THE OCCURRENCE AND SIGNIFICANCE OF "MOTILE" ERYTHROCYTES IN HUMAN BLOOD AND MARROW IN ANEMIC STATES*

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It has frequently been observed that fixed films of blood from macrocytic anemias, e.g., pernicious anemia, are characterized by poikilocytosis to a degree not seen in other anemic states. The study of living blood presents new facts of interest to the cytopathologist which contribute to the explanation of this phenomenon.

The dark-field microscope is particularly suited to the study of living blood cells since the smallest organoids are visible without stains or other alternative treatment and the most delicate of membranes appear as lines of varying thickness and refractivity. Consequently, all descriptions in this paper, unless otherwise specified, are of the dark-field appearance of the structures concerned. Because of better reproducibility, the photographs are given as negative rather than positive prints. Artifact formation was kept at a minimum by using only freshly drawn material and by handling it with the greatest care.

In a number of anemias of varying etiology (Hodgkin's disease, leukosarcoma, pernicious anemia et al.) it was noted that some erythrocytes of the peripheral blood exhibited irregular cell surfaces. The membrane of these cells (figs. 1–5, plate I) was less thick and refractive than that of the red blood cells of typical discoid shape. This appearance of the cell surface denotes a lower concentration of hemoglobin and a more primitive condition of the cytoplasm. Although most of these cells contained mitochondria and neutral red-staining granules not all of the cells containing such organelles exhibited motility. Few if any erythrocytes from normal blood contain such structures.

It was noted that this irregular form of the cytoplasm was constantly changing. In the least primitive of these cells the alterations took the form of small shallow notches that repeatedly appeared in their surface at from one to four places and slowly disappeared. In the more primitive forms (figs. 1–5, plate I) these constrictions were much deeper and divided the cell into lobes or pseudopods which were slowly protruded and retracted. Coincident with these surface changes the organoids of the cell, sometimes tangled into a knot, slowly moved about as though carried by currents within the cytoplasm. Occasionally in the most primitive erythrocytes a tiny granule-free area was present, surrounded by a group of neutral red-staining granules about which the mitochondria were loosely aggregated—the centrosphere. The sum of all these activities constitutes something that simulates

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‡ Not to be confused with the vacuome or granules of reticulum induced by high concentrations of stain.
PLATE I

Fig. 1. Motile primitive erythrocyte from a patient with pernicious anemia showing thin, irregular outline, rod-like mitochondria and neutral red-stainable granules. 1500 X.

Fig. 2. Same cell as in fig. 1, one minute later
Fig. 3. Same cell as in fig. 1, two minutes later
Fig. 4. Same cell as in fig. 1, four minutes later
Fig. 5. Same cell as in fig. 1, six minutes later

Fig. 6. Megaloblast from bone marrow of patient with Hodgkin's syndrome showing the formation of a large pseudopod with a partial constriction of the cell membrane at its base. 1500 X.

Fig. 7. Same cell as in fig. 6, three minutes later. Note completion of cytoplasmic constriction
Fig. 8. Same cell as in fig. 6, six minutes later. Note apparent effort of the pseudopod to detach itself from the parent cell.

Fig. 9. Same cell as in fig. 6, thirty-six minutes later. Bud is attached to parent cell by thin, slightly refractive thread.

Fig. 10. Same cell as in fig. 6, eighty-seven minutes later. Bud has become detached and continues to exhibit motility.
amoeboid movement. It differs from amoeboid movement in that the bud does not move from place to place and that the motility is not related to any vital function such as food getting, phagocytosis of debris, etc.

