Henry Ford Hospital Medical Journal

Volume 27
Number 3 *John W. Rebuck Testimonial Issue*

Article 10

9-1979

Contributions of Electron Microscopy to the Study of Platelets

J. W. Rebuck

Jeanne M. Riddle

S. A. Johnson

R. W. Monto

Ruth M. Sturrock

Henry Ford Hosp Med Journal
Vol 27, No 3, 1979

Contributions of Electron Microscopy to the Study of Platelets†

J. W. Rebuck, MD, PhD,* Jeanne M. Riddle, PhD,* S. A. Johnson, PhD,* R. W. Monto, MD, and Ruth M. Sturrock***

Introduction

In 1939, the work of Wolpers and Ruska (1) launched the study of platelet ultrastructure and at once made far-reaching contributions to this subject. Immediate osmium fixation revealed that the normal platelet was free of processes and electron opaque. After a 12-minute sojourn in citrated plasma, long and threadlike protoplasmic processes appeared, some of which possessed knobbed tips. Fifty-four hours after the blood had been taken, an extensive protoplasm possessed plump, lobular processes. Partial fixation unmasked a chromomere with more than 40 osmiophilic granules of varying size, the granulomere. Loosening of the protoplasm by addition of distilled water revealed a framework of protoplasmic threads 25 to 30 mμ in width. Vacuole formation was depicted adjacent to the chromomere. Hypotonicity led to the formation of a single, extremely long, pointed process. In the formation of the fibrin clot, the fibrin was attached to the granulomere while only a remnant of the protoplasm remained. Almost a decade elapsed before these findings were confirmed and amplified by the work of Bessis and his associates (2-6) in France. To a lesser extent electron microscopy of platelets has also been carried on in our laboratories (7).

Bessis and his group (2-6) depicted successive stages of platelet change, labelling them round or discoidal, dendritic, transitional and spread, according to their shapes. In the latter stages, shadowing technique revealed that the hyaloplasm contained fibrils, themselves composed of chains of small granules; some were radiating fibrils connecting the opaque center to the outer rim; some were circular or arc-like; others were irregularly arranged. Mechanical destruction led to a microsomal residue. Retraction of the hyalomere occurred after platelets were kept at 4°C (6). Gurevitch and his fellow workers (8) confirmed the single, sword-like protrusion of the platelet effected by an increasingly hypotonic saline environment, and found the inner structure of the sword to be made up of small, dense granules. Braunsteiner and Febvre (9) and Braunsteiner (10) prepared a platelet microsome fraction by ultracentrifugation. The microsome ranges from 60 to 200 mμ in diameter and possesses "thrombokinase" activity.

Bessis and Burstein (6) had reported coalescence of spread platelets as did Braunsteiner (10), who unfortunately early labelled such aggregates "agglutinates," although no antibody was concerned in the process. Braunsteiner noted that shortly after aggregation of the platelets, hyaloplasmic dissolution set in, at first in large round fragments which later broke down completely or remained as small granules 60 to 150 A in diameter. De Robertis, Paseyro and Reissig (11) also observed that in a normal clotting process, certain platelets, which were closely bound to the fibrin, underwent disintegration, while others which had no connection with the network remained intact. They were able to reproduce this phenomenon of platelet disintegration by the addition of purified thrombin in amounts as low as 2.5 units per ml even when after washing out the fibrinogen, there was no formation of a fibrin network. All stages except some of the extended type showed disintegration from the periphery to the center. The hyalomere gave rise to long, fibrillar elements formed of rows of microvesicles linked together. The fibrils broke up into microvesicles while simultaneously the granulomere was reduced to a round, opaque amorphous mass. Bloom (12, 13) also studied hyaloplasmic disintegration in heparinized blood after the platelet chromomere was covered with fine quartz particles. Between the hyaloplasmic strands, and apparently budding out from them, large numbers of granules 100 to 250 mμ in diameter appeared. The chromomere fused into a so-called pseudonucleus but usually did not disintegrate. In some instances, however, the chromomere disappeared without other platelet change. In the disintegrating hyalomere a ground structure was revealed with rays of hyaloplasmic substance of a fibrillary appearance arranged in such a way as to indicate that they served as the skeleton of the pseudopodia-like protrusions typical of normal dendritic-stage platelets. Braunsteiner (14) noted in addition that exposure of platelets to thrombin caused marked aggregation and destruction of the platelets with fibrin formation. Hutter (15), in doing differential counts, found no significant difference between platelets exposed to crude thrombin and those in controls. Lysed forms comprised less than 1% of the platelets counted.

Study of chromomeric granulations in intact platelets has not been entirely unrewarding. Bloom (12, 13) found 50 to 100 granules in the chromomere. At times chromomeric granular release in toto

---

† This article has been reprinted from the Henry Ford Hospital Medical Bulletin 1960;8:273-92, copyright 1959.

Supported in part by research grant H-3636(R-1) from the National Heart Institute, U. S. Public Health Service

* Department of Laboratories, Henry Ford Hospital

** Division of Hematology, Henry Ford Hospital

was observed. In disintegrating platelets, where individual granules could be studied, a surrounding coating was seen. Studies of electron microscopic sections of platelets by Rinehart (16) revealed a background cytoplasmic matrix containing poorly defined gray densities as well as aggregates of small vesicles and short-paired lamellae which suggested the equivalent of a Golgi apparatus. Of greater importance was the advance made in our knowledge of chromomeric ultrastructure. The most numerous granules were round or ovoid in structure and of considerable electron density. A few exhibited internal cristae indicative of mitochondria; a few granules were empty. Section studies by Bernhard and Leplus (17) and Watanabe (18) confirmed the mitochondrial structures and emphasized the presence of forms intermediate between the mitochondria and the dense granules. Feissley and his associates (19) noted clear granules in addition to the dense granules and mitochondria in normal platelets. Clear, dense and mitochondrial granules were also apparent in the micrographs of Aleksandrowicz and his group (20), as was endoplasmic reticulum (microvesicles and canaliculi) of Palade. Schulz, Jürgens and Hiepler (21) distinguished a platelet membrane 60 A thick and plain in contour. These workers described and depicted still another type of chromomeric granule, granulomere delta, which they interpret as a siderosome. The interior of these granules is clear but their margins show many contrasting granules 55 A in size probably representing the iron component of ferritin. They also confirmed the presence of the common oval granules with a dense homogeneous ground substance measuring 0.195 x 0.12 μ with an external membrane 50 A thick; these are called granulomere alpha. The mitochondria, which are called granulomere beta, are much smaller than those in many other cells measuring only 0.16 to 0.22 μ and frequently only one or two cristae are cut. The external mitochondrial membrane appears to be simple and is 55 A in thickness; the internal membranes have an osmiophilic layer of 55 A and an osmiophobic interval of 50 A. They found microvesicles 240 to 440 A in size and tubuli forming the endoplasmic tubular system previously observed but designated by them as granulomere gamma. Johnson and her group (22) obtained a granule-rich sediment from frozen and thawed normal platelet extracts treated extensively in a sonic oscillator and ultracentrifuged at 10,000 g for 10 minutes. Under the electron microscope, the granules so obtained were about 350 m μ in diameter. The platelet factor 3 activity was in this suspension. Smears of this active preparation stained by Leishman's stain and viewed under the light microscope showed the same azurophilic properties as the chromomeres of intact platelets. These isolated active platelet granules under further treatment in the sonic oscillator were replaced by increasing numbers of small, submicroscopic particles. With the disappearance of the granules proper, platelet factor 3 activity also disappeared.* The mitochondrial origin of the common dense granules has been proposed by several workers (16, 17, 18, 20).

Schulz, Jürgens and Hiepler (21) agree with most investigators that the pseudopodia extend from the peripheral hyalomere and do not contain granulomere. Kautz and De Marsh, (24) however, noted a small particulate component located at the base of the processes in several platelets. Bessis (4) described the dendritic processes adherent to the slide and at that point a terminal enlargement developed. Haydon (25) reported that the rounded or clubbed ends appeared to be a stickier portion of the platelet surface. Rinehart (16) noted that when the process of aggregation had been initiated, the projecting pseudopodia abutted upon those of neighboring platelets and the platelets were drawn together finally comprising a compact mosaic of interlocked units. Aleksandrowicz and fellow workers (20) further depicted disappearance of limiting membranes and confluence of adjacent platelets as aggregation progressed. Next, there was elongation of both dense and mitochondrial granules (21) and finally the granules largely disappeared from the center of the platelet thrombus (20).

