphocytes, after the lymphocytes had ingested those organisms. In another series, in which various lipids were injected into the body cavities, Bergel emphasized that the lymphocytes underwent structural changes as they assumed phagocytic and digestive functions toward lipids. As this physiological function was exerted by the lymphocyte, its nucleus enlarged, often becoming slightly indented; a parallel change went on in the cytoplasm with gradual ingestion of one, two, or more fat droplets; and the protoplasm of the cellular body also increased gradually. As more and more droplets were ingested, the cytoplasm became large, the nucleus became plumper with a looser structure, and the macrophage stage was attained.

In the same year, a comprehensive resume of Dominici's (59) experimental and observational works was published in three lengthy papers. As a background for his views on the lymphocytic origin of the phagocytes, he included a great deal of human material: a scrotal fistula, tertiary lesions of syphilis, mycotic infections, and normal lymph nodes, as well as those with carcinomatous metastases.

Latta (1921) reported that large lymphocytes in the lymphatic tissue of the intestine could take on abundant acidophilic cytoplasm and ingest lymphocytic remnants (60). In 1922, Danchakoff and Seidlin (61) injected a mass of the protein, edestin, into the mesenchymal tail plate of tadpoles. In reference to lymphocytic functions, the authors stated: "As these cells advance toward the injected mass they exhibit a series of changes, which made them rapidly acquire a digestive activity little expected from the small lymphocytes. A rapid transformation of a small lymphocyte into a histiotopic wandering cell thereby takes place" (p. 105).

In 1923, Maximow (62) added to the list of techniques by which the developmental potencies of lymphocytes had been studied. Lymphocyte production of macrophages in tissue cultures of mammalian lymphoid tissue is well depicted (Taf. XVIII, 5, 6; Taf. XXI, 13). In the same year, Maximow (63) also investigated blood formation in the Selachii and found that histiocytic cells of the embryonic forms as well as of adults arose from large lymphocytes which, in turn, arose from body mesenchyme. A year later, this indefatigable investigator (64) had managed to produce tuberculosis in explants from mesenteric lymph nodes, omentum, and intermuscular connective tissue in tissue culture. In the lymph nodes, reticulum cells were, of course, the most active phagocytes. However, lymphocytes also played an active part, hypertrophying and transforming into polyblasts and, later, sometimes acquiring an epithelioid character "joining the reticular cells in the process of tubercle and giant cell formation."

In the same year, 1924, Alfejew (65) reported on the origin of the wandering cells of the connective tissues of the embryonic forms and newborn of many different species, including man. He concluded that the wandering cells arose from lymphocytes. In 1925, Maximow (66) inoculated tissue cultures of buffy coats of the blood of rabbits and found that "the monocytes respond promptly to the stimulus and, being larger and better prepared for the defense reaction, sooner reach the fully developed epithelioid stage. The lymphocytes are slower, but nevertheless they follow in the same way and, sooner or later, join the monocytes in their transformation into epithelioid cells" (p. 429). In the same year, Timofejewsky and Benewolenskaja (67) reported similar findings. In this year too, Jordan (68) injected India ink into the dorsal lymph sac of frogs and concluded that the monocytes observed developed from lymphocytes.

In 1926, Stillwell (69) injected India ink and fresh egg yolk into the tongues of adult live frogs and observed that the living mononuclear exudates were almost exclusively hematogenous in origin: "They represent lymphocytes and monocytes of the circulating blood which have emigrated out of the blood vessels and have undergone a rapid hypertrophy in the tissues" (p. 90).

In the same year, Vierling (70), too, studied living cells in Amphibian larvae. He injected vital dyes into the heart or lymph vessels, and then used the supravital dye, neutral red, to follow the changes in his preparations. He saw that lymphocytes, already tagged with the vital dyes which they had ingested within the vessels, migrated out of the blood and lymph vessels, and he concluded that these cells were capable of forming any of the elements of connective tissue.

In 1926, Lang (71) introduced bits of sponge containing lecithin and agar into subcutaneous tissues of animals into which he had already introduced India ink intravenously. In this way, he demonstrated that, although the endothelial cells, at times, contained intracellular carbon aggregates, they did not transform into phagocytic elements. Instead, the macrophages arose from lymphocytes and monocytes. This work stressed that the hypertrophy of the emigrating lymphocytes and monocytes began while these cells were still *within* the capillary lumen: "Transitional forms between lymphocytes and monocytes, ordinarily missing or rare in the circulating blood, seem to become common in the stagnant blood of the vessels . . . (of the inflammatory site)."

In 1927, Timofejewsky and Benewolenskaja (72) repeated their previous work with tubercle bacilli; but, at this time, they used the cells of human blood. The lymphocytes and monocytes of man were likewise seen to transform into epithelioid cells.

In that same year, Michels and Globus (73) studied the lymphocyte reaction in the central nervous system of 12 human beings. Eight cases of polioencephalomyelitis and four cases of acute epidemic encephalitis were studied. The authors pointed out the favorable opportunity for determining the origin of perivascular infiltrations in inflammatory lesions in the central nervous system: "There is a strong tendency for the inflammatory elements to be restricted to the adventitial spaces for relatively long periods, undergoing various morphological changes."

Here, in an inflammatory site almost devoid of mesenchymal elements, the compound granular corpuscles (macrophages) were found to arise largely from lymphocytes and monocytes emigrated from the blood stream and, to some extent, from the mesenchymal glial elements as well. Because of the numerous lymphocyte-

migration-pictures, in some cases, and their paucity in others, one of their conclusions was that such cells must be extravasated in showers.

In 1928, Watson (74) studied phagocytosis in a case of histoplasmosis. Although reticulum cells were the chief source of phagocytes in the lymph nodes, spleen, liver, adrenals, and lung; in the lymph nodes, liver, and lung, lymphocytes also became phagocytic for the yeastlike invaders.

In the decade 1915-1925, there was set forth the hypothesis by Mallory, McJunkin, Foot, Herzog, and Marchand that simple endothelium, lining the common blood vessels, gave rise to the monocytes of the peripheral blood and, to those cells, they applied the name "endothelial leukocytes." This group also believed that macrophages or histiocytes corresponded to these same "endothelial leukocytes." As soon as the reticuloendothelial system had been delineated by Aschoff (52), Downey (50,-56,74a,74b) and Maximow (75), the hypothesis of "endothelial leukocytes" as a source of macrophages was abruptly abandoned, for it became obious that the littoral reticulum cells lining the blood or lymph spaces of the hematopoietic and sinusoidal organs were the only cells lining blood or lymph spaces which possessed true phagocytic and occasionally hematopoietic powers.

In the decade 1920-1930, there arose groups of investigators who emphasized cell types other than lymphocytes and reticuloendothelial cells as the cellular origins of the macrophages. One of these was von Möllendorf, who claimed the fibroblast as the sole source of the phagocytes. He denied the hemtogenous origin of all exudative cells, including neutrophilic leukocytes.

Another group of investigators claimed the monocyte as the most important of the hematogenous macrophage sources. The investigators who attached prior importance to the monocytes can be divided into two groups according to their interpretation of monocyte-macrophage relationships. Lewis (76) and Carrel and Ebeling (77) held that the moncyte could enhance its phagocytic powers and transform into the macrophage. The other group has been called the "supravitalists" because its members have classified living blood cells according to the way they react to neutral red and Janus green. Simpson (78) noted neutral red granule groups in the cytoplasm of the monocyte and believed they were characteristic of monocytes. In 1925, Sabin, Doan, and Cunningham (79) distinguished two types of cells in the field of inflammation: a clasmatocyte which they derived from the endothelium and a "monocyte" which developed in the spleen and in "wide areas of the body" from a "primitive cell." They explained that "the monocyte of the tissues arises like a blood cell and has to undergo a maturation before it becomes a typical adult type." They also stated of the monocyte that "its remarkable power of cell division often makes it the predominating functional cell after it has time to mature."

In the same year, Cunningham, Sabin, Sugiyama, and Kindwall (80) reported on the role of their "monocyte" in tuberculosis in rabbits. They concluded that successive waves of nonphagocytic reticular cells and epithelioid cells appeared and reappeared in tuberculosis.

In 1926, Murray, Webb, and Swann (81) reported on the appearance of a high monocytosis in the blood of many rabbits suffering from a disease from which they isolated the causative organism termed *Bacterium monocytogenes* (now *Listeria monocytogenes*). These workers performed six brief one-stage experiments on the white cell response in pleural exudates. They injected into the pleural cavities of successive normal rabbits: peptone broth, *B. coli*, and *L. monocytogenes*. At six hours, they observed in all a predominant polymorphonuclear leukocytic response. Next, they injected the same antigens into animals which were running high blood monocytoses due to systemic infection with *L. monocytogenes*. In these latter animals, the response to peptone broth and *B. coli* was as before, but local injection of *L. monocytogenes* elicited 30.4% of large mononuclears at six hours. Witts and Webb (82) (1927) utilized this organism in an attempt to establish the origin of monocytes. They concluded that monocytes arose in the spleen and bone marrow.

Bloom (83) (1928) made an extensive study of the origin of the monocyte in animals suffering with this disease, using section, supravital, dry and wet smear techniques. He concluded that the monocytes arose through transformation of lymphocytes occurring in areas of sluggish circulation, such as the sinusoids of the liver and sinuses of the spleen and bone marrow.

In the same year, Bloom (84) studied focal necroses in rabbits which had been given large doses of a virulent strain of *L. monocytogenes* intravenously. These abscesses found in the liver, spleen, adrenals, and lymph nodes consisted largely of macrophages (his polyblasts) connected by a series of morphological transitions with blood lymphocytes and monocytes at the edges of the abscesses.

Again, in 1928, Bloom (85) made supravital studies of the blood cells, subcutaneous connective tissues, and mesenteric lymph nodes of the white rat and found that lymphocytes, monocytes, and plasma cells all contained a "segration apparatus" of neutral red vaculoes and that such a configuration could not be used as a criterion in differentiating the agranular leukocytes. By an ingenious method, he stained the lymphocytes of the subcutaneous tissues intravascularly with neutral red, and these cells, while still within the vessel lumen, developed the segregation apparatus within their cytoplasm.

In the same year, in tissue cultures of rabbit thoracic duct lymphocytes, Bloom (86) traced the lymphocytes through a "monocyte" stage to the macrophage and then to the fibroblast. His supravital studies of such cells also revealed a neutral red rosette in the small early lymphocytogenous macrophages.

Maximow, (86a) too, utilized tissue cultures to study the leukocytes of the chick, rabbit, guinea pig, and *Macacus rehesus*. He employed supravital stains, as well as customary histologic techniques. To his tissue cultures, he at times added inflammatory extracts, Lithium carmine, and neutral red. Within four to eight hours, the lymphocytes had eccentric nuclei, increased cytoplasm, and increased pericentriolar neutral red vacuoles. By 11 hours, the limits between the lymphocytes and monocytes had been effaced and, just as in inflammation, the lymphocytes had gradually hypertrophied to form elements which could not be distinguished from monocytes.

Caffier (87), in the same year, observed lymphocytic transformation into monocytes at 12 hours, and later into histiocytes and epithelioid cells, in his cultures of normal human blood cells. Timofejewsky and Benewolenskaja (88) continued their work on lymphocytic formation of macrophages, in 1928, reporting on the introduction of tubercle bacilli, type BCG, into their cultures. They confirmed their earlier findings.

Also in 1928, Lacassagne and Gricouroff (89) exposed in vitro cultures of rabbit blood leukocytes to the action of roentgen rays and radon. These cells were observed at 2, 4, 5.5, 7, 12, 24, 29, and 53 hours on up to eight days. Whereas beta rays rapidly destroyed the cells, gamma rays in proper dosage permitted migration of lymphocytes and transformation of survivors into macrophages.

Michels and Globus (90) (1929), in their second series of investigations, described the transformation of lymphocytes and monocytes, migrated from the blood stream, in the encephalon of cases of paresis, meningovascular syphilis, and vascular syphilis. Mitoses among the emigrating lymphocytes were frequently noted, one of the few references found describing this process in vivo, in inflammation.