In order to study the origin of such cells, sternal marrow aspirations were obtained from selected patients whose peripheral blood contained large numbers of "motile" erythrocytes. All such specimens of marrow contained megaloblasts* with reduced amounts of hemoglobin in their cytoplasm, displayed the same cell membrane refractivity and types of mitochondria and neutral red-stainable granules that distinguished the motile erythrocyte in the peripheral blood. Some were polynucleate (fig. 14) and contained more than one central apparatus. In some cases multipolar mitoses could be seen, with as many as seven centrospheres clearly marked out by the clustering of neutral red-stainable granules. These cells gave a positive reaction to the benzidine test for hemoglobin. After a short time for adjustment to in vitro environment, these cells begin to extrude slowly and to retract one or more large blunt pseudopodia. Gradually the base of such pseudopods might narrow (fig. 6, plate I) and when constriction became sufficiently acute, an actual cleavage of the cytoplasm occurred (fig. 7, 8, plate I; fig. 13, plate II). This cleavage at first involved only the hemoglobin-laden endoplasm of the cell. The thin, weakly refractive ectoplasmic membrane at the surface** might remain intact for some time and be visible as a thin gray thread holding the budded erythroplastid to its parent cell (fig. 9, plate I). This strand eventually broke and the newly formed red cell left the mother cell by amoeboid-like movement (fig. 10, plate I). These buds might occur at any place on the cell surface, but there was a tendency for them to be formed near the centrosphere, in which case frequently not only mitochondria but some of the neutral red-stainable granules (fig. 13, plate II), and a part or all of the centrosphere were contained in the bud.

The movement of the newly formed erythroplastid differs from that of the more mature units in the circulation, in that it appears to be more active and the cell moves about from place to place. The motility gradually diminishes in in vitro preparations, but it has been seen to persist in individual plastids for as long as five hours after separation from the parent cell.

The formation of buds from leukocytes and connective tissue cells, described as clasmatoctylosis by Ranvier* and as pictured by Cunningham, Sabin, Doan**, may be of several types and due to a variety of causes. Although a detailed description of these changes is the subject of a further communication, they are here briefly characterized for the sake of comparison.

When exposed to in vitro conditions for considerable lengths of time, dark-field illumination demonstrates that the cytoplasmic membrane of living cells becomes much more refractive, thicker and less labile. Eventually the cell becomes permeable to its milieu and imbibes fluid. The cytoplasm becomes markedly less viscous, as evidenced by the acceleration of Brownian movement of organoids within it, and many tiny new granules may be precipitated in the otherwise homogenous

* The term megaloblast is here intended to include the megaloblast as described by Jones, et al. and the morphologically similar giant erythroid elements found in a variety of dyscrasias. They both exhibit the motility and budding described in this paper.
298 SIGNIFICANCE OF "MOTILE" ERYTHROCYTES

Plate II

Fig. 11. Same cell as in fig. 6, one hundred and seventy minutes later. Motility continues.

Fig. 12. Brightfield photograph of megaloblast from patient with Hodgkin’s syndrome supravitally stained with Janus green B and neutral red. Note mitochondria surrounding nucleus and cluster of neutral red-staining granules about central apparatus at upper edge of nucleus. 1500X.

Fig. 13. Brightfield photograph of bud forming from megaloblast in pernicious anemia. Note that base of bud is opposite the central apparatus and that the bud contains rod-shaped mitochondria and neutral red stainable granules. 1500X.

Fig. 14. Tri-nucleate megaloblast from patient with Hodgkin’s disease forming three erythrocytes by budding. 1180X.

cytoplasm. Amoeboid movement may persist in cells in which these changes have taken place, although it is sluggish and altered qualitatively. Portions of the cell...
membrane frequently round up and form vesicles which bud from the parent cell. They contain fluid of very low viscosity, organoids and precipita, and are not capable of amoeboid movement. Clasmatocytes are characterized by the irreversible extrusion of long tubular, worm-like pseudopodia which may or may not separate from the parent cell. This lytic type of budding is due to alterations in permeability of the cell membrane, and is a part of the death process. The budding of the megaloblast differs from that just described, in that it takes place in the living body, the bud is motile, carries out its normal function, i.e., transport of oxygen, and the parent cell does not exhibit moribund changes.

Primitive cells from connective tissue, lymph nodules, spleen and marrow, leukemic and sarcomatous cells and normal, but immature cells mobilized into the peripheral blood by unusual stimuli, are frequently delicate and at the same time turgid and incapable of amoeboid movement. They often fragment without exhibiting many of the lytic changes previously described. The bud produced contains the organoids of the parent cell and exhibits the characteristics of the intact parent cytoplasm. It differs from the budding described for the red cell in that the bud is not motile, serves no useful function and is produced by the mechanical action of an unsuitable environment upon the cell.

