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MODERN CONCEPTS OF THE PLATELET IN HEALTH AND DISEASE*

PART I

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THE platelet has long been regarded as a mysterious quasi-cell that appears in the blood in numbers that vary with certain diseases, and with a multiplicity of incompletely described or appreciated functions and characteristics. It has been the subject of a great deal of research, speculation and literature of uneven quality of hypothesis and interpretation. This paper represents an attempt to collect the most useful knowledge about the platelet into a description of its normal and abnormal states, with a view towards what might be called a "unified theory of the platelet", with emphasis on its pathophysiology. As will be seen, there are still many incompletely answered questions about the platelet, but it is my contention that it is not merely a conglomerate of loosely related functions, as one might suspect from the attitude of the literature, but rather a highly specialized cell or cell particle, the various functions and diseases of which can be discussed as minor variations on a few major themes.

Though the literature referred to in this paper probably is not exhaustive, it does include, in my opinion, the most significant advances in the study of the platelet since 1950. No attempt has been made to discuss the historical development of theories about the platelet¹. This review has been designed as a report on concepts of the platelet at the time of writing.

STRUCTURE AND FUNCTION

The megakaryocyte, the precursor cell of the platelet, has been studied in vivo and in vitro, and with phase contrast and standard microscopical technics²⁻¹¹. The earliest cell of the series is the myeloblast, which differentiates in the thrombocytic line to a megakaryoblast. This form may undergo complete

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mitosis once and develop further, or it may undergo up to four mitoses in the nucleus only, resulting in a mature megakaryocyte with from two to thirty-two nuclei. There is probably an intermediate promegakaryocyte. The young megakaryocyte has a round or oval homogeneous, finely granular cytoplasm with an irregular, blurred cell membrane, and a slightly lobulated or round nucleus. A mature megakaryocyte is large, about 80 micra in diameter, with irregularly shaped basophilic cytoplasm containing nonrefractile mitochondria and coarse moderately refractile granules, and a thin nuclear membrane; the large lobulated nucleus contains heavily packed coarse chromatin. Fluorescein-labelled antibody technics demonstrate an antigen common to both megakaryocytes and platelets¹⁰.

Megakaryocytes are found intra- and extra-vascularly, primarily in the bone marrow, but also in the spleen and lymph nodes; they are found intravascularly in the lungs, liver, kidney and other organs, rarely in the circulating blood. Half of those found in the lungs and three-fourths of those in the spleen lack the usual large amount of cytoplasm.

These cells exhibit no mobility, but they may be capable of phagocytosis. They turn over at a rate of 10 per cent per day. Only seventy per cent of those in the body produce platelets, the production of which occurs within 48 to 60 hours of megakaryocyte maturation; in the other thirty per cent, the cells undergo nuclear disintegration within the same amount of time. It has been estimated that 28 to 36 per cent of the actual number of megakaryocytes appear in a bone marrow aspirate smear.

Thrombocytopoiesis occurs either by simultaneous disintegration of the entire megakaryocyte cytoplasm, or by detachment of cytoplasmic pseudopod fragments. Microcinematographic technics⁹ have shown that the megakaryocyte contains long thin fila-

ments all through its cytoplasm; these filaments have periodic enlargements of which platelets are formed when the filaments are extruded from the cell. Since the platelets themselves apparently contain no nuclear material, they are incapable of further regeneration.

The regulation of thrombocytopoiesis is influenced by several factors^{3,12-18}. One of these may be the number of circulating platelets *per se*, since acute stimulation may be induced by thrombopheresis¹², causing a release of megakaryocyte cytoplasm with an increased platelet count after three to four days, and chronic stimulation of the same kind causes an increased number of megakaryocytes. There is considerable evidence for humoral substances which are capable of stimulating and inhibiting platelet production. These substances can be fractionated from the plasma¹⁵, and it is probably a balance between them that normally maintains platelet levels. There is also good evidence¹⁸ that a single humoral factor may be responsible for the proliferation of all blood cell type precursors. Other factors which have been reported³ to mobilize platelet reserves are ACTH, epinephrine, serotonin and hypothermia; pyridine, fats, batyl alcohol, and citrovorum factor¹⁶ will also increase thrombopoiesis.

The morphology of the mature formed circulating platelet as seen with the electron microscope¹⁹ is much as it appears in my experience with the phase contrast microscope, to the limits of resolution of the latter instrument; the routine blood smear is of little use in differentiating the various features of the platelet, since the distortion produced by the smear is too great. The earliest stage is a dense, discoid form, about one to two micra in diameter. It contains clear hyalomeric cytoplasm within a double-layered membrane, and several granules, constituting the chromere (granulomere), also within double membranes. Dendritic processes around the periphery of the cytoplasm appear next, followed by a form with one long pseudopod-like process. The final stage before disintegration is a ballooning of the entire platelet, with loss of the intracytoplasmic detail. Platelets probably function as intact units, not requiring disintegration for normal activity; this will be discussed in various appropriate sec-

tions later in this paper.

Osmotic fragility has been shown to begin at 0.44 per cent sodium chloride, and is complete at 0.34 per cent sodium chloride, as measured by the release of serotonin into the supernatant solution²⁰. Magnesium and colloids are required for maintenance of the normal discoid shape²¹.

Using various labeling technics, the *life span of platelets* has been rather uniformly estimated at five to eleven days, with a half-life of two to three days²²⁻²⁷. It has been suggested that platelets show an aging process similar to that found in red blood cells, but most investigators postulate their random disappearance from the circulation, probably due to a continuous intravascular process of coagulation. The hypocoagulable state has been found²⁵ to show a more linear curve of platelet disappearance resulting from less random destruction of platelets; similarly, hypercoagulability has been described as a state in which platelet utilization is accelerated. The measurement of platelet life span has been proposed as a basis for the reclassification of the thrombocytopenias²³, and will be used for that purpose in clinical sections of this report.

The physical alterations of the platelet during the clotting process have been subject to much discussion and confusion^{3,28-41}. The definitive terminology recently proposed by Anstall and Hawkey⁴² will do much to clarify the situation, and is accordingly summarized and used in the present discussion.

At the time of injury to a tissue, including its blood vessels, red blood cells release a substance, "Factor R,"³⁴ which transforms non-adhesive circulating platelets to platelets which will adhere immediately to the wound surface. Platelet adhesiveness is directly proportional to the hematocrit, the bleeding time is inversely proportional to the number of adhesive platelets, and there is no relation between the bleeding time and the total number of platelets³⁴. An alternative suggestion is that this adhesion is promoted by a thermolabile non-dialyzable plasma protein called Thrombocyte Agglutinating Factor (TA_g)⁴⁰. This adhesiveness, or stickiness, is a function of the viscosity of the platelet surface, is reversible, will occur in the presence of cooling, and requires certain divalent cations, primarily magnesium and manganese, but probably not calcium. The entire process

is apparently unrelated to the mechanism of coagulation as defined by the chemical reactions leading to the formation of fibrin; it is independent of calcium ion, antihemophilic factor (AHF), prothrombin, fibrinogen, and perhaps Platelet Factor 3³⁸.

