THE BLOOD-PLATELETS IN TROPICAL AND OTHER FORMS OF ANÆMIA.

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The morphology and variations in number of these blood elements have not received due attention in the study of the blood changes of the various anæmias and other conditions affecting the bone marrow. The principal reasons of this neglect are an erroneous notion of the histogenesis of the platelets, and the fact that relatively few laboratory workers in the temperate zones use the modified Rowmonowsky stains in their examination of blood films.

In the Tropics, laboratory workers, of necessity, in staining fresh blood films and autopsy smears for malaria, plasmodia, *Leishmania*, filaria and the secondary anæmias of hæmoglobinuric fever, uncinariasis, *Verruga*, etc., have come to use these polychrome stains more frequently than their fellows in the temperate zones; they have learned by constant use their great value in staining blood elements and protozoa, and they have, it is believed, developed some resourcefulness in the handling of these beautiful and invaluable, though sometimes capricious, stains.

An interesting commentary on the relative value of polychrome blue stains over triple stains is that seen in the last edition of EHRLICH and LAZARUS's work on Anæmia,* in which four of the five plates show the blood elements stained by GIEMSA and only one plate stained by the triacid method.

The general use of these stains is not always a simple matter, for while the value of a particular stain may remain constant for weeks, the character of the films is changing constantly; by this I mean that the stain gives varying results, depending on whether the blood film is damp or old, and whether the smear from tissue was taken from fresh material a few hours old or from a cadaver after twenty-four or more hours, when the reaction may be acid. The staining method must be varied to meet the requirements of the film or smear. In

* EHRLICH and LAZARUS, *Anæmia*. REBMAN, LTD., London, 1910.

dealing with marrow spheres very fresh material should be used, for while it is possible to get well-stained specimens from old autopsy material, the picture is almost always ill-defined and blurred.

Various writers have attributed the origin of platelets to the extrusion from red cells of masses or chains of globular material which give many of the reactions of the nucleo-proteids; *e.g.*, MAXIMOW, ARNOLD, WLASSOW, BREMER, ENGEL, KLEBS. KING* believes that practically every red-blood cell in the blood of normal adults, contains stainable remnants of a nucleus, and he says that in a single preparation he has seen as many as ten ruptured erythrocytes, from each of which from two to six blood-platelets were emerging. He can hardly escape the conclusion that the blood-platelets are simply the extruded fragments of the erythrocytic nuclei.

With regard to this observation of KING's, I may say that in very fresh dog-marrow films when stained faintly with Hastings' stain, so that there was little differentiation of the various elements—all staining bluish—I have noticed large blue dots and rings (Howell's bodies and rings) in the red-blood cells, particularly in those red-blood cells showing more basophilia than the rest. These are undoubtedly the remains of nuclear material and are entirely different from platelets. If these same films are immediately restained and thus well differentiated, the blue nuclear remnants are entirely obscured. The diffuse basophilia of red-blood cells would seem to be due to the distribution of colloid nuclear material uniformly throughout the cell. In preparations from the marrow of the dog, cat and rat there may occasionally be seen alongside the red-blood cells, which contain Howell's rings and bodies, platelet-mother-cells—the megalokaryocytes, with clumps of platelet and platelet-like cytoplasm, just ready for distribution to the peripheral blood stream.

By some it is believed that blood-platelets (*a*) are fragments of the nuclei of disintegrated leucocytes; by others, that they are (*b*) pre-erythrocytes; and still by others, that they are (*c*) precipitated globulin particles; or, that they are (*d*) special cells. Finally, according to WRIGHT,† the platelets are detached portions or fragments of the cytoplasm of the megalokaryocytes. WRIGHT's views, I believe, represent

* KING (R. W.), *Jour. of Med. Research*, Boston, 1910. "Erythrocytic nuclei of normal human blood."

† WRIGHT (J. H.), *Boston Medical and Surgical Journal*, 1906, 154, p. 643.

the truth of the question of the histogenesis of the blood-platelets, and they may be summarised in his own words: "All of the blood-platelets are detached portions or fragments of the cytoplasm of the megalokaryocytes, which are in such relation to the blood channels in the marrow that detached portions of their cytoplasm are quickly carried by the blood current into the circulation. The breaking up of the cytoplasm platelets occurs only in the cells which have reached a certain stage of growth and development, and is probably rapidly completed when once begun. It takes place in various ways, but usually by the pinching off of small rounded projections or pseudopods from the cell body, or from larger pseudopods, or by the segmentation of slender pseudopods, or by the pinching off of longer or shorter pseudopods which may not undergo segmentation later. . . . Before the separation of a platelet takes place, the red to purple granules in that portion of the cytoplasm which is to form the platelet are separated from the rest by a zone of hyaline cytoplasm, and this sharply outlined mass of granules becomes the central granular mass of the blood-platelet, which had been regarded by some observers as a nucleus."

