Structural Changes in the Platelets as Observed by Electron Microscopy

By H. Braunsteiner, M.D., K. Fellinger, M.D., and F. Pakesch, M.D.

Aside from the decrease of the number of platelets in idiopathic thrombocytopenic purpura (ITP), an additional qualitative defect has been suspected for a long time. Such a defect is also thought to cause hemorrhagic manifestations in a group of "thrombocytoasthenic" diseases characterized by normal platelet number and apparently normal plasma coagulation factors.

Electron microscopic investigations by appropriate technic have resulted in a better understanding of morphology and functional behavior of normal platelets. Some characteristic features have also been observed in pathologic states. The following results are based on observations in more than sixty normal persons and fifty patients with various hematologic and hemorrhagic diseases. Recent advances in serologic aspects of ITP and the application of new methods in this field influenced these experiments.

Methods

Method 1

Approximately 10 cc. of venous blood were collected directly in a silicone-coated tube, containing small glass slides covered with Formvar membranes (0.1 per cent solution of polyvinyl-formaldehyde in ethylene dichloride). In some experiments 50 units of heparin were added. The temperature was kept at 37 C. After various times (30 seconds to 30 minutes) the slides were withdrawn and briefly dipped several times in Ringer solution at 37 C. to wash off the erythrocytes and plasma proteins; leukocytes and thrombocytes remained on the membrane. For fixation, the slides were then held some 5 to 10 minutes in 1 per cent osmium tetroxide (OsO₄) buffered saline. The Formvar membranes were then washed with distilled water, removed from the glass slides and mounted on copper screens. This method was suitable in case of normal adhesiveness of platelets; for studies of agglutination the following modification was necessary.

Method 2

Suspensions of normal platelets and platelet-free serum to be tested for its agglutinating effect were prepared according to the technic of Stefanini, et al. Blood was collected through silicone-coated needles in silicone-coated test tubes. Sequestrene Na₂ was used as an anticoagulant and Triton WR 1939 was added to prevent clumping of platelets during the different steps of low speed centrifugation for platelet isolation. The platelet-free serum was decalcified with Amberlite IR-100 and deprophosphinized with tricalcium phosphate gel.

Only serum from one case of ITP exhibiting a platelet agglutination titer higher than 1:16 was employed, in order to eliminate the possibility of a nonspecific platelet damaging effect, which may be seen occasionally with undiluted serum. Such a relatively high dilution was also necessary to prevent a massive coagulation of plasma proteins by the following fixation with OsO₄ which would make the preparation unsuitable for electron microscopic observation. Two-tenths cc. of the fresh platelet suspension and 0.2 cc. of the pa-
STRUCTURAL CHANGES IN PLATELETS

Fig. 1.—Normal platelets in contact with a Formvar membrane after 4 minutes. (6500 X.)

Fig. 2.—Thrombocytopenic platelets in contact with a Formvar membrane after 5 minutes. Only a few platelets adhere. Numerous leukocytes on the membrane. (6500 X.)

Fig. 3.—Thrombocytopenic platelet. The granulomere is diffusely distributed in the hyalomere. No pseudopods, no spreading of the hyalomere. (6500 X.) This picture was taken at high tension (100 KV), with a better penetration.

Fig. 4.—Thrombocytopenic platelet showing a single pseudopod. (6500 X.)

tient’s serum, diluted 1:16 with saline, were mixed and very small droplets immediately transferred to copper meshes coated with a parlodion film. A control series was started using platelet suspension and normal serum, diluted 1:16 with saline. The preparations were kept in a wet chamber and incubated for 1 to 15 minutes at 37 C. A small droplet of 1 per cent \( \text{OsO}_4 \) in buffered saline was then added for fixation and the preparations were allowed to dry. The excess of salt was removed by one careful washing with distilled water.

Most preparations were shadowed with palladium. A Philips electron microscope (model 1951) was used.