Vacuolar structures in normal platelets described originally by Wolpers and Ruska (1) have been studied by Bessis (26), Bloom (12, 13), and Goodman and his associates (27). Bessis (26) described them as hyaloplasmic structures. Bloom (12, 13) perhaps related them to the clear granulations of sections, noting that when granules from the chromomere had been released from the platelet in some way, a number of small, empty round spaces were left. Goodman, et al (27) observed them in sectioned platelets as numerous vacuoles or droplets of very low staining material. Aleksandrowicz and his group (20), too, observed in some platelets an extreme vacuolization in the course of the functional transformation.

Beginning with Wolpers and Ruska's (1) classic observation of the close attachment of fibrin to the platelet granulomere, fibrin-platelet relationships have been the subject of controversial studies. Braunsteiner (10) early depicted the platelets as both the support and the retraction center of the fibrin net. Haydon (25) observed fibrin formation apart from the platelets and believed that fibrin and platelets stick together because of their mutual stickiness. In Hutter's (15) preparations, integration of fibrin into the substance of the platelet was not observed. It should be recalled that De Robertis and his group (11) had depicted fibrin fibers converging on the platelet, and that those platelets so intimately bound to the fibrin underwent disintegration in preference to those which had no connection with the network and remained intact. The most detailed micrographs of this relationship have been supplied by Bloom (12, 13) which depict platelets as the supporting center for the fibrin network. From the hyalomeres of still other structurally intact platelets fibrils were found interlacing with the fibrin surrounding the platelets. Finally, thick fibers of fibrin linked the chromomeres of platelets attached to the network of the clot.

It is the purpose of this report to relate the known but complex ultrastructure of the platelet to ultrastructural changes in a series of platelet diseases studied by correlated electron microscopic, physiological and clinical methods.

* Thus the platelet factor 3 activity appears to be associated with the intact large granules as originally suggested by Fonio (23).

Materials and Methods

The platelets which served as the basis of the electron microscopy in this study were obtained from 27 patients and eight normal individuals. Control platelets were obtained from seven normal volunteers; the stored platelets were obtained from a normal Group O, Rh negative professional donor. The abnormal platelets were obtained from eight patients with uncomplicated thrombocytopathy A, from two patients with thrombocytopathy complicated by AHG deficiency, from seven patients with pernicious anemia in relapse, from three patients with idiopathic thrombocytopenic purpura, from four patients with uremia, from two patients with acute granulocytic leukemia and from one patient with a demonstrable deficiency of platelet fibrinogenlike factor. Correlative coagulation studies were performed by one of us (S.A.J.). Pertinent summaries will be reported in the next section. For further methods and details relating to these studies the reader is referred to the appropriate previously published studies (22,28,29).

Basic method for electron microscopy of platelets

Fifteen ml of peripheral blood were obtained by venipuncture using a sterile, siliconized 19 gauge needle and a siliconized 20 ml syringe. The blood specimen was immediately placed in a siliconized 6 oz rectangular vessel which contained 1 ml of liquid heparin (Liquaemin, 1,000 USP units per ml). After the blood and anticoagulant were gently mixed, a slide previously coated with a 1% solution of formvar was introduced and the slide was covered with the blood specimen. The vessel containing the slide was placed horizontally into a 37°C constant temperature oven and a stopwatch was started. This system was incubated at 37°C for eight minutes. The slide was then withdrawn and washed briefly in Tyrode's solution heated to 37°C. The slide was next placed into 1% buffered osmium tetroxide and allowed to fix for 15 minutes at room temperature. After fixation, the slide was thoroughly washed with distilled water at room temperature and allowed to air-dry. This method of preparation was originally suggested by Braunsteiner and his associates (30,31). The final specimen mounting was easily achieved in the manner reported by us previously (32,33). The slide was scraped with a sharp scalpel at both ends and at each side to remove the thickened portion of the formvar film, thus freeing the film from the glass slide. Representative areas on the film were selected by use of the phase microscope and the specimen screens were placed over the desired regions. After exhaling lightly onto the film's surface, the entire formvar film, including the specimen screens, was smoothly covered with Scotch tape. This tape was pressed firmly against the slide and each specimen screen. When the Scotch tape was removed, the now adherent formvar film with interposed specimen screens was also removed. A careful dissection of the formvar film about the screen's circumference and removal of the screen from the tape allowed the specimen trapped between the specimen screen and formvar film to be studied.

Method for the electron microscopic examination of platelet agglutination

(Modified from the light microscopic methods of Tullis (34,35). To a small siliconized tube, labelled C, 0.15 ml of treated control serum and 0.05 ml of diluted complement (1:32 dilution with saline) were added. To another small siliconized tube, labelled P, 0.15 ml of treated patient serum and 0.05 ml of diluted complement were added. Both the control serum (O, Rh negative donor) and patient's serum were treated by allowing them to drop through an Amberlite XE-128 resin at the rate of one drop every five seconds. This procedure removed calcium and inactivated the plasma thromboplastin component, serum prothrombin conversion accelerator, and residual prothrombin, thereby preventing their interference with platelet agglutination. To each of the two tubes, 1 ml of concentrated, preserved platelets was added. The tubes C and P were incubated in a 37°C constant temperature waterbath for 90 minutes. Each specimen was washed twice using 4 ml of room temperature saline for each washing. The washed control and patient's specimens were then resuspended in 0.5 ml of room temperature saline and by means of siliconized pipettes transferred in drop-wise fashion onto two slides previously coated with formvar films. Fixation was achieved by exposure to osmium vapors for 15 minutes. The specimens were next thoroughly washed in distilled water and allowed to air-dry. They were mounted for electron microscopy as in the basic procedure above.

Method for the examination of stored platelets in the electron microscope

A tube of preserved platelets prepared for use in the platelet agglutination test, as described by Tullis (34,35), was removed from the refrigerator and allowed to reach room temperature. This standard platelet suspension was dispensed in 1 ml quantities into a clean, sterile, siliconized Kahn tube. The preserving media contained glucose, sodium chloride, sodium acetate, sterile gelatin, and sterile water. The platelets were washed twice using 4 ml of room temperature saline for each washing. The washed platelets were then resuspended in 0.5 ml of room temperature saline and by means of siliconized capillary pipette they were placed in drop-wise fashion onto a slide previously coated with a formvar film. The slide was lightly centrifuged to throw the platelets down onto the formvar film. Fixation was achieved by exposure to osmium vapors for 15 minutes. The specimen was next thoroughly washed in distilled water and allowed to air-dry. Screens were mounted for electron microscopy as in the basic procedure above.

Method for the examination of a normal control without the introduction of an anticoagulant

Twenty ml of peripheral blood were obtained by venipuncture using a siliconized 20 ml syringe and a siliconized 19 gauge needle. The specimen was immediately placed into a siliconized vessel which contained three slides previously coated with a 1% solution of formvar and the slides were covered with the blood specimen. A stopwatch was started and the system was placed into a 37°C constant temperature oven. The slides were withdrawn at varying intervals — the first at three minutes, the second at four minutes, and the third at five minutes. As each slide was removed from the blood specimen, it was thoroughly washed in Tyrode's solution. All three slides were fixed in a 1% solution of buffered osmium tetraoxide for 15 minutes at room temperature. The specimens were washed in distilled water and allowed to air-dry. They were counted for electron microscopy as in the basic procedure above.

Results

Control preparation

Heparinized preparations from normal controls fixed in osmic acid eight minutes after withdrawal presented four classical stages of platelet change (Figs. 2-4). The circulating round or oval form is smallest in diameter and largely electron opaque (Fig. 1*). The next, the dendritic stage (Fig. 2), is the extrusion of at first a few, then many hyaloplasmic processes leaving a dense osmiophilic center which corresponds to the chromomere of light microscopy. The third transposition (Fig. 3) of the shed platelet, the intermediate stage, is marked by hyaloplasmic webbing between the dendritic processes and broadening of the pseudopodial bases. At the same time there is noted peripheral spread of the central chromomeric substances. This latter process is just under way in Fig. 3; in other control intermediate forms there was considerable loss of osmiophilic chromomere at this stage. Further expansion of the platelet hyalomere leads to the spread form (Fig. 4). At this stage normally there has been considerable leaching out of the chromomeric substance although some osmiophilic substance remains, often in an eccentric position. Leaching out of chromomeric material may be accompanied by considerable vacuolar formation in its vicinity. Loss of the central osmiophilic granular material was even more pronounced when intermediate and spread platelet forms were observed in native unheparinized preparations at the fifth minute in their formation of a fibrin clot.

