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Editorial: Blood Platelets

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BLOOD PLATELETS

Rewarding experiences with the blood platelets have been the fruit of multiple disciplinary talents devoted to understanding these, the least of the formed elements of the blood. In 1882 Bizzozero not only described the platelets but demonstrated their part in the coagulation of the blood, their adhesiveness, even their participation in thrombi. The succeeding decades were marked, platelet-wise, by Wright's discovery (1906) that the platelets were the shed cytoplasmic fragments of the megakaryocytes of the marrow; by Tocantins (1938) masterly updating of the literature in the same year that Michels performed a similar monumental service for the mast cells, and by the scholarly but almost unnoticed monograph of Fonio (1942).

Although sharpening methodology and increasing complexity of instrumentation were the accidental bases of the new platelet experiences, elucidation and stabilization of one after the other of the plasma and serum clotting factors were the prerequisites for appreciating the dynamic transformations of the platelets themselves in the flooding, transient medium which forms their environment. That worthwhile stability of the coagulation factor system has been nearly attained, if only for present platelet purposes, is evidenced by the identification of the new and at once powerful prothrombin activator and prothrombin derivative (autoprothrombin C) with factor X.

With transformational possibilities recognized and non-transforming media available, the ultrastructure of the platelet revealed a protoplasm bounded by a membrane 60 Angstrom units thick and apparently plain in contour, although segmental interruption of this membrane becomes apparent very early in clotting. Within the platelet and against a cytoplasmic matrix containing poorly defined densities are four well-defined structures: among 50 to 100 granules, the common round or oval granules of considerable density predominate (granulomere alpha); next appear a few small mitochondria (granulomere beta); microvesicles and tubuli (granulomere gamma); and, finally granules with clear interiors (granulomere delta).

Concurrent physiologic studies added increasing numbers of platelet factors (listed by arabic numerals) to the clotting scheme. Platelet factor 1 is an accelerator of...
prothrombin conversion to thrombin and is known to be platelet-adsorbed plasma factor V. Platelet factor 2 aids in the interaction of thrombin and fibrinogen. Platelet factor 3 has the triple role of synergism with coagulation factor VIII to catalyse the conversion of prothrombin to thrombin, with plasma factor IX to function similarly and of facilitating the conversion of prothrombin to plasma factor VII. Platelet factor 4 neutralizes the activity of heparin. Platelet factor 5 is a clottable factor, platelet factor 6 an antifibrinolysin and platelet factor 7 a cothromboplastin. In addition there can be found histamine, serotonin, contractile protein, ATP, fibrinolysin activity, a platelet procoagulant inhibitor and an inactivator of plasma factor V. An endothelial supporting role has long been manifest. Even the unused, disintegrating platelet is an important source of plasma lipids. The stickiness of the platelet originally noted was soon found to be crucial in hemostasis for adhesion to injured vascular linings and for formation of the platelet plug. Under the light microscope, platelet fusion became known as the phenomenon of viscous metamorphosis. Without platelets, clot retraction was found to fail. Furthermore platelets were demonstrated to have a surprisingly active metabolism aimed especially at the preparation of high energy bonds.

With this background, function gradually became related to structure. Johnson and her associates obtained granules about 350 m\(^2\) in diameter with platelet factor 3 activity from platelets treated in a sonic oscillator. Schulz, Jürgens and Hiepler related platelet factor 3 activity and platelet factor 1 activity similarly to the common dense granules. Platelet factor 1 activity was also associated with the mitochondria and microvesicles, but platelet factors 2 and 4 were associated with the hyalomere.

Fixed but timed electron-microscopic studies revealed far-reaching ultrastructural transformations further related to platelet function. As seen in electron micrographs, platelets in their normal circulating status were disk-like and electron opaque but soon after venipuncture, if clotting was slowed for their study, they rapidly protruded long, sticky dendritic processes. At this pseudopodial stage they quickly aggregated in the various stages of viscous metamorphosis. Study of the relatively few platelets persisting unaggregated, revealed further transformation leading to spreading of the hyalomere between the platelet processes accompanied by loss of much of the central granular content.