Stieve (1929) studied the uteri of more than 100 women in all stages of pregnancy, labor and the puerperium, as well as nonpregnant human uteri (91,92). He observed the lymphocyte as it progressively transformed into the histiocyte, then the macrophage and, ultimately, the fibroblast or smooth muscle cell, both in the enlarging uterus of pregnancy with its physiological inflammatory process and in the early puerperium with its true local inflammation. In the early stages of pregnancy, he observed the migration of the lymphocyte, a readily available mobile source of the histiocyte, into the uterine tissue. This process dwindled at the fourth month of pregnancy, but was revived at the second day of the puerperium, where again a mobile source of hematogenous macrophages was sorely needed to bolster the local defences of a uterine wall so recently subjected to the birth trauma.

In 1929, Higgins and Palmer (93) injected autogenous blood into the subserous spaces of the stomach and cecum of rabbits. They traced the development of blood monocytes and lymphocytes into histiocytic elements in the experimental hematomas so produced. In the same year, Kreyberg (94) cultured exudate cells from a case of tuberculous pleurisy. Lymphocytes comprised 98.4% of the agranular leukocytes; monocytes and macrophages the remainder. The lymphocytes rapidly transformed into macrophages, epithelioid cells, Langhans' giant cells, and fibroblasts. Supravital and vital staining techniques confirmed the observations made from stained smears. In the same year, Timofejewsky and Benewolenskaja (95) cultured mature and immature lymphocytes from five cases of lymphatic leukemia. Both mature and immature lymphocytes were seen to develop into macrophages, epithelioid cells, and giant cells. A year later, Silverberg (96) confirmed the earlier work of Babkina (46).

In 1930, Sabin, Doan, and Forkner (97) studied the reaction of the connective tissues of the rabbit to chemical fractions derived from tubercle bacilli. Their index of fractional activity was the stimulation of "monocytes" and their formation of epithelioid cells.

In the same year, Cappell (98) made an extensive study of the cytology of the inflammatory exudate of the peritoneal cavity. He observed: "By the end of six to nine hours the lymphocytic cells . . . exhibit commencing neutral red storage, coincidentally with an increase in the amount of cytoplasm" (p. 433). After 12 hours: "while many cells of the lymphocyte type are present the majority of the mononuclears exhibit nuclear indentation, a more abundant and basophilic cytoplasm and increased storage of neutral red, the granules being in some aggregated in rosette form, in others scattered throughout the cell. No clear distinction can be made by supravital staining between lymphocytes, monocytes, and macrophages; while typical examples of each can readily be identified, the three types are connected by all intermediate stages and it is clear that a progressive development is going on in the smaller vessels, by which they are rapidly acquiring the functional characters of young macrophages." Cappell also obtained corresponding results after the use of vital stains.

In 1931, Ekola (99) studied inflammation in the subcutaneous tissues of rabbits into which trypan blue, sodium ricinoleate, and diphtheria toxin had been previously introduced. At timed intervals, she was able to trace the origin of the macrophage from the blood lymphocyte, the tissue clasmatocyte, and the fibroblast.

In 1932, Pierce (100) cultured human leukemic lymphocytes in vitro and observed that lymphoblasts produced monocytelike cells which, in turn, transformed into polyblasts (macrophages). In 1935, Seki (101) studied cells from the heart's blood of various reptiles and amphibia stained vitally with trypan blue, and noted transitional forms between lymphocytes and monocytes.

In 1936, Taliaferro and Cannon (102), in describing the cellular reaction in the spleen of Panamanian monkeys infected with malaria, stated that: "The increase in macrophages can be easily explained on the basis of their transformation from lymphocytes and our evidence is overwhelmingly in favor of such a conclusion" (p. 122). As evidence, they described an intense lymphocytopoiesis: "the cells in the activated follicles, particularly in the transitional zone, look as if they were developing into macrophages. After continued malarial stimulation, the sinuses of the red pulp also show every gradation from lymphocyte to monocyte, to monocyte with abnormal nuclear lobes, to macrophages, and somewhat the same picture is seen within the splenic cords." In 1937, Taliaferro and Mulligan (103), studying malaria in Rhesus monkeys, noted that the greatest number of new macrophages in spleen, bone marrow, liver, and adrenals arose from medium lymphocytes.

In 1938, Hertzog (104) incubated blood of human patients suffering from acute and chronic lymphatic leukemia, absolute and relative lymphocytoses, and infectious mononucleosis with nonvirulent *Staphylococcus aureus* and *Streptococcus viridans*. He demonstrated that lymphocytes, even in their "prephagocytic stage" showed some phagocytic activity and that phagocytosis was more marked in the leukocytoid lymphocytes of infectious mononucleosis with their larger cell bodies. His photomicrographs of this process stand as objective evidence of this phase of lymphocytic function (his Figures 3 to 5).

In 1939, Kolouch (105) introduced a radically new technique for demonstrating the lymphocytic origin of macrophages. After in-

jection of egg white into the subcutaneous tissues of rabbits, he made dry-fixed spreads of the inflamed connective tissues at timed intervals and stained his preparations in the manner of blood smears. This technique permitted accurate and detailed cytologic examination of the lymphocytes as they transformed into macrophages. Kolouch pointed out that, even with his improved technique, inflammation must be studied within the first 14 hours or else the lymphocytic origin of the mononuclear cells is largely obscured.

In 1940, Taliaferro and Klüver (106) made air-dried impressions of the liver, spleen, and bone marrow of Panamanian monkeys with malaria. Intermediate stages between lymphocytes and monocytes and between monocytes and macrophages were increased. In the same year, Finlayson and Lattla (107) studied the early reaction of the leptomeninges of the rabbit to injections of trypan blue at timed intervals. They reported: "... it was found that within 30 minutes there occurred in the pia mater a perivascular infiltration of lymphocytes, which gradually became transformed into monocytes and plasma cells. Such cells in turn increased in size; became more and more vacuolated; and segregated increasing quantities of dye, becoming typical macrophages" (p. 286).

Also, in the same year, Plimpton (108) injected ventriculin subcutaneously into guinea pigs and rabbits. Within three hours, she observed lymphocytes hypertrophying into hematogenous macrophages (her polyblasts).

The works of Tompkins (109-112), concerning the role of large mononuclear leukocytes in inflammation, will be considered as a separate topic in this monograph. Her student, Gray (113) in the same year (1940) followed the hematogenous origins of "monocytes" with differential supravital counts after injection of various synthetic triglycerides into the subcutaneous tissue of guinea pigs. The large majority of these cells hypertrophied and became macrophages.

In 1942, Berman (114) described a method for obtaining dry films from tissue cultures of rabbit lymph nodes. He found transformation of lymphocytes to macrophages marked after 31 hours. He stated: "Typical localized vacuolation seen in the lymphocytes of dry films is replaced by diffuse vacuolation at the time when both the directional polarity of the cell and the lymphocytic character of the nucleus are lost. This accompanies a change in the type and location of pseudopodia and represents the point of transition from a lymphocyte to a polyblast of indifferent origin." Berman was the first to emphasize that the lymphocyte, as it transformed into the macrophage, showed a progression of transitional modes of locomotion and pseudopodial formation.

Clark, Clark, and Rex (115) and Ebert, Sanders, and Florey (116) had made use of windows or chambers in rabbit ears to observe leukocytic functions. They were unable to observe the transformation of living lymphocytes into macrophages. However, in 1942, Harper (117) reported on fibrous wound healing as seen in transparent chambers inserted in the ears of rabbits. He studied the process of wound healing from a period shortly after the production of the wound until the formation of the completed scar. His pertinent conclusions were: "The formation of new cells and tissue back from the edges of the wounds by mitotic division and subsequent migration into the wound was not observed to occur in these studies. It was not possible to account for the fibroblasts or macrophages appearing in the healing area by mitotic division from pre-existing cells in the wound area. A process of differentiation through which lymphocytes undergo progressive transformation into macrophages in the tissues at the wound edge was reported. This process was observed to correspond closely to the differentiation of lymphocytes which is reported to occur in tissue culture."

Frerichs (1943) studied the influence of a monocytosis within the blood stream produced by *Listeria monocytogenes* upon the character of the cellular exudate (118). He found a high percentage of polymorphonuclears at six hours in his controls, but, in his test animals, the ratio of polymorphonuclears to monocytes was usually 1 to 1.

In the same year, Rey (119), in studying the reactions of the hamster associated with natural immunity to *Leishmania brasiliensis*, noticed migration of lymphocytes and monocytes and their transformation to hematogenous macrophages in the third and fourth hours, with peak activity in the 5th to 12th hours. Destruction of the invading organisms by the hematogenous macrophages occurred between the 13th and 24th hours.

In 1944, Dougherty (120) introduced the dry-fixed impression technique to the study of inflammatory reactions of the brain and cerebrospinal fluid. In addition, he employed vital and supravital staining, as well as ordinary section techniques. He observed that: "... the first cells reacting to experimental brain wounds... were lymphocytes which transformed to macrophages" (p. 85).

In 1945, De Bruyn (121) studied the locomotion of lymphocytes cultured from the abdominal lymph nodes of rabbits and guinea pigs by means of motion pictures. The lymphocyte presented two phases, a locomotion phase, in which the activity of the cells was polarized, and a depolarized phase of activity. A gradual hypertrophy of the lymphocytes took place while, concomitantly, the depolarized phase of the lymphocytes changed progressively into the motion of macrophages which were continuously depolarized.

In 1949, Campbell and Good (122) studied the antigen-antibody mechanisms in neurotropic virus diseases and observed lymphocytic transformation into intermediate polyblasts, which, in turn, formed macrophages.

In the same year, Townsend and Campbell (123) utilized Kolouch's (105) technique in mice and rabbits to observe the effects of roentgen rays on the inflammatory cells of those animals. They reported: "Within two hours after the initial infiltration began, some lymphocytes were acquiring an increased nuclear and cytoplasmic volume which characterized early intermediate polyblast development. In the inflammatory site the evolution of the nuclei of the lymphocyte to that of the intermediate polyblast and finally to the macrophage could be followed in detail" (p. 1349).

In 1950, Good (124) studied experimental allergic brain inflammation in rabbits, utilizing egg white as the antigen. In nonallergic inflammation in the brain, he noted: "Soon after their initial diapedesis the lymphocytes undergo changes in both their nu-

clear and cytoplasmic structure which eventually results in a transformation through the intermediate polyblast to large fat-filled phagocytic macrophages of the inflammatory exudate" (p. 84). In the sensitized rabbits, there was some delay of the lymphocytic diapedesis but similar structural and functional changes eventuated.

In the same year, Dougherty and Schneebeli (125), also making use of Kolouch's (105) technique, observed lymphocytic transformation into macrophages in connective tissue spreads of the inflammatory tissue of the mouse.

In summary, the transformation of monocytes and reticuloendothelial cells and their counterparts in the connective tissues appears to have been well established in the literature dealing with inflammation. The transformation of the lymphocyte to the macrophage has been reported to have been accomplished by the following morphologic and functional changes: increase in nuclear size; breaking up of coarse nuclear chromatin masses into fine angular pieces; assumption of a nuclear chromatin pattern with chromatin-parachromatin distinction; increase in parachromatin; increase in cytoplasm; increase in phagocytic ability for bacteria, cellular debris, and vital dyes; increase in cytocentric size; increase in number of cytoplasmic neutral red vacuoles with aggregation into a rosettelike apparatus; and increasing evidences of depolarization-locomotion.

It is the purpose of this investigation to report on a technical procedure which has permitted evaluation of the relative importance of the role played by lymphocytes and monocytes as macrophage producers in acute inflammation in man.

## Materials and Methods

The site chosen for our tests in man is either the volar surface of the forearm or the anterior surface of the thigh, although any other convenient region may be employed. The skin is shaved and cleansed with alcohol. Then, by means of a sterile scalpel or razor blade, the epithelium is scraped away from an area three or four millimeters in diameter. When the papillary layer of the corium is reached, fine bleeding points are in evidence. If the lesion is further deepened, bleeding increases, and another site had rather be chosen. A small amount of bleeding is to be desired as evidence that the corium has actually been reached.

The time at which the epithelium is removed denotes the time at which the experimental inflammation begins, because the trauma of the technique itself serves as an inflammatory stimulus. A solution or suspension of the desired nonlethal inflammatory agent is next applied to the denuded corium with a platinum loop. The lesion is then immediately covered with a sterile, chemically clean cover slip, which is surmounted, in turn, by a square of cardboard, cut slightly larger than the cover slip. The cardboard is covered by surgical adhesive tape measuring approximately two by four inches. The tape is so applied that its center overlies the lesion. If further tension is required, leverage is obtained by placing a flat cork disc over the tape lying above the lesion and affixing the disc with a second and narrower piece of adhesive tape. We have employed 15 mm square glass cover slips.