Hyalomeric disintegration was not observed in control or abnormal, heparinized or native platelet preparations that we have studied, with two important exceptions. Addition of two and twenty units per ml of bovine thrombin, purified from the Parke-Davis material, resulted in platelet hyalomic dissolution as originally described by De Robertis and his associates (11). Platelets stored for a year by the method of Tullis (34,35) also showed considerable hyaloplasmic distortion (Fig. 16).

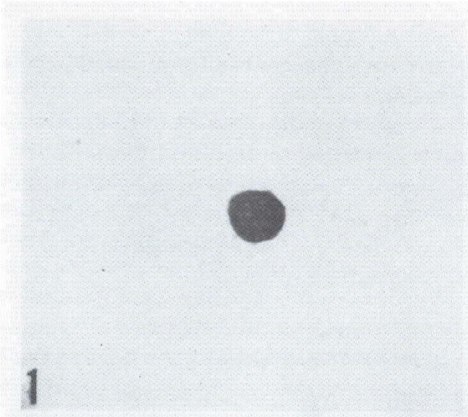

Figure 1. Circulating or round form. Heparinized fixation OSO4 eight minutes after venipuncture. X5000.

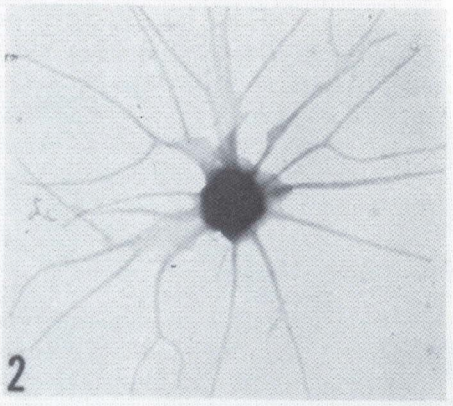

Figure 2. Dendritic form, control. Treatment as in figure 1. X5000.

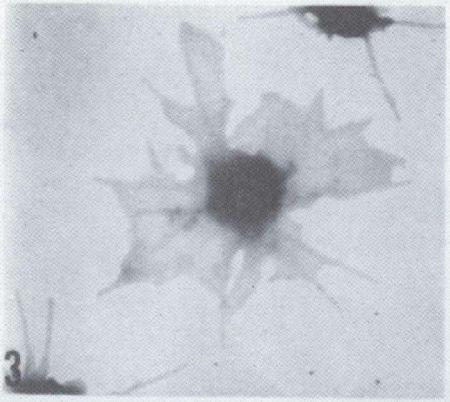

Figure 3. Intermediate form, control. Treatment as in figure 1. X5000.

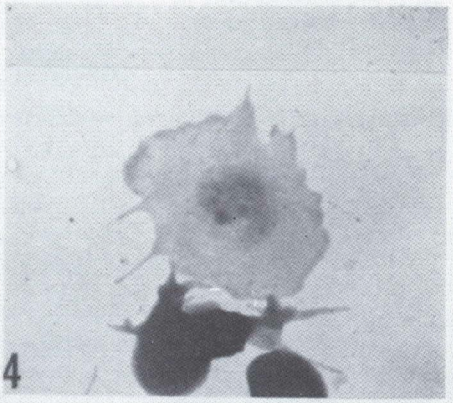

Figure 4. Control spread form. Treatment as in figure 1. X5000.

* This platelet, structurally identical to control forms of this stage, was obtained from the more abundant representatives of this stage found in acute granulocytic leukemia.

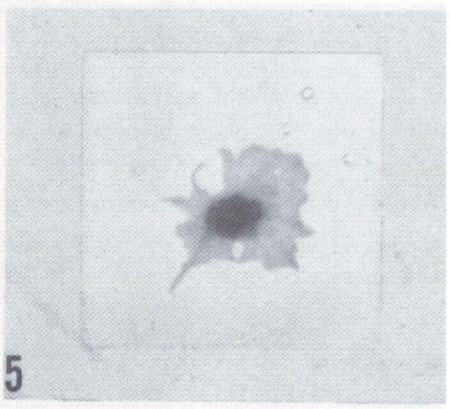

Figure 5. Intermediate, thrombocytopathy A. Treatment as in figure 1. X5000.

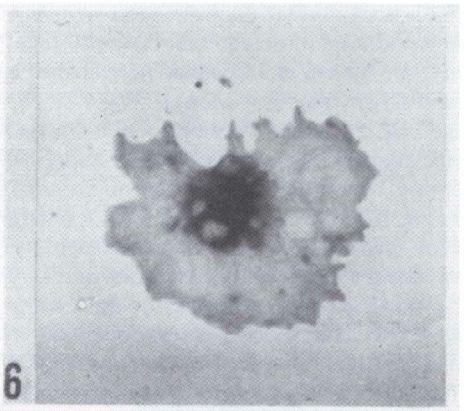

Figure 6. Spread, thrombocytopathy A. Treatment as in figure 1. X5000.

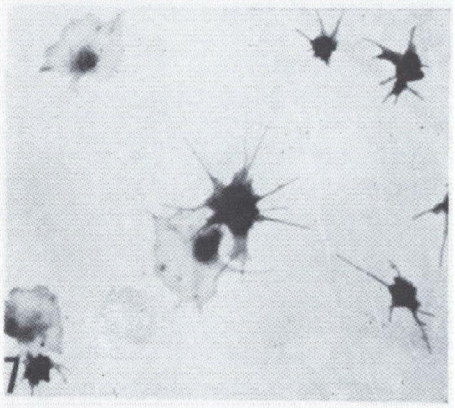

Figure 7. Dendritics and intermediates. Treatment as in figure 1. X2000.

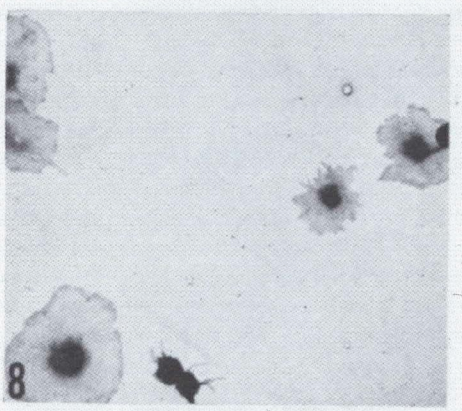

Figure 8. Thrombocytopathy A, predominance of intermediates and spreads. Treatment as in figure 1. X2000.

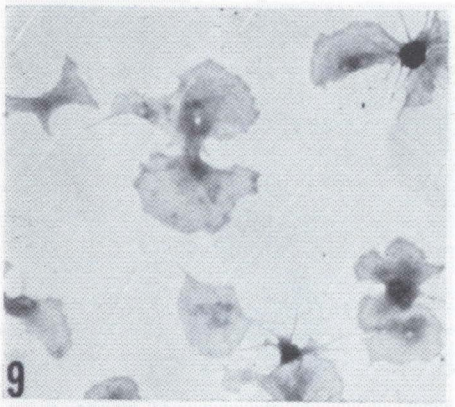

Figure 9. Thrombocytopathy, predominance of early spread-aggregates (VMs). Treatment as in figure 1.

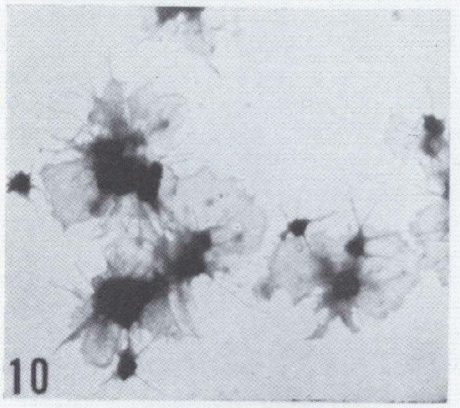

Figure 10. Thrombocytopathy, predominance of spread-aggregates (VMs). Treatment as in figure 1. X2000.