Structural metamorphosis, traditionally referred to as viscous metamorphosis, is a series of progressive irreversible changes in the structure of the platelet. It occurs late in the clotting process, and is often not complete until after fibrin has been formed. It requires thrombin, magnesium, manganese, Hagemann Factor, and probably calcium ion. It is independent of plasma thromboplastin component (PTC, Christmas factor), plasma factor V (proaccelerin), plasma factor VII (proconvertin), fibrinogen and Platelet Factor 3, exogenously administered anticoagulants, and possibly AHF and plasma thromboplastin antecedent (PTA). Much more careful investigation is required on these points, since some of these factors are reported to have some delaying effect.

The effectiveness of the hemostatic plug is due to the structural metamorphosis of platelets. The plug adheres more readily to venous endothelium than to that of the arterial vessels, though the mechanism is not understood; in fact, a thrombus may be considered as an exaggeration of normal compensatory hemostatic mechanisms.

There is some as yet undefined relationship between platelets and capillary fragility (for ease of expression in a pathophysiologically oriented discussion, the term capillary fragility is preferable to its equally frequently used opposite, capillary resistance). Increased fragility is always found with depressed platelet levels, no matter what the cause of the thrombocytopenia. This may be due to the antigenic relationship between platelets and endothelial cells, permitting any antibody which attacks platelets to attack the endothelium also. This relationship may be more direct, since platelets have been observed to interact with the capillary bed cells and contribute some S³⁵O₄-labeled material to the latter³. Adrenal cortical hormones, stimulated by the release of endogenous epinephrine or by ACTH, will reduce capillary fragility²⁸; the catechol amine content of platelets may contribute to this phenomenon.

The vasoconstrictor serotonin, which is car-

ried by platelets and released with thrombocytolysis, may also play an important role in hemostasis. It is discussed more fully in a subsequent section.

Structural metamorphosis is the result of the fusion of the chromomeres of the already aggregated platelets, resulting from the loss of protection normally afforded by the intact hyalomere in unshed blood, possibly an anticoagulant form of protection⁴¹. The exact mechanism initiating breakdown of the hyalomere is not known, but it may be secondary to the polymerization of fibrinogen on its surface, concomitant with the coagulation process. Sulfhydryl groups on the platelet surface are probably involved, since sulfhydryl inhibitors will stimulate platelet breakdown³³. When fibrin strands first form, they do so at a distance from the platelet, intact or previously disintegrated, but are seen to attach to the clumped chromomeric granules soon after, while the hyalomeric material remains lying loosely in the fibrin mesh³⁶.

At this point, the other forms of platelet aggregation should be differentially defined from those described above⁴². Cold-induced adhesion is an aggregation of platelets induced by cooling and agitation, involves no structural change, is independent of any metal ions, and is reversible upon rewarming. Platelet clumping is due to a stickiness induced in the presence of certain divalent cations and plasma factors upon agitation at 37°C., and is reversible upon removal of the ions. Platelet agglutination is an immunological phenomenon due to platelet antibodies (anti-platelet agglutinins); these will be discussed in considerable detail within the context of the thrombocytopenias in Part II.

Platelet metabolism is very active, as we have shown in our laboratory at the Massachusetts General Hospital⁴³, in respect to both anaerobic and aerobic processes, even though the platelet has been regarded as rather an incomplete cell. It has been found to contain most of the enzymes and coenzymes for the major pathways of intermediary metabolism, particularly those involved in glycolysis; hexokinase is the rate-limiting system, as it is in other cell types^{21,44-46}. Fifty per cent of the glucose metabolized is converted to pyruvate and lactate; twenty per cent is lost as carbon dioxide and water; twenty-five per cent is converted to glycogen, lipids and amino acids.

All of the human amino acids have been found in human platelets, with particularly high concentrations of the monoaminodicarboxylic acids. Measurable amounts of acid phosphatase are liberated at clotting, but there is very little alkaline phosphatase. The individual platelet shows an endogenous oxygen consumption equal to about two per cent of that of the individual white blood cell⁴⁷. Approximately sixty-five per cent of its nucleic acid is ribonucleic acid⁴⁵, though the total amount of nucleic acid is reportedly insignificant⁴⁸. Platelets have a considerable amount of sulfated acid mucopolysaccharides^{21,42}, probably of a structural nature; they have about one-half as much sulfatase activity as megakaryocytes, which possess the greatest such enzyme activity of all blood cell types⁴⁹.

About 8.5 per cent of the dry weight of platelets is carbohydrate, including glycogen, glucose and six-carbon sugar polymers⁴⁷. The carbohydrate components are primarily in the form of glycoprotein⁴⁸, and may be associated with the coagulation factors as such. There are also high concentrations of adenine, hypoxanthine and adenosine triphosphate (ATP) (21.5 micromoles ATP per 10^{11} platelets)^{46,48}. Almost all of the energy produced by ATP is used in structural metamorphosis and clot retraction. There is no creatine phosphate or creatine phosphate kinase in platelets.

The *platelet lipids* contain the thromboplastic activity of the platelets⁵⁰⁻⁵⁵. Most of this activity is due to phosphatidyl serine, and may be enhanced by lecithin. Phosphatidyl ethanolamine shows some activity only in the presence of the individually inactive lipids lecithin and sphingomyelin. Inositol phosphate and cholesterol are also present in platelets, but have no thromboplastic effect. Very high lipid concentrations have an anticoagulant effect. The free phosphatidyl ethanolamine released from chylomicra postprandially may be partly responsible for the hypercoagulable state seen shortly after eating. It has been suggested⁵³ that if the phospholipid molecule is incorporated in the active thromboplastin of the thromboplastin generation test, it is in some form different from that in which it is found in the platelets originally.

All these lipids are present in approximate-

ly the same concentrations as in red blood cells; there is somewhat more lecithin and slightly less phosphatidyl serine in platelets. Calcium is complexed with the platelet lipids in an unusual form of combination; it is non-ionic, and is not exchangeable with radioactive calcium⁵⁵. The binding site is probably in the cell membrane. It has been postulated⁵⁶ that the reactive site of the platelet is a membrane lipoprotein, which would be easily available for utilization without requiring any prior reaction causing the disintegration of the platelet, and is compatible with the observation made above that structural metamorphosis and platelet disruption occur independently of the coagulation process.

Serotonin, histamine and the catechol amines are present in significant quantities in platelets⁵⁷⁻⁷⁶.

