EXPERIMENTAL STUDY OF THE MONONUCLEAR CELLS OF THE BLOOD AND TISSUES

WITH SPECIAL REFERENCE TO THE SO-CALLED TRANSITIONAL CELL *

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The uncertainty that exists in regard to the large mononuclear white blood cells is probably in large part due to the diverse forms they present with the usual stains, and the scarcity of each of these forms in normal blood. The several distinct types represented in this group do not have constant morphologic characteristics demonstrable by the usual stains that admit of a differential classification among them; and only occasionally is a case encountered in which one type stands out predominantly.[1] Similarly the mononuclear wandering cells of the tissues, intimately associated with the mononuclear cells of the blood, are seen in many different forms, and although widely distributed throughout the body, each form is encountered alone in appreciable numbers only in rare pathologic conditions. The relations obtaining among these different mononuclear cells of the blood and tissues is, therefore, in some confusion and the status of each uncertain.

Some of these cells, the so-called transitionals, stand out from all the rest by reason of their content in granules of oxydase ferment, as demonstrable by the indophenol-blue reaction; and some may be recognized wherever encountered by their ability to take vital stains. It has seemed that advantage might be taken of these biologic reactions in clearing up the confusion that exists as to the identity of the different types of mononuclear cells in the blood and tissues. The following study was undertaken with this end in view.

The different forms presented by the mononuclear cells of the blood and tissues has caused a wide variation in nomenclature for cells, many of which are probably identical or closely related to each other, and this tends to deprive us of the advantage of cumulative observation by different authors. But much work has been done, and in view of

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1. Van Nuys, F.: An Extraordinary Blood, Boston Med. and Surg. Jour., 1907, clvi, 390. Netousek, M.: Endothelium in Stromenden Blute, Folia haematol., 1913-1914, xvii, 407. Bartlett, W. B.: Localized Leukocytosis Associated with Atypical Phagocytic Cells, Pub. Massachusetts Gen. Hosp., 1908-1909, ii, 390. Evans, F. A.: Observations on the Origin and Status of the So-Called Transitional White Blood Cell, THE ARCHIVES INT. MED., 1916, xvii, 1.

that on which these experiments to be reported are based, a brief résumé of the nomenclature, with discussion of the more prominent hypotheses thus far advanced, is essential.

1. Primitive Wandering Cell: This term is applied to an undifferentiated small mononuclear cell of the embryo arising from the original mesenchyme cells recognized by Saxer[2] and Maximow[3] as the mother cell of all the hematogenous and histogenous wandering cells of the body.

2. Resting Wandering Cell: By this name Marchand[4] designates an undifferentiated small round cell considered by him as lymphoid in origin and which, similar to the one mentioned above, is found in the tissues and gives rise to many different cell forms.

3. Clasmatocyte: Ranvier[5] first described under this term a wandering cell of the tissues characterized by a coarse polychrome protoplasmic granulation and frequently long processes that may become separated from the cell. When first described by Ranvier this cell was believed by him to be a derivative of circulating blood elements.

4. Plasma Cell: The small round cells thus named and first described by Unna,[6] which are seen in the tissues in great numbers under some conditions, may be readily recognized by their oval shape and excentrically placed nucleus, with coarse chromatin threads. These are considered as one type of lymphocyte[7] which has either wandered out from the blood stream, or at least is closely related to the lymphoid elements of the blood.

5. Polyblast: Maximow, in his classic studies[8] on the wandering cells of the tissues in inflammatory reactions, identified one type of medium-sized mononuclear constantly present, which he named polyblast. This cell is characterized by great variation in form, which can best be appreciated by reference to the pictures accompanying his articles, but it is predominantly in active tissue reactions a mononuclear round cell having a fairly heavily stained nucleus often irregular in shape, and moderately abundant protoplasm that commonly does not contain any granulation. He asserted that the polyblasts arise in part from emigrated lymphocytes and in part from clasmatocyte-like forms previously in the tissues. He further stated that these cells might become "resting wandering cells" of the tissues, might assume forms resembling the clasmatocytes or those difficult to distinguish from connective tissue elements, all of which have the power to again become round mononuclear wandering cell forms. Under the name of polyblast, Maximow postulates a definite relation between the lymphocytes of the blood and many diverse types of wandering cells, including the clasmatocyte of the tissues.

6. Adventitial Cell: This is a small, round cell, described by Marchand[4] as occurring in the tissues, especially in the region of the blood vessels. He includes in this group the mast cell, the plasma cell, the cell of small round-cell infiltration and others, and maintains that they have the power to produce

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2. Saxer, F.: Ueber die Entwickelung und den Bau der normalen Lymphdrusen und die Entstehung der roten und weiszen Blutkorperchen, Anatomische Hefte, 1895-1896, vi, 349.

3. Maximow, A.: Ueber die Entwickelung der Blut- und Bindegewebezellen beim Saugetierembryo, Folia haematol., 1907, iv, 611.

4. Marchand, F.: Ueber Klasmatocyten, Mastzellen, Phagocyten des Netzes, Verhandl. d. deutsch. path. Gesellsch., 1902, iv, 124.