For convenience in handling, a hundred or so kits are prepared at a time, each kit consisting of a cover slip and a corresponding cardboard square wrapped together in a paper wrapper. These are sterilized in covered Petri dishes and can be removed individually as needed.

The cells of the inflammatory exudate migrate to the undersurface of the cover slips, flattening themselves out as they do so. When this much has been accomplished (in 30 minutes to an hour, depending on the nature of the inflammatory exudate), the cover slip is removed and rapidly air-dried. At the same time, another sterile cover slip is placed over the same lesion and the process is repeated as often as desired at timed intervals. In this way, a series of permanent, fixed preparations of in vivo samplings of the cellular exudates of man have been obtained.

The cover slips exhibit but a single layer of exudative cells on their undersurfaces. Having been rapidly air-dried, they are treated like a blood slide and stained with May-Grünwald-Giemsa or Wright-Giemsa stain, modified as follows: about four to six drops of May-Grünwald are put on the cover slip for one minute; the stain is then diluted with the same number of drops of Haden's buffer solution, and the mixture is allowed to remain for two minutes; the mixture is then drained off and, without washing, six to eight drops of 1.5 strength Giemsa stain (1.5 drops Giemsa stain to each 1.0 cc of buffer) are added and allowed to remain six minutes. The cover slip is then washed rapidly in distilled water, blotted dry, and mounted in Clarite on a slide.

In staining, the cover slips were either held in cover-slip holders or mounted on an ordinary glass slide by means of a drop of rubber cement. If the latter method is employed, the entire slide can be covered with the staining solutions and correspondingly larger amounts of the reagents are employed.

Our photomicrographs (Figs. 1-13) are demonstrations of cells which have migrated to the undersurfaces of such preparations. Single lesions can thus be sampled for their cytologic content at short intervals throughout the first 48 hours of acute inflammation in man. The preparations are permanent, and their improved cytological detail permits careful comparison with the cells seen in ordinary dry smears of the blood or bone marrow aspirates.

Forty-two different lesions of this type were studied in healthy human volunteers and they composed over 250 separate cover-slip preparations at 1.75, 2, 3, 3.75, 5, 6, 7, 9, 10, 12, 14, 15, 16, 16.5, 18, 20, 21, 22, 23, 24, 28, 31, 40, and 47.5 hours of inflammation. In addition to the trauma of the technique alone, nonpyogenic antigens were employed (egg white, diptheria toxoid, triple typhoid vaccine, old tuberculin, and first-strength PPD), to which human subjects were not systemically immunized.

That the participation of blood monocytes in local inflammation is in proportion to their numbers in the blood has been demonstrated previously in experimental animals by Murray, Webb, and Swann (81) and by Frerichs (118). Study of functional and structural changes in monocytes in acute inflammation in man was achieved by application of the technique described above to the study of a patient suffering from monocytic leukemia, Naegeli type (126), a form of chronic granulocytic leukemia in which functionally maturated monocytes, approximately 15 $\mu$ in diame-

ter, numbered more than 6,000 cells per cu mm (Fig. 14). Prominent nuclear indentations "tagged" 85% of these monocytes. Our photomicrographs (Figs. 15-18) depict cells which migrated to the undersurfaces of the preparations obtained at timed intervals from a single lesion inoculated with egg white.

Concomitant, high monocytoses, in the peripheral blood of rabbits, were produced by experimental *Listeria monocytogenes* infection. Forty half-grown male rabbits were used in this series. Thirty-five of these animals were used to establish the proper dosage and virulence of *L. monocytogenes* required to obtain the monocytic conversion of the nongranular leukocytes of the blood. Five animals were used for the actual experiments on local inflammation. A strain of high virulence, *L. monocytogenes*, American Type Culture, was employed. Its morphology was that of strongly gram positive, small straight or curved motile rods. This strain produced leukopenia and death in rabbits within 15 hours after a single administration of 0.1 cc in 0.9% sterile saline (No. 1 nephelometer) from a 36 hour Difco-liver-veal agar slant growth. After four months' passage on artificial media with monthly transplants on Difco-liver-veal agar, the virulence of this strain was reduced to levels at which 0.3 cc to 0.8 cc of our No. 1 nephelometer reading diluted in sterile 0.9% NaCl of a 36 hours' growth on the same agar was then nonlethal when given intravenously. At 60 to 100 hours, a peak monocytosis was thus produced in the rabbit blood (Fig. 19).

At the height of the above-described monocytoses, a local inflammation was produced on the rabbit's ear with appropriate modification of the technique described above for man, as follows: a tongue depressor was fitted as a splint into the hollow of the inner side of the shaved ear and fixed in place by two strips of adhesive tape, one encircling the ear at its base and the other two centimeters from its tip. The outer ear of the rabbit then presented a smooth, flat bare area whose vascular supply was in no way impaired. The lesion was produced and studied in the center of this area in the manner described above for the volar surface of the forearm of man.

(Figs. 20-27) are demonstrations of cells which have migrated to the undersurfaces of such preparations. Five rabbits, with varying normal and converted blood pictures, were tested as to their local inflammatory response to local *L. monocytogenes* alone, to the liver-veal agar, to avirulent *Staphylococcus albus*, and to the virulent strain of *L. monocytogenes*.

## Results

### A. Nonimmune acute inflammation in man

This group consisted of 42 experimental lesions in which an antigen to which the human subjects were not systemically immunized was applied locally. Experiments H-8 and H-11 are representative of this group and their protocols will be reported in detail.

**Experiment H-8.**

The lesion was prepared in the skin of the forearm of O.E. as described above, and 0.05 cc of 1:1000 tuberculin (O.T.) and 0.05 cc of 1% solution of trypan blue were applied to the lesion. O.E. has had a consistently negative tuberculin test. Blood samples were taken before the start of the experiment and were within normal values. In the next 24 hours, seven consecutive cover-slip preparations were obtained from this single lesion.

At three hours, neutrophilic leukocytes were present in great numbers. Fig. 1 is a photomicrograph of the 3-hour preparation from this lesion. The neutrophils were larger than normal and appeared edematous. Their cytoplasm was colorless, and their specific granules were wide spaced. A few intracytoplasmic vacuoles were present. Moderate numbers of rounded-up portions of neutrophilic leukocytic cytoplasm had broken away from the leukocytic cell body. Such fragments were 6 to 8 $\mu$ in diameter. Their specific granules were the same as those found within intact neutrophils. A few eosinophils were present, and a rare, free portion of eosinophilic cytoplasm was noted.

A few lymphocytes were likewise present at this stage. Such a lymphocyte is depicted near the center of our Fig. 1. This lymphocyte measures 11 X 12 $\mu$ and is swollen like the neutrophils, although the latter in Fig. 1 varied from 15 to as high as 20 $\mu$ in diameter. The lymphocyte nucleus was composed of large, dark chromatin masses poorly demarcated from scant, colorless parachromatin. The cytoplasm was sparse and consisted for the most part of basophilic spongioplasm. Three or four azurophil granules were present in one area of the cytoplasm.

A few macrophages with round, oval, or irregular nuclei were also present. Their nuclei possessed a coarsely stippled or ringed chromatin pattern, each chromatin piece being anuglar and irregular, with frequent clumping of these pieces. The colorless or light blue parachromatin was separate from the purple chromatin. Nucleoli, when seen, were sparse, irregular in outline, and basophilic. The cytoplasm was abundant with irregular outlines and consisted of a pale blue background containing mottled and vacuolated areas of almost colorless hyaloplasm. Some of the vacuoles contained particles of ingested trypan blue. These cells were the clasmatocytes or resting wandering cells of the connective tissue.

At six hours, neutrophilic leukocytes were still the most numerous of the cells. They were smaller, now measuring only 10 to 11 $\mu$ to their greater diameters. The cytoplasm, in many areas peripheral to their nuclear lobes, was scant or missing. Many more free cytoplasmic fragments were observed. A few neutrophils possessed cytoplasmic vacuoles and some had ingested trypan blue.

About a third of the exudative cells were lymphoid in character. Many of these cells were small lymphocytes, such as those shown in Fig. 2. They measured from 7 to 11 $\mu$ in diameter. These cells were frequently fixed in ameboid motion, and, as a result, the cellular outlines were irregular. Intense activity was exhibited by the cytoplasmic-nuclear interface of these small lymphocytes. The lymphocytic nucleus, which usually appears to be round in smears of peripheral blood, possessed a more or less deeply grooved indentation near the cytocentrum of the cell body. The nuclear membrane exhibited, in addition, irregularity of outline here and there along its entire extent. The cytoplasm was deeply basophilic. Some of the small lymphocytes contained ingested portion of the neutrophilic cytoplasm described above in their cell bodies. The neutrophilic granules, in such protoplasmic bits, were acidophilic.

Clasmatocytes, too, were greatly increased in number, and frequently they exhibited kidney-shaped or horseshoe-shaped nuclear outlines bearing superficial resemblance to monocytes of the circulating blood of man, although their cytoplasm contained no azurophilic dust. There were occasional vacuoles and particles of ingested trypan blue, but ingestion of bits of neutrophilic cytoplasm was infrequent in contrast to such activity of the small lymphocytes.

At nine hours, neutrophilic leukocytes made up about 50% of the cells of the exudate. The neutrophils showed profound changes. Although a few were large and edematous, the majority were shrunken. Their cytoplasm was scanty and some were miniatures, measuring only 7 $\mu$ in diameter. Vacuolation of cytoplasm was sometimes noted. The nuclear lobes had clumped. Often the chromatin stained darker than normally and the chromatin masses showed evidence of clumping. Free cytoplasmic portions of neutrophils were not much in evidence at this stage.

The most striking change in the inflammatory picture, at nine hours, was an increase in the lymphocytes which could be found in this preparation. Fig. 3 depicts such a field. Many of the lymphocytes in this field had been fixed in ameboid motion. As a result, they were wormlike in their elongated migratory pattern. Although the more elongated forms rarely measured more than 15 $\mu$ in length, they were correspondingly narrower in breadth (7 $\mu$). An interesting additional feature of the nucleus, other than its irregularity of outline, was the breaking up of the coarse chromatin pieces ordinarily associated with the mature lymphocytic nucleus observed in the peripheral blood. Although numerous, coarse chromatin masses remained in the lymphocytic nuclei at this stage, the parachromatin had increased in amount and was distinct in some nuclear areas. An examination of Fig. 3 reveals that the cytoplasm is still scant, forming but a thin rim about the rounded or elongated nucleus. In the actual specimen, it was deeply basophilic, flaky and occasionally vacuolated. At times, it contained ingested trypan blue particles, as shown in one of the lymphocytes in Fig. 3. Another one of the lymphocytes in Fig. 3 presents a deep cleft in its nucleus. The small size of this cell and its scant, deeply basophilic cytoplasm characterize it as a lymphocyte.