Whole blood contains 0.1-0.2 micrograms per cubic centimeter of *serotonin* (5-hydroxytryptamine)⁵⁸, all of which is carried by the platelets to the extent of 0.2-0.4 micrograms per 10^9 platelets⁵⁹. The platelets have no serotonin attached when they leave the megakaryocyte; they pick it up primarily during their circulation through the gastrointestinal tract, where it is manufactured by argentaffin cells. A platelet retains the same molecules of serotonin during its entire life span. There is little or no serotonin in the plasma (except in patients with carcinoid syndrome), but all of that in the platelets is released into serum at clotting.

Exogenous serotonin is taken up by platelets both in vivo and in vitro. Slow passive diffusion occurs at physiologic pH, while active transport occurs at more acid pH (5.7-6.5), and is limited by the passive process. The platelet serotonin content does not appear to be complexed within any cellular compartment. Most of it is actively transported into the platelet by some mechanism which is coupled with the energy-generating systems. ATP is the primary factor which stimulates serotonin absorption in a stoichiometric relation of two serotonin molecules to one ATP. In this regard, it is most interesting to note that there is evidence of lowered serotonin levels associated with deficiency of pyridoxol, as well as diseases of folic acid deficiency and megaloblastic anemias. Serotonin absorption is facilitated by glucose, phosphate and potassium, and inhibited by inhibition of

glucose oxidation and uncouplers of oxidative phosphorylation (though there is some disagreement about the latter). Iproniazid, and, to a lesser degree, isoniazid, both monoamine oxidase inhibitors, which reduce serotonin metabolism, will induce a rise in platelet-bound serotonin. There is no relationship between blood serotonin levels and the quantity of its metabolite, 5-hydroxy-indoleacetic acid, in the urine.

Reserpine blocks active transport of serotonin into the platelet at the cell boundary, and blocks whatever form of intracellular binding exists, though it does not affect passive diffusion. The reserpine block is effective even at acid pH and in the presence of potassium and phosphate. Digitoxin and ouabain also block serotonin uptake. Platelets preincubated in saline for two hours at 37°C. cannot bind serotonin, even with the addition of glucose, ATP, potassium and phosphate; if potassium or phosphate, but not glucose or ATP, is added to the saline suspension, serotonin uptake will proceed.

The role of serotonin in hemostasis, in particular the platelet-dependent phases of coagulation, is not known. There is no evidence that it takes any active part in the control of bleeding, and its usefulness in the hemorrhagic disorders is doubtful. There is evidence that serotonin participates in clot retraction (it is known that structural metamorphosis and clot retraction, induced by thrombin, will decrease the ATP concentration — upon which serotonin depends — by 50 per cent; cold-induced clumping does not alter the ATP concentration), and that it inhibits fibrinolysis by modifying either the fibrin substrate or the fibrinolytic enzyme, plasmin, or both. It was first studied as a vasoconstrictor; primarily, it increases vascular resistance and may shorten periods of traumatic bleeding, but these effects are short-lived. However, though a group of patients who were treated with reserpine had no serotonin in their platelets, they showed no defect in their coagulation properties, as measured by all the standard clinical laboratory and bedside tests.

The release of serotonin from platelets is independent of the clotting process, though the disruption of platelets brought about in clotting will bring about its release. Throm-

bin appears to release platelet serotonin; this effect is reduced by heparin or calcium-complexing agents. An alkaline extract of kidney, called nephrosin, will release platelet-bound serotonin. Certain antigen-antibody reactions will also cause its release in vitro if calcium ion is present.

Almost all of the histamine in the blood is carried by the platelets, less than 0.1 microgram per 10⁹ platelets⁵⁹. As in the case of serotonin, its release is independent of coagulation but is induced by coagulation, thrombin, nephrosin, and in vitro antigen-antibody reactions; a factor present in fresh serum, most likely thromboplastin, has recently been shown to release rabbit platelet histamine. Histamine, and tryptamine, have been found unable to be actively transported into the platelet. Other factors which are known to release histamine from platelets are peptone and trypsin; but release does not occur in plasma in which an antigen-antibody reaction has previously occurred, nor with histamine liberators such as 48/80, or streptokinase. If the release of histamine from platelets does take part in anaphylaxis, it is probably due to a local vascular effect with increased capillary permeability, but not dependent on platelets *per se*.

Platelets are able to bind and concentrate epinephrine and about one third as much norepinephrine. The catechol amine uptake is slower than that of serotonin, but is similarly dependent on ATP, is enhanced by potassium and phosphate at low pH, and is blocked by reserpine.

Clot retraction is one of the best documented functions of platelets^{65,77-86}. It is proportional not only to the logarithm of the total number of intact viable platelets⁷⁷, but also to the number of adhesive platelets⁷⁹. Further, it is directly proportional to the hematocrit, and to the concentration of fibrinogen. But, clot retraction is good in severe anemia and weak in polycythemia. Calcium (or barium, magnesium or strontium), glucose (or mannose) and thrombin, at appropriate pH (at least 6.0) and ionic strength are required for clot retraction. Excess calcium will suppress clot retraction. It is not inhibited by heparin. Serotonin is thought to be involved, especially in conjunction with an unidentified plasma protein.

As noted before, the energy for clot retraction is supplied by ATP. The clot-retracting activity of platelets is in the hyalomere, and the coagulation-promoting activity in the chromomere, which probably accounts for the fact that freezing and thawing destroys the former, but has a much lesser effect on the latter.

Clot retraction is not due to any physical change in fibrin, but to an active role of platelets, including structural metamorphosis. However, the strength and firmness of the clot depend not on the platelet but on the concentration of fibrinogen and fibrinolysin, though the latter has no effect on clot retraction. The force exerted by the process of clot retraction is insufficient to draw the vessel walls together to achieve hemostasis. The phenomenon of clot retraction seems to be largely responsible for restoring the patency of blood vessels after thrombosis⁸⁰. This mechanism may increase the degree of thrombosis as well; when the clot retracts and serum is expressed from it, the thrombin which is found in the serum may induce even more clotting, with enlargement of the thrombus. Further evidence of this possible mechanism is afforded by the observation that, in surgical patients, the platelet count is highest about ten days postoperatively or postpartum, the period associated with the greatest incidence of venous thrombosis — it will be remembered that platelets adhere most readily to venous endothelium, and at this time the outpouring of new platelets provides more adhesiveness. Hypercoagulability has been shown^{86a} in ischemic heart disease patients, associated with, among other factors, increased thromboplastin generation and platelet adhesiveness, but not with increased numbers of platelets.

No functional defect of clot retraction alone has been reported. Some diseases of the lungs, kidneys, liver and intestines are reported to cause deficient clot retraction, but this probably is due to increased fibrinogen levels in relation to other plasma proteins, though abnormalities in serotonin uptake or release may be responsible. The majority of deficiencies of clot retraction are due to conditions involving thrombocytopenia.