5. Ranvier: Des clasmatocyte, Compt. rend. Acad. d. Sc., 1890, cx, 165.

6. Unna: Ueber Plasmazellen insbesondere biem Lupus, Monatsh. f. prakt. Dermat., 1891, xii, 296.

7. Hertz, R.: Ueber Vorkommen, Natur und Herkunft der Plasmazellen in der Milz, Folia haematol., 1912, xiii, 177.

8. Maximow, A.: Beitr. z. path. Anat. u. z. allg. Path., 1903, xxxiv, 153; Ibid., 1904, xxxv, 93; Ibid., 1905, xxxviii, 301.

highly phagocytic cells and by further change to become leukocytoid and lymphoid cells. They migrate from the tissues into the blood stream and are in turn constantly renewed to the tissues from the blood and blood-forming organs. Marchand questions the close relationship of any of these cells to some forms included by Maximow with the polyblasts, namely, those polyblasts which, remaining in the tissues, assume forms difficult to distinguish morphologically from true fixed tissue elements, and considering the clasmatocyte a fixed tissue derivative does not include it in the class of his adventitial cells. He, in disagreement with Maximow, thus excludes from what he considers the lymphoid mononuclear wandering cells of the tissues any cell that is not readily recognized as a round mononuclear cell; but in addition to the lymphoid reaction in which the adventitial cells are prominent, Marchand recognizes a histogenous reaction,[9] in which cells definitely related to the fixed tissue elements and entirely independent of any of the lymphoid mononuclear cells, are concerned. The cells of this histogenous reaction may also, as those of the the other group, the lymphoid mononuclear wandering cells of the tissues, go over into the blood and appear there as large mononuclear cells. Whereas Maximow included with his polyblast, considered of lymphoid origin, certain wandering cells difficult to distinguish from fixed tissue elements. Marchand sharply divided these into two groups, which may roughly be classified as round cells, lymphoid, and irregular cells, histogenous in origin.

7. Pyrrhol Cell: These cells were first recognized as a distinct entity by their ability to take vital stains and were so named by Goldmann, who used pyrrhol blue among the first of the successful vital stains. They are intimately related to the fixed tissue elements, are of diverse form and ubiquitous in origin, but are specially conspicuous in the milky patches of the omentum, in the liver as the Kupffer cells, and in the reticulum of spleen, lymph glands and bone marrow.[10] These are the cells concerned in the histogenous reaction of Marchand mentioned above, and possibly are the irregular forms included in Maximow's polyblasts.

8. Carmin Cell: Kiyono[11] used lithium carmin as a vital stain, and by this name speaks of essentially the same cells as those described by Goldmann and named pyrrhol cells. He also, with Marchand, maintains that they are active wandering cells of the tissues, concerned in various types of inflammatory reaction, and that they also wander into the blood and are seen there as part of the mononuclear group. In one communication he is inclined to include the transitionals with these histogenous cells.

9. Histiocyte: Thus Aschoff[12] designates those cells which he considers of histogenous origin. He includes in this group the vitally staining cells mentioned above and in agreement with Marchand believes they are seen in the blood as large mononuclears, and that they are not related to the lymphoid cells. Which of the large mononuclear cells of the blood are lymphoid and which are histogenous in origin he does not assert; but his pupil Kiyono later states[13] that although the mother cell of the histiocytes in the tissues and the histiocytes seen in the blood is identical, the cells of this group in the blood are very scarce and a differentiation between them and the other large mononuclear cells is impossible without the vital stain. Kiyono suggests that some

9. Marchand, F.: Ueber die Herkunft der Lymphocyten und ihre Schicksale bei der Entzundung, Verhandl. d. deutsch. path. Gesellsch., 1913, xvi, 5.

10. Goldmann, E. E.: Cellular Activity in Health and Disease; Biochemical Studies Based on New Methods of Intravitam Staining, Lancet, London, 1912, i, 1183.

11. Kiyono, K.: Die vitale Karminspeicherung, Jena, Gustav Fischer, 1914.

12. Aschoff, L., and Kiyono, K.: Zur Frage der groszen Mononuclearen, Folia haematol., 1913, xv, 383.

13. Kiyono, K.: Zur Frage der histiozystaren Blutzellen, Folia haematol., 1914, xviii, 149.

of the large mononuclear and transitional cells of the blood are transition forms of the histiocytes.

10. Macrophage: This term, familiar since Metchnikoff[14] introduced it to indicate any mononuclear phagocytic cell, has been restricted by H. M. Evans[15] to those cells capable of taking the vital stains. While admitting their location and supposed origin in the body as already discussed, Evans calls attention to the fact that these diverse cell forms, sometimes appearing as fixed tissue elements, sometimes as mononuclear wandering cells, have little in common except their ability to take vital stains; and by this only are differentiated from some other wandering cells and fixed tissue elements. He states that these are the cells concerned in tubercle giant cell formation,[16] and, although not venturing any opinion as to their relation to any of the mononuclear wandering cells of the blood, does assert that only very rarely may they be encountered in the peripheral circulation.

11. Endothelial Leukocyte: Mallory[17] in his studies on typhoid fever described the activity of certain cells believed by him to be of endothelial origin, but some of which can now be recognized as those cells capable of taking a vital stain (Pyrrhol cell, Goldmann; Macrophage, Evans). Other cells to which the term endothelial leukocyte was applied by Mallory[18] are those concerned in tubercle formation, osteoclasts of bone, the mononuclear cells of serous surfaces and blood, etc. He maintains that in the tissues they are derived in part from those already in the blood as large mononuclears and in part from the endothelium of the lymphatic system.

More recently, Tschaschin,[19] similarly trying to classify all the mononuclear cells of the body under one head, includes the cells of the milky patches of the omentum (vitally staining cells), those of the serous surfaces and of the spleen and blood forming organs, the small round cells abundant in the region of the blood vessels (Marchand's adventitial cell), in fact all the cells concerned in the lymphoid and histogenous reaction of Marchand, Aschoff, etc., in one group. This composite group, corresponding closely to Mallory's endothelial leukocyte classification, Tschaschin speaks of as histogenous in origin, in that they are all derived from the fixed tissue elements of the primitive mesenchyme. He believes, however, that they are also closely related to the lymphoid system, as evidenced by the appearance in inflammation of the same polyblasts from lymphocytes and these tissue wandering cells.