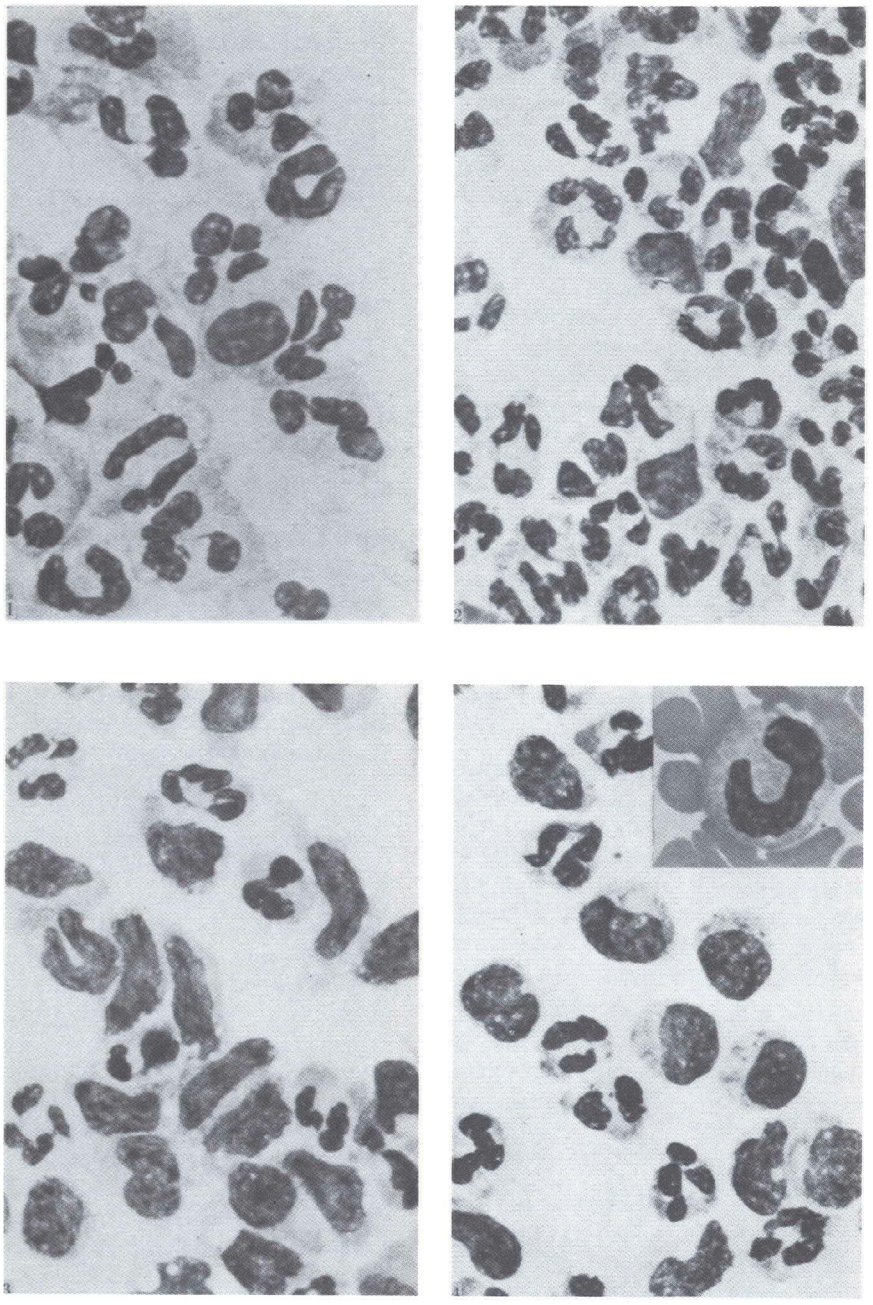
For ready comparison of such monocytoid lymphocytes with true monocytes of man as observed in his peripheral blood, in the inset of our Fig. 4, we have depicted a monocyte from the peripheral blood of O.E. from a smear taken at the beginning of this experiment. The magnification is the same and the staining techniques were identical. This monocyte was a large cell measuring 16 X 18 $\mu$, yet it was small among monocytes. This larger size is an important distinguishing feature of the monocyte as opposed to the small phagocytic lymphocytes in these preparations. The large cytoplasmic-nuclear ratio of the blood monocyte is likewise apparent in the inset in Fig. 4. The cytoplasm was a muddy-blue gray and contained fine, dustlike azurophil granules. The nucleus showed a large nuclear bay. The nuclear pattern was characterized by fairly coarse chromatin clumps and strands which, nevertheless, were well demarcated by distinct parachromatin. Cells with all the characteristics of the cell shown in Fig. 4 (inset) were rarely observed in our cover-slip preparations taken from acute inflammatory lesions in subjects without increased monocytic populations in their peripheral bloods. Fig. 5 (inset) depicts another monocyte from the blood of O. E. for further comparative study of these cell types.

At 12 hours, neutrophilic leukocytes comprised less than half the cells of the exudate. As depicted in Fig. 5, the neutrophils are shrunken and their nuclei were pyknotic and clumped, and most of their cytoplasm peripheral to their nuclear lobes was missing. They measured only 6 to 8 $\mu$ in diameter with a few of these cell types relatively more intact.

The lymphocytes of the type depicted in Fig. 5, which is a photomicrograph of this preparation, when rounded up as they are in this figure, measured only 10 to 12 $\mu$ in diameter. Their small size and lymphocytic characteristics were much more apparent and resembled the well-known characteristics of lymphocytes of the peripheral blood. They made up about 50% of the cellular exudate at this stage. The nucleus of these lymphocytes, although not as yet enlarged, showed increasing irregularity of outline and breaking up of denser chromatin masses into finer and finer, distinct, angular pieces. This process more sharply delineated chinks of parachromatin (nuclear juice) which was apparently increased. The cytoplasm in two or three of the more central lymphocytes in Fig. 4 was slightly increased in amount. This increase was probably more apparent than real for, when viewed under the microscope, this cytoplasm was bulging with ingested particles of trypan blue, bits of neutrophilic cytoplasm, and other types of cellular debris. In other areas in this field, lymphocytes with remnants of neutrophilic nuclear lobes in their cytoplasm were found occasionally.

Clasmatocytes were as numerous as in the preceding stage but, due to the great influx of lymphocytes evidenced at this period, they were, numerically, relatively less important. During the early stages of inflammation, they were quite prominent and an important early source of macrophages, but as the hematogenous defense elements continued to migrate into the area in increasingly large numbers, the activation of most of the available clasmatocytes already having been accomplished, such clasmatocytic activity was gradually overshadowed by the hematogenous elements.

At 14 hours, pyknotic, degenerated remnants of neutrophilic leukocytes comprised less than one-fourth of the cells present. One such remnant is depicted on the border of Fig. 5. Although a noticeable number of clasmatocytes persisted, the greatest number of cells present in this preparation were slightly hypertrophied lymphocytes like those depicted in Fig. 5. Twelve lymphocytes are shown in this photomicrograph which, like the remaining photomicrographs of this experiment, was taken at the same magnification as the preceding figures. Both nucleus and cytoplasm showed structural evidence of increasing activity. Although four or five of the lymphocytes in this figure possessed typical lymphocytic nuclei with large, coarse chromatin massed, the remainder presented irregularity of nuclear outline and smaller, broken-up chromatin particles. Cell diameters now ranged from 11 to as high as 13 $\mu$. The cytoplasm was more abundant than in the preceding stage. Occasional large, clear, intracytoplasmic vacuoles made their appearance. The cytoplasmic increase was due to parallel increases in both



* Fig. 1. A lymphocyte and numerous neutrophils in the lesion of a normal male (H-8) in the third hour of inflammation in man (X2000).

Fig. 2. Four lymphocytes and numerous neutrophils in the same lesion as Fig. 1 in the sixth hour of inflammation in man (X2000).

Fig. 3. Eighteen lymphocytes, six neutrophils in the same lesion as Fig. 1 (H-8) in the ninth hour of inflammation in man (X2000).

Fig. 4. Nine lymphocytes and six neutrophils in the same lesion as Fig. 1 in the 12th hour of inflammation in man. Inset: a monocyte from the blood of the same individual to be compared with lymphocytes as to size and structural characteristics (Both X2000).

* All figures have been reduced by one half.

basophilic spongioplasm and colorless hyaloplasm. The basophilic material seemed to be arranged as delicate network against a colorless background. The cytoplasmic outline was indefinite; and numerous thin delicate process protruded from its periphery.

At 21 hours, there was evidence of a second migration of structurally intact neutrophilic leukocytes in moderate numbers. Although a number of the macrophages or histiocytes in this preparation still presented structural evidence of their lymphocytic origin as described above for the 14-hour stage, the majority of these cells now resembled those of our Fig. 6, a photomicrograph of eight such macrophages from this preparation. These cells possessed diameters of from 14 to 17 $\mu$. There had been an increase in the size of both nucleus and cytoplasm. The nucleus was round, oval, or slightly indented. It was larger than in the preceding stage. The chromatin pieces were small, angular, and irregularly distributed. They left distinct, irregular, parachromatin spaces increased in extent, as though the nuclear juice, increased in quantity, had widened the chromatin interstices. The cytoplasm, too, was much more abundant than in the preceding stage, and this latest increase was due largely to a rather diffuse increase in the colorless portion of the cell body. One of the cells in Fig. 6 presented a nucleus with a deeper indentation than the others and thus superficially resembled the monocyte in Fig. 4 (inset).

Observation of this preparation at the 21-hour stage emphasized the need for careful evaluation of the structural features of the mononuclear exudative cells, in general, during the first 12 to 14 hours of acute inflammation. If the inflammatory process had been observed for the first time, beginning with the 21-hour stage represented by Fig. 6, there would have been little to indicate the structural and functional modification undergone by the lymphocytes in the earlier hours of the process (shown in Figs. 1-5).

This experiment was concluded at the end of the first day. At 24 hours, the majority of the exudative cells were sessile macrophages with diameters up to 20 $\mu$ as depicted in Fig. 7. One of the better preserved neutrophils, mentioned in the preceding stage is also shown.

**Experiment H-11.**

The lesion was prepared in the skin of the anterior thigh of J.R. as described above, and 0.05 cc of diphtheria toxoid was applied to the local lesion. J.R. has had a consistently positive Schick test. In the next 40 hours, seven consecutive cover-slip preparations were obtained from this single lesion. Blood samples taken from J.R. revealed that he ran a monocytosis for short periods of from 9 to 10%; otherwise they were within normal values.

At 2 hours, a rim of red blood corpuscles at the lesion's edge was evidence of the previous trauma. Neutrophilic leukocytes were present in great numbers. They were edematous and measured up to 17 to 18 $\mu$ in diameter. A few clasmatocytes, eosinophils, and lymphocytes were likewise observed. The lymphocytic structure was identical with that of an ordinary medium-sized lymphocyte, as found in peripheral blood.

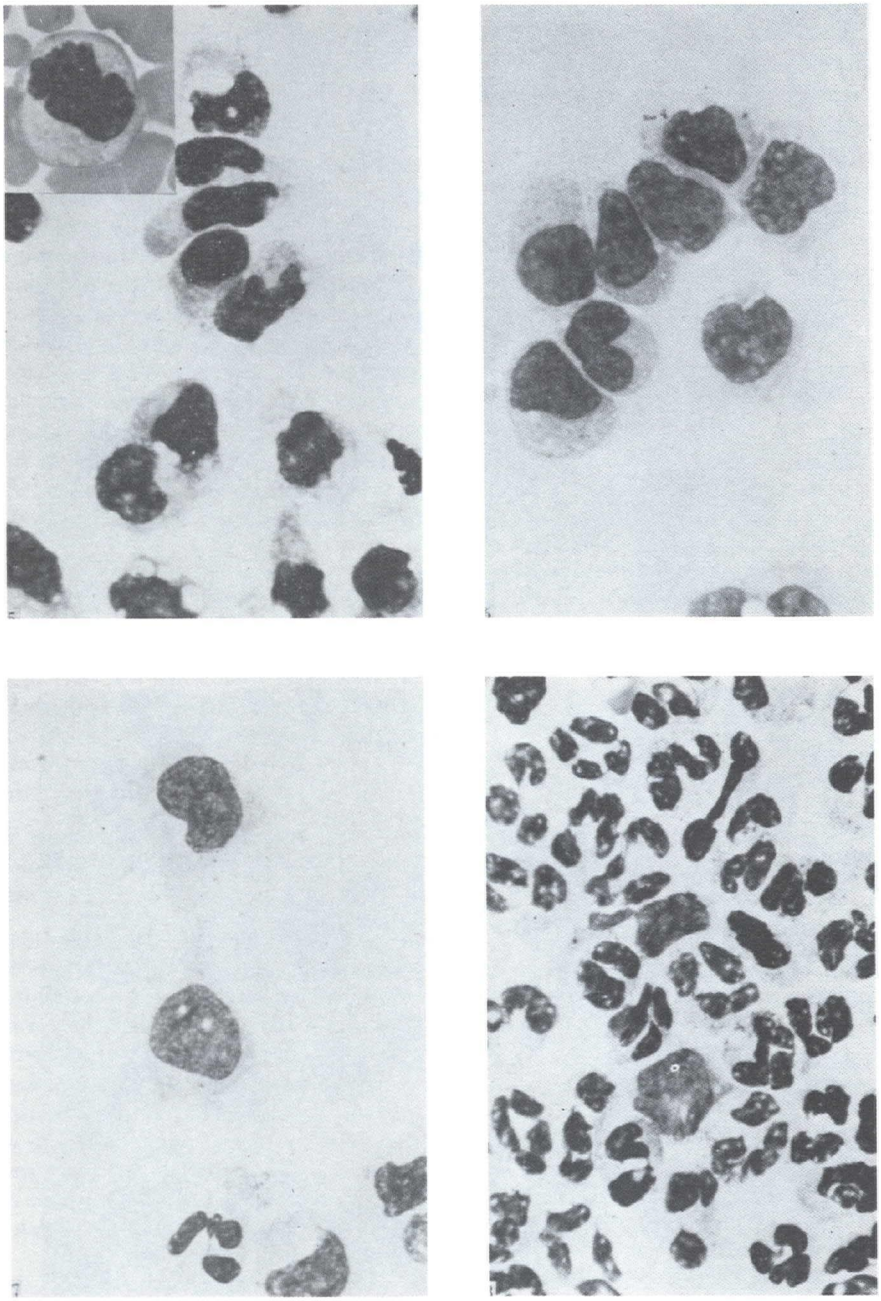
At five hours, a scattering of red corpuscles remained. Neutrophils were the predominant cell type. They had regained their normal size, a few were phagocytic, while others had early pyknotic changes in their nuclear lobes. A few eosinophils were noted. The clasmatocytes were increased in numbers. In some areas, such cells were found frequently with horseshoe-shaped nuclei.