RESULTS

The normal thrombocyte in a siliconed tube is round or oval with no processes; the granulomere is distributed in the hyalomere. This form also probably obtains
in the normal vascular system, ("circulatory form"). As with most biologic specimens thicker than 0.3 μ, platelets in this state are generally impermeable to electrons and appear as dense disks. When the thrombocyte comes in contact with a wettable supporting surface (a Formvar or parlodion membrane in our case), numerous pseudopods form after a few seconds, and the granulomere coalesces to a pseudonucleus. A few (1 to 4) minutes later the hyalomere spreads into a thin layer (fig. 1). The hyalomere of a very large number of thrombocytes disintegrates shortly before and during the coagulation and the coalesced granulomere forms the retraction centers of the fibrin net. As a consequence, the development of spreading forms is better seen in slightly heparinized or in hemophilic blood.

In normal, hemophilic, and, to a somewhat lesser extent, in slightly heparinized plasma the thrombocytes in the pseudopod or spreading form stick together and form agglutinates of varying size.

In contrast to this physiologic behavior are the observations on platelets in eleven cases of typical chronic ITP and two cases of thrombocytoasthenia. In these conditions, the number of platelets adhering to the Formvar membrane is greatly reduced. This is easily demonstrable in the cases of thrombocytoasthenia with normal platelet count, but can also be shown by comparing the number of adherent platelets in a normal, 10 times diluted blood and the blood of patients with chronic ITP. Whereas normally 30 to 100 platelets can be counted for one

---

*One of these was a case of chronic ITP which, following splenectomy, exhibited over years a platelet count of 300,000 to 500,000/cu. mm. but the same hemorrhagic manifestations as before; the other case was reported elsewhere.*

---

Plate II

Fig. 5.—Extremely large and dense platelet in chronic ITP after splenectomy. Note the defective pseudopods and absence of spreading. (6500 X.)

Fig. 6.—Disintegrating platelet in chronic ITP. Reduced granulomere. The bulbs of hyalomere may be mistaken for pseudopods. (6500 X.)
STRUCTURAL CHANGES IN PLATELETS

Plate III.- Normal Platelets Under the Influence of a Pathologic Agglutinin in the Serum of a Case of Chronic ITP

Fig. 7.—Pseudopod formation and spreading of hyalomere is inhibited. The length of shadows demonstrates the spheroidal shape of these platelets. (2600 X.)

Fig. 8.—Agglutinates are formed. (2600 X.) (Larger agglutinates are difficult to observe, because they break the thin supporting membrane.)

Fig. 9.—The agglutinated platelets often show marked vacuolization. (5200 X.)

leukocyte, this relation is now reversed and more leukocytes than platelets are found (fig. 2).

These adherent platelets also show characteristic pathologic features. In some cases, even after several minutes of contact with the membrane, they remain in their round, circulatory form, the granulomere diffusely distributed in the hyalomere (fig. 3). When this form of paralyzed platelet prevails, the clot retraction is also generally inhibited. In other cases a single and often bizarre pseudopod may be found (fig. 4). Spreading platelets are never seen. Often extremely large forms can be observed (fig. 5). Some platelets also disintegrate on the membrane.
and parts of the hyalomere may be mistaken for pseudopods under the normal microscope (fig. 6).

This aberrant behavior is quite specific for chronic ITP and thrombocytoas-thenias. Somewhat similar forms may however be observed occasionally in acute leukemias and have been interpreted as immature forms.7

Inhibition of spreading and adhesiveness was also observed in one case of cryoglobulinemia, especially at lower temperature (below 35 C.). In this case the adhesiveness and mobility of leukocytes were also strongly inhibited. This indicates a nonspecific action of the cryoglobulin.

From these observations arose the interesting question whether this aberrant behavior in thrombocytopenic diseases was linked to an intrinsic platelet defect or to the action of an extrinsic serum factor. Several attempts to modify the behavior of normal platelets with the serum of patients with chronic ITP or thromboctoassthemia failed. Only with improved methods and a patient* with chronic ITP and relatively high titer of platelet agglutinins (1:32) could this effect be clearly demonstrated.

Figures 7, 8, and 9 show the isolated platelets of a normal individual incubated for 1 to 15 minutes with the 1:16 diluted serum of this patient. The action of the pathologic serum had three distinct components: (1) the normal platelets exposed to the diluted serum showed neither pseudopods nor spreading forms, and they did not adhere to the membrane so that a change to method 2 was necessary; (2) the platelets form agglutinates; (3) degenerative changes in form of vacuolization of the hyalomere occurred.