Differential platelet counts under the electron microscope, the time of fixation remaining constant at eight minutes, revealed striking differences between transpositional stages of control in contrast to abnormal platelets. Differential platelet counts in seven normals revealed 0.5% at the round stage with a range of 0 to 1%; 87.58% at the dendritic stage with a range of 82 to 94%; 4.14% were intermediate forms with a range of 1 to 8%; and 7.71% were expanded or spread forms with a range of 3 to 11%.

Aggregation of platelets leading to early viscous metamorphosis (VMs) was not lacking in controls. Such aggregation of the predominant dendritic forms was accomplished by fusion of their chromomeres to form a central osmiophilic mass and by fusion of their hyalomeres which retain their peripheral pseudopodial process (Fig. 14). However, only four early VMs were normally present per 100 platelets counted.

Thrombocytopathy

This mild-to-moderate hemorrhagic disease (29,36), occurring in either sex and showing a familial tendency, is characterized by only one abnormal clinical test, a prolonged bleeding time. Laboratory tests reveal a paradoxically normal platelet count but the blood from these patients forms serum which supports poor prothrombin consumption although there is little or no actual prothrombin in this serum as measured by the two-stage prothrombin determination. The platelets from these patients afford poor platelet thromboplastin generation. These platelets contain normal amounts of platelet factor 3 which, unlike that of normal platelets, is not liberated by freezing and thawing the platelets once, but is liberated by sonic oscillations (29) or by extraction with distilled water (37). The thrombocytopathy of at least some of the Aland islanders (v. Willebrand-Jürgens) is apparently complicated by an additional partial deficiency of AHG (21,38).

Heparinized preparations from patients with thrombocytopathy were fixed in osmic acid eight minutes after withdrawal as were the control preparations. In controls the largest number of platelets were in the circulating and dendritic stages, while in thrombocytopathy patients, although the classical stages of platelets were all present, the largest numbers were in the intermediate (Fig. 5) and spread stages (Fig. 6). This latter platelet presents radial hyaloplasmic fibrils. Furthermore, there was retention of much of the chromomere substance through the spread stage far into the stage of viscous metamorphosis (Figs. 8 and 10).

Differential platelet counts under the electron microscope in eight patients with uncomplicated thrombocytopathy, fixation again being eight minutes after withdrawal, revealed 0.8% at the round stage with a range of 0-3%, and only 28.4% at the dendritic stage with a range of 8-55%. The intermediate and spread forms (Fig. 8), on the other hand, showed steep increases over the controls (Fig. 7). Intermediates accounted for 28.4% of all the abnormal platelets with a range of 9-57%, and spread forms 42.4% with a range of 34-67%. Another deviation from the normal was the marked tendency for the thrombocytopathic forms to aggregate their chromomeres and hyalomeres first in twos, "Butterfly forms" (Fig. 9), then into larger and larger aggregates of later viscous metamorphosis (Fig. 10). For this reason, 43.2 VMs were observed per 100 platelets counted.

Platelets from two cases of thrombocytopathy complicated by AHG deficiency were studied by us under the electron microscope. In neither were round forms visible at fixation after eight minutes. Dendritic, intermediate, and spread forms were 33%, 25%, and 42%, respectively, in the first, and 18%, 10%, and 72% in the second. VMs were 14/100 platelets counted in our first case and 16/100 in the second. This striking increase is spread forms resembled severe uncomplicated thrombocytopathy. In one of the complicated cases, however, the numerous spread forms contained diminished osmiophilic substance when compared to uncomplicated thrombocytopathic spread forms.

Shadow-casting

Figure 11 depicts dendritic, intermediate and spread forms shadow-cast with chromium at an angle of 25°. This three-dimensional study confirms the gradual depletion of chromomeric substance as the dendritic forms, with very long chromomeric shadows, give way to intermediate and spread forms with little or no central shadows.

Idiopathic thrombocytopenic purpura (ITP)

Direct electron microscopy of platelets from three patients with idiopathic circulating antiplatelet antibodies revealed qualitative changes as well as the established quantitative depletion. Differential platelet counts under the electron microscope reading: round 4% (range 0-11%); dendritic 33.6% (range 16-78%); intermediate 10% (range 2-14%); and spread forms 52.4% (range 15-71%). These differentials were intermediate between those of controls and those of thrombocytopathic preparations. Study of dendritic formations revealed malformed, abortive pseudopodial processes (Figs. 12 and 13). In Fig. 12 the scant hyalomere presents a serrated, gearlike border on which the antiplatelet antibody appears to have brought up small but distinct plateaus at the affected sites similar to those produced by known antierythrocytic antibodies on the sensitized erythrocytic surface (39). This antibody effect leads to shortened, deficient pseudopodia (Fig. 13) as found in this untreated disease. Some agranular platelets were noted among the dendritic forms. Although the impression was gained that platelet agglutinates were bound by their hyalomeres in contrast to the aggregation and fusion of both remaining chromomeric material and hyalomeres in viscous metamorphosis (Fig. 10), because of the difficulty in distinguishing platelet aggregates (VMs) from platelet agglutinates resulting from antibody activity, the studies reported in the second and third sections below were undertaken. Ablation of the antiplatelet antibody-forming cells through splenectomy may restore the platelets of such patients to approximately normal numbers, structure, and function (Figs. 14, 20).

Pernicious anemia

In seven patients suffering with true pernicious anemia in relapse, differential platelet counts under the electron microscope revealed 15% round forms (range 0-52%); 60% dendritic forms (range 42-76%); 9% intermediates (range 0-12%); and 16% spread forms (range 0-48%). 8.2 VMs were observed per 100 platelets counted. In this condition, too, there was defective, sparse, and blunted pseudopodial formation at the dendritic stage (Fig. 15). The platelets themselves were smaller than normal. Coagulation studies were obtained in six of the seven patients in

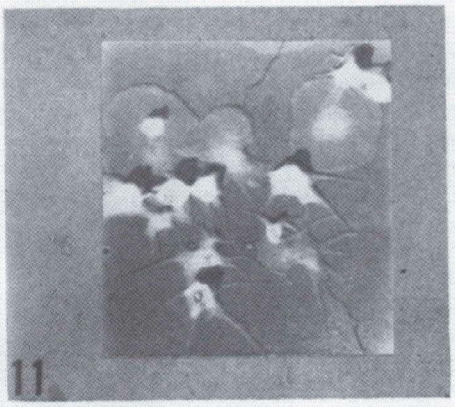

Figure 11. Control platelet forms shadow-cast. Eight-minute stage. X2000.

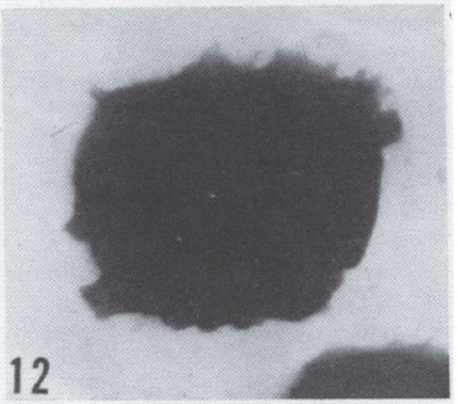

Figure 12. Serrated border produced by antibodies in ITP. Treatment as in figure 1. X13,200.

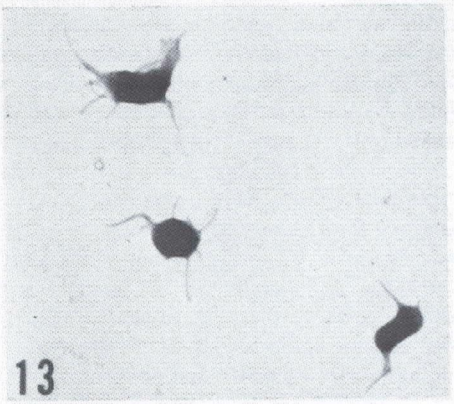

Figure 13. Defective pseudopodial formation in ITP. Treatment as in figure 1. X2000.