12. Splenocyte: Pappenheim[20] recognizes as histogenous macrophages (Aschoff's histiocytes) cells which he believes are descendants of the vitally staining cells of the omentum and elsewhere. Some of these which are fixed in the spleen and never go over into the blood he speaks of as splenocytes, and ascribes to them the function of local phagocytosis and elaboration of hemolysin.

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14. Metchnikoff, E.: Die Lehre von den Phagocyten und deren experimentelle Grundlagen, Handbuch der Pathogenen Microorganismen, Kolle u. Wassermann, Ed. 2, 1913, ii, 655.

15. Evans, H. M.: The Macrophages of Mammals, Am. Jour. Physiol., 1915, xxxvii, 243.

16. Evans, H. M., Bowman, F. B., Winternitz, M. C.: An Experimental Study of the Histogenesis of the Miliary Tubercle in Vitally Stained Animals, Jour. Exper. Med., 1914, xix, 298.

17. Mallory, F. B.: Histological Study of Typhoid Fever, Jour. Exper. Med., 1898, iii, 611.

18. Mallory, F. B.: The Principles of Pathologic Histology, W. B. Saunders Company, Phila., 1914, p. 37.

19. Tschaschin, S.: Ueber die "Ruhenden Wanderzellen" und ihre Beziehungen zu den anderen Zellformen des Bindegewebes und zu den Lymphozyten, Folia haematol., 1913-1914, xvii, 317.

20. Pappenheim, A.: Einige Worte über Histiocyten, Splenozyten und Monozyten, Folia haematol., 1913, xvi, 1.

13. Monocyte: Pappenheim also recognizes a hematogenous macrophage, a circulating blood mononuclear that is also a descendant of the vitally staining cell, but does not arise from those in the spleen. These he speaks of as monocytes, and although he does not admit unreservedly their identity with the wandering cell of Marchand, the macrophage of Metchnikoff, the polyblasts of Maximow, the histiocyte of Aschoff, or the Goldmann pyrrhol cell, he does class together all those mentioned by stating that his monocyte is a descendant of them.

14. Transitional Cell (Uebergangsform): This cell, incorrectly so named by Ehrlich,[21] because, when first recognized by him as a definite entity, it was thought to be a transition form between the large mononuclear and the polymorphonuclear cells of the blood, constitutes an important part of the large mononuclear group of blood cells. It is capable of specific stimulation,[22] but is not even yet generally considered as an independent cell type. When recognized at all it has been ascribed various sources of origin, endothelial (Mallory[18]), histogenous (Aschoff[12]), lymphoid (Pappenheim[23]), and myeloid (Naegeli[24]). It is the only mononuclear cell that contains an oxydase ferment, and to avoid confusion throughout the rest of this paper, it will be designated oxydase mononuclear.

From this brief résumé of part of the literature it is apparent that although all observers quoted admit the close embryologic relation between the connective tissue elements and the wandering cells of the tissues and blood, they are in no way in agreement as to the exact relations existing in the mature organism. Maximow, under the classification of polyblasts, relates the mononuclear cells of the tissues to the lymphoid cells of the blood, and under the same classification describes forms of wandering cells hard to distinguish morphologically from fixed tissue elements. Marchand, through his adventitial cells and derivatives, likewise associates many of the mononuclear cells of the tissues to the blood lymphocytes, but in this group includes, it seems, only mononuclear cells with regular outline, and places the irregular forms, not in the lymphoid group, as Maximow[25] would have it, but in a distinct histogenous system of cells. Marchand believes that these histogenous cells, although entirely distinct from the

21. Ehrlich, P.: Farbenanalytische Untersuchungen zur Histologie und Klinic des Blutes, A. Hirschwald, Berlin, 1891, p. 126.

22. Footnote 1, last reference.

23. Pappenheim, A.: Ueber verschiedene Typen von Lymphozyten und Monozyten, zum Teil im scheinbar normalen Blut, Folia haematol., 1911, xii, 26.

24. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, Veit & Co., Leipzig, 1913, p. 180.

25. One cannot be certain, of course, that the irregular cell forms seen by Maximow and classed with polyblasts and therefore to be considered of lymphoid origin, are identical with those of Marchand's histogenous group and therefore vitally staining cells, but from the description and pictures such an assumption seems justified. On the other hand, recalling that Maximow describes transformation of cells readily recognized as emigrated lymphocytes to the irregular forms under discussion, one hardly dares assume that Maximow, even in his careful study, had seen a transformation from a blood lymphocyte to a vitally staining cell, an observation so far not recorded by anyone studying these cells by means of the vital stains.

lymphoid cells, occur in the blood among the large mononuclears. Aschoff, Pappenheim, Kiyono and others similarly see distinct lymphoid and histogenous systems of wandering cells in the tissues and present in the blood in the large mononuclear group; but Pappenheim, while recognizing the cells of this histogenous group by means of the vital stain as the other observers mentioned, thinks those in the blood do not come from the vital staining cells of the spleen (splenocyte); and Kiyono believes that they are not present in great numbers in the blood. In contrast to this division of mononuclear cells into two groups, lymphoid and histogenous, may be mentioned the hypotheses of Mallory and Tschaschin, who include all the different cells in one group, according to Mallory, of endothelial, and to Tschaschin, of histogenous origin. From the literature, then, although it is manifestly unfair to interpret the opinions of the more prominent students of the subject along such narrow lines and without further exposition, one may formulate in general the following different hypotheses: that the different types of mononuclear cells of the blood and tissues are (1) lymphoid (Maximow), (2) endothelial (Mallory), (3) histogenous (Tschaschin), (4) in part histogenous and in part hematogenous (Marchand, Aschoff, Pappenheim, Kiyono).