The coagulation factors associated with platelets present a confusing array of data

and speculation; an extremely lucid recent report by Seegers⁸⁷ has been followed closely in the present discussion, and supplemented by the work of others⁸⁸⁻⁹².

The following are generally accepted as activities of platelets which contribute to the coagulation process:

- Platelet Factor 1 — Plasma factor V (proaccelerin)
- Platelet Factor 2 — Fibrinoplastic factor
- Platelet Factor 3 — Prothrombin activator (thromboplastic activity)
- Platelet Factor 4 — Antiheparin factor
- Platelet Factor 5 — Clottable factor (fibrinogen)
- Platelet Factor 6 — Antifibrinolysin
- Platelet Factor 7 — Cothromboplastin (plasma factor VII)
- Platelet Cofactor I — AHF
- Platelet Cofactor II — exact nature undetermined
- Autoprothrombin I — Plasma factor VII (proconvertin)
- Autoprothrombin II — PTC
- Serotonin (function unconfirmed)
- Clot retraction
- Capillary fragility (mechanism undetermined)
- Free phospholipids (contribute to thromboplastic activity)
- Calcium

Most of these factors appear to be adsorbed and concentrated from the plasma by platelets, the adsorbing surface of the platelet thereby becoming the link between platelet aggregation at the site of injury and small vessel hemostasis, via the process of coagulation. Thrombin, which is generated on the platelet surface, initiates structural metamorphosis; this is followed by release of the coagulation factors carried by the platelets and subsequent clotting. At the same time a substance known as "S-protein" clots under the influence of thrombin and ATP (fibrinogen, which is similar, does not require ATP for its coagulation), and imparts firmness to the platelet clumps, probably by an action on the platelet cell membrane. "S-protein" is similar in contractile properties to actinomycin, and traps fibrinogen in its interstices⁸⁸.

Platelet Factor 1 represents about 6 per cent⁹⁰ (another report⁸⁸ gives a figure of 37 per cent) of the total plasma concentration of plasma factor V, strongly bound to the platelet. Platelet Factor 2 is the ability of platelets to accelerate the clotting of fibrinogen in the presence of thrombin.

Platelet Factor 3 is lipoprotein material which is not adsorbed but rather found in the granules of the chromomere. Its function is as a prothrombin activator, but it does not seem to be a true thromboplastin, even though it may be measured in the thromboplastin generation test. The substance produces a prothrombin derivative which takes part in prothrombin activation, and itself uses another prothrombin derivative in the conversion of prothrombin.

Platelet Factor 4, which is known to be present in the hyalomere, antagonizes the action of heparin, and has rarely been found to be deficient in patients. Platelet Factor 5 is probably fibrinogen, which has been discussed above. Platelet Factor 6 represents a large proportion of the plasma antifibrinolytic activity, loosely bound to the platelet surface. Platelet Factor 7 is plasma factor VII (proconvertin) adsorbed to the extent of about 2.1 per cent.

Platelet Cofactor I is very tightly bound AHF, adsorbed from plasma. Platelet Cofactor II is a derivative of Autoprothrombin II which is adsorbed PTC. Autoprothrombin I is adsorbed plasma factor VII; its differentiation from Platelet Factor 7 is unknown.

The other factors listed above have been discussed previously. Additional endogenous factors known to be adsorbed on platelets are Stuart Factor (plasma factor X), certain glycolytic enzymes, blood group antigens, certain phospholipids in uremic states, and some atypical proteins found in the dysproteinemias. Adsorbable exogenous factors include some bacteria and viruses, dextran, and India ink. Fibrinolysin and antithrombin are not adsorbed by platelets, though the latter may be an intracellular component.

It is readily seen that many of the activities heretofore ascribed to platelets are in reality those of the plasma clotting factors, and not entitled to specific unique association with the platelet. Figure 1, modified from Seegers³⁷, presents the current concept of the role of the platelet in the conversion of prothrombin to thrombin, the major function of the platelet in coagulation, giving it the appearance of possessing thromboplastic activity. This involves the recognition of a diversity of similar but nonidentical prothrombin derivatives as intermediates; it will be

noted that Platelet Factor 3 is the most important factor derived from platelets, and is involved in all three pathways of prothrombin conversion.

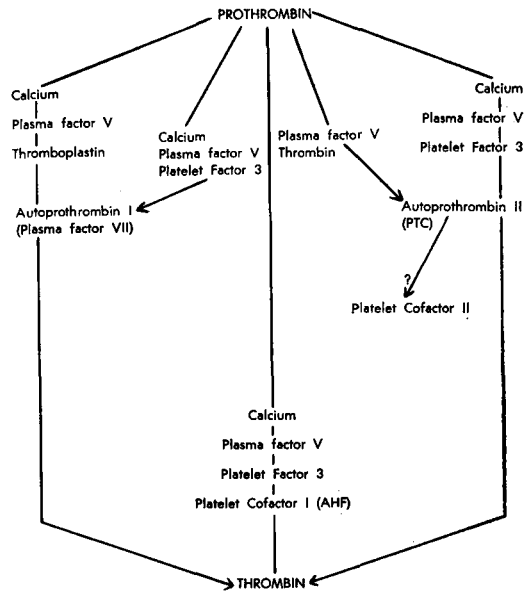


Figure 1 — Role of the Platelet in the Conversion of Prothrombin to Thrombin.

It is difficult for this writer to understand the origins or biological necessity for such an overlapping multiplicity of prothrombin conversion pathways. Future researches may well show that the basic mechanism is much less complicated, though the present scheme is by far the simplest and most rational to date.

Blood group antigens appear in platelets as well as in red blood cells^{93,94}, but they are in general of considerable less antigenicity than those of the red blood cells. Blood group antigens known to be found on platelets include the A₁A₂BO system, C and D of the Rh system, K, and Tj^a. Those known not to be present include P, Le^a, Le^b, Lu^b or MN systems, though platelets do possess, in common with red blood cells, an antigen which is related to and associated with the MN system.

Platelets also possess, or may possess, several antigens which are unrelated to the blood group antigens. One of these, known as Zw, is inherited as a Mendelian dominant and is present in 98 per cent of the Dutch

population⁹⁵; it has not yet been reported elsewhere. "Cold" and "warm" platelet agglutinins have been described, but they are of no known clinical significance. Non-agglutinating platelet antibodies have been described, and may be responsible for decreased platelet survival in a few patients; they are identifiable with an antiglobulin consumption test.

With this last exception, all of the foregoing platelet immunological phenomena play an extremely rare role in disease, if at all. The great and important group of platelet antibodies which do cause platelet dyscrasias will be discussed later in the course of this review.