The lymphocytes were also increased in numbers. Fig. 8 depicts two medium-sized lymphocytes, one of which was fixed while in motion, amid a mass of neutrophils.

At 12 hours, neutrophils still comprised nearly half of the exudate cells. Most of them were shrunken and had lost much of their cytoplasm peripheral to their nuclear lobes (Fig. 9). The nuclear lobes themselves were frequently clumped and pyknotic. Lymphocytes were likewise numerous and many of them were phagocytic. Fig. 9 depicts ten such lymphocytes, demonstrating that even small lymphocytes, measuring only 8 to 10 $\mu$ in diameter, were capable of marked phagocytic activity. One of the small lymphocytes, in the center of the photomicrograph, has ingested a portion of neutrophilic cytoplasm almost as large as its nucleus, while another nearby has ingested a large portion of a pyknotic nuclear remnant.

Fig. 10, also taken from the same preparation, illustrates the marked lymphocytic content of the cellular exudate at this stage. Seven of these lymphocytes were quite small. Four more showed slightly hypertrophied cytoplasm. The nuclear membranes were usually irregular. In many of the cells, it can be seen that the coarser chromatin masses have been divided into finer, angular chromatin pieces providing a distinct, more prominent parachromatin. Reference to the monocytes depicted at the same magnification in the inserts to Figs. 4 and 5 or actual measurement of the lymphocytes in our photomicrographs with a centimeter scale will assure our readers that the cells which we have designated as lymphocytes, in these preparations, were well under 14 $\mu$ in diameter. Neutrophilic leukocytes, however, cannot be used for size orientation in such preparations at any time. Early in acute inflammation in man, they appeared to be edematous and enlarged. Soon afterwards, they diminished in size, gave up portions of their cytoplasm as broken off cytoplasmic fragments, and ultimately degenerated. In many of these preparations, some lymphocytes have become shrunken, were almost devoid of cytoplasm, and, at times, even possessed pyknotic nuclei. An occasional lymphocyte was thus unable to survive in the field of inflammation, even when the irritant was a relatively mild one such as we have employed.

At 14 hours, the neutrophils presented the same features as described in the preceding stage. Several of them are depicted in Fig. 11. Lymphocytes now comprised almost half of the exudate cells. Fig. 11 depicts 12 lymphocytes, many of them slightly hypertrophied. There has been a slight increase in the parachromatin of the nucleus, distinctly separating the angular, finer chromatin pieces. In addition, the cytoplasm was more abundant, due to a diffuse increase in colorless hyaloplasm, vacuoles, and ingested material. One of the smallest lymphocytes, shown in Fig. 11, which still possessed the typical pachychromatic nuclear pattern, has ingested a red corpuscle. The cytoplasmic outline of several of the larger lymphocytes in Fig. 11 presented numerous blunted pseudopodia. An occasional lymphocyte (not depicted) has ingested a large portion of neutrophilic cytoplasm.



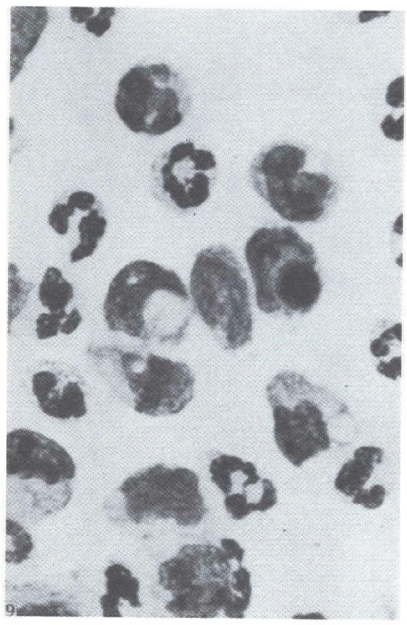
* Fig. 5. Lymphocytes and hypertrophied lymphocytes and a single pyknotic neutrophil in the same lesion as Fig. 1 in the 14th hour of inflammation in man. Inset: a monocyte from the blood of the same individual to be compared with the lymphocytes as to size and structural characteristics (Both X2000).

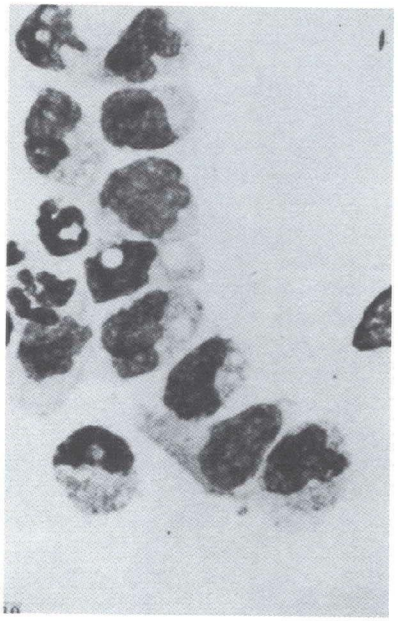
Fig. 6. Lymphocytogenous macrophages in the same lesion as Fig. 1 (H-8), in the 21st hour of inflammation in man (X2000).

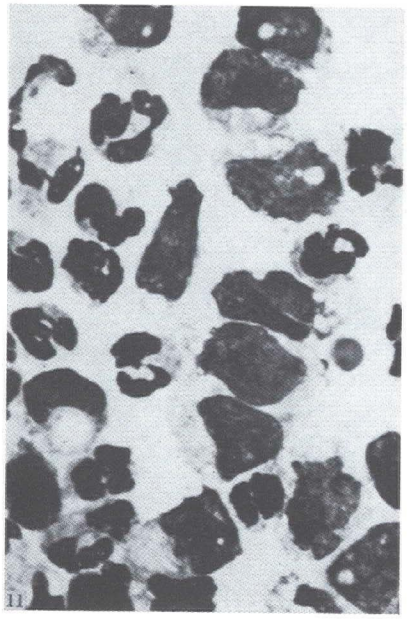
Fig. 7. Three macrophages and a neutrophil in the same lesion as Fig. 1 (H-8) in the 24th hour of inflammation in man (X2000).

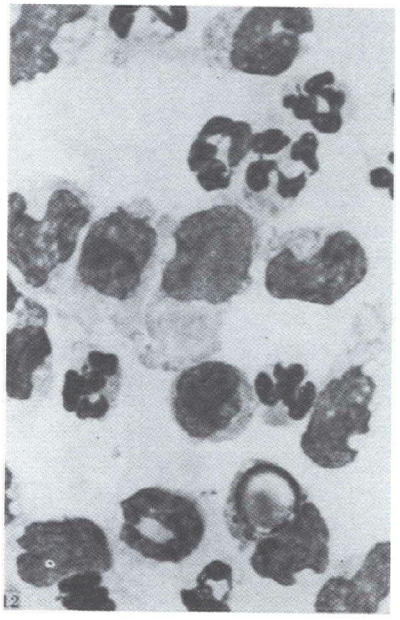
Fig. 8. Two lymphocytes and numerous neutrophils in the lesion of a normal male (H-11) in the fifth hour of inflammation in man (X2000).

* All figures have been reduced by one half.









* Fig. 9. Ten lymphocytes, several of them phagocytic, and nine shrunken neutrophils in the same lesion as Fig. 8 (H-11) in the 12th hour of inflammation in man (X2000).

Fig. 10. Seven lymphocytes, four hypertrophied lymphocytes, and two degenerating neutrophils in the same lesion as Fig. 8 (H-11) at the 12th hour of inflammation in man (X2000).

Fig. 11. Twelve hypertrophied lymphocytes and 13 degenerating neutrophils in the same lesion as Fig. 8 at the 14th hour of inflammation in man (X2000).

Fig. 12. Two lymphocytes, seven lymphocytogenous macrophages, and six degenerating neutrophils in the same lesion as Fig. 8 (H-11) after 16.5 hours of inflammation in man (X2000).

* All figures have been reduced by one half.

Clasmatocytes were numerous, but there has been but little addition to their numbers since the early stages. None of the cells of the inflammatory exudate observed in any of our preparations exhibited any evidence of mitotic activity or cellular division.

At 16.5 hours, the lymphocytes were so hypertrophied that they were no longer clearly distinguishable from histogenous macrophages which were also present. Degenerating neutrophils comprised about a third of the cells at this stage. Fig. 12 depicts a representative field from this preparation. Two of the exudative mononuclears were still obviously lymphocytes. The remainder had increased amounts of cytoplasm and increased amounts of nuclear parachromatin, so that they were designated as lymphocytogenous macrophages or simply macrophages. The chromatin pieces were fine and angular and irregularly distributed, and there was chromatin-parachromatin distinction. One of these macrophanges had ingested a large nuclear remnant. These larger cells had long diameters of from 15 to as high as 22 $\mu$ or more. One of these cells on the border of the field has ingested a portion of neutrophilic cytoplasm.

At 28 hours, there was evidence that a second wave of neutrophils had appeared in the field at some time after the preceding stage. Almost all the mononuclear cells were macrophages. A group of these is depicted in Fig. 13. They are particularly phagocytic for bits of neutrophilic cytoplasm. Their structure at this time afforded no clue to their lymphocytic origin as depicted in Figs. 8 to 12. Basophilic leukocytes were occasionally observed with fairly intact structure at this time.

At 40 hours, macrophages predominated. The remaining degenerated neutrophils were being phagocytosed in toto. A single binucleate giant cell was found in the preparation.

The succeeding experiments of this group presented similar findings except for the cellular responses to the trauma resulting from the technique alone. In this latter group, the leukocytic cycles were of less intensity and more transient than when an antigenic stimulus was applied. Although there was an abundance of neutrophils, there was a less marked response of histogenous macrophages and a minimal response of lymphocytogenous macrophages. Apparently, in the absence of antigenic stimulation, a mobile source of macrophages was not needed to accomplish the simple removal and reparative functions required for the healing of the experimental lesion itself.

### B. Acute inflammation in man with concomitant monocytosis

The patient studied suffered from a form of chronic granulocytic leukemia (monocytic leukemia, Naegeli type) (126) in which, in addition to developing granulocytes, functionally maturated monocytes numbered more than 6,000 cells per cubic mm (Fig. 14). These monocytes were approximately 15 $\mu$ in diameter and 85% of them possessed prominent nuclear indentations which served to "tag" them in the field of inflammation. Serial preparations, stained like blood smears, were obtained, at timed intervals, from a single lesion inoculated with egg white.

At four hours, monocytes were present in small numbers in the field of inflammation, and were phagocytic and slightly hypertrophied (Fig. 15). At seven hours, their numbers were increased (Fig 16). At times, their nuclei showed polymorphous changes with only thin strands connecting individual lobes but amitotic division was not in evidence. At 12 hours, the monocytes were the predominant mononuclear cell. They were further hypertrophied (Fig. 17), and had ingested neutrophilic cytoplasmic fragments. At 12.75 hours, the numerous monocytes, approximately 18 $\mu$ in diameter, were identifiable as monocytogenous macrophages (Fig. 18).