The oxydase mononuclear (transitional) cell has not been recognized as a distinct entity in many of these studies, and has either been overlooked or included in one or the other group, and thus attributed to every possible source of origin. Pappenheim, speaking of the oxydase mononuclear cells, particularly describes them as myelolymphoidocytes,[23] yet subsequently states that they arise from the vitally staining cells in the milky patches of the omentum, where they may be seen side by side with the vitally staining cells. Kiyono suggests that the large mononuclear transitional cells are transition forms of the histiocytes. Türk[26] denies the relations of these oxydase mononuclear cells to the lymphoid system, and Naegeli[24] considers them specific myeloid elements.

EXPERIMENTAL PART

In view of the uncertainty about the genetic relations of the various mononuclear wandering cells of the tissues and the different large mononuclear cells of the blood, and the difficulty of definitely establishing this relationship by methods so far available, it was deemed advisable, ignoring this phase of the subject entirely, to attempt a classification of the adult cells of the mature organism based on the ability of the different cells to take the vital stain and to give a positive oxydase reaction. Although not definitely proved, it seems likely and is

26. Türk, W.: Vorlesungen über klinische Haematologie, W. Braumiller, Wien und Leipzig, 1904, p. 304.

generally accepted that all the cells capable of vital staining are histogenous (as compared with hematogenous) in origin and will be here so considered without further question; and for lack of a more accurately descriptive term they will be designated throughout what is to follow as *histogenous macrophages*. For the same reason any cell that does not take the vital stain will be considered of hematogenous origin and, if lacking in oxydase ferment, will be considered as ultimately derived from the lymphoid (as opposed to the myeloid) system. By the application of these two biologic reactions under conditions outlined below, whereby cells considered as histogenous (in the common acceptation as described above) may be recognized, and the commonly accepted diagnostic criteria of the lymphoid cells demonstrated, an attempt has been made to determine (1) which of the mononuclear wandering cells of the tissues, if any, are lymphoid and which histogenous; (2) which of the large mononuclear cells of the blood are lymphoid and which, if any, histogenous; (3) if the oxydase mononuclear (transitional) cells of the peripheral blood can be identified on the basis of the reactions employed with any other mononuclear cell of the blood or tissues for which well-recognized diagnostic criteria are available; and (4) whether all the mononuclear wandering cells of the body (exclusive, of course, of the true small lymphocytes) take the vital stain and are therefore after all, as some would have us believe, of histogenous origin.

Technic.—Rabbits were used in all the experiments. The total and differential white blood cell counts in these animals are subject to great variation, as already noted by Moss,[27] but the same cell types as in human blood are present, and there is always a good number of mononuclear cells, a large percentage of which (about 5 per cent. of the total) are typical oxydase mononuclears. Lithium carmin was used as the vital stain because of the contrast accorded with the dark-blue granules of the indophenol-blue reaction. The lithium carmin solution was used in 5 per cent. strength, and made up as advised by Kiyono.[11] The individual dose employed was uniformly 6 c.c. The animals were vitally stained by both intraperitoneal and intravenous injections. While the supply on hand lasted, Grubler's carmin was used, as by Kiyono in his exhaustive studies. In later experiments, because of inability to obtain Grubler's, Merck's carmin was substituted for it. As a vital stain the Merck preparation compared very favorably with that of Grubler, except that the former was more irritating and had a tendency to cause thrombosis of the ear veins after repeated injections and to call out a mild leukocytic reaction in the peritoneal cavity when

27. Moss, W. L., and Brown, G. L.: Variations in the Normal Leukocyte Count in Rabbits, Bull. Johns Hopkins Hosp., 1911, xxii, 258.

injected intraperitoneally. For the demonstration of oxydase ferment the indophenol-blue reaction was used.[28]

Intravenous Vital Staining.—To see which, if any, of the large mononuclear cells of the blood are vitally staining histogenous macrophages, animals were stained acutely and chronically by intravenous administration of the dye, and smears of blood from various vessels, tissue sections, and the omentum were studied microscopically and submitted to the oxydase reaction.

Animals chronically stained by repeated daily doses of filtered lithium carmin showed the usual distribution of carmin-stained cells in the spleen, liver, milky patches of the omentum, and bone marrow as described by Kiyono. But even when the animal was very heavily stained by more than twice the amount of stain used by Kiyono (two courses of seven daily doses separated by six days, and followed in four days by two more doses just before death) no carmin cells were seen in smears of the peripheral blood after careful search of many smears made at different periods during the course of the vital staining. This finding is in accord with results recorded by H. M. Evans,[16] working with trypan blue, Goldmann,[29] using isamin and pyrrhol blue, and Pappenheim[20] and Netousek,[30] who state that rarely do these cells appear in the peripheral capillary circulation. Kiyono and Aschoff,[13] using different substances, including colloidal metal suspensions, although insisting that these cells are normally seen in the peripheral blood,[31] admit that they make up only a very small part of the large mononuclear group. However, with these heavily stained animals, or in those less heavily stained, an occasional carmin cell was seen in the smears made from the portal and splenic vein blood, but even in the most heavily stained animal they were very rare and did not seem to increase appreciably in proportion to the degree of staining. In animals thus stained none of the many carmin cells in the tissues or the

28. Evans, F. A.: The Practical Significance of the Oxydase Reaction as Applied to Blood Cells, Proc. New York Path. Soc., 1915, xv, 143.

29. Goldmann, E. E.: The Process of Digestion Illustrated by the Action of Stains on the Living Tissues, Lancet, London, 1913, ii, 69.

30. Netousk, M.: Ueber Endothelien und ihre Beziehung zu den Monozyten, Folia haematol., 1914-1915, xix, 1.

31. Kiyono did not study smear preparations of the blood, but sections of the various vessels after fixing the animal in toto in formaldehyd. By this method confusing artefactions are likely to occur, for Shipley and Cunningham (Am. Jour. Physiol., 1916, xl, 75) have shown that vital stain may be absorbed through the blood vessels of the omentum and large amounts be suspended free, entirely independent of any cell, in the portal circulation. It is readily conceivable that some of this suspended material might be so placed in relation to the white blood cells in microtome sections of a formaldehyd-hardened vessel full of blood, as to simulate vital staining where in reality none was present. The possible error when intravenous injection of the stain is employed is obvious.

occasional one seen in the blood of the portal system showed a positive oxydase reaction.