(To be concluded)

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MODERN CONCEPTS OF THE PLATELET IN HEALTH AND DISEASE*

PART II

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CLINICAL PATHOLOGY

LABORATORY diagnosis and study of platelet dyscrasias is not difficult. Though new methods of determining the platelet count are published with alarming frequency, the procedure of choice at this time is that of Brecher et al.,^{96,97} which involves the enumeration of platelets in blood diluted with 1 per cent ammonium oxalate under the phase-contrast microscope. This method, with an error of 10-12 per cent in my experience, as well as of others,⁹⁷ shows the normal platelet count to be in the range of 150,000-450,000 per cubic millimeter. The values so obtained are higher than those found with the older indirect count, based on the number of red blood cells on the Wright-stained smear; the discrepancy is due to the lack of random distribution of the red blood cells on the smear, since most of these cells concentrate at the periphery rather than the center of the cover slip.⁹⁸ Gross estimation of the number of platelets on smear is not a useful clinical tool.

Other quantitative and qualitative methods of study of platelets are discussed and outlined in several recent volumes,^{97,99,100} and will not be detailed here. The *in vitro* methods for studying platelet function in our laboratory have recently been published,⁴³ and various modifications of the standard technics are published, with most of the clinical papers referred to in this review.

One new method, which, though it requires special apparatus, seems to be useful in the assessment of the thrombocytopenias, is the use of chromium⁵¹-labeled platelets in measuring platelet life-span.²³ This technic permits a reclassification of the platelet deficiencies on the basis of decreased production or increased destruction, but is most useful in describing the course of the diseases and evaluating treatment.

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Diseases of platelets have been the subject of much discussion and many schemes of classification over the past ten years. We are only now sorting them all out and studying their pathophysiology with a systematic approach. The outline to be followed here is perhaps oversimplified, but it should serve to tie together some of the widely scattered fragments of the literature. We will not be concerned with those diseases which, although they show purpura as a major manifestation, are not diseases of the platelet itself, or which do not affect the platelet, such as Henoch-Schönlein purpura.

THROMBOCYTOPENIAS

The thrombocytopenias, or quantitative defects of platelets, may be considered most conveniently as due either to production deficits or to increased peripheral destruction, or to combinations of these two mechanisms. The traditional separation into congenital and acquired thrombocytopenias does not emphasize the pathogenetic aspects to be of use clinically. It is of interest that in a recent study of 3,342 patients referred to one clinic for evaluation of their bleeding diatheses, thrombocytopenia was the most frequent coagulation defect found.¹⁰¹

Thrombocytopenia due to a primary production deficit has been attributed to many causes. These diseases are essentially amegakaryocytic; the bone marrow is hypoplastic, and the platelets show a normal life-span,²³ unless previous isoimmunization has occurred due to platelet or whole-blood transfusions.

There are many well-studied exogenous causes of bone-marrow hypoplasia.^{102,103} Those which regularly depress thrombopoiesis, in sufficient dosage, include whole-body irradiation, the oncolytic drugs and benzol. The latter two agents manifest their effects largely as aplastic anemia and are well described in various texts.

The effect of *irradiation* on platelets has been well studied by several investigators from different points of view. Purpura was

found to be one of the best clinical indications of severity of exposure to the gamma rays and neutrons of the 20-kiloton atomic bombs dropped in Japan in 1945.¹⁰⁴ The platelet count remained stable for three to four days after the explosions, and thereafter dropped rapidly to 0 in the second week, as the circulating platelets were depleted and not replaced by the bone marrow, the primary site of radiation damage. The platelet count remained extremely low for two to four weeks, during which time various hemorrhagic states appeared, characterized by increased capillary fragility, clotting time and bleeding time. Patients exposed to about 400 roentgens or less began to show improvement after two to five weeks; those exposed to higher doses failed to respond to any therapy. The same clinical pattern was found in victims of two major postwar nuclear accidents,¹⁰⁵⁻¹⁰⁷ although bone-marrow transfusion was effective in 4 patients who received estimated doses of 600 to 1000 rem. At the same time, increased levels of plasma factors V and VII were found, due not to a direct effect of the irradiation but secondary to the thrombocytopenia.

Irradiation of horse platelets in vitro has been shown to depress certain enzyme systems, resulting in decreased oxygen consumption by the platelets.¹⁰⁸ Disintegrated platelets are capable of correcting the coagulation deficit but not the bleeding of irradiated dogs; intact platelets are required to do both.¹⁰⁹ The total hemostatic defect produced by whole-body irradiation has been postulated to be caused by loss of the following platelet functions;¹¹⁰ ability to form hemostatic plugs, vasoconstrictor substance, interaction with AHF or other plasma factors, acceleration of prothrombin conversion, and clot retraction.

There are many agents which produce amegakaryocytic thrombocytopenia alone or as one manifestation of acquired aplastic anemia, occasionally: several antibiotics and antimicrobials (chloramphenicol, sulfonamides, streptomycin, the tetracyclines; arsenicals; para-aminosalicylic acid); the hydantoins; some of the antithyroid compounds; phenylbutazone; several minerals (arsenic, gold, bismuth, silver, mercury, lead); certain dyes and polishes (especially the aniline

dyes), insecticides, organic solvents and various nitrophenyl compounds.¹⁰² To track down the exact cause of the patient's disease, expert search must be made for all of these agents, and others, in his history.

Endogenous causes of platelet production deficits are more difficult to delineate. Thrombocytopenic purpura is a frequent first clinical manifestation of "congenital" *aplastic anemia*, or may present by itself. Defects apparent at birth include *Fanconi's syndrome*,¹¹¹ in which thrombocytopenia is associated with anemia, cutaneous pigmentation, microcephaly, mental retardation, retarded growth, testicular hypoplasia, and other congenital abnormalities. Other, probably related, congenital amegakaryocytic thrombocytopenias have been reported in association with other varieties of anatomical abnormalities,¹¹² especially absent radii. A maturation disorder of megakaryocytes, associated with panmyelopathy and erythroblastosis, has been reported on rare occasions, *Heggin's syndrome*.^{113,114} The platelets found in this disease show abortive dendritic forms, increased platelet volume, and defective structural metamorphosis. The thrombocytopenia of *DiGuglielmo's syndrome*^{115,116} is probably due to megakaryocytic deficiency.

