MORPHOLOGY AND PATHOMORPHOLOGY

THROMBOCYTES UNDER THE ELECTRON MICROSCOPE

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Lately, hematologists are being attracted more and more to the use of the electron microscope in their studies of the formed elements of the blood and bone marrow. The study of the finer cell structures in normal and in pathological states may help refine our understanding of some phases of the pathogenesis of aplastic and hypoplastic anemias, leucoses and other diseases of the hematological system. In particular, the morphology of thrombocytes and an investigation of their finer structure is of special interest as they play such a well known role in blood coagulation.

The first data on the electron microscopic structure of blood platelets are associated with the work of Wolpers and Rusca [10]. Their studies were followed by the works of Bessis [4], Porter and Hawn [8], Alexandrowicz and Blicharski [3], Braunsteiner, Fellinger and Pakesch [6] and many others. The data accumulated by all these authors permit not only formation of an opinion on the structure of the thrombocytes but also unfold some of their significance in the complex process of blood coagulation.

In the present communication, which is our first on the work we have done using the electron microscope in analyzing the cells of the blood, we offer the results of our study of the thrombocytes in 20 healthy people, 10 patients suffering with leucoses and 12 patients ill with aplastic and hypoplastic anemias. We chose this particular group of patients as hemorrhagic diathesis is frequently the most important feature of their entire clinical picture.

EXPERIMENTAL METHODS

The preparations for investigation were made in the following fashion: the heparinized blood was centrifuged for 10 minutes at 1000 rpm, then the plasma was carefully pipetted off and centrifuged again for 20 minutes at 1500 rpm. The thrombocytes, which were separated by this method, were placed on special copper nets which had been first covered with a colloidal membrane. After remaining in a thermostat at 37° for 10-15 minutes, the preparations were rinsed with Ringer's and then fixed in a 2% solution of osmic acid and finally rinsed with distilled water.

Thrombocyte preparations vary according to the time elapsed between the drawing of the blood and the preparation for electron microscope studies, so that we always employed blood prepared 30-35 minutes after it had been drawn.

The thrombocytes were examined under an electron microscope EM-3 of native construction giving a magnification of 7,000 times.

EXPERIMENTAL RESULTS

According to our observations, which coincide with the findings in the literature (G. M. Abdullaev [1], F. Alexandrowicz [3], M. Bessis [4], G. Braunsteiner [6], G. I. Roskin [2] and others), the thrombocytes of healthy people when examined under the electron microscope present many polymorphic formations with innumerable pseudopodia and outgrowths.

In the thrombocytes of donor bloods there is easily distinguished a peripheral zone called the hyalomere and a central portion called the granulomere (Fig. 1).

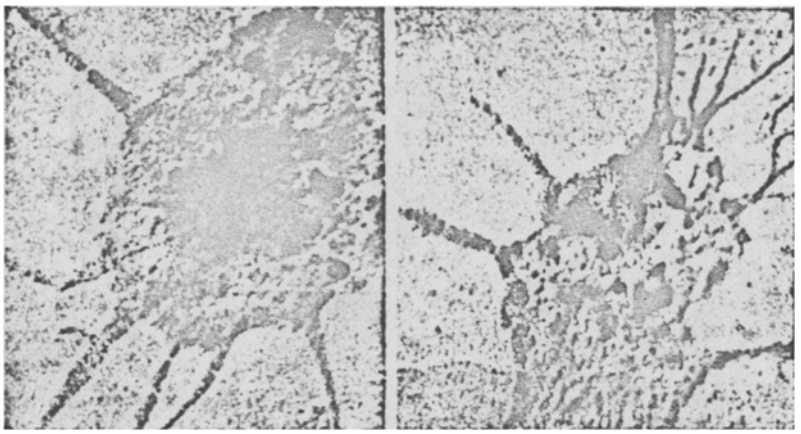

Fig. 1. Electron microscopic pictures of blood thrombocytes taken from healthy people. Magnification 7,000 times.

The hyalomere is not homogenous, structurally speaking, and consists of numerous fibrils intertwined with each other (M. Bessis [5]). This fibrillar network protrudes at the periphery of the thrombocyte forming outgrowths and protrusions of variable dimensions; from tiny serrations to pseudopodia (Fig. 2). The pseudopodia have different thicknesses and lengths, although the thickness is always greatest at the point of attachment to the body of the thrombocyte. Also encountered are pseudopodia which branch further and then intertwine with each other. The number of pseudopods present in individual thrombocytes varied, in our studies being within the limits of 2 to 12.

The thrombocytic granulomere consists of numerous grain-like granules, packed closely together in specific places and seeming to form a single conglomerate. Usually, the granulomere assumes a central position (see Fig. 1) but it may be eccentric (see Fig. 2). However, we saw individual instances when the granulomere granules were distributed evenly over almost the entire surface of the platelet.

Concerning the roles played by the hyalomere and the granulomere in the complex blood clotting process, there exists a diversity of opinion in the literature. Most authorities (M. Bessis [4], G. Braunsteiner [6] and others) believe that, when the platelets disintegrate, f m the hyalomere which has a fibrillar reticular structure the microsomal grains which are the carriers of thrombokinase are liberated. These authors believe that the granulomere represents the center from which the fibrin threads radiate.