A few animals were acutely stained by intravenous injection of 20 c.c. 5 per cent. filtered lithium carmin, a toxic dose that killed the animal in thirty minutes. In these no carmin cells were seen in smears made during the thirty minutes preceding death and after death from the peripheral capillary blood, from arterial blood, or from blood of the splenic and portal veins, although the plasma was a deep pink color. A few minutes after the smear was made many of the nuclei of the leukocytes took on a pink tint as they died, thus proving that the carmin was abundant enough in the plasma, even in the small amounts of it in a smear, to act as a stain.

When unfiltered lithium carmin solution, which was always opaque and showed minute particles under the microscope, was very slowly injected intravenously to avoid death by multiple embolism, often after one and almost always after two doses carmin cells might be seen in the circulating blood. But these cells uniformly gave the oxydase reaction, and with appropriate stains could be identified as polymorphonuclears and as the oxydase mononuclears of the circulating blood.

Intraperitoneal Vital Staining.—To supplement the foregoing and to determine the relations existing between the cells of the omentum and serous cavities to other wandering cells of the body and blood, animals were stained by intraperitoneal injections and studies similar to those described above were made.

Intraperitoneal injection of the filtered lithium carmin solution, even after two courses of twelve daily doses, alone or in combination with intravenous injection, gave the same results as those discussed above for the animals stained by intravenous administration of the filtered stain, except that the milky patches of the omentum were more densely stained. In several animals thus stained the omentum showed many heavily stained carmin cells that did not give the oxydase reaction, side by side with polymorphonuclear and oxydase mononuclear cells which contained no carmin stain; and in two cases the omentum was also infiltrated with many typical plasma cells which showed no carmin staining[32] and did not give the oxydase reaction.

32. As already stated, cells of many different forms take the vital stains and H. M. Evans (Footnote 15) calls attention to the fact that a classification of cells based on their ability to take vital stains crosses all other lines of classification so far employed. In this connection it should be said that in these preparations containing typical plasma cells with no vital staining there were also some mononuclear cells of almost the same size that were vitally stained. This bears out Evans' statement in part and suggests that some such transition as is discussed in Footnote 25 may occur. But the observation made on these preparations can only be considered a remote suggestion of this and admits of no definite conclusion.

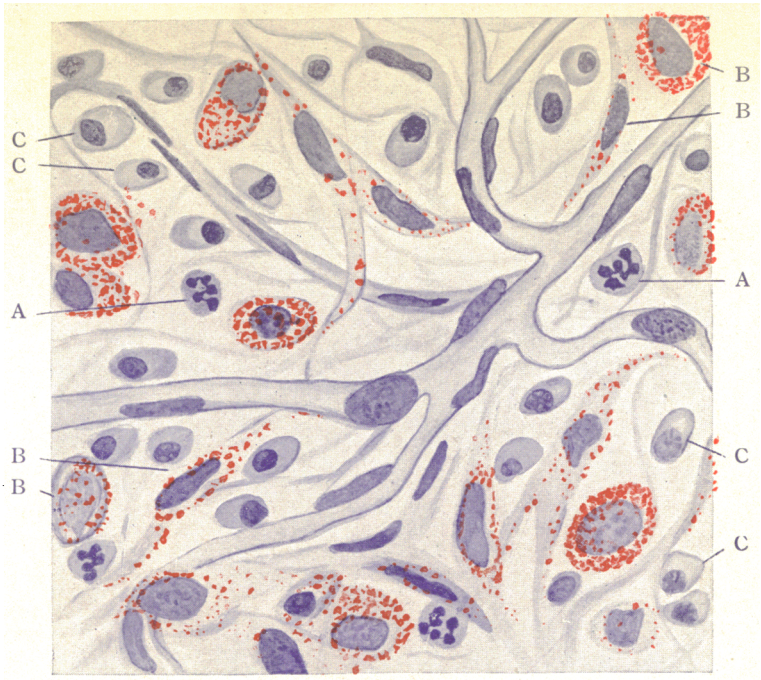

Fig. 1.—Portion of omentum from rabbit vitally stained by intravenous and intraperitoneal injection of filtered lithium carmin solution. The omentum was stretched out on a slide and stained with hematoxylin. It will be noticed that although several different types of both fixed and large wandering cells are heavily stained, the polymorphonuclear and many plasma cells present are entirely free of the stain. *A*, polymorphonuclear cells; *B*, vitally staining histogenous macrophages; *C*, plasma cells.