WRIGHT's conclusions have been confirmed by BUNTING with regard to the rabbit.*

WRIGHT offers, also, an hypothesis that the thrombocytes of birds and reptiles, regarded by some as the morphological equivalents of platelets, are rather the homologues of the megalokaryocytes, and there is no doubt of the correctness of this view. It would seem, then, that as the nucleated red cells in reptiles, amphibians and birds leave the blood-forming organs to circulate peripherally, so do the homologues of the megalokaryocytes—the thrombocytes; but while the thrombocytes circulate as true cells, they function as platelets.

Observations on the thrombocytes of *Bufo marinus* show that they have a small amount of perinuclear and polar cytoplasm staining like platelet material. The thrombocytes of *Iguana tuberculata* show agglutination of one another into strands and clumps of two or three to as many dozen. Polar pseudopodia can be seen projecting out into the plasma staining like platelet material, but none was ever seen detached as a free platelet. The same is true of the blood of the turtle (Panaman fresh-water species).

*BUNTING, C. H., "Blood-platelets and Megalokaryocyte Reactions in the Rabbit," *Journal of Experimental Medicine*, 1909, XI., 541.

Observations on the fresh blood of the green-spotted frog of Panama show the thrombocytes to have a small amount of amœboid cytoplasm, throwing out pseudopodia exactly like parasitic amœbæ at intervals from all sides of the cell, becoming attached to cells, and also separating from them and joining others near by. These pseudopodia never appear to become detached as platelets, but seem to furnish an extra glutinous surface for the formation of the clot.

WRIGHT further notes that a "comparison of the numbers of blood platelets per c.mm of blood in certain diseases, as estimated by various observers, with the histological findings in the bone marrow in the same diseases, suggests a relationship between the blood platelets and the megalokaryocytes. Thus, in pernicious anæmia and lymphatic leukæmia the blood has been found to contain abnormally few platelets, while the marrow in typical cases of these diseases—as far as can be inferred from the reports in medical literature and from my own observations, undergoes profound changes in the character of its cellular constituents with marked diminution in the number of megalokaryocytes. On the other hand, in post-hæmorrhagic and secondary anæmia the blood-platelets are increased in number, and there is also an increase in the amount of red marrow, with consequent increase in the total number of megalokaryocytes in the body. In so-called myelogenous leukæmia, the blood platelets are also increased in number, and in the cellular accumulations of this disease megalokaryocytes do not seem to be an uncommon finding, although but little attention has been paid to them by pathologists. In view of the enormous increase in the marrow cells in this disease, it must be obvious that the presence among them of a relatively small proportion of megalokaryocytes means a great absolute increase in the number of such cells in the body."

BUNTING has shown* that in the rabbit an increased number of platelets in the blood stream is synchronous, or appears just after an increased number of megalokaryocytes in the marrow.

An experiment of COLE's† is very suggestive in showing that there is, as he expressed it, "no genetic relationship between red-blood cells and platelets." COLE says, "to approach this problem we produced an

* *Journ. Exp. Med.*, XI., 551.

† COLE (R. I.), *J. H. U. Bulletin*, XVII., p. 261, 1907, "Note on the Production of an Agglutinating Serum for Platelets."

hæmolytic serum by the injection into two rabbits of human red-blood cells obtained by defibrination. The platelets are always caught in the fibrinous mesh-work, and it is probable that such corpuscles have mixed with them few or no platelets. By the injection of these platelet-free corpuscles into a rabbit, a serum having hæmolytic action in dilution of 1 : 600 was obtained. The agglutinating power for platelets was now tested, and it was found that such a serum was entirely inactive, even in dilution of 1 : 1. These experiments seem to speak quite definitely against a genetic relationship between platelets and red-blood cells . . . and offer additional evidence against the view of KEMP, and others, that the platelets are derived from red-blood cells."

Normally, then, in man the blood-platelets are undoubtedly formed from the megalokaryocytes in the bone marrow. In myelogenous leukæmia they may be formed in the spleen, liver and other collections of myeloid cells. In neoplasms of the myeloid type, such as Hodgkin's disease, which histologically contain giant cells of the megalokaryocyte type, it would appear that platelets might be formed from the latter.