A bizarre amegakaryocytic thrombocytopenia occasionally occurs in *heat stroke*.^{4,117} At body temperatures over 106° F., the megakaryocytes show damage and disappearance of the nuclei before cytoplasmic dissolution into platelets occurs (though fibrinolysis has been implicated as the direct cause of the thrombocytopenia more recently).¹¹⁸

Onyiai^{119,120} is a form of thrombocytopenic purpura, characterized by distinctive hemorrhagic bullae on the mucous membranes of the mouth and nose, occasionally progressing to exsanguination due to hemorrhage of the urinary tract, gastrointestinal tract and uterus. The disease is found predominantly among the Bantus, and usually runs a self-limiting course, although it may require repeated blood transfusions. Though megakaryocytes appear normal on smear, the disease appears to be one of platelet production, since there is no evidence of platelet malfunction, only of thrombocytopenia. The specific cure for the disease is lemon juice or vitamin C, but it is not yet known

whether the basic lesion involves increased renal ascorbic acid excretion, or perhaps an unknown toxic factor. Onyalai may be related to the bleeding defect seen in the more important vitamin C-deficiency disease, *scurvy*. A defect in Platelet Factor 3 has been postulated in *scurvy*,¹²¹ but a more recent investigation¹²² presents evidence for occasional thrombocytopenia, and suggests a possible connection between vitamin C and the folic acid system in hematopoiesis, with an imbalance of this relation in some cases of *scurvy*. In this connection, it may be noted that thrombocytopenia is infrequently associated with pernicious anemia, and bone-marrow aspiration reveals few megakaryocytes and rare thrombocytopoiesis; the condition is reversed with liver-extract therapy.^{114,123} The platelets that are formed are increased in volume and show decreased dendrite activity.^{114,124}

One physiological cause of depressed platelet levels is *menstruation*.¹²⁵ During the fourteen days prior to the period of a normal woman, the platelet count slowly decreases, and returns to normal rapidly after the onset of menses. It is not known whether this is due to changes in the hormones, the bone marrow or the reticuloendothelial system, but the change should be considered in evaluating the platelet count in women of the childbearing age group.

Thrombocytopenia due to predominant peripheral destruction includes the most puzzling of all platelet dyscrasias. There is still considerable controversy over some of these diseases, and the literature is full of conflicting and inconsistent data, but some general principles may be discerned, and more advanced investigative technics will eventually elucidate the various disease mechanisms. The basis for the classification of these thrombocytopenias is a decrease in *in vivo* life-span, with normal megakaryocyte activity.²³ A production deficit may be associated, but it is probably secondary to bone-marrow exhaustion following hyperplasia induced by the increased peripheral destruction.

The major disease in this category is so-called *idiopathic thrombocytopenic purpura*. We probably should abandon the first word of the name, since many definitely proven etiologies have been described, and in fact,

these various causes may all precipitate the same disease.

Idiopathic thrombocytopenic purpura has been differentiated into two clinical forms¹²⁶: an acute, self-limited thrombocytopenia which usually shows recovery within four months of onset, and a chronic form exhibiting remissions and frequent exacerbations, and showing decreased platelet levels (less than 50 per cent of normal) even during periods of clinical remission. In the differential diagnosis of these two forms of idiopathic thrombocytopenic purpura, the following points should be considered: the chronic form is more frequent in females and in older age groups, almost always presents with a past history of untoward bleeding, rarely shows any accompanying lymphocytosis or eosinophilia, more frequently gives a positive family history of bruising or thrombocytopenia, and about two-thirds of the patients will respond to splenectomy, the major form of therapy.

The cause of the platelet depression is generally considered to be immunological in origin, but the nature of the antibody is in dispute, and, in fact, several antibodies may be involved. Tullis^{127,128} has shown that the platelet antibodies of patients with idiopathic thrombocytopenic purpura are true antibodies, found in the gamma-globulin fraction of idiopathic thrombocytopenic purpura plasma; these antibodies are selectively removed by absorption with normal human platelets, and their electrophoretic pattern shows them to lie in the region of electrical neutrality. A platelet agglutinin is found in the beta₂-globulin fraction.¹²⁹ In the presence of complement, platelets that have been agglutinated by antisera undergo lysis.¹³⁰ Certain unrelated antigen-antibody complexes may be adsorbed on platelets, leading to their agglutination, and their lysis in capillaries, with release of serotonin and histamine.¹³⁰ The direct antiglobulin consumption test gives similar results and permits the demonstration of intracellular antibodies.^{131,132} The Coombs test may also be positive on platelets from patients with idiopathic thrombocytopenic purpura^{129,133} and may be positive with platelets from patients with hemolytic anemia but without thrombocytopenia.¹³⁴ It is of interest to note, in this connection, that in patients with active

red-blood-cell hemolysis due to deficient red-cell glucose-6-phosphate dehydrogenase, their platelets also show a deficit of the enzyme, but no thrombocytopenia.¹³⁵

Plasma from patients with idiopathic thrombocytopenic purpura reduces the platelet count of normal recipients.^{129,136,137} This property may remain after splenectomy, but a normal platelet count may also be maintained.¹³⁶

The problem of false-positive platelet antibody tests occurs but infrequently. Those found in patients with cirrhosis or congestive splenomegaly may actually be due to an unmasking of nonantibody cytotoxicity by the associated hypoalbuminemia.¹²⁸ Other false positives have been eliminated by the removal of calcium and barium sulfate-adsorbable serum factors.¹³⁸ A very recent study finds no evidence for any platelet antibodies in idiopathic thrombocytopenic purpura,¹³⁹ and suggests that the platelets contain a separate protein moiety which acts as an antigenic stimulus. An incomplete antibody has been shown in 50 per cent of platelet antibody serums.¹⁴⁰

The frequency of finding platelet antibodies varies.^{127,131-133,138} They can be demonstrated in 0 to 4 per cent of normal subjects, in 21 to 70 per cent of patients with idiopathic thrombocytopenic purpura, in 2 to 23 per cent of cases with secondary thrombocytopenic purpura, in 66 per cent of hypersplenic syndromes, and in 99 per cent of patients with systemic lupus erythematosus.

The bone marrow of patients with idiopathic thrombocytopenic purpura shows an increased number of megakaryocytes, predominantly young forms, with decreased granularity and platelet production; degenerative changes are seen in nucleus and cytoplasm.¹²³ Platelets that are formed are frequently large and bizarre, and have been shown to bind less serotonin than normally.^{123,141} This evidence, and other data discussed above, have suggested that idiopathic thrombocytopenic purpura attacks both the platelet and its parent cell.^{123,129}

The role of the spleen in idiopathic thrombocytopenic purpura is still open to question, though it probably does remove sensitized platelets, and perhaps produces a platelet agglutinin.¹³⁷ However, no differences were

found in the platelet counts of splenic-artery and splenic-vein blood in idiopathic thrombocytopenic purpura or any other condition investigated.¹⁴² It has been suggested that the spleen regulates the maturation and release of platelets, possibly under adrenal cortical influence, and explaining the efficacy of ACTH in some cases of idiopathic thrombocytopenic purpura.¹⁴³ Further evidence for this role of the spleen is the finding that after the injection of an aliquot of chromium⁵¹-labeled platelets, they do not appear in the circulation for several hours, indicating some sort of temporary sequestration.²³ A few cases of idiopathic thrombocytopenic purpura subjected to splenectomy have shown abnormal histiocytes, filled with phospholipids (largely sphingomyelin).^{144,145} This phenomenon may be due to reticuloendothelial phagocytosis of platelets, but apparently occurs so very rarely as to leave its etiology in considerable doubt.