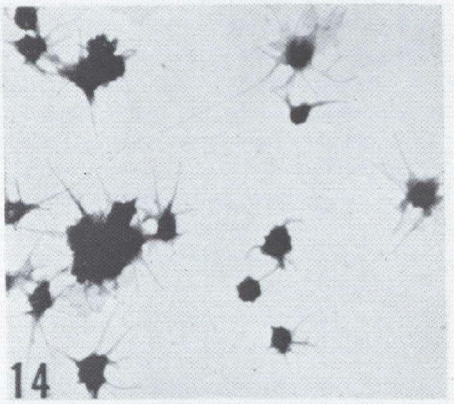

Figure 14. ITP, almost complete return to normal after splenectomy. Treatment as in figure 1. X2000.

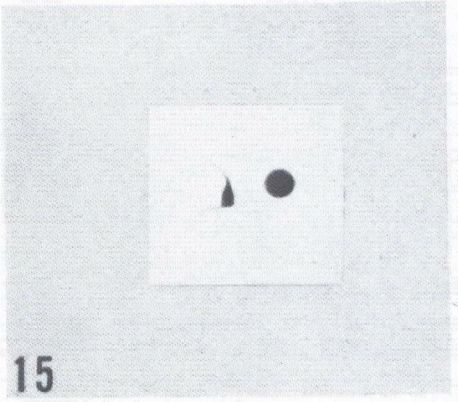

Figure 15. Small platelets with defective pseudopodial formation in P.A. Treatment as in figure 1. X2000.

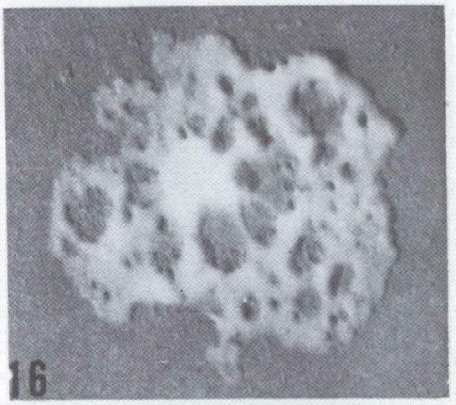

Figure 16. Platelet stored one year. Shadow-cast. X16,000.

this group. In all, the prothrombin consumption was poor. In two of the six, the poor prothrombin consumption could be accounted for on the basis of decreased platelet numbers (50,000/cmm or less). In the remainder, the poor prothrombin consumption could not be accounted for solely on the basis of platelet numbers although there was moderate depression of the platelet count (100,000 to 125,000/cmm).

Stored platelets

Test platelets from Group O, Rh negative donors were stored according to the method of Tullis (34,35) and were employed as platelet antigens for the platelet-antibody tests described below. Fig. 16 depicts such a test platelet removed after a year's storage and shadow-cast with chromium. The platelet is small, has retained only a portion of its central chromomere, and shows an extended, vacuolated hyaloplasm. Such platelets retained only 25% of their platelet factor 3 activity when tested functionally but they were antigenically active in platelet-agglutinin studies.

Platelet-antibody relationships

Platelets (from Group O, Rh negative donors) stored for five weeks according to the method of Tullis (34,35) served as platelet procedures of the same author. A positive test with serum from a patient with ITP was obtained and the agglutinates were studied under the electron microscope (Fig. 17). Note the delicate strands, presumably hyaloplasm and antibody, connecting the three major agglutinated platelet masses. Note, too, their distinction from early viscous metamorphosis (Figs. 9, 10, 14). Further insight into platelet-antibody relationship was gained by analysis of the agglutinates produced by exposure of control platelets to macroglobulins (Waldenström) followed by a second exposure of the same platelets to rabbit antimacroblobulin serum.* Adherence of respective platelet processes characterizes such agglutinates (Fig. 18) and distinguishes them from viscous metamorphosis (Figs. 9, 10, 14, 22).

Platelet-fibrin relationships

In heparinized preparations with slowing of fibrin deposition, platelet-fibrin relationships are relatively uncomplicated. In Fig. 14, eight minutes after venipuncture, a simple, long strand connects the processes of two widely separated platelets. In Fig. 19, 15 minutes after venipuncture, shadowcasting reveals one broad process of the central, expanded platelet in contact with the nearest fibrin strand and further protrusion of a fingerlike extension of the same pseudopod to the second nearest fibrin strand; a second footlike process nearby has also contacted the first fibrin strand. As a result, the nearest fibrin strand forms a loop about the expanded platelet.

In native, unheparinzed preparations from a patient with ITP restored to normal by splenectomy, the classical fibrin clot is observed in Fig. 20. It consists of intertwined fine and coarse fibrin threads, both extrathrombocytic and radiating from platelet aggregates which are composed predominantly of massed and osmiophilic material and of lesser amounts of peripheral hyaloplasm. Evidence that platelet aggregates rather than individual platelets serve as these major foci of platelet-fibrin interplay can be seen in the early, fibrin-poor clot depicted in Fig. 21. This native, unheparinized preparation was obtained from a patient whose platelets had demonstrable functional deficiency of platelet fibrinogen-like or clottable factor, although his plasma fibrinogen levels were normal. In this inhibited version of fibrin-clot formation, the individual platelets are aggregating and fusing to form the major foci.

Platelets in uremia

Platelets in uremia have been shown to be deficient in platelet factor 3 activity (28). Furthermore, this deficiency was not restored by exposure of the affected platelets to sonic oscillations. Differential counts in four such patients revealed 8.25% round forms; 69.5% dendritic forms; 7.25% intermediates; and 15% spread forms. These were similar to normal differential counts. Study of a rare viscous metamorphosis accomplished at eight minutes by fusion of four spread forms and one dendritic form in uremia (Fig. 22) reveals aggregation of each of their centrally situated osmiophilic areas. The density of the massed chromomeres is noteworthy when one considers the deficient platelet factor 3 activity they manifest. Aggregation of extended forms here in contrast to control aggregation at the dendritic stage (Fig. 14) recalls similar aggregation of extended forms in thrombocytopathy (Figs. 9, 10). Arcuate fibrils can be made out in the hyaloplasm of one of the component spread forms in Fig. 22.

Platelets in acute granulocytic leukemia

In the two cases studied, there was a retardation of spreading noted at eight minutes. Round forms (Fig. 1) were more numerous than in controls or in the other conditions described above. Differentials in the two cases revealed, respectively, round forms 21.5 and 27%; dendritics 76 and 68%; intermediates 2 and 0%; and spread forms 0.5 and 5%.

Discussion

In 1953, Braunsteiner and his associates (30) described two cases of thrombasthenia characterized by a history of bleeding from the mucous membranes. Clotting and prothrombin times were normal, but the bleeding times were prolonged. In the first case clot retraction was poor, in the second, the prothrombin consumption was positive. Of importance for this study was their use of the electron microscope to demonstrate an ultrastructural platelet defect, in each case marked by lack of pseudopodia formation. Exposure of normal platelets to the patient's plasma did not affect their customary transformations and, conversely, exposure of the defective platelets to normal plasma did not restore them to proper spreading. The following year, Alatas and Ulutin (40) published a confirmatory case in which electron microscopy again yielded poor pseudopodial formation and absence of platelet spreading. In 1956, Braunsteiner and Pakesch (36) added three additional cases, redefining this important group of qualitative platelet diseases as thrombocytoasthenia, a designation we deem advisable to adopt. Thrombocytoasthenia presents a uniform clinical picture with a tendency to bleeding from the mucous membranes, normal platelet numbers, normal coagulation factors, and lacking circulating anticoagulants and platelet agglutinins. Clot retraction shows manifest or latent disturbance.

* These studies were performed in collaboration with Dr. R. Pachter and T. R. Neblett and Miss J. Caldwell.

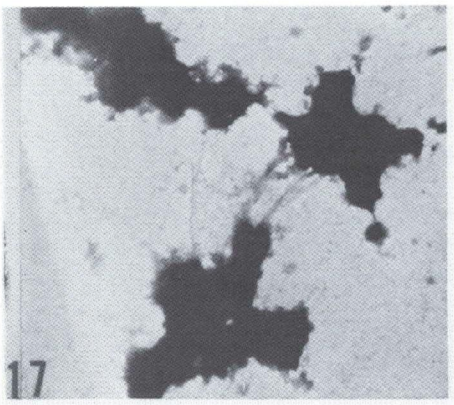

Figure 17. Positive agglutination test. Heparinized. X5000.