Structural modifications accompanying transformation of blood monocytes (Fig 14) into monocytogenous macrophages (Figs. 15-18) were: increase in cytoplasm, particularly hyaloplasm; gradual diminution of the azurophilic granulation characteristic of the monocyte in man; and increase in nuclear size, in parachromatin, and in distinctness of chromatin network with *pronounced accentuation of the nuclear indentations*. Hypertrophying monocytes, an important mobile source of macrophages in this patient with a marked monocytosis in his peripheral blood, at key stages of transformation were, hour for hour, 6 $\mu$ larger than comparable lymphocytes (cf. Figs. 1-5 and 8-11).

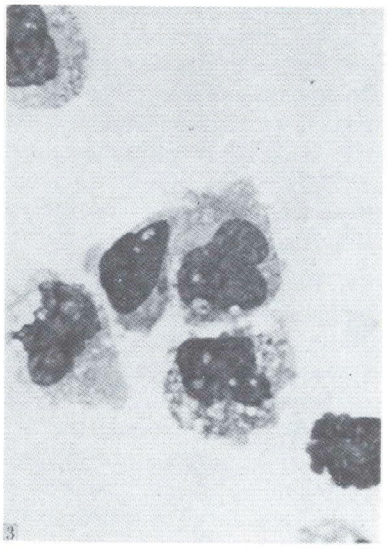
### C. Acute inflammation in rabbits with concomitant monocytoses

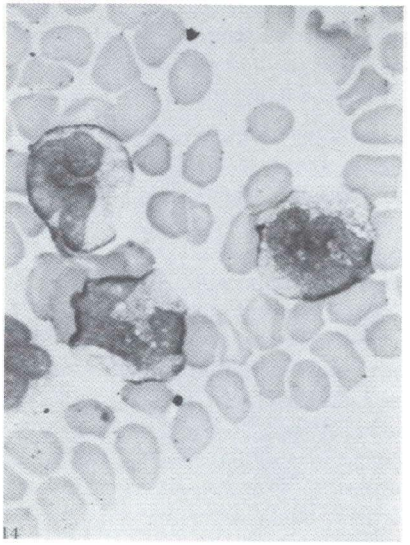
Experiment L-40 is representative of this group, and its protocol will be presented in detail. Before experimentation, the blood of this half-grown male rabbit was shown to be normal. Next, 0.8 cc of a 5 cc suspension of the growth of one plain agar and one liver agar slant (24 hour) of a virulent strain of *L. monocytogenes* was injected intravenously into this animal. The blood was checked 44 hours later, and a sharply rising monocytosis was noted. At this time, the monocytes comprised 21% of all the white blood cells and numbered 2,600 per cu mm.

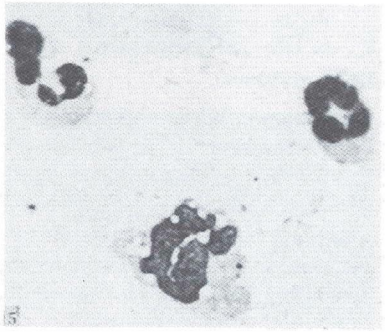
A denuded area of the ear was prepared as described above and was swabbed with a 70-hour culture of the same virulent *Listeria* strain, suspended in sterile physiological saline. A successful attempt was made to obtain the peak blood monocytosis during the time of study of the local inflammation.

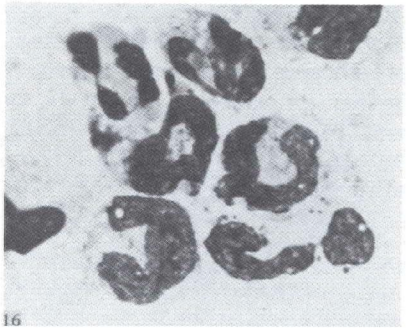
At four hours, red cells were seen indicative of minute traumatic hemorrhage in the field. Many polymorphonuclears (rabbit heterophils) had already started their migration. At seven hours, the inflammatory site showed large numbers of intact and ameboid heterophils. Some were vacuolated, others were losing their granules, and the cytoplasm of some was swollen. Lymphocytes were not infrequent. They had accumulated more hyaloplasm and, in some cases, their nucleus was larger, while their chromatin pattern became a little more distinct. A few lymphocytes were in process of hypertrophy, but the majority still had the appearance of blood lymphocytes. A few macrophages were also observed.

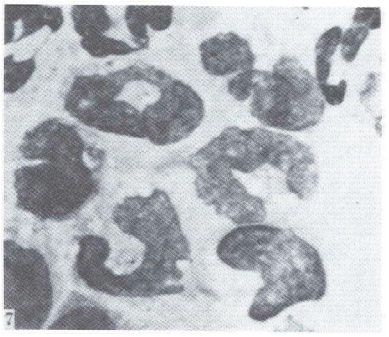
At eight hours, the blood was re-examined and the monocytes had now increased to 5,250 per cu mm. An extremely low lymphocyte percentage (7%) should also be noted. At 9 hours, the heterophils of the exudate were swollen with cytoplasmic and nuclear vacuoles. The cells themselves were clumped and disintegrating. Some of their nuclei had lost their staining affinity. The lymphocytes, also present, showed an increase in hyaloplastic

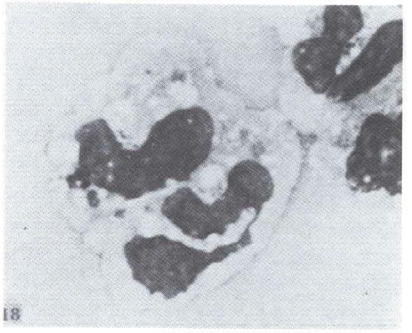












* Fig. 13. Four macrophages and a portion of a fifth in the same lesion as Fig. 8 (H-11) at the 28th hour of inflammation in man (X2000).

Fig. 14. Three monocytes in the blood of a patient suffering from Naegeli type, monocytic leukemia. More than 6000 such cells were found per cu mm (X1800).

Fig. 15. A monocyte in the lesion (L-1) of the patient with monocytic leukemia depicted in Fig. 14 at the fourth hour of inflammation in man (X1800).

Fig. 16. Four monocytes and two neutrophils in the same lesion as Fig. 15 (L-1) of the patient with monocytic leukemia at the seventh hour of inflammation in man (X1800).

Fig. 17. Five hypertrophied monocytes in the same lesion as Fig. 15 (L-1) of the patient with monocytic leukemia at the 12th hour of inflammation in man (X1800).

Fig. 18. Two monocytogenous macrophages in the same lesion as Fig. 15 (L-1) of the patient with monocytic leukemia at 12.75 hours of inflammation. The cells in Figs. 14-18 should be compared with the control cells in Figs. 1-13 (X1800).

* All figures have been reduced by one half.

areas. The nucleus showed separation of chromatin from increased parachromatin and the nuclear membrane appeared to be thinner. "Monocytoid" cells and monocytes or their derivatives were lacking.

At 12 hours, many of the numerous heterophils present were still intact, but others were clumped and in various stages of disintegration. Small lymphocytes were present but they were hypertrophying. A few macrophages had already developed.

At 16 hours, the blood showed a peak monocytosis for this animal of 7,300 monocytes per cu mm (Fig. 19). In the lesion itself, ordinary small lymphocytes had hypertrophied still further. Small early macrophages and larger macrophages were showing signs of phagocytic activity. Very few cells with slightly indented nuclei ("monocytoid" cells) were seen.

At 22 hours, the heterophils were in clumps, some had disintegrated, while groups of others had been invaded by the large lymphocytogenous macrophages with their customary, round, oval, or slightly irregularly shaped nuclei. A small number of the lymphocytogenous macrophages were "monocytoid"; that is, they had slightly indented nuclei. At this time, the first few monocytes (Fig. 20) made their appearance at the site of inflammation. Note that the chromatin masses already showed a slight tendency to splitting and that vacuoles were beginning to form in the cytoplasm.

At 26 hours, more monocytes had taken their place alongside the lymphocytogenous cells. The heterophils had all but disintegrated. Great numbers were amorphous, poorly stained clumps. Many had been ingested by the numerous lymphocytogenous macrophages. Lymphocytes were still found in small numbers. By the time the monocytes had reached the under surface of the cover slip, the nuclear pattern had undergone a slight breaking up of its clumps and strings of chromatin into small, irregular, angular pieces. A concomitant increase of parachromatin in the monocytes tended to distend the nucleus, so that eventually a fine nuclear pattern with sharp chromatin-parachromatin distinction was reached which resembled the pattern of the lymphocytogenous macrophage or reticuloendothelial cell nucleus. The monocytic nucleus retained its characteristic horseshoe shape and, as the parachromatin of the nucleus continued to increase, the large nuclear bay was even accentuated. This facilitated the identification of these cells.

At 31.5 hours, the monocytes had increased in number. They, along with the lymphocytogenous macrophages, were beginning to take on the characteristics of large macrophages. The chromatin of the monocytes was splitting up from the coarser arrangement seen in the blood, the parachromatin was distinct and on the increase, and the amount of cytoplasm had increased, especially the hyaloplastic and vacuolar portions. Fig. 21 shows three such monocytes whose nuclei are still greatly indented. Two macrophages of either lymphocytogenous or histiocytic origin are also shown in Fig. 21 to aid in the comparison of the two-cell types.

At 41 hours, the heterophils were of interest only in showing further disintegration, disappearance, or phagocytosis by the macrophages. Lymphocytogenous and histogenous macrophages were present and were large and active. Often they had banded together to form giant cells in which the nuclei were all oval, round, or slightly irregular in shape, just as Maximow (32) had described them in 1902. In addition, there were many monocytes present hypertrophying into monocytogenous macrophages, and this hypertrophy tended to dominate the picture. Fig. 22 shows a monocyte from the lesion at this time and two monocytogenous macrophages. Their nuclei are characteristically deeply indented. The small cells in the figure were disintegrating heterophils. These monocytogenous macrophages comprised an important portion of the macrophage cell group. Blood studies taken at these later stages showed a continual fall in both absolute and relative monocyte counts.

At the same time, another phase had begun in which the large histogenous and lymphocytogenous macrophages, arranged in groups, had settled down after "digesting" their phagocytosed particles. Long processes extended from their cytoplasm and their cell outlines had a stellate appearance. Their vacuoles were small, sharply outlined, and regular in size and distribution. The remainder of their cytoplasm was more basophilic and homogeneous. At the same time, the chromatin pattern of their nucleus had become more finely reticulated. In 1902, Maxinow (32) had described such a phenomenon in the process of scar tissue formation in vivo, and later (1928) he made a similar observation in his tissue culture experiments (86a).

At 46 hours, entire groups of monocytogenous macrophages were assembled with the nonmonocytic forms. Fig. 23 shows four such monocytogenous macrophages. With their increased phagocytic activity they had attained huge proportions. Their finely stippled chromatin pattern was similar to that of ordinary macrophages, but, in each case, the horseshoe-shaped indentation of the nucleus has remained a constant and even accentuated feature. A non-monocytic macrophage is shown for comparison in Fig. 23.

At this stage, the monocytogenous macrophages exhibited two further potentialities: they banded together with similar cells to form purely monocytogenous giant cells and with nonmonocytic macrophages to form hybrid giant cells.

At 57 hours, the phagocytic giant cells were present in three forms among the numerous monocytogenous macrophages. They were of the ordinary lymphocytogenous or histogenous type, all of the nuclei being round or oval; or they were made up of purely monocytogenous macrophages with all their nuclei deeply indented; or they were hybrid and represented the fusion of histogenous or lymphocytogenous components with monocytogenous components. Fig. 24 represents such a hybrid giant cell found at this time. The one monocytogenous component, with its indented nucleus, stands out from the three nonmonocytic components also making up the cell.

At 70 hours, the macrophages and their derivatives dominated the picture. As a large portion of these cells were monocytic in origin, our remaining descriptions will be confined to the part they played. In addition to many purely monocytogenous macrophages which were present in large clumps, purely monoctyogenous giant cells or hybrid giant cells (Fig. 25) were seen. From this field, the figure portrays a typical phagocytic hybrid giant cell with one monocytogenous and one nonmonocytic component. Such cells add, by the distinction of their nuclei, to the concept that fusion of macrophages is, at least, one important method of giant cell formation.

The monocytogenous macrophages exhibited still further potentialities through formation of monocytogenous clasmatocytes (Fig. 26) with elongated sessile processes and very fine chromatin nuclear pattern, the nucleus still retaining its large indentation. They even approached the fibroblast stage depicted in Fig. 27 with its outstretched stellate processes, fine and regular vacuolization, and characteristic nuclear indentation. This cell represents a transitional stage between a monocytogenous clasmatocyte and a fibroblast.