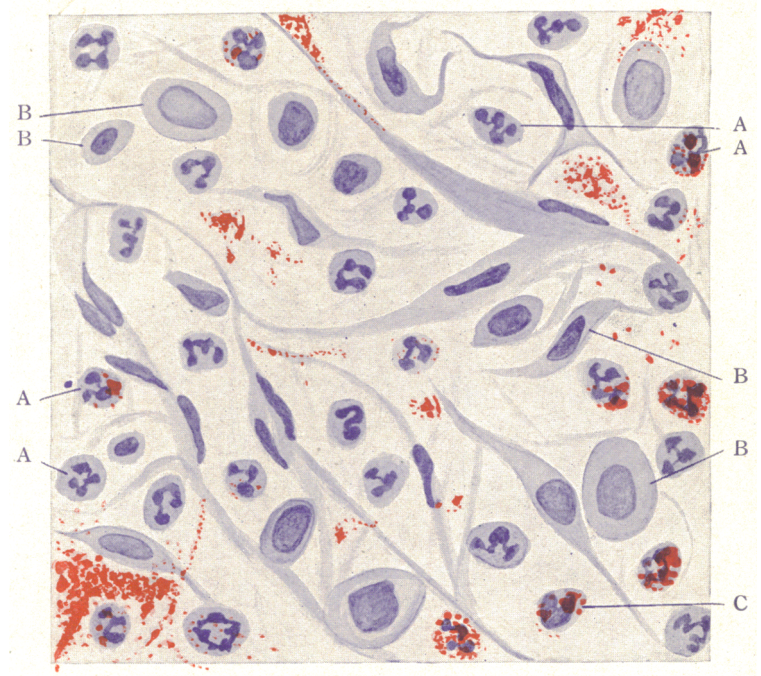

Fig. 2.—Portion of omentum from rabbit killed one hour after intraperitoneal injection of 10 c.c. unfiltered lithium carmin solution. The omentum was stretched out on a slide and stained with hematoxylin. The stain can be seen in abundance in many of the polymorphonuclear cells present, and in an oxydase mononuclear cell. The histogenous macrophages are unstained. It will be noticed that there is much free stain in the tissues. *A*, polymorphonuclear cells; *B*, histogenous macrophages; *C*, oxydase mononuclear cell.

Never were oxydase mononuclear cells seen except when the polymorphonuclear cells were numerous, and then only after careful search.

It is of further interest that in a vertical section through heavily stained milky patches of the omentum an uninterrupted layer of endothelial cells, reacting differently to carmin and having no oxydase ferment, may be seen running over their peritoneal surfaces, so that these carmin cells are separated from the peritoneal cavity by a continuous sheet of cells of a different type. These peritoneal cells do not contain carmin as compared with the abundant coarse granules of the specifically stained cells, but only a very few fine particles of this stain may be seen in their protoplasm on careful examination with highest magnification. This is in agreement with the observations of Kiyono[11] and of Evans[16] in regard to trypan blue.

When the unfiltered stain was injected intraperitoneally, however, even after one dose and before the vitally staining histogenous macrophages had taken the stain to any extent, the polymorphonuclear and oxydase mononuclear cells in the omentum and peritoneal fluid showed many carmin granules, and under these conditions occasionally an oxydase cell with carmin granules could be found in smears of the splenic vein blood. But even when a mild leukocytic reaction in the peritoneal cavity was called out by a previous injection of weak turpentine solution, no oxydase cells were seen to contain the stain after large doses of filtered lithium carmin.[33]

Mononuclear Cells in Reaction to Localized Bacterial Invasion; Experimental Pneumonia.—To study the different mononuclear cells

33. Similar experiments were undertaken with trypan blue with surprisingly different results, an observation already recorded by Pappenheim (Footnote 20). Trypan blue, a coarse colloidal suspension, differs from lithium carmin in that no particles are seen under the microscope in its unfiltered solution and yet when filtered it is not translucent as is the filtered lithium carmin. Yet no living polymorphonuclear or oxydase mononuclear cell takes up trypan blue when injected intravenously or intraperitoneally, filtered or unfiltered. Evans (Evans, H. M., and Schulemann, W.: The Action of Vital Stains Belonging to the Benzidine Group, Science, 1914, xxxix, 443), bases a hypothesis of the mechanism of vital staining on the size of the colloidal particle, the coarser colloidal suspensions not acting as vital stain only because of their impermeability. Colloidal metal suspensions also act as vital stain (Footnote 12), and Buxton and Torrey (Buxton, B. H., and Torrey, J. C.: Absorption from the Peritoneal Cavity, Jour. Med. Research, 1906, x, 5) have shown that histogenous macrophages will in time take particles as large as red blood corpuscles if made available for them by intraperitoneal injection. Thus we have the histogenous macrophage taking the finest colloidal particle or the red blood cell, and the polymorphonuclear cell refusing any colloidal particle however coarse and not active in the phagocytosis of red blood cells, yet taking microscopic masses of carmin and bacteria. Does this mean that the histogenous macrophage will take any material mass regardless of size, while the polymorphonuclear cell is selective for particles not too large or too small?

in reaction to localized bacterial invasion advantage was taken of the mononuclear character of the early exudate in pneumonia.[34]

In the early exudates in pneumonic processes induced in rabbits by intratracheal injection of various organisms and substances after the method of Meltzer,[35] not only polymorphonuclear but also oxydase and nonoxydase mononuclear cells are to be seen in the exudate in great abundance.[34] The oxydase mononuclears are the same as those seen elsewhere; the nonoxydase mononuclears are made up of a few typical lymphocytes and other larger round cells greatly resembling the polyblast of Maximow. These cells no doubt belong to that great group of mononuclear cells seen in various inflammatory reactions throughout the body. Yet in pneumonia induced in rabbits after they had been heavily stained by combined intravenous and intraperitoneal injections of lithium carmin, neither the oxydase nor nonoxydase mononuclear cells contained the stain, and the few irregularly shaped, vitally stained cells present were either in the alveolar walls or in close association with them.

As further proof that the mononuclear cells in the early exudate in experimentally induced pneumonia in rabbits showing oxydase mononuclear and other cells resembling the polyblasts of Maximow are not vitally staining cells, pneumonia was induced in rabbits poisoned with benzol. Benzol destroys the leukopoietic tissue,[36] but spares the histogenous macrophages, as evidenced by the fact that in vitally stained animals rendered aleukemic by benzol administration, and in which the splenic pulp and bone marrow were largely aplastic, the milky patches of the omentum were as numerous, as cellular, and as heavily stained as in normal vitally stained animals. Yet in pneumonia induced in animals with their leukocytes thus destroyed and with their vitally staining histogenous macrophages unaffected, no cells were seen in the exudate, as already recorded by Winternitz and Hirschfelder.[37]

Mononuclear Cells of Splenic Vein Blood.—To determine, if possible, how active a contributor to the large mononuclear cells of the blood the spleen may be, white cell counts were made of the splenic vein blood at different periods throughout these experiments; and several of these were made simultaneously with counts from the femoral veins, the right and left heart, and the peripheral capillary circulation. Tremendous variations were encountered in both the total

34. Evans, F. A.: The Cytology of the Exudate in the Early Stages in Experimental Pneumonia, Jour. Infect. Dis., 1916, xix, 440.