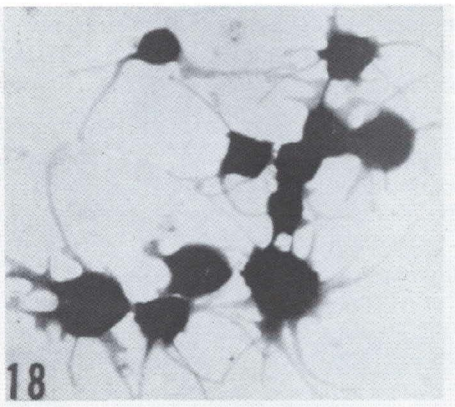

Figure 18. Positive agglutination test. Heparinized. X5000.

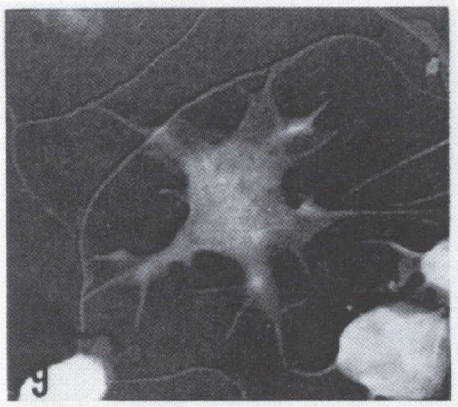

Figure 19. Adherenece of spread form to fibrin strands. Shadow-cast. Heparinized. X5000.

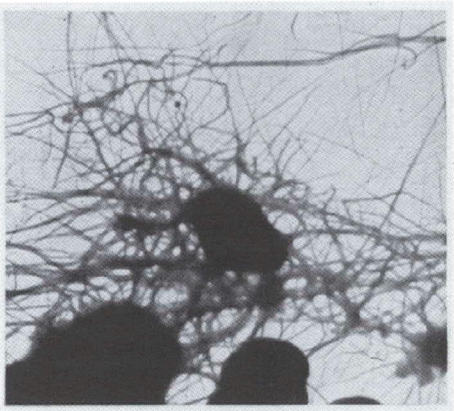

Figure 20. Normal fibrin clot. Eight minutes after venipuncture. No heparin. X2000.

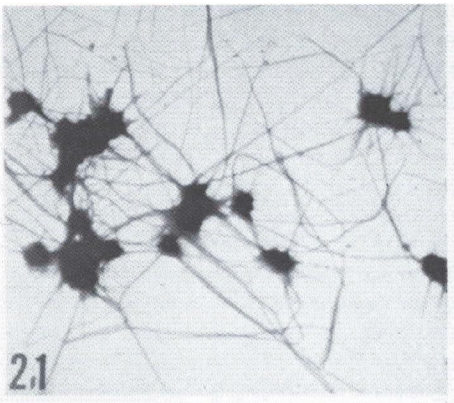

Figure 21. Defective fibrin clot. Four minutes after venipuncture. No heparin. X2000.

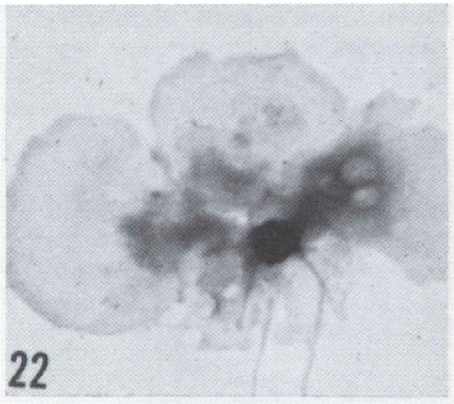

Figure 22. Rare VMs in uremia. Same treatment as in figure 1. X5000.

Electron microscopic examination reveals a severe defect of pseudopodial formation and a lack of platelet spreading. Almost as important a contribution was their delineation, in which we concur, of pseudopodial formation and spreading (Figs. 2-4) as structural manifestations of the adhesive property of platelets. Aggregation or cohesion, then, is the ability of platelets to fuse with one another, viscous metamorphosis (Figs. 9, 10, 14, 22), and is to be distinguished from agglutination, a serologic phenomenon effected by antiplatelet antibodies (Fig. 17).

With idiopathic or constitutional thrombocytoasthenia so nicely pinpointed it has become apparent from ultrastructural and companion studies that a similar qualitative platelet defect can be brought about secondary to other diseases, the secondary thrombocytoasthenias. Alatas and Gurturk's (41) electron microscopic studies of platelets from two cases of tuberculosis revealed defective pseudopodial formation and lack of platelet spreading. After successful treatment of the primary condition, the platelet structural spectrum returned to normal. Haydon (25) similarly observed shortened dendritic processes in the platelets from a patient with pancytopenia of unknown etiology. As early as 1950 and 1951, Braunsteiner and his group (10,42) had reported on a similar secondary thrombocytoasthenia, as it would now be called, in acute leukemia in which no or few platelet pseudopodia and no aggregations were present in an electron microscopic survey. This finding was depicted by Gurturk and Alatas (43) in micrographs of platelets from a case of acute myeloblastic leukemia and in our own two cases of actue granulocytic leukemia reported above (Fig. 1). In this present study we have described, in addition, defective, sparse and blunted pseudopodial formation in unusually small platelets from seven patients suffering from pernicious anemia in relapse. An analysis of our correlated coagulation studies reveals that qualitative platelet defects may accompany quantitative platelet deficiencies. In 1957 and 1958, Frank and Ulutin and their associates (44-46) reported the first and second cases of thrombocytoasthenia complicated by AHG deficiency. Their electron micrography revealed both defective pseudopodial formation and lack of platelet hyalomeric spreading.

From consideration of the intrinsic ultrastructural defects in the transformational states of thrombocytoasthenic platelets as revealed by electron microscopy, it became apparent that dendritic formation and/or spreading could be inhibited and distorted by harmful extrinsic agents. Early in 1950 and 1951, Braunsteiner and his fellow workers (10,42) observed in their electron micrographs that the platelets from cases of ITP had no pseudopods or incompletely developed ones. Their hyalomere disintegrated immediately upon contact with the film. The granulomere remained dispersed and at times fell out of the platelet. Further electron microscopic studies by these authors (31) in 1954 of 11 cases of ITP revealed the same inhibited and bizarre pseudopodial deficiency of the so-called paralyzed platelets. The extrinsic basis of these abnormal changes was brought out by study of normal platelets which had been exposed to serum containing a high titer of platelet agglutinins. The normal platelets so treated failed to develop into dendritic and spread forms, failed to adhere to the membrane, formed agglutinates, and presented vacuolization of their hyalomere. Electron microscopy of platelets from the three cases of ITP which we have reported above also revealed malformed and abortive pseudopodial processes. Although demonstrable idiopathic circulating antiplatelet antibodies were present, intermediate and spread forms were also present in considerable numbers. The serrated border of the affected platelet with its elevated plateaus (Fig. 12) is reminiscent of the ultrastructural changes by antierythrocytic antibodies on the sensitized erythrocytic surface (39). Modification of Tullis' platelet agglutination test (34,35) for electron microscopy revealed agglutinates of aged platelets unlike the aggregates of viscous metamorphosis and presenting delicate strands, presumably of hyaloplasm and antibody between the agglutinated platelet masses (Fig. 17). That appropriate, nonspecific, extrinsic factors were capable of similarly distorting normal platelet process extrusion became known when the effect of macromolecular cryoglobulins was observed with the electron microscope (31,37). Pachter and his associates (7) are reporting similar findings related to Waldenström's macroglobulinemia at this conference. Exposure of control platelets first to macroglobulins (Waldenström) and secondly to rabbit antimacroglobulin antibodies produced agglutinated platelets bound process to process (Fig. 18) and distinct from the aggregates of viscous metamorphosis (Figs. 9, 10, 14, 22).