At 98 hours, the cytology was such that both lymphocytogenous and histogenous macrophages could still be distinguished from the monocytogenous forms. The giant cells were present in the three forms found in this lesion.

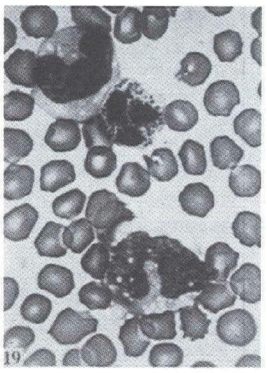
Control lesions testing the inflammatory response to the liver-veal agar, avirulent *Staphylococcus albus*, and, to local but not systemic administration of *L. monocytogenes* revealed lymphocytogenous and histogenous formation of macrophages as described by Kolouch (105), but a relatively sparse monocytic origin. In this respect, our Figs. 20 to 27 should be compared with Kolouch's figures 3 to 6 depicting comparable control lymphocytogenous formation of macrophages in acute inflammation in the subcutaneous connective tissues of the rabbit.

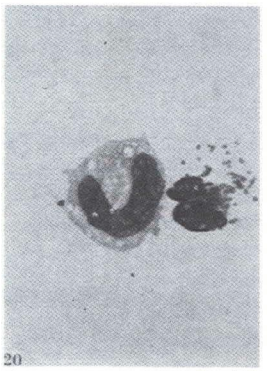
## Discussion

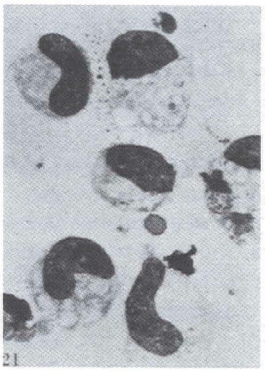
The transformation of the lymphocytes of man into macrophages proceeds in an orderly fashion in the simple cycle of acute inflammation. When the exciting antigen is one to which the human subject is not systemically immunized, migration of the lymphocytes and early transformational changes reach their peak between 12 and 14 hours. At this same stage, the lymphocytes and their hypertrophied forms are usually numerically in excess of the exudative neutrophils for the first time in the cycle. This transformation of lymphocytes into macrophages involves modifications of a functional and structural nature in both lymphocyte nucleus and cytoplasm. These modifications are usually so advanced by the 14th to 16th hour of inflammation that the lymphocytic origin of the macrophages is largely obscured at any later stage in the dynamic process.

In our present report, by means of an original technical procedure, the cellular exudate of single lesions in man has been sampled hour by hour. In a large series of experiments, cover-slip preparations, dried and stained like blood smears, were obtained which allowed detailed comparison of the cells obtained in acutely inflamed tissues of man with the cells of blood smears. In this same series, we have noted mean structure and function of large numbers of lymphocytes of single lesions concurrent with the chronological progression of inflammation. In a simple non-immune cycle, in from two to nine hours, the lymphocytes were present as lymphocytes, but in ever-increasing numbers. In from 9 to 14 hours, the great mass of lymphocytes presented cytoplasmic and nuclear hypertrophy. In from 14 to 18 hours, lymphocytogenous macrophages dominated the field. There was no need to resort to the study of intermediate forms or transitional stages within the cells of a single sampling. It was the mass of lymphocytes that changed progressively hour by hour. It was the structural and functional condition of the majority of the lymphocytes in each preparation, during each stage of inflammation, that most concerned us. This description can be substantiated by a review of Figs. 1 to 13, which present liberal portions of the cellular exudates of single lesions in consecutive stages.

The majority of the lymphocytes studied in each stage progressively changed in structure and function until a stage was reached at which the majority of the mononuclear cells were no longer lymphocytes as such, but were larger phagocytic forms. It is also true that, in most preparations, there were some lymphocytes which lagged behind the majority and remained unaltered. The contrasting possibility cannot be excluded that some lymphocytes may be capable of precocious transformation and of anticipating their more slowly changing but preponderant fellow lymphocytes. To demonstrate the lymphocytic origin of the macrophages in vivo then, attention has been directed to gradual



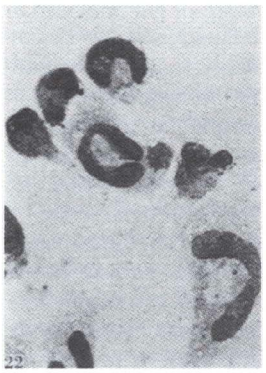
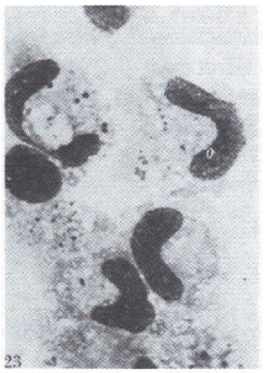
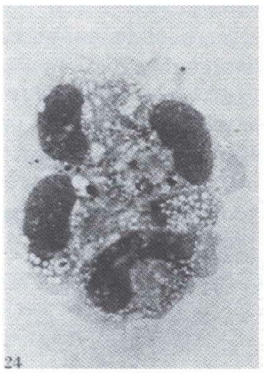
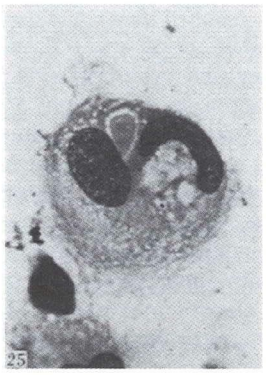
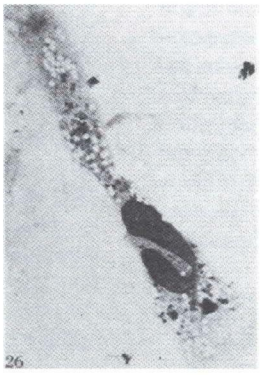
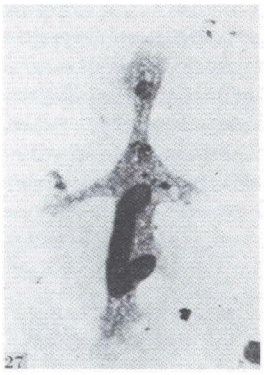




*** Fig. 19. Two monocytes and a heterophil in the blood of a rabbit (L-40) representative of the peak monocytosis reached (7,300 monocytes per cu mm) 60 hours after intravenous administration of L. monocytogenes (X1800).**

**Fig. 20. A monocyte and a disintegrating heterophil in the lesion of rabbit L-40 (wtih peripheral blood monocytosis as depicted in Fig. 19) at 22 hours of inflammation (X1800).**

**Fig. 21. Three monocytogenous macrophages and two macrophages not of monocytic origin in the same lesion of rabbit L-40 depicted in Fig. 20 at 31.5 hours of inflammation (X1800).**

*** All figures have been reduced by one half.**

*** Fig. 22. A monocyte and two monocytogenous macrophages and several disintegrating heterophils in the same lesion of rabbit L-40 (with peripheral blood monocytosis depicted in Fig. 19) at 41 hours of inflammation (X1800).**

**Fig. 23. Four monocytogenous macrophages and a macrophage not of monocytic origin in the same lesion of rabbit L-40 depicted in Fig. 20 (with peripheral blood monocytosis depicted in Fig. 19) at 46 hours of inflammation (X1800).**

**Fig. 24. Hybrid giant cell with three nonmonocytic components and one monocytic component in the same lesion of rabbit L-40 depicted in Fig. 20 (with peripheral blood monocytosis depicted in Fig. 19) at 57 hours of inflammation (X1800).**

**Fig. 25. Hybrid giant cell with a monocytic and nonmonocytic component in the same lesion of rabbit L-40 depicted in Fig. 20 (with peripheral blood monocytosis depicted in Fig. 19) at 70 hours of inflammation (X1800).**

**Fig. 26. Sessile monocytogenous macrophage now a clasmatocyte in the same lesion of rabbit L-40 depicted in Fig. 20 (with peripheral blood monocytosis depicted in Fig. 19) at 70 hours of inflammation (X1800).**

**Fig. 27. Clasmatocytic fibroblast from a sessile monocytogenous macrophage in the same lesion of rabbit L-40 depicted in Fig. 20 (with peripheral blood monocytosis depicted in Fig. 19) at 70 hours of inflammation (X1800).**

*** All figures have been reduced by one half.**

alterations in the majority of the lymphocytes in each lesion. Little mention has been made, therefore, of the occasional lymphocytes which were out of phase with the functional conditions of the greater number of their fellow exudative cells.

Of fundamental importance is the problem of relating physical and chemical alterations in the lymphocyte nucleus and cytoplasm to their structural and functional modifications as the transformation into the macrophage proceeds. Furthermore, any understanding of alterations within the cell presupposes a clear knowledge of the functional relationship of the lymphocyte to the changing conditions of its environment.

One of the first structural modifications which the lymphocytic nucleus undergoes, as it transforms into a macrophage nucleus, is a change in the size and distribution of its chromatin. The lymphocyte, as it reaches the field of inflammation, usually possesses a nucleus which is pachychromatic; the chromatin pieces are coarse and large; the parachromatin or nuclear-juice content of the nucleus is scanty. Furthermore, the parachromatin, which is colorless in Romanowsky stains, is poorly delimited from the chromatin masses whose borders blend imperceptibly with the parachromatin. Within 9 to 12 hours, and often before this period, the large chromatin pieces which stain deeply basophilic

are observed to be divided into smaller and smaller, irregular, angular pieces. At first, this process is accomplished with only a relative increase in parachromatin but, as the process of chromatin subdivision proceeds, there is an actual increase in the colorless parachromatin. At the same time, the smaller, angular chromatin pieces begin to form an irregular but definite chromatin network, and the parachromatin becomes more and more distinct from this chromatin framework. This nuclear change may precede or accompany cytoplasmic modifications.

The next nuclear modification which may be noted is increasing irregularity of the shape or outline of the nucleus. Even the lymphocytes of the blood, when observed in wet smears, possess a nuclear indentation (127). In the field of inflammation, this lymphocytic indentation is usually noticeable as the cleft becomes deeper in the outstretched cell. Changes in the nuclear membrane are not confined to the region of the widening indentation. Other areas of nuclear membrane, at some distance from the indentation, show definite undulation suggestive of increased activity at the nuclear-cytoplasmic interface. If vacuoles or ingested substances are in contact with the nuclear membrane, they are able to indent the nucleus at the point of contact, suggesting again an increased fluidity of the nucleus. By the 14th hour, the nucleus of the lymphocyte has increased in size. This increase is apparently due to the increased amounts of nuclear juice or parachromatin, which latter becomes increasingly distinct (Fig. 11).

Little is known concerning the functional changes in the lymphocytic nucleus. A beginning in this direction has been made by Thorell (128), who, in studying metabolism in the lymphocytes, found that the intracellular nucleic acid metabolism of large lymphocytes indicated a high intensity of growth.

Lymphocytic cytoplasm undergoes more apparent structural and functional changes. The most remarkable and earliest of these changes in lymphocytic cytoplasm is the assumption of the phagocytic function (*cf.* Figs. 4 and 9). This ability is possessed by lymphocytes within the blood stream, but is usually a latent one. Downey (55,56) demonstrated that lymphocytes within the blood itself possessed the ability of phagocytosing vital dyes if the dyes were made available to these cells by the simple expedient of double ligature of a vessel, followed by study of the lymphocytes in the interposed segment. Included in these phagocytic powers, then, is the ability to ingest vital dyes (*cf.* experiment H-8).

Equally rapid in its assumption by the lymphocyte, in the area of inflammation, is the ability to form a rosette of numerous neutral red vacuoles in the cytoplasm of the nuclear bay. This property of the lymphocytes has been demonstrated by Maximow (86a), Bloom (86), Hall (129,130) and Cappell (98). Interestingly enough, the lymphocytes also become peroxidase positive (131) and Nadi positive (132) after a short sojourn in the field of inflammation.