The budding of platelets from megakaryocytes, although poorly understood is differentiable from the budding of megalo- and normoblasts because of qualities of the thromboplastid itself. The platelet is an extremely labile thixotropic system which changes irreversibly from a rigid gel to a sol on contact with any wettable surface. Eventually vesicles bud from its surface similar to those formed by the action of in vitro conditions upon normal cells.

The question then arises, are these "motile" erythrocytes to be construed as specific entities pathognomonic of the disease in which they are found or are they merely evidence of a greater or lesser "left shift" of erythroid elements in the marrow with a larger percentage of the circulating cytoplasmic units derived from more primitive cells. The mouse (Klieneberger) and the rabbit (Seyfarth) normally have many more circulating reticulocytes than does the adult human. In a study of normal adult rabbits the author found some quite motile erythrocytes in every sample of blood examined. They contained mitochondria and neutral red-staining granules and were in every way similar to those found in human macrocytic anemia except that there was not the great variation in size and shape nor the pronounced hypochromia. The same phenomenon was found in the mouse except that fewer of the cells showed motility. Young animals of both species showed more motile erythrocytes than did the adults.

**Discussion**

The normal process by which a mammalian erythrocyte is produced is one of budding in which the parent cell, i.e., the normoblast, becomes separated into two fragments. One contains a pyknotic nucleus, a shred of cytoplasm, and perhaps a few cellular organoids; the other is a disc of hemoglobin-laden cytoplasm with a cell membrane and occasionally a few mitochondria and neutral red-staining granules. This process has been seen to occur in vitro by the author. It is, therefore,
SIGNIFICANCE OF "MOTILE" ERYTHROCYTES

perhaps inaccurate to speak of the normoblast as "extruding" its nucleus merely because the division is usually unequal and the nucleus-containing fragment smaller and sometimes apparently devoid of cytoplasm. In the normal human adult the reticulocyte exhibits no motility. This is because the normoblast from which it buds is so mature as to have lost this quality. Under conditions in which the marrow is unable to supply erythrocytes from mature normoblasts, younger and younger cells, with less hemoglobin and with other evidences of immaturity such as the presence of mitochondria and neutral red-staining granules, a more fluid cytoplasm, and amoeboid movement are called upon to supply these small packages of respiratory pigment, the erythrocytes. Naturally, these red cells will to a certain extent exhibit the characteristics of their parent, i.e., hypochromia, motility, content of organoids, etc.

Werner Schultz in a report based on a single case of untreated pernicious anemia described the formation of erythrocytes (blastopodiocytes) by localized budding of the cytoplasm of megaloblasts. He regarded this budding as the true origin of the poikilocyte, and as a "pathological regeneration" limited to the megaloblast of pernicious anemia in the human adult and, consequently, a point of "functional" differentiation between this cell type and the "macrolast" of some other anemic states. We cannot support his conclusions. First, we have found this budding to occur in a variety of conditions other than pernicious anemia. Second, poikilocytosis and "motile" erythrocytes are by no means limited to pernicious anemia but may occur in the human adult in a variety of anemic states and in the rabbit and mouse under normal conditions. The presence of large numbers of poikilocytic and motile erythrocytes is due to the absence of normal blast forms from which they may be budded.

Summary

1. With the aid of supravital studies and dark-field illumination, "motile" erythrocytes (capable of self initiated amoeboid-like change in form and, in some cases, movement from place to place) may be found in the peripheral blood of cases of anemia, particularly those of the macrocytic variety.

2. These motile erythrocytes are usually hypochromic, contain mitochondria and neutral red-stainable granules, all of which are attributes of normal but immature red cells.

3. Motile erythrocytes similar to those found in the blood of human patients with anemias are found in the blood of normal rabbits and mice.

4. In the marrow of patients with macrocytic anemias, megaloblasts and megalocytes may be found which give rise to motile erythrocytes by budding.

5. Poikilocytosis, motility, paleness, content of mitochondria and neutral red stainable granules, and origin by budding from a large parent cell do not differentiate the erythrocytes of pernicious anemia from those of other anemic states.

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