Another important group of qualitative platelet diseases was emphasized in 1956 by Braunsteiner and Pakesch (36) under the acceptable term of thrombocytopathy. This group of patients with clinical hemorrhagic disease presents with all known coagulation factors normal, yet with poor prothrombin consumption and platelet thromboplastin generation tests. The original abnormality revealed by the electron microscope in three of five patients was featured by excessive platelet spreading. This group was further linked to deficient thromboplastin activity of the platelets, that is, deficient platelet factor 3 activity. Johnson and her associates (29) demonstrated that this group of patients with a familial tendency to the disease and with the idiopathic qualitative defect in their platelets (thrombocytopathy A) actually possessed platelets with poor platelet factor 3 activity which paradoxically became normal after treatment of the platelets with sonic oscillations. These workers concluded that the platelets of thrombocytopathy A contained adequate amounts of platelet factor 3 but were resistant to disintegration, and the activity was liberated with difficulty. The electron microscopic studies of these cases have been reported in abstract (48) previously and in this report above in detail (Figs. 5, 6, 8, 9, 10). Johnson and her associates (29) have published the clinical and functional studies in this series. Differential platelet counts under the electron microscope now document the excess of intermediate and spread platelet forms among eight patients with this uncomplicated thrombocytopathy. In addition, we have observed an excess of early viscous metamorphosis (Figs. 9, 10) accompained by increased retention of osmiophilic chromomeric substance (Figs. 6, 8, 10).

Hemmeler (49) has recently reported an ultrastructural variant of familial thrombocytopathy. The platelet sections revealed the customary, abnormally large platelets, but the granules were sparse and of low density.

Although Jürgens and his group have recently observed a complicating partial AHG deficiency in the Aland island group of

consitutional thrombocytopathy (v. Willebrand-Jürgens), there is little doubt of the presence of a qualitative platelet defect in these patients due to deficient functioning of platelet factor 3. Sections (21,50) of the platelets from this group viewed with the electron microscope revealed bizarre, drumstick forms in the dense granules of the platelet chromomeres (their granulomere alpha). In only one of the six patients studied were the drumstick granular aberrations absent. Our two cases of thrombocytopathy complicated by AHG deficiency have been described above. Frank and Ulutin (37,51) restudied their original case of thrombocytoasthenia and revealed that although the ultrastructural defect was one of deficient pseudopodial formation, the platelets which natively lacked factor 3 activity released this activity after exposure to distilled water. They now designate this as a combined thrombocytoasthenic-thrombocytopathic defect.

Secondary thrombocytopathy was described by Cetingil and his associates in sprue (52) and in scurvy (53). In each instance platelet factor 3 activity was deficient, but exposure of the platelets to distilled water did not release platelet factor 3 activity. Unfortunately, electron microscopic studies were not made. Cahalane and his associates (28) found a similar, acquired thrombocytopathy in platelets from a patient with uremia. Factor 3 activity was not restored by exposure of the deficient platelets to sonic oscillations; furthermore, normal platelets became deficient after prolonged exposure to uremic plasma. The electron microscopic studies in these cases have been described above in this report with only a small increase in round forms distinguishing their differentials from controls. Elucidation of their ultrastructural defect awaits thin section studies of affected uremic platelets.

Ultrastructural abnormalities in the area of platelet-fibrin relationships await further knowledge of normal platelet-fibrin interplay. It has become apparent from our own studies and the introductory survey of the literature that rapid disintegration is not the only role played by the platelet. Release of platelet factor 3 may well be gradual rather than by platelet disruption; in any event, more work is needed to pinpoint the mechanism of factor 3 release. Our studies reveal that intact platelets in controls follow the steps of 1) round, 2) dendritic, and 3) dendritic viscous metamorphosis formation, with few isolated intermediates and spreads. The literature indicates that hyalomeric disintegration takes place with platelet aggregation and not necessarily before it (10-13). In thrombocytopathy, what was at first thought to be normal succession of platelet stages—1) round, 2) dendritic, 3) intermediate, and 4) spread—takes place, and viscous metamorphosis at the dendritic stage as in normals does not occur. We have emphasized that such an abnormal succession of changes leads to a fifth stage, that of spread-aggregations or spread-VMs. The ultrastructural study of viscous metamorphosis is herewith emphasized because abnormalities may be more pronounced at this stage (Fig. 10) than in the four classical preceding stages. Study of the aggregations of viscous metamorphosis is even more important as a prelude to distinguishing abnormalities of platelet-fibrin interrelationships. There is now abundant evidence (10,13) that platelet aggregates (VMs) serve as the central supports (Fig. 20) of the fibrin network, and because of their close association with the fibrin fibrils as the platelets aggregate (Fig. 19), they serve as retraction centers as well. That qualitative platelet defects may well lead to further faulty platelet-fibrin relationships has been shown by Braunsteiner (10,14) in the thrombocytoasthenic states, leading to loose, deficient fibrin networks. A similar sparse, defective fibrin network was observed in relation to the platelets of our patient whose platelets were low in platelet-fibrinogenlike or clottable factor, although his plasma fibrinogen levels were normal. This draws attention to the need for further investigation of the possible function played by the platelet in general and by this platelet factor in particular in direct participation in fibrin formation.

The array of ultrastructural qualitative platelet defects known to date has resolved certain classical questions and at the same time propounded many more.

Summary

The literature concerning the contribution of electron microscopy to the study of platelets has been reviewed.

The known but complex ultrastructure of the platelets has been related to the electron microscopic changes in a series of platelet diseases in which functional and clinical studies have been correlated with the ultrastructural changes.

Control platelets were obtained from seven normal volunteers. Stored platelets were obtained from a normal Group O, Rh negative professional donor. Abnormal platelets were studied from eight patients with uncomplicated thrombocytopathy A, from two patients with thrombocytopathy complicated by AHG deficiency, from three patients with ITP, from four patients with uremia, from two patients with acute granulocytic leukemia, from one patient with a demonstrable deficiency of platelet-fibrinogenlike factor and from seven patients with true pernicious anemia in relapse. Electron microscopic platelet differentials were performed on controls and abnormal preparations.

The platelets in thrombocytopathy A have poor platelet factor 3 activity which paradoxically became normal after treatment of the platelets with sonic oscillations. Differential platelet counts under the electron microscope confirmed previous reports of excess intermediate and spread platelet forms. New abnormal findings were an excess of early viscous metamorphosis and an increased retention of osmiophilic chromomeric substance. Similar findings were observed in cases of thrombocytopathy complicated by deficiency of AHG; viscous metamorphosis was not as pronounced as in uncomplicated thrombocytopathy.

Acquired thrombocytopathy was observed in platelets from patients with uremia. Factor 3 activity was lacking and was not restored by exposure of the deficient platelets to sonic oscillations. Electron microscopic studies in these cases were distinguished with difficulty from control differentials, the uremic platelets presenting a small increase in round forms over those of controls.

Platelets stored for a year and viewed with the electron microscope were small, retained only a portion of their central chromomere, and presented vacuolated hyaloplasm. Such platelets retained 25% of their platelet factor 3 activity and were antigenically active in platelet-agglutinin studies.

Platelets from patients with ITP yielded differential counts intermediate between normals and thrombocytopathic studies. Dendritic formations were abortive and malformed. A new finding was a serrated, gearlike border on which the antiplatelet antibody brought up small plateaus at the affected sites. This effect led to the shortened, deficient pseudopodia. Ablation of the antiplatelet antibody-forming cells tends to restore such platelets to approximately normal numbers and structure. Electron microscopy of the positive platelet agglutination test itself revealed agglutinates of the stored platelets with delicate strands of hyaloplasm and antibody between the agglutinated platelet masses. Exposure of control platelets first to macroglobulins and then to anti-macroglobulin antibody produced agglutinates of platelets bound process to process. Agglutinates could be distinguished from viscous metamorphosis.

Defective sparse and blunted pseudopodial formations in unusually small platelets were observed in patients with pernicious anemia in relaspse. Correlated coagulation studies revealed a qualitiative platelet defect in association with quantitative platelet deficiencies in this disease. Similar findings were observed in acute granulocytic leukemia.

Emphasis was placed on viscous metamorphosis because abnormalities may be more pronounced at this stage than in the four classical preceding stages of platelet change previously studied under the electron microscope. These platelet aggregates also serve as the central supports of the fibrin net and because of their close association with the fibrin fibrils as retraction centers as well. Qualitative platelet defects may lead to faulty fibrin-platelet relationships. Patient whose platelets were low in platelet fibrinogenlike factor although his plasma fibrinogen levels were normal.