In some mammals the platelets may be formed in the spleen, as in the coati; for instance, I found in experimental trypanosomiasis of that animal a considerable degree of anæmia, and the spleen contained many megalokaryocytes.

Little has been written on the subject of blood-platelets in disease. CABOT* says, "they are increased in number in diseases which lead to a multiplication of giant cells (post-hæmorrhagic anæmia and leukæmia), and diminished in conditions like pernicious anæmia, which are associated with a marrow poor in giant cells."

Their number is increased in post-hæmorrhagic anæmia and myeloid leukæmia, and they are notably diminished in most cases of pernicious anæmia, in many fevers, including typhoid fever, and especially purpura hæmorrhagica.

It is important to know the approximate number of platelets in the peripheral blood in various diseases, and it is of equal importance to note any changes in morphology or in the tinctorial characters of the platelets. This last is best done with one of the polychrome stains—LEISHMAN'S, HASTINGS' or WRIGHT'S.

As usually seen in well-stained films of normal blood, the platelets

* CABOT (R.), "Diseases of the Blood," *Osler's Modern Medicine*.

are small, round or oval, flattened bodies, two or three mm. in diameter, made up of slightly refractile clear ground substance, staining very faintly blue, and dotted over with fine purple granules, having a tendency to clump towards the centre, leaving a clear margin. There is no nucleus nor chromatoid material of any kind demonstrable by the usual staining methods. Occasionally the margin of the platelet has a fine purple staining rim, and sometimes faintly staining ciliate or flagellate processes, or pseudopodia, extend out from the margin, such as have been described by SWINGLE. Normal platelets in films are usually agglutinated into clumps of two or three to several dozen or more elements.

In anæmia, on the other hand, peculiar forms may be encountered. Some are much larger than normal, and their outline may be regular and entire and of an oval or round shape, or they may be very irregular in form with a twisted elongated or nodose form, almost resembling trypanosomes or the flagella of malarial gametes. In a case of pernicious anæmia, the blood findings of which are reported here, there was noted, in addition to all types of bizarre and giant shapes, a remarkable tendency towards polychromasia and diffuse staining. The peculiar changes in the platelets were noticeable before there was any marked degree of poikilocytosis of the red-blood cells, and before nucleated red cells appeared in definite numbers. These changes pointed unmistakably to some profound intoxication of the hæmopoietic organs, and the very large size of the platelets in this case suggests very strongly that they could not, under any circumstances, have been formed from red-blood cells. The changes in the platelet in the case seemed to be analogous to those in the red-blood cells, when the latter are subject to poikilocytosis, microcytosis and polychromasia.

No attention seems to have been paid to the changes in the blood-platelets in some of the diseases in the Tropics, particularly those associated with severe anæmia, as malaria, hæmoglobinuric fever, *Verruga Peruviana*, hookworm disease, etc.

In malaria there is during some phases of the disease a well-marked diminution in number of the platelets, and some tendency towards the formation of bizarre forms. This appears to be limited to the period of the febrile paroxysm, when parasites are in the peripheral blood and when there is a leukopenia; and also in untreated cases during the course of the infection as the secondary anæmia is developing.

Case 18-1.—Malaria transmission experiment. This patient was given no quinine for several days. In a number of blood films the platelets were greatly diminished in number, but all were of normal size, and they were agglutinated.

Case L.—Latent tertian malaria. Films taken during an attack of tertian malaria while patient was on leave in the United States. Platelets much diminished in number.

Case 23.—Tertian malaria. On admission parasites were numerous, while platelets were reduced in number. On successive days, as the parasites disappeared, the platelets reappeared in increasing numbers until a normal number was reached.

Blood films from several cases of latent quartan malaria among labourers and from the native village of Chame, R.P., showed marked diminution in the number of platelets, and there was a number of large forms and some bizarre forms.

Cases 21-41 and C 26.—Pernicious malarial fever with marked secondary anæmia. Films from these cases contain a diminished number of platelets—less than 30,000 per c.mm.—some of which were of large size.

Blood from school children in the native villages of La Chorera, Chame, La Ville de los Santos, and Guararé, R.P., showing a malarial infection, also showed in most cases a diminished number of platelets, while those in which malarial plasmodia could not be demonstrated usually presented a normal appearance so far as platelets were concerned.

In a number of other cases of tertian and estivo-autumnal malaria the platelets were reduced in number, and some of large size were seen.