35. Meltzer, S. J., and Lamar, R. V.: Experimental Pneumonia by Intrabronchial Insufflation, Jour. Exper. Med., 1912, xv, 133.

36. Selling, L.: Benzol als Leukotoxin, Beitr. z. path. Anat. u. z. allg. Path., 1911, li, 576.

37. Winternitz, M. C., and Hirschfelder, A. D.: Studies on Experimental Pneumonia in Rabbits, Jour. Exper. Med., 1913, xvii, 657.

and differential counts, so that the results were not convincing. But in agreement with Morris,[38] these counts all showed a higher mononuclear percentage in the splenic vein than elsewhere, quite independently of the other conditions of the experiment. Of these, a goodly number were typical oxydase mononuclears, some were suggestive of them in appearance but contained no oxydase ferment, and over half were nonoxydase mononuclears. As already stated, carmin cells in the splenic vein blood after staining with filtered lithium carmin were so rare as to be negligible in counting the total of nonoxydase mononuclear cells.

Tissue Culture of Spleen.—In the splenic pulp may be seen many oxydase and many nonoxydase mononuclear cells. In addition, many histogenous macrophages may be recognized by means of the vital stain. For the better differentiation of these cells tissue cultures of spleen[39] from vitally stained animals were grown in a clear medium, and cultures of spleen from a normal animal were grown in a medium containing carmin. In each of these the polymorphonuclear, oxydase mononuclear, and nonoxydase mononuclear cells that wandered out from the planted tissue during the first few days, neither contained carmin nor took it up from the medium. After these cells had died, however, on the second and third day of the original inoculation and in the transplant, many larger mononuclear cells, predominently round, but often angular and spindle shaped, and all heavily stained with carmin, wandered out from the transplanted tissue into the surrounding medium. These cells continued to wander out even in the transplants and after active growth of the connective tissue elements which showed no stain was in progress. In many of these tissue culture preparations giant cells were formed on the cover slip. All of these were heavily stained with carmin and none showed any oxydase ferment.

Foreign Body Giant Cells in Living Animal.—For the more complete study of cells taking part in the formation of foreign body giant cells and to control the observation made in regard to them in tissue cultures, lycopodium spores were injected into the omentum and portal vein of animals that were vitally stained and received several doses of the stain after the introduction of the foreign bodies. In microscopic sections from these animals the carmin cells were relatively scanty, but in close apposition to the foreign bodies, often spread out over their surfaces. And in addition there was frequently an outer layer composed of small, round cells and larger, more irregular mono-

38. Morris, D. H.: The Rôle of the Spleen in Blood Formation, Jour. Exper. Med., 1914, xx, 379.

39. For these tissue culture preparations and help with their interpretation I am indebted to Dr. R. A. Lambert.

nuclears that did not contain the stain. None of these cells showed an oxydase ferment.

In brief, these experiments admit of the following observations:

1. Cells capable of specific vital staining by lithium carmin, the histogenous macrophages, contain no oxydase ferment; and conversely, none of the oxydase-containing cells, the polymorphonuclears and oxydase mononuclears, is specifically stained intra vitam by lithium carmin.

2. The polymorphonuclear and oxydase mononuclear cells phagocyte particles of carmin from the unfiltered solution long before the histogenous macrophages are vitally stained by it.

3. No normal circulating blood cell, either after acute or chronic staining of the animal by the intravenous or intraperitoneal route, takes the carmin stain; and the vitally staining histogenous macrophages are never encountered normally in the peripheral circulation, and only very rarely in the blood of the portal system.

4. In the tissues, in addition to oxydase cells of accidental occurrence, and the vitally staining histogenous macrophages normally present, there may be seen, normally and in reaction to inflammation, foreign bodies, etc., mononuclear wandering cells that contain no oxydase ferment and do not take the vital stain or phagocyte undissolved particles of it. Among these are small round cells resembling tissue lymphocytes and Marchand's adventitial cells, typical plasma cells, and other larger irregular mononuclears resembling pictures of some of Maximow's polyblasts.

5. The blood of the splenic vein contains more large mononuclear cells than blood from other sources. Many of these cells are oxydase mononuclears, but the great majority are nonoxydase mononuclears.

6. In the splenic pulp there are present polymorphonuclear, oxydase mononuclear, nonoxydase mononuclear, and vitally staining cells, each entirely distinct from all the others. The first three types are active, wandering cells and do not survive long in tissue culture preparation. The last does not migrate so actively as the other three under these conditions, survives a relatively long time in tissue culture preparations, and takes part in the formation of foreign body giant cells.

SUMMARY AND CONCLUSIONS

The application of the biologic reactions under the conditions just described, although not affording any information about the remote embryologic relation of the different mononuclear wandering cells or their complete genetic classification, does prove that they are not all of histogenous or lymphoid origin, by the generally accepted criteria, and does justify classification of the adult cells based on biologic

properties. The confusion of names encountered in the literature makes it impossible to place with certainty in this proposed classification many of the cells described, but a few forms of definite morphology, such as the plasma cell of Unna and the *Uebergangsform* of Ehrlich (oxydase mononuclear) can be assigned to one group or another without equivocation.

1. Cells containing an oxydase ferment and not taking the vital stains, the polymorphonuclear and oxydase mononuclear cells (so-called transitionals or *Uebergangsformen* of Ehrlich), primarily of the blood, but seen in the tissues under some conditions.