References

1. Wolpers C, Ruska H. Strukturuntersuchungen zur blutgerinnung. Klin Wchnschr 1939;18:1077,1111.
2. Bessis M. Ultra-structure du protoplasma des thrombocytes au microscope electronique. Compt Rend Soc Biol 1948;142:647.
3. Bessis M. Les applications du microscope electronique en hematol. Press Med 1949;57:954.
4. Bessis M. Studies in electron microscopy of blood cells. Blood 1950;5:1083.
5. Bessis M, Bricka M. Nouvelles etudes sur les cellules sanguines au microscope electronique avec une etude particuliere de leur ultrastructure. Arch Anat Mic 1949;38:190.
6. Bessis M, Burstein M. Etudes sur les thrombocytes au microscope electronique. Rev hemat 1949;3:48.
7. Pachter MR, Johnson SA, Neblett TR, Truant JP. Bleeding, platelets and macroglobulinemia (in press).
8. Gurevitch J, Nelken D, Danon D. Osmotic fragility of human blood platelets. Phase contrast and electron microscopic studies. Blood 1958;13:773.
9. Braunsteiner H, Febvre H. Etude au microscope electronique de la coagulation du sang humain dans ses rapports avec les thrombocytes. Compt Rend Soc Biol 1949; 143:1582.
10. Braunsteiner H. Uber morphologie, physiologie und pathologie der thrombocyten. Klin Wchnschr 1951;29:335.
11. De Robertis E, Paseyro P, Reissig M. Electron microscopic studies of the action of thrombin on blood platelets. Blood 1953;8:587.
12. Bloom G. The disintegration of human blood platelets after taking up fine quartz particles. Ztschrf Zellforsch 1954;40:222.
13. Bloom G. The morphology of human blood platelets and the coagulation of human blood in vitro. An electron microscopical examination. Ztschr Zellforsch 1955;42:365.
14. Braunsteiner H. Thrombopathie und thromboasthenie. Wien Ztschr Inn Med 1955;36:421.
15. Hutter RV. Electron microscopic observations on platelets from human blood. Am J Clin Path 1957;28:447.
16. Rinehart JF. Electron microscopic studies of sectioned white blood cells and platelets. Am J Clin Path 1955;25:605.
17. Bernhard W, Leplus R. La methode des coupes ultrafines et son application a l' etude de ultrastructure des cellules sanguines. Schweiz Med Wchnschr 1955;85:897, 921.
18. Watanabe Y. Observations of white blood cells with electron microscope. J Electron Micro 1957;5:46.
19. Feissly S, Gautier A, Marcovici I. Nouveau procede d'examen des thrombocytes au microscope electronique. Rev Hemat 1957;12:397.
20. Aleksandrowicz J, Blicharski J, Feltynowski A. Mikroskopia elektronowa krwinek badanych metoda ultracienkich skrawow. Haematol III Clin Med Intern Acad Med Cracoviensis 1957;1:3.
21. Schulz H, Jurgens R, Hiepler E. Die ultrastruktur der thrombozyten bei der konstitutionellen thrombopathie (v. Willebrand-Jurgens) mit einem Beitrag zur submikroskopischen orthologie der thrombozyten. Thromb Diath Haem 1958;2:300.
22. Johnson SA, Sturrock RM, Rebuck JW. Morphological location of platelet factor 3 activity in normal platelets. Proc 4th Internat Congr Biochem Symposium X Blood Coagulation. Vienna, 1958.
23. Fonio A. Uber das funktionelle Verhalten der isolierten Skrukturelemente der Thrombocyten, des Hyalomers und des Granulomers. Acta Haemat 1951;6:207.
24. Kautz J, De Marsh QB. Electron microscopy of sectioned blood and bone marrow elements. Rev Hemat 1955;10:314.
25. Haydon GB. Electron microscopic observations of blood platelets and fibrin formation. AMA Arch Path 1957;64:393.
26. Bessis M. Cytology of the blood and blood-forming organs. (E. Ponder, trans.) New York:Grune & Stratton, 1956:629.
27. Goodman JR, Reilly EB, Moore RE. Electron microscopy of formed elements of normal human blood. Blood 1957;12:428.
28. Cahalane SF, Johnson SA, Monto RW, Caldwell MJ. Acquired thrombocytopathy. Observations on the coagulation defect in uremia. Am J Clin Path 1958;30:507.

29. Johnson SA, Monto RW, Caldwell MJ. A new approach to the thrombocytopathies. Thrombocytopathy A. Thromb Diath Haem 1958;2:279.

30. Braunsteiner H, Fellinger K, Pakesch F. Elektronenmikroskopische Beobachtungen der Blutplattchen bei Thrombasthenie. Klin Wchnschr 1953;31:21.

31. Braunsteiner H, Fellinger K, Pakesch F. Structural changes in the platelets as observed by electron microscopy. Blood 1954;9:595.

32. Rebuck JW. A simple direct method for the electron microscopy of peripheral blood cells. Am J Clin Path 1949;19:217.

33. Rebuck JW, Woods HL. Electron microscope studies of blood cells. Blood 1948;3:175.

34. Tullis J. Preservation of platelets. Am J Med Sc 1953;226:191.

35. Tullis J. Identification and significance of platelet antibodies. N Engl J Med 1956;255:541.

36. Braunsteiner H, Pakesch F. Thrombocytoasthenia and thrombocytopathia. Old names and new diseases. Blood 1956;11:965.

37. Ulutin ON, Karaca M. A new way of considering the pathogenesis and investigations about the platelet defect in cases of thrombopathia. Forum Medicum 1956;2:257.

38. Jurgens R, Lehman W, Wegelius O, Eriksson AE, Hiepler E. Mitteilung uber den mangel an anti-haemophilem globulin (Faktor VIII) bei der aalandischen thrombopathie (v. Willebrand-Jurgens). Thromb Diath Haem 1957;1:257.

39. Rebuck JW. Structural changes in sensitized human erythrocytes observed with the electron microscope. Anat Rec 1953;115:591.

40. Alatas S, Ulutin ON. Etude au microscope electronique des thrombocyte d'un cas de thrombopathie congenitale. New Istanbul Contr Clin Sci 1954;3:22.

41. Alatas S, Gurturk S. Etude des thrombocytes au microscope electronique dans 2 cas de thrombopenie secondaire survenue au cours de 1 infection tuberculeuse. New Istanbul Contr Clin Sci 1955;3:268.

42. Braunsteiner H, Febvre HL, Klein R. Observation des thrombocytes au microscopes electronique et etude de leurs rapports avec la coagulation a l, etat normal et dans quelques etats pathologiques. Proc III Internat Congr Hemat. Cambridge, England, 1950:511-13.

43. Gurturk S, Alatas S. Etude des thrombocytes au microscope electronique chez le normal et dan quelques cas de thrombopenie symtomatique. New Istanbul Contr Clin Sci 1955;3:254.

44. Frank E, Ulutin ON. Hemofili baksine yeni bulumlar muvacehesinde tophu bir bakis ve bu hususda sahsi fikirlerinuiz. Turk Tib Cem Mec 1957;23:301.

45. Frank E, Ulutin ON. A study concerning two cases of vascular haemophilica (athrombia Haemophilica). Proc VII Congr Internat Soc Hemat. Rome, Italy, 1958:288-9.

46. Ulutin ON. Haemophilia-like states in girls. Lancet 1958;2:591.

47. Braunsteiner H, Falkner R, Neumayer A, Pakesch F. Makromolekulare kryoglobulinamie. Klin Wchnschr 1954;32:722.

48. Riddle JM, Rebuck JW. Correlation of ultrastructural platelet abnormalities with platelet factor 3 defects. Fed Proc 1958;17:454.

49. Hemmeler G. Thrombopathie familiale. Schweiz Med Wchnschr 1958;88:1018.

50. Schulz H, Jurgens R. Die ultrastruktur der thrombocyten bei der konstitutionellen thrombopathie (v. Willebrand-Jurgens). Verhandl Deutsch Gesellsch Inn Med 64 Kongr, 1958:232-6.

51. Frank E, Ulutin ON, Karaca M. Die isolierten und assozierten Athrombien. New Istanbul Contr Clin Sci 1956;4:62.

52. Cetingil AI, Ulutin ON, Karaca M. Coagulation defects in a sprue case. New Istanbul Contr Clin Sci 1956;4:95.

53. Cetingil AI, Ulutin ON, Karaca M. A platelet defect in a case of scurvy. Brit J Haemat 1958;4:350.