In hæmoglobinuric fever there is great diminution in the number of platelets with very little agglutination. The platelets, however, are of normal size and tinctorial characters.

Case 1.—Hæmoglobin, 65 per cent. Platelets diminished in number.

Case 2.—Hæmoglobin, 75 per cent. Platelets diminished in number, and show chiefly single discrete forms without agglutination.

Case 3.—Hæmoglobin, 40 per cent. Platelets greatly reduced in number.

Uncinariasis.—Blood films from a number of cases of this disease, in which the red-blood cells were reduced to two or three millions, showed

a much diminished number of blood-platelets, most of which were isolated or in clumps of only two or three. A few platelets of rather large size were seen.

Relapsing Fever, European type (Sp. recurrentis).—Smears taken during the paroxysm contain a diminished number of platelets, though of normal size and tinctorial character.

Relapsing Fever, American type (S. Novyi).—The platelets are diminished in number, though of normal size and tinctorial character.

Verruga Peruviana.—In this disease there is a profound anæmia, and a very marked tendency to hæmorrhage from the "warts." There is also great diminution in the number of the platelets during the febrile period and when the anæmia is most severe.

In case 1, a patient who was admitted to the hospital very ill and died in nine days, the platelets were normal on admission but became greatly reduced in number, and the power of agglutinating was lost on the day of death.

In case 3, a patient admitted with profound anæmia improved slightly from day to day, and ultimately recovered. On admission the platelets were very much diminished in number, agglutination was practically abolished, and the anæmia was very severe. From day to day, as the patient improved, the platelets increased in number, and the inability to become agglutinated returned after six or seven days. When the temperature had become normal and the blood picture had improved, the platelets were present in normal numbers and had normal staining characters.

Yellow Fever.—In this disease there is no anæmia unless there is black vomit or hæmorrhage from the intestines. Smear preparations from the spleen show considerable phagocytosis of platelets by large mononuclear cells and there are numerous free platelets.

It will be recalled that during the epidemic of yellow fever at New Orleans, in 1905, bizarre platelet-like bodies in the peripheral blood of yellow fever patients were described as being the pathogenic agents of the disease.

In *Kala-Azar*, judging from films from the peripheral blood and spleen, there is a very marked reduction in the number of platelets.

Through the kindness of Drs. DEEKS, MONROE and VALLELEY, I recently had an opportunity to examine blood films and to make a

complete blood examination of a patient who had a very rapidly progressive and fatal attack of pernicious anæmia; and as the platelets in this case presented practically all the pathological forms met with in the various anæmias, I shall describe them in some detail.

Case W. B. C.: January 30-31: the occasion of the first examination. The platelets are diminished in number, sometimes only one to four or five fields being seen, and they show no tendency whatever toward agglutination. Many are very large, measuring 4·5, 7·5, 9 and 12 mu. in diameter. Bizarre forms are usually seen, there being very few normal platelets. Some are long and narrow, 30 mu. in length by $1\frac{1}{2}$ mu. in width, with knobbed ends. Others have flagella-like processes, suggestive of the flagella of malarial gametes. Dumb-bell forms are seen. Some of the platelets have fine, eosin-staining flagella-like processes, radiating out from the periphery, for a distance of $\frac{1}{2}$ to $\frac{3}{4}$ of the diameter of the platelet. Others have a clean entire margin. Some are oval and pale with entire margins and contain very few purple granules. There is very little basophilia of the red-blood cells, but a number of shadow corpuscles. There is poikilocytosis and a number of microcytes. One nucleated red cell (normoblast) was seen in one film.

March 10: The platelets in the film present an appearance very much like those of January 30, but the blood shows much more marked evidences of anæmia. Normoblasts are infrequent, though more readily found than on January 30. There is more poikilocytosis and more microcytes and shadow corpuscles.