In control series the platelets were incubated with diluted normal plasma and with saline alone. They showed normal behavior, spreading forms prevailing.

**DISCUSSION**

Formation of pseudopods and subsequent spreading of normal platelets in contact with a wettable surface, as shown repeatedly by electron microscopy were constant and reproducible under the standard conditions in our experiments.

The mechanism of this process is poorly understood. Further studies must concern the influence of physicochemical factors such as ionic strength, osmotic pressure, oxygen tension, etc. There is some evidence that form and length of pseudopods may be slightly influenced by different anticoagulants. Cocaine reportedly prevents the formation of pseudopods.14

None of these factors seems to underly the inhibitions of pseudopod formation and spreading that were encountered in our standardized experiments. These inhibitions were seen with platelets from patients with chronic ITP or thrombocytoassthemia, and repeatedly with normal platelets incubated with 1:16 diluted serum of a patient with chronic ITP and proven platelet agglutinin. As this agglutinin caused both agglutination and inhibition of pseudopod formation and spreading in vitro, it is tempting to speculate that this inhibition could be caused by a weak agglutinin in all cases, even when not detectable by agglutination in

---

*The patient was a 41 year old male with the typical clinical manifestation of a chronic ITP. The onset of the disease occurred eight years ago. Splenectomy was unsuccessful. The injection of 100 cc. of the blood of this patient into a normal subject provoked a marked (80 per cent) thromboctopenia persisting twelve hours.
vitro. However, in two cases of chronic ITP with negative agglutinin test and in the two cases of thrombocytopenia no definite inhibitory action of diluted serum on normal platelets was seen.

Inhibition of pseudopod formation and spreading are probably rather non-specific phenomena and may be caused by different exogenous platelet-damaging agents, as platelet agglutinins; in other cases it may be due to an inherent defect in platelets, as in thrombocytopenias. It may also be that pathologic and giant platelets (fig. 5), probably released from immature megakaryocytes, are incapable of normal pseudopod formation and spreading. Recently a very similar behavior of cells in tissue culture under the influence of specific antibodies has been described.

The physiologic significance of these phenomena is of interest. Lack of pseudopod formation and spreading were generally associated with strongly diminished adhesiveness of the platelets to the wettable membrane. A close relationship therefore appears probable. In terms of hemostasis, adhesiveness of platelets with secondary agglutination in situ may help shorten bleeding time. It seems to be absent in chronic ITP and thrombocytopenia.

However, it is important to point out that adhesiveness and agglutination, or at least pathologic agglutination, are not necessarily identical. This is clearly shown by the agglutination of normal platelets by serum from a chronic ITP where at the same time pseudopod formation and spreading were inhibited. This dissociation of adhesiveness and agglutination seems to be a characteristic feature of platelets, at least in those cases of chronic ITP where an agglutinin can be detected.

**Summary**

Deficient pseudopod formation and spreading of platelets on Formvar films were encountered in cases of chronic idiopathic thrombocytopenic purpura and thrombocytopenias. The same defect together with agglutination was induced in isolated normal platelets by a platelet agglutinin from the serum of one case of chronic ITP. The possible significance of these results is briefly discussed.

**Summario in Interlingua**

Per microscopia electronic il esseva constatat-ta que thrombocytos ab patientes con chronic idiopathic purpura thrombocytopenic o con thrombocytopenias devia specificamente del reaction de thrombocytos ab patientes normal quando illos es ponite in contacto con un superficie permittente lor adhesion. In thrombocytos normal le formation de pseudopodios e le diffusion del hyalomero in un tenue strato es disveloppamentos caracteristic. In le casos hic presentate le formation de pseudopodios esseva deficiente e le diffusion del hyalomero non se presentava. Le mesme defecto (insimul con agglutination) esseva produciti in isolate thrombocytos normal per un agglutinin thrombocyte prendite ex le sero de un paciente con chronic idiopathic purpura thrombocytic. Le possibile signification de iste resultatos es discutite.

**REFERENCES**


Structural Changes in the Platelets as Observed by Electron Microscopy

H. BRAUNSTEINER, K. FELLINGER and F. PAKESCH