The other point of view is held by F. Alexandrowicz and I. Blicharski [3]. They consider that the disintegration of the hyalomere frees the granulomere and as a result of this the thrombocytes liberate the lipoidal factor which participates in the formation of thromboplastin into the surrounding plasma. This is the precondition for the beginning of the blood coagulation process. In the opinion of these authors, the hyalomere participates in the retraction of the blood clot.

In the light of new data published lately on the blood clotting mechanism (P. Owren [7], M. Stefanini and V. Damechek [9]), we are inclined to prefer the second view point.

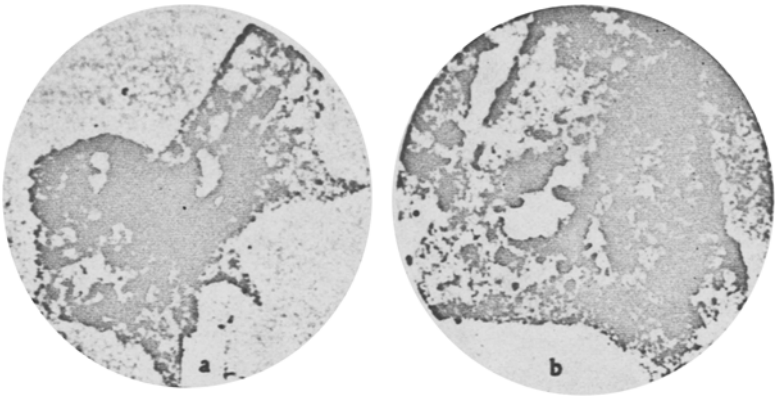

Fig. 2. Electron microscopic pictures of thrombocytes taken from patients suffering with aplastic (a) and hypoplastic (b) anemias. Magnification 7,000 times.

Examination of the thrombocytes, obtained from patients ill with aplastic and hypoplastic anemias, by means of the electron microscope reveals that the thrombocytes of these patients have a morphology distinct from the morphology of the thrombocytes obtained from healthy people. The first difference lies in greater morphological uniformity of the platelets. The numerous serrations and pseudopodia observed in donor thrombocytes are either altogether absent or else much less marked (Fig. 2).

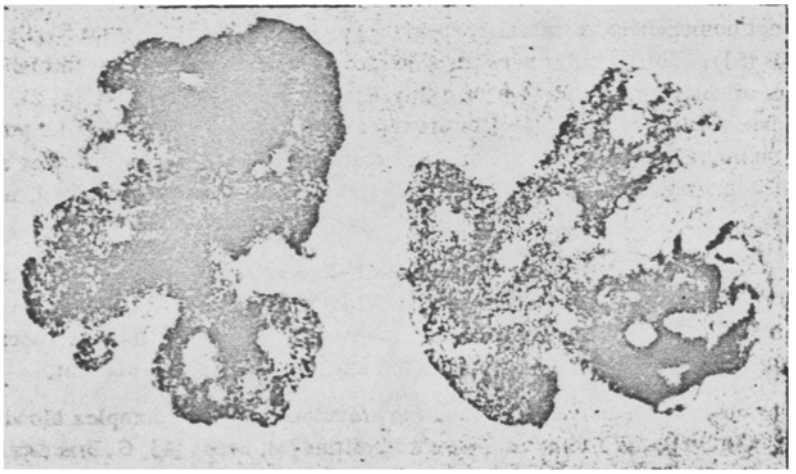

Fig. 3. Electron microscopic picture of the thrombocytes in the blood of a patient ill with leucosis. Magnification 7,000 times.

The surface of the thrombocytes obtained from these patients is smoother, frequently shows no outgrowths or serrations, and may possess only 1 or 2 pseudopods which are usually quite short. Similar to the thrombocytes

of healthy people, the thrombocytes of hypoplastic anemia patients have 2 zones, namely, granulomere and hyalomere. However, in the case of the patients' thrombocytes, the boundaries between these zones tend to become erased. Within the platelets is seen a considerable number of sharply demarcated vacuoles of various forms and sizes; this may possibly be an indication of degenerative alterations.

Distinct morphological changes are also to be found when the thrombocytes of patients suffering with various forms of leucosis are examined. In these patients the degenerative alterations within the platelets are quite marked. Vacuolization, absence of serrations and pseudopods, as well as the almost total inability to determine the boundaries between the hyalomere and granulomere within the thrombocytes of leukemic patients appear to be indicative of marked abnormalities (Fig. 3). These findings agree with the findings in the literature [2].

The present paucity of observations makes it difficult to correlate the precise morphological alterations in the structure of the thrombocytes with the exact clinical course taken by the disease. There is the over all impression that in specimens from the patients having severe hemorrhagic manifestations, there are demonstrated in the electron-microscope the sharpest deviations from normal in the structure of their thrombocytes.

The electron microscopic picture presented by the thrombocytes of patients ill with leucoses, aplastic and hypoplastic anemias, in our opinion, may be of aid in understanding the mechanism underlying the development of hemorrhagic tendencies in these diseases.

SUMMARY

The electron microscope was used to study human thrombocytes at magnification of 7,000 times. Thrombocytes taken from healthy donors were compared with thrombocytes obtained from patients having leukemias and various aplastic and hypoplastic anemias.

The details of the granulomere and hyalomere structures were compared. It was pointed out that they may have a bearing on the hemorrhagic tendencies these diseases show clinically.

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