March 17: On this date a more thorough examination was made:—

Erythrocytes	...	...	...	...	1,290,400
Leucocytes	...	...	...	...	1,720
Platelets (calculated)	...	...	...	...	41,000
Hæmoglobin	...	...	...	...	32 %
Color index...	...	...	...	...	1·24

Differential—

Polymorphonuclears	...	...	...	...	55·7 %
Eosinophiles	...	...	...	...	2·8
Small mononuclears	...	...	...	...	22·8
Large	„	...	...	...	3·5
Intermediate	„	...	...	...	4·2
Transitionals	„	...	...	...	10·7

There was approximately one nucleated red cell (normoblast) to every 100 leucocytes. There is some poikilocytosis and a considerable number of microcytes. A number of the erythrocytes have a diameter of 9 to 12 μ . The platelets are very much diminished in number and they are not agglutinated. There are numerous large forms—giant forms, 10 to 12 μ . in diameter, staining rather diffusely purple, with some very fine granules. These are definitely not degenerated red-blood cells, for all sizes, from those slightly larger than normal platelets, up to the giant sizes, are seen. A number of bizarre, irregularly elongated forms are seen and the ground substance of many has a tendency to stain purple instead of faintly blue with polychrome stains. Some of those that have blue staining ground substance contain very few purple granules, merely a dot or two. There was a superficial resemblance of some of the platelets to trypanosomes, malarial gametes and piroplasmata.

This mimicry by platelets of protozoa has been noticed in the case of normal platelets by SWINGLE.*

Hodgkin's Disease.—The peripheral blood of two cases falling into the clinical category of Hodgkin's disease has been examined with regard to the platelets.

Case H.: Sections of the enlarged cervical lymph nodes presented the picture of Hodgkin's disease as described by REED and LONGCOPE, with giant cells, megalokaryocytes and eosinophilic cells—rib marrow elements, in other words—and the peripheral blood contained a great increase in the number of normal sized platelets, suggesting that the increase was due to the formation of platelets from megalokaryocytes in the myeloid lymph node.

Case D.: Clinically, this case presented the typical appearance of Hodgkin's disease, with a large cervical collar consisting of enormously enlarged lymph nodes. In sections of tissue, however, there is an entirely different picture from that seen in case H, for giant cells, megalokaryocytes and eosinophilic cells are absent, the lesion being a lympho-sarcoma. Upon examination of the patient's blood it was found that the platelets were of normal appearance and number.

BUNTING† has recently published his findings in several cases of

* SWINGLE (L. D.), "On the similarity of Blood Platelets and Certain Hæmatozoa," *Journal of Infectious Diseases*, 1908, V., 46.

† BUNTING, *Johns Hopkins University Bulletin*, 1911.

Hodgkin's disease in which there was an increased number of platelets in the peripheral blood, some of them being of unusual size. In his cases microscopic examination of the sections of the glands showed the picture described by REED and LONGCOPE.

It would seem, then, that in doubtful cases of Hodgkin's disease, where removal of tissue for diagnosis is impossible, a differential diagnosis might be made by an examination for platelets.

A further suggestion from these cases is that true Hodgkin's disease is a myeloma of the lymph nodes.

On account of the neglect into which the study of platelets has fallen, there appear to be no satisfactory terms available to describe the various changes in their numbers, morphology and tinctorial characters. The following terms, therefore, are suggested :—

Plastocyte . . .	...	A blood platelet.
Orthoplastocyte	...	A normal blood-platelet.
Microplastocyte	...	An undersized blood-platelet.
Megaloplastocyte	...	A platelet larger than normal.
Poikiloplastocyte	...	An irregularly shaped platelet.
Polyplastocytosis	...	An increase in the number of platelets above normal.
Plastocytopenia	...	A decrease in the number of platelets below normal.

CONCLUSION.

Blood-platelets, according to WRIGHT, are the detached portions of the cytoplasm of certain large marrow cells, called by HOWELL megalokaryocytes. They are diminished in number in certain diseases, such as malaria, hæmoglobinuric fever, uncinariasis, Verruga Peruvian, relapsing fever, kala-azar, and typhoid fever at certain phases of the disease ; and they are also diminished in number in Addisonian anæmia, lymphatic leukæmia and purpura hæmorrhagica.

The platelets are increased in number in myelogenous leukæmia and in myeloid neoplasms, such as Hodgkin's disease. This suggests that the giant cells described by REED and others in Hodgkin's disease are megalokaryocytes, and that Hodgkin's disease is a myelomatosis of

certain lymphoid elements ; as a diagnostic corollary, the clinical separation of true Hodgkin's disease from lympho-sarcoma follows when the latter does not contain megalokaryocytes.

The size and staining characters of the platelets are sometimes altered, this being analogous to such changes in the erythrocytes as microcytosis, poikilocytosis, macrocytosis and polychromasia.

Several terms are suggested for use in describing the normal and pathological changes of platelets.

It is believed that if the changes in numbers and morphology to which the platelets are subject in various diseases will be observed more carefully, they will furnish material help in the diagnosis of diseases affecting the blood-forming organs.