Similar sequential electron micrographs of sectioned early phase clotting related fibrin to the tridimensional process and permitted interior observation of the larger platelet aggregates. In such sections the individual platelets, at first round or oval, put out short, stubby projections. The outer membrane, single and originally dense, became segmentally interrupted. As time elapsed the projections became more and more numerous and elongated, the limiting membrane was once more sharply defined and the granules became centrally concentrated. At this stage a few fibrin strands appeared and the platelets began to fit together like the pieces of a jigsaw puzzle. With small aggregates formed, membranes were still intact, separated by a space a few hundred Angstroms in width. Fibrin was apparent between most of the
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platelets, closely opposed to the surface of some and a great distance from others. As the filmy clot became macroscopically visible, micrographs revealed larger and more complex platelet aggregates until single platelets were rare and fibrin was deposited in larger and larger amounts. The larger platelet aggregates were of two types: the first with easily identifiable platelet limiting membranes presented specifically recognizable granulomeres; the second large aggregate type, increasingly apparent in the retracting clot, showed absence of identifiable membranes in their interiors and poorly defined granular material, while peripherally there were sac-like protrusions containing finely granular material and microvesicles. Schulz, Jürgens and Hiepler noted that the common dense granules emerged from the platelets at the beginning of clotting. Bloom and Aleksandrowicz noted that when the granules from the chromomere had been released from the platelets, numerous small empty, round spaces were left. Sequential section micrographs of the clotting process emphasizing granular changes revealed progressive loss of the common dense granules, on the average to 40 per cent of the original number, indeed some platelets became virtually empty of granules and contained only hyaloplasm. When fibrin was related to more intact platelets, a crescentic gathering of the granules along the surface adjacent to the fibrin took place. Membrane defects appeared and in some areas of the clot there was dense intermingling of fibrin strands, platelet debris and free granules. The number of vesicles also decreased during viscous metamorphosis.

When the entire clotting process was examined by sectioning and staining the normal platelet-plasma clot for light microscopy step-wise from its incipiency through final retraction, remarkable insight into platelet-fibrin relationships was obtained. The platelets enlarged, sent out pseudopodial process and joined to form larger and larger aggregates. Normal fibrin patterns were remarkably geometric in configuration. The platelet aggregates acted as focal points for the construction of a coarse fibrin lattice, the coarse fibrin strands connecting one group of platelets with all neighboring groups as the major structure. In the intervening spaces in addition there was a fine fibrin network. The dendritic process of the platelet aggregates extended into the fibrin nets joining the appendages of other platelet masses producing a platelet lattice overlying the fibrin. Indeed after fibrinolysin, the fibrin networks, both fine and coarse, were gone but the cast of the platelet network remained. Poole has properly emphasized the more static nature of the clot and the churning environment of the thrombus, however his electron micrographs revealed that the apparently structureless areas of the thrombus studied under the light microscope were again composed of interdigitating, closely packed platelets.

Of very particular importance has been the realization that each and every functional facet of the platelet has been disclosed as a source of platelet disease when functional failure ensued. Braunsteiner and his associates described the first of a long series of hemorrhagic diseases attributable to intrinsic platelet abnormalities in their delineation of thrombocytopenia, a constitutional disease in which ultrastructural studies demonstrated the inability of platelets from such patients to extend pseudopodial process. Next platelet aggregation failed and clot retraction was impeded. Fibrin attachment, when it occurred, was to single platelets, not aggregates.
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Gross found that clot retraction and platelet transformation were dependent on a high energy potential in platelets with ATP as the main source of that energy. In patients with thrombocytoaesthenia he further uncovered hereditary platelet defects in two glycolytic enzymes: glyceraldehyde phosphate dehydrogenase and pyruvate kinase, the lack of the former being largely responsible for the diminution of ATP in the affected platelets.

Subsequently, a number of additional secondary thrombocytoaesthenias with platelets similarly affected by impairment of process formation and aggregation have been reported in leukemia, tuberculosis, pernicious anemia and other conditions, although their metabolic faults have not as yet been delineated. Braunsteiner, Riddle, Johnson and their associates demonstrated a second important group of qualitative platelet diseases in which platelet numbers and coagulation factors were again normal but in which platelet factor 3 activity was deficient. Ultrastructurally the platelets spread excessively within fixed time limits but they were unable to release their granular chromomeres. Platelet factor 3 activity of such platelets could be restored to normal in the test tube by sonic oscillation. Secondary thrombocytopenias, differing in one or more respects from the idiopathic group have already been reported in sprue, scurvy and in some cases of uremia. Hemmeler recently reported an ultrastructural familial variant in which platelet sections presented abnormally large platelets with greatly diminished granule numbers and low granule density.