Neonatal thrombocytopenic purpura¹³⁷ may be found in the infants of mothers with idiopathic thrombocytopenic purpura, as a result of the transplacental transfer of maternal platelet agglutinins. More rarely, it may be due to the development of isoagglutinins arising from fetal and maternal platelet incompatibility. Platelet antibody tests are positive in about two-thirds of these mothers and babies, but not always in both at the same time.¹²⁷

Idiopathic thrombocytopenic purpura has been found to be the forerunner of systemic lupus erythematosus in growing numbers.^{146,147} In one series of 51 splenectomized patients with idiopathic thrombocytopenic purpura at least 31.4 per cent developed other manifestations of systemic lupus erythematosus, and 15.7 per cent developed the full-blown syndrome. These patients are preponderantly females. It is, therefore, of the utmost importance to consider virtually every case of idiopathic thrombocytopenic purpura as a potential case of systemic lupus erythematosus, and to maintain long-term follow-up studies on all patients with idiopathic thrombocytopenic purpura. Though it has been thought in the past that other rheumatoid diseases occasionally exhibited thrombocytopenia, it is most likely that such cases were actually systemic lupus erythematosus which had not been fully diagnosed. The

finding of platelet antibodies in 99 per cent of patients with systemic lupus erythematosus¹³² may reinforce the autoantibody concept of the basis for idiopathic thrombocytopenic purpura. It should be mentioned here that no platelet abnormality has been reported in patients with the discoid form of lupus erythematosus.

Clinically, idiopathic thrombocytopenic purpura and most other forms of thrombocytopenia, as well as the qualitative defects of platelets, present with petechiae and ecchymoses. Hematomas, cerebral and gastrointestinal hemorrhage, and hemarthroses, are other frequent findings. Screening of the patient's coagulation status reveals increased bleeding time and capillary fragility, decreased prothrombin consumption and clot retraction, and normal clotting time. Prothrombin consumption and clot retraction are directly proportional to the platelet count.¹⁴⁸ Recent work¹⁴⁹ indicates that there is little correlation between the platelet count and the occurrence of purpura and hemorrhage. Instead, an obscure link between hemorrhagic phenomena and platelet thromboplastic factor, probably identical or similar to Platelet Factor 3, is postulated, with purpuric manifestations first occurring at platelet thromboplastic factor levels of 25 to 55 per cent of normal, and hemorrhage with less than 25 per cent of normal platelet thromboplastic factor levels. This work requires more extensive and carefully controlled confirmation.

It may turn out to be of interest in the study of the pathogenesis of idiopathic thrombocytopenic purpura, and of aplastic anemia with thrombocytopenia, that the leukocyte alkaline phosphatase levels are moderately low in these diseases.¹⁵⁰ Supranormal levels of this enzyme are obtained with ACTH and steroid therapy.

Besides the "idiopathic" form, there are several forms of thrombocytopenic purpura of known etiology. Many *drugs* induce immunological thrombocytopenia. The mechanisms were first elucidated for Sedormid (allyl-isopropyl-acetyl-carbamide).^{151,152} Sedormid combines with the platelets of "sensitive" patients to form an antigen complex; this antigen and its antibody induce platelet lysis in the presence of complement. A similar mechanism occurs with capillary endo-

thelium, so that the resulting hemorrhagic tendency is due not to the thrombocytopenia alone. Those patients who are sensitive to Sedormid are also sensitive to other chemically related compounds.

Similar evidence has been presented for thrombocytopenia due to quinidine hypersensitivity.¹⁵³ In this case, the antibodies attach to a platelet-quinidine complex, but complement is fixed between two antibody molecules only when both antibodies have exactly two (not one or three) quinidine molecules attached. This high degree of specificity led to the suggestion that the adsorbed antibody so changed the platelet membrane as to cause its removal from the circulation, by phagocytosis or other splenic action; this mechanism may be very like that which occurs in idiopathic thrombocytopenic purpura. Quinidine-induced thrombocytopenia¹⁵⁴ is an acute self-limiting disease with a good prognosis if the drug is stopped early in its course. The diagnosis may be suggested by the clinical appearance of hemorrhagic bullae on the oral mucosa.

Other drugs known to produce what are probably similar phenomena include certain barbiturates, pyrazolones, sulfonamides and arsenobenzols; quinine; chlorprophenpyridamine; digitoxin; PAS; and DDT. An even longer list has been incriminated at one time or another.¹⁰² One last common drug that is known to induce thrombocytopenia, but rather due to some undetermined type of megakaryocyte suppression, is prednisone in high dosage,¹⁵⁵ used for maintenance of patients with idiopathic thrombocytopenic purpura. In preliminary clinical trials, intravenous fat emulsions have produced several thrombocytopenic conditions^{156,157}; these are thought to be due to overloading of the antibody mechanism of the reticuloendothelial system with fat, with subsequent activation of a pathologic virus-platelet antigen complex.

A large group of *infectious diseases* has been implicated in thrombocytopenic purpura. Remission of the disease usually is accompanied by a return to normal platelet levels, unless hemorrhage intervenes. It may be seen in tuberculosis,¹⁵⁸ usually when there is marrow involvement by the invading organism. A fulminating purpura may occur after scarlet fever, and mild thrombocyto-

penia may occur with various streptococcal infections.¹⁵⁹ The anticoagulant effect of certain bacterial polysaccharides appears to be enhanced in the presence of platelet-deficient plasma, as in leukemia.¹⁶⁰

Viruses have been responsible for some instances of thrombocytopenic purpura and purpura fulminans. Among those viruses so far incriminated are varicella,^{161,162} mumps,¹⁶³ rubeola,¹⁶⁴ and rubella.^{165,166} In uncomplicated rubella, platelet counts were found to be low in most cases, and capillary fragility high, at the onset of the disease; these findings reversed during convalescence.¹⁶⁶ In most cases, a hypersensitive mechanism has been postulated. Influenza virus¹⁶⁷ is thought to be an antigenic link between platelets and red blood cells, inducing their mixed agglutination. Human bone-marrow pancytopenia has been produced experimentally with Venezuelan equine encephalitis virus, though the responsible mechanisms were not discussed.¹⁶⁸ Thrombocytopenia has been noted in one disease of possible viral origin, infectious mononucleosis, though it was ascribed to hypersplenism.^{169,170} Several cases of a syndrome of hemolysis, thrombocytopenia and renal disease have been reported^{171,172}; an allergic response to a viral infection, possibly of the lower intestinal tract, was postulated for some of these patients. It has been speculated¹⁶⁵ that if an immunological activity is responsible for these cases, with a virus-platelet complex acting as an antigen, may not many cases of "idiopathic" thrombocytopenic purpura be due, in reality, to a virus, especially following unrecognized infections? Though the long course of most thrombocytopenias argues against this hypothesis, it certainly warrants further careful, prospective investigation.