The cytoplasm gradually increases in size until the large cell-body size of the macrophage is attained. In this connection, it is pertinent to recall Lang's studies of aseptic inflammation in the subcutaneous tissue of rabbits. Lang (71) observed that this hypertrophy of the emigrating lymphocytes began while they were still within the capillary lumen. He had previously injected India ink intravenously into his animals. He observed that the hypertrophied cytoplasm of lymphocytes in the stagnant blood of the vessels of the inflammatory site began to ingest small particles of India ink. Probably, many of the early functional and structural modifications of the lymphocyte, as it grows into a macrophage, have already begun, or are capable of beginning, within the capillary lumen at the inflammatory site. The increase in lymphocytic cytoplasm is probably due, fundamentally, to a gradual increase in hyaloplasm as described by Kolouch (105), although occasionally an equal increase in basophilic spongioplasm is also noted. Jones (133) has furnished evidence that the hyaloplastic portion of blood-cell cytoplasm represents mitochondria. The cytoplasm of the lymphocytes undergoes further enlargement, of course, through phagocytosis of protein antigens, red blood corpuscles, and fragments of free neutrophilic cytoplasm.

The observation of ingestion of cytoplasmic fragments of neutrophilic leukocytes was made possible by the clarity of the cellular details reached by a technique which permitted study of the tissue exudate, dried and stained like a blood smear. This relationship between hypertrophying lymphocyte and shedding neutrophilic cytoplasm may afford amino acid residues, lipids, carbohydrates, and other building stones needed by the lymphocytic cell-body in its rapid and relatively great cytoplasmic structural reorientation and growth, preparatory to the assumption of its macrophage function. The changes in the submicroscopic particulates, mitochondria, micellae and Golgi net, as the lymphocyte transforms into the macrophage await further studies with the phase and electron microscope. Changes in lipids and carbohydrate content await the application of methods as utilized by Gomori (134) and Wislocki and Dempsey (135).

With reference to cytoplasmic organelles, it is interesting to recall Wallgren's (49) observations on the growth of the cytocentrum as the lymphocytes transformed into macrophages. His Fig. 1, b, d, g, h, Taf. XIII, depict the customary two centrioles in the cytoplasmic bay of the lymphocyte. As the lymphocyte hypertrophied, at first three centrioles, later six or seven, were present in the same area; finally, as the macrophage stage was attained, 12 or so centriolar bodies were present. Concurrently, astral rays were elaborated and ran centrifugally from the aggregated centrioles.

Early opposition to Metchnikoff's concept of the lymphocytic origin of the macrophage was based on the mistaken view that lymphocytes were not motile and therefore were incapable of migration. Later, differences in modes of locomotion were seized upon to differentiate lymphocytes from other cellular sources of macrophages. However, Berman (114) had observed a gradual transition from lymphocytic to macrophage pseudopodial activity, but in fixed preparations. De Bruyn (121) made motion picture studies of lymphocytes as they hypertrophied toward the macrophage stage. Their mode of locomotion gradually changed from the polarized "hand-mirror" method to that of continuous depolarization, characteristic of the monocyte.

As observed by the early critics of Metchnikoff and more recently by the Clarks (115), Hall and Furth(136), and by Florey and his group (116), some lymphocytes in the field of inflammation or in tissue culture may either fail to hypertrophy or to become phagocytic, and retain their coarse, clumped, nuclear pattern or even ultimately degenerate in situ. Again, in areas of chronic inflamma-

tion, sessile lymphocytes are a common finding. Metchnikoff (22) gave the first of several explanations for such findings. He held that there is a constant struggle in the field of inflammation between the migrated lymphocytes and the admittedly deleterious environment of the inflammatory lesion, and it is to be expected that some of the lymphocytes will succumb. The works of Dougherty and White (137,138) and Harris, Harris, and Farber (139,140) and Wesslen (141) afford another explanation for the presence of sessile lymphocytes. Inasmuch as lymphocytes have been demonstrated to be a source of antibodies, local aggregates in areas of inflammation may be functioning in the direction of antibody production rather than macrophage transformation. Ehrich and his group (142), furthermore, have suggested a trophocytic function for the apparently inactive lymphocytes.

In summary, transformation of the lymphocyte into the lymphocytogenous macrophage in acute inflammation is accompanied by the following structural and functional modifications: increase in phagocytic ability for cellular debris, bacteria, and vital dyes; increase in nuclear size; division of coarse chromatin masses into fine, angular pieces; increase in parachromatin; increase in chromatin-parachromatin definition; increase in cell-body size; enlargement of cytocentrum; increase in centrioles; appearance of astral rays; increase in number of neutral red vacuoles with aggregation into a rosettelike apparatus about the cytocentrum; assumption of positive peroxidase (131) and Nadi reactions (132); and increasing evidences of depolarization locomotion.

The importance of the role of the monocyte in acute inflammation appears to be proportional to the number of monocytes available for emigration or mobilization at the time of the inflammation. The morphology of the hypertrophied lymphocytes in Figs. 2 to 5 and 8 to 11 bears little or no resemblance to that of the monocytes in the insets of the photomicrographs of Figs. 4 and 5, or in the series represented by Figs. 14 through 27.

The experiments of Murray, Webb, and Swann (81), Bloom (84), Frerichs (118), and those of our third series, described above, upon local inflammation in animals with high blood monocytoses were in agreement, in that only in such animals did the migration of monocytes to the inflammatory site occur in appreciable numbers. If the blood of the animals studied contained only the meager, normal numbers of monocytes, then few were available for migration into the local area of inflammation. If the blood of the animals studied contained several thousands of monocytes per cu mm, then monocytes migrated in ever-increasing numbers into the inflammatory sites. In addition, our utilization of air-dried preparations of the exudative cells of lesions in experimental animals with concurrent high blood monocytoses permitted direct comparison of migrating monocytes with those of blood smears. The large monocyte of Pappenheim and Ferrata (143), with its indented nucleus, functioned quite like the lymphocyte in the event of migration into the site of inflammation. However, monocytes remained structurally distinct from the lymphocytes and retained their horseshoe-shaped nucleus as they progressively transformed into monocytogenous macrophages, monocytogenous giant cells, monocytogenous clasmatocytes and, finally, monocytogenous fibroblasts (Figs. 19-27).

Proponents of a more intensive monocytic origin of macrophages, in general, submit that the admittedly small number of monocytes available for migration or mobilization at inflammatory sites is offset by the large number of mitoses among the monocytes. In our entire series of preparations from lesions in human volunteers, mitoses have been absent in the hematogenous exudative cells, lymphocytes as well as monocytes.

Structural modifications accompanying transformation of blood monocytes from a patient suffering from an intense but maturated, leukemic monocytosis into monocytogenous macrophages were: gradual loss of the azurophilic granulation characteristic of the monocyte in man; increase in cytoplasm, particularly hyaloplasm; increase in nuclear size, in parachromatin, and in distinctness of the chromatin network with a striking accentuation of the nuclear indentations (Figs. 14-18).

The role of clasmatocytes, resting-wandering cells or histogenous macrophages, in local inflammation has been established by the schools of Maximow, Downey, Sabin, Doan, and Tompkins. Our study of responses of these cells in acute inflammation in man adds little that is new to the accepted concepts of clasmatocyte function. They were readily phagocytic in the earliest stages of acute inflammation and were the earliest source of the macrophage but, because of their limited numbers, their inability to migrate great distances, and their limited powers of cell division, they were of necessity reinforced either by the more mobile monocytes (when available) and/or the more numerous lymphocytes in most lesions.

The intact organism, in a few strategically situated locations along the lymph and blood streams, masses great numbers of histogenous macrophages in order to ingest foreign antigens which make their way into the lymph and blood stream. These potential histogenous macrophages are the reticuloendothelial cells, and the aggregates which they form are the spleen, lymph nodes, lymphocytic tissues, and bone marrow, as well as the sinusoids of several of the endocrine glands. The events of inflammation in reticuloendothelial tissues with such massed, readily available histogenous macrophages, are quite different from those in other regions of the body. In such reticuloendothelial organs, the histogenous macrophages, of course, dominate the inflammatory field and hematogenous elements usually play only a secondary role.

The great, mesothelial-lined cavities of the body lie midway in the scale of relative histogenous-hematogenous macrophage participation, in the event of inflammation. In the exudative responses in these cavities, both sources of macrophages are of almost equal importance.

It is well to point out that most regions of the body contain relatively fewer histogenous macrophages. In the central nervous system, they are called "microglia"; in the interstitial connective tissue of the myocardium, "Anitsckhows's myocytes"; in the lung, "septal cells"; in the general connective tissues, "clasmatocytes" or "histiocytes," rarely "adventitial cells." Once the intensity of an inflammatory stimulus exceeds the strength of the normal wear and tear process, neutrophilic leukocytes, lymphocytes and/or monocytes migrate from the vessels reinforcing the histogenous macrophages.

Metchnikoff discovered the phagocytic powers of the neutrophilic leukocytes, and Opie (144-146) did much to help in our understanding of their enzymatic activity. Our own preparations, obtained in acute inflammation in man and described above, have demonstrated an additional, subsidiary function of neutrophilic leukocytes. Prior to shrinkage and degeneration in the area of inflammation, they lost much of their granule-containing cytoplasm in the form of small fragments, which for a time were free in the inflammatory fluids. These free portions of neutrophilic cytoplasm were readily ingested by lymphocytes as well as histogenous macrophages. In the process of transformation from lymphocyte to macrophage, these ingested fragments may be of service in the elaboration and reorientation of the scant lymphocytic cytoplasm into the abundant cytoplasm of the macrophage.

In our preparations, the polymorphonuclear basophilic leukocytes of the blood appeared late in inflammation, but were structurally intact. Their migration into the field of acute inflammation in man is thus established, but further study of their function in such areas awaits the clinical occurrence of high blood basophilias.

Finally, because important functions of normal leukocytes are exerted in the tissues, application of technical procedures described above to the study of cellular responses in patients affords an additional method for detecting abnormal or distorted leukocytic functions (147-149).

## Summary

By means of an original technical procedure, the cellular exudate of single lesions in man has been sampled hour by hour. In 42 lesions, cover-slip preparations, dried and stained like blood smears, were obtained which allowed detailed comparison of the exudative leukocytes obtained from the acutely inflamed tissues of man with the leukocytes of blood smears. A modification of this technique permitted similar observations of leukocytic functions in the rabbit.

When the exciting antigen was one to which the human subject was not systemically immunized, transformation of the lymphocytes of man into macrophages was accompanied by the following functional and structural changes in lymphocytes as such: increase in cytoplasmic size, increase in phagocytic ability for cellular debris and vital dyes; assumption of a positive peroxidase reaction; assumption of a positive Nadi reaction; increase in division of coarse chromatin masses into fine angular pieces; increase in nuclear juice or parachromatin; increase in irregularity of nuclear membrane; and increase in chromatin-parachromatin definition.

The participation of blood monocytes in local inflammation was in proportion to the number of monocytes available for emigration or mobilization at the time of inflammation. In man, the role of monocytes in acute inflammation was followed by the technique described in a patient with monocytic leukemia, Naegeli type, in which functionally mature monocytes in the blood outnumbered the lymphocytes. Structural modifications accompanying transformation of blood monocytes tagged with prominent nuclear indentations were: an increasingly prominent accentuation of the nuclear indentation; gradual loss of characteristic dustlike azurophilic granulation; increase in cytoplasm; increase in nuclear size, in parachromatin, and in parachromatin-chromatin distinctness. The monocytes hypertrophied as an important mobile source of the macrophages in the subject, with a concomitant monocytosis in his peripheral blood, and key stages of inflammation were, hour for hour, six $\mu$ larger than comparable lymphocytes and their hypertrophied forms.

In rabbits with high blood monocytoses brought about by systemic *L. monocytogenes* infections, a slight modification of the technique employed in man permitted study of the large monocytes with their indented nuclei at the site of inflammation. Such monocytes, present in great numbers, remained structurally distinct from the lymphocytes and their hypertrophied forms in controls, and progressively transformed into monocytogenous macrophages, monocytogenous giant cells, monocytogenous clasmatocytes, and monocytogenous fibroblasts.

Neutrophilic leukocytes of man migrated at an early stage into the lesions and, after performing their functions of phagocytosis and enzyme elaboration, shrank and degenerated. Before degeneration, they lost their granule-containing cytoplasm to the exudate in the form of small fragments which, for a time, were free in the inflammatory fluids, but which were ultimately ingested by the lymphocytes and hypertrophied lymphocytes as well as by the histogenous macrophages.

The literature of leukocytic participation in the inflammatory response was reviewed.

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