2. Cells containing no oxydase ferment and specifically stained intra vitam. These cells, recognized by all as intimately associated with the fixed tissue elements and variously termed histiocytes (Aschoff, Pappenheim, Marchand), pyrrhol cells (Goldmann), and macrophages (Evans), are normal constituents of the tissues and of rare and accidental occurrence in the peripheral blood. They present diverse forms in the tissues, some of which can be differentiated from cells included in the next group only by reason of their vital staining.

3. Cells containing no oxydase ferment and not taking the vital stain, the lymphoid elements. To this group belong the true lymphocytes and probably most of the nonoxydase, large mononuclears of the blood (Pappenheim's monocytes); and, in the tissues, the plasma cell of Unna, the polyblasts of Maximow, and the cell of small, round-cell infiltration.

Thus the observations made in these experiments refute the hypotheses of Mallory and Tschaschin that all the adult mononuclears of the blood and most wandering cells of the tissues are of one class, endothelial or histogenous in origin, and support the contention of Aschoff, Pappenheim, and Marchand, that there is a histogenous (capable of vital staining) and lymphoid (incapable of vital staining and containing no oxydase ferment) group distinct from each other. That many vitally staining histogenous cells are included in the large mononuclear group of blood cells, however, as asserted by Aschoff and Pappenheim, seems unlikely from the results obtained in these experiments, in which no vitally stained cells were seen in the peripheral circulation; and the fact that none of these cells anywhere contained an oxydase ferment does not support Kiyono's suggestion that the oxydase mononuclear cells are a type of the vitally staining histogenous macrophages. The higher percentage of mononuclear cells in the splenic vein blood than elsewhere is not in accord with Pappenheim's statement that the large mononuclear cells of the blood (monocytes) are not supplied in any way from the spleen; but our finding that even in the splenic vein the vitally staining histiocytes occur very rarely is in support of his belief that those of the spleen (splenocytes) are not

concerned with the large mononuclear cells of the blood. The observation here recorded that plasma cells, polyblast-like cells, cells of small round-cell infiltration, and some others in reaction to inflammation, and making up part of the cells in the region of foreign bodies, etc., do not stain vitally is not in entire agreement with Marchand, who includes many of these cells in the histogenous group; but rather, although no definite proof has been made available, since they do not contain an oxydase ferment, it appears likely that they are lymphoid cells or even changed forms of emigrated blood lymphocytes. The results obtained by applying the methods of study here employed to tissue cultures of the spleen proves a complete differentiation between the polymorphonuclear and oxydase mononuclear cells in one group, the lymphocytes and nonoxydase mononuclears in another, and the vitally staining histogenous cells in a third, as entirely justifiable in the spleen in accordance with Steudemann's views.[40]

The transitional cell reacts constantly as those of the granulocyte series, in that it contains an oxydase ferment, is not specifically stained intra vitam and takes up carmin particles from the unfiltered solution. It may be seen in the spleen and other blood-forming organs, as admitted by all observers, but is not strikingly more abundant in the splenic vein blood than elsewhere, and is never seen in the omentum, serous fluid or tissues when no polymorphonuclear cells are present. Already sufficient evidence has been adduced that cells of this type are not seen in the uncontaminated lymph of the thoracic duct.[41] These observations, therefore, lead to conclusions contrary to the opinion of Aschoff, Kiyono, Pappenheim, Mallory, Tschaschin and others, that these cells belong to the histogenous group, are lymphoid elements, or of endothelial origin; but in accordance with Naegeli, that they are of the granulocyte series, and together with the neutrophil, eosinophil and basophil, should be considered as descendants of the myeloblasts in the bone marrow and splenic pulp.

In regard to the large mononuclear group of cells as seen in the peripheral blood under normal condition, it may be said that many of them are these transitionals (oxydase mononuclear) of myeloid origin, and none are histogenous, as demonstrated by the power of vital staining. No further statement, however, can be made as to the cells making up this group. No doubt many of them are lymphoid in relation (lymphoblast, Aschoff) and probably some are small lymphocytes that have wandered in the tissues a while, have become changed in form and returned again to the blood. And there is no

40. Steudemann, K.: Phagozytose in der Milz, Folia haematol., 1914, xviii, 140.

41. Davis, B. F., and Carlson, A. J.: Contributions to the Physiology of Lymph, Am. Jour. Physiol., 1909-1910, xxv, 173. Lejeune, E.: Die Zellen im Ductus lymphaticus, Folia haematol., 1914-1915, xix, 371.

reason to believe that endothelial cells may not occur in the vascular system as in other serous cavities,[42] although no morphologic characteristics can be brought forward at present to differentiate them from the lymphoid elements.

In conclusion, therefore, although not venturing any conjecture as to the genetic relation of any one cell type to another, in regard to the adult cells seen in the mature organism it may be definitely stated that (1) the vitally staining histogenous macrophages are not normally encountered in the peripheral blood and occur in the blood of the portal system in such small numbers as to be negligible; that (2) most of the nonoxydase large mononuclear cells of the blood, and those mononuclear wandering cells of the tissues designated as plasma cells, polyblasts, and cells of small round-cell infiltration are not identical with the vitally staining histogenous macrophages, or of myeloid origin, but are probably lymphoid cells or even emigrated blood lymphocytes; and that (3) the so-called transitional cell is not a lymphocyte, any type of histogenous macrophage, or of endothelial origin, but is, with the polymorphonuclear cell, a descendant of the myeloblast and might conveniently be termed the oxydase mononuclear of normal blood.

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42. It is not intended to convey the impression that all the cells of the serous cavities are endothelial, even under normal conditions. H. M. Evans asserts (Footnote 15) that most of them are vitally staining cells.