Distorted platelet-fibrin relationships leading to sparse, defective fibrin networks have now been described in thrombocytoaesthenia, platelet clottable factor deficiency, polycythemia vera and macroglobulinemia of Waldenstrom. The latter condition underlines the concept of extrinsic platelet abnormalities in contrast to the intrinsic forms previously considered. Non-specific coating of the platelets with the monstrous polymers of globulins in patients with macroglobulinemia leads to abnormal bleeding by preventing dendritic process formation and interfering with normal platelet-fibrin relationships. Platelets of normal persons are similarly affected when exposed to macroglobulins in vitro.

Immunologic thrombocytopenic purpura (ITP) dramatically demonstrated by Harrington and co-workers to be caused by anti-platelet antibodies is still another example of extrinsically produced thrombocytoaesthenia. In this condition specific attachment of anti-platelet antibody to the platelet antigens leads to pseudopodial deficiency over and above the well known reduction in platelet numbers. Recent improvements in the methodology of anti-platelet antibody detection especially the production of increased test platelet membrane permeability induced by cathode irradiation and the preparation of control serum in an equivalent thrombocytopenic milieu have further improved the reproducibility of this important procedure. Utilization of fluorescein labeled antoglobulin serum revealed its affinity for the megakaryocytes in the marrows of patients with chronic ITP to be of a non-gamma globulin nature but indicating adherence of a humoral substance to megakaryocytes.
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in this condition. Cr$^51$ labeling for measuring platelet life-span has shown that thrombocytopenic states belong to three broad categories: primary production deficit, predominant production deficit and predominant peripheral destruction. However, in 20 per cent of the cases of chronic ITP so studied, a dual mechanism of peripheral destruction and production deficit was operative.$^{12}$

Time relationships of platelets are being recognized for their true value in respect to the transiency of their origins and functions. Schulman$^{27}$ in a painstaking study of a thrombocytopenia due to an amegakaryocytic marrow which responds to plasma injections by manifestations of megakaryocytopenia and thrombocytopenia has timed the human platelet cycle with reproducible rises in platelet counts to 700,000 platelets per cu. mm. ten days after each plasma transfusion with returns to previous thrombocytopenic levels in three weeks. Platelet survival times must be thought of both in terms of platelet survival in storage in vitro and platelet survival in the transfused recipient in vivo. These important concepts have been ably covered in the formative years of this journal and to such pages the reader is referred. For our present purpose a few observations will suffice. Transfusion therapy with ACD banked blood stored at 4 C. for more than 24 hours may lead to the loss-dilution syndrome$^{29}$ of hemorrhage from low platelet levels since viable platelet levels in stored blood fall appreciably after three hours of such storage and precipitously after 24 hours of storage. The continued administration of small amounts of similarly banked bloods over periods of days or weeks does not lead to dilution thrombocytopenia but may eventuate in the stimulation of anti-platelet antibody formation on the part of the persistent recipient. The blood platelets of man survive in his own blood stream, if not put to use, for from eight to 14 days.$^{29}$ However, removed from his body, maximum storage of platelet-rich plasma is ten hours and that of concentrates is four hours if loss of less than 50 per cent of transfused platelets is to be achieved.$^{22}$ Stored platelets thus lose their ability to survive in direct proportion to their storage time.$^{26}$ Here, extended survival times for viable platelet storage as glycerolized frozen platelets$^2$ leave a loop-hole for the future. Fresh platelets transfused into thrombocytopenic recipients survive normally providing there is no thrombocytolytic factor in the recipient and providing the recipient is not undergoing excessive hemorrhage.$^{26}$ Transfused platelets may survive only a matter of a few minutes or hours in recipients with circulating anti-platelet antibodies or in recipients suffering from undue bleeding who lose not only large numbers of their own platelets but their borrowed ones as well. It should be cautioned that low platelet survival in the recipient could also mean effective and proper utilization in hemostasis at the focal bleeding sites precluding further circulation for the sole purpose of being counted.

The future of the blood platelets promises to be exciting in the areas of: concurrent complexities of platelet dysfunctions plus plasma or serum coagulation factor deficiencies; more effective transfusions of viable platelet suspensions; and appropriate platelet substitutes. Recalling the long list of platelet functions it would only be fair to demand of the pioneer platelet substitutes the performance of one platelet activity at a time to avoid the disappointment of failed simulation of a megakaryo-
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cytic extrusion that is at once transport, trephone and hemostatic constellation.—
—John W. Re buck, M.D., Ph.D., The Henry Ford Hospital.

REFERENCES