Thus, the nature of the platelet antibodies is still a mystery. It is not known whether they are different for each precipitating case, or whether they are homogeneous. The appearance of thrombocytopenia with viral diseases may not be due to any hypersensitive mechanism, but may represent some extreme form of the disease. In this regard, it may be remembered that in most of these cases, at least in those in which a virus is recoverable at all, the infecting agent cannot be isolated by the time clinical manifestations appear, presumably because by that time

the body's defenses have produced enough antibody to neutralize the invading virus, and yet this is the time when the thrombocytopenia appears. Does the virus enter the platelet and/or megakaryocytes and produce its effects intracellularly, and thus be outside the range of antibody action?

Finally, there is a question as to the origin of the platelet antibody. Current thought is that it is autoimmune in nature, but it cannot be proven by any technics now available and only Hashimoto's disease of the thyroid has so far fitted all criteria for autoimmune disease. The evidence so far presented in a few well-studied cases argues in favor of an isoantibody in response to a complex of platelet and exogenous agent, perhaps unidentifiable, such as a virus or drug. Antibodies similar to those of the blood groups cannot be ruled out, and neither can cross-reactivity between platelets and the foreign material. We can only hope that there will be less precipitous opinionating and more recourse to sophisticated technology in the answering of these questions. It should be remembered that currently available laboratory methods cannot elucidate any responsible mechanisms.

Aldrich's syndrome is a rare fatal disease manifested as thrombocytopenia, eczema and infection in infants.^{173,174} Platelet production is normal, as is the immune response. The basic condition seems to be a genetically determined liability to infection, with a secondary thrombocytopenia.

The remaining forms of thrombocytopenia due to increased peripheral removal are more mechanical in their pathogenesis. One rare entity is thrombocytopenia due to sequestration of the platelets within a giant hemangioma in infants.¹⁷⁵ The resulting purpura is reversed with x-irradiation to the tumor.

Thrombotic thrombocytopenic purpura is a bizarre syndrome which includes hemolytic anemia and neurological symptoms.¹⁷⁶⁻¹⁷⁹ Pathological studies show endothelial proliferation and platelet thrombi in terminal arterioles and capillaries. The disease is rapidly progressive and fatal and is usually diagnosed only at autopsy. It appears to be the result of a hypersensitive state of the red blood cells, platelets and endothelium, though no platelet agglutinins or red-blood-cell hemolysins have been demonstrated; a shortened platelet life-span has been shown. More recently, it has

been suggested that thrombotic thrombocytopenic purpura is pathogenetically similar to eclampsia, and that the thrombi are composed not of platelets but of fibrin.¹⁸⁰

The role of the platelets in *shock* has been explored to some extent. In experimental hemorrhagic shock in the rabbit,¹⁸¹ three phases are noted: an initial hypercoagulable stage, with increased platelet counts and thromboplastin generation and a rapid decrease in fibrinogen; this is followed by abnormally low platelet levels, fibrinogen concentration and plasma coagulation factors; the final stage is a return to normal levels of these parameters if the bleeding is stopped. Widespread intravascular clotting was seen histologically, possibly around foci of platelet microthrombi. Studies on anaphylactic and peptone shock¹⁸² in the rabbit reveal that the degree of thrombocytopenia is a constant indication of antigen sensitization and severity of shock. At the same time, there is a correlation between the disintegration of white blood cells and platelets and the liberation of histamine. The intracellular reaction between antigen and antibody apparently results in the activation of a proteolytic enzyme, probably trypsin, which releases histamine bound to intracellular protein. The disintegrated platelets appear to clump and, with the added influence of histamine, obstruct the small blood vessels. However, the hemorrhage seen in thrombocytopenic shock is not due to the fall in circulating platelets, but to products of their disintegration. These mechanisms may not operate in man, whose platelets contain less histamine than those of rabbits, but they nevertheless provide a model for future investigation.

Surgery and some of its special technics have measurable effects on human platelet levels. A 10 per cent increase in platelet count is found immediately postoperatively in nontransfused normothermic patients.¹⁸³ The platelet count returns to preoperative levels in about three days. Marked thrombocytopenia is usually seen in operations under hypothermic conditions at 25° C. or below, associated with increased clotting time and decreased prothrombin consumption.¹⁸⁴ *Extracorporeal circulation* technics destroy large amounts of both donor and recipient platelets.¹⁸⁵⁻¹⁸⁷ The abnormal bleeding observed after some of these operations does not seem

to be due to the thrombocytopenia so much as to the hypercoagulability and subsequent intravascular clotting due to platelet disruption. As a result, plasma coagulation factors are depleted and increased fibrinolysis occurs, leading to a hemorrhagic diathesis. Platelet Factor 4 deficiency may be involved as well.

Thrombocytopenia and bleeding are frequently induced by exchange transfusion or the *massive transfusion* of banked blood stored for more than one day.¹⁸⁸⁻¹⁹⁰ This effect is enhanced in patients with pre-existing coagulation abnormalities, as in liver disease and hypersplenic syndromes, and is largely due to the inability of stored blood platelets to circulate. It is also possible that platelets are consumed during storage by slowly progressive thromboplastic activity which is not completely prevented by standard anticoagulating technics. All patients in whom massive transfusion is deemed probable, especially those with hepatic damage, should have preoperative studies of their platelet levels and prothrombin-complex factors performed, and fresh blood should be made available. *Transfusion reactions* have also been associated with thrombocytopenia,¹⁹¹ secondary to intravascular clotting and release of platelet thromboplastin.

THROMBOCYTOSIS

Increased numbers of platelets are also associated with bleeding dyscrasias.

*Hemorrhagic thrombocythemia*¹⁹²⁻¹⁹⁴ is a rare and unusual myeloproliferative disorder of the platelets, in which the severity of the bleeding manifestations is roughly proportional to the platelet count. The platelets are probably functionally normal, since they will correct the bleeding of a thrombocytopenic recipient. The platelets of patients afflicted with hemorrhagic thrombocythemia have been found to bind less serotonin than those of normal subjects, but this may be because the number of platelets in the body exceeds the available serotonin stores. In some patients, the spleen has been found to be absent or atrophic, but it has been enlarged in others. The hemorrhagic episodes have been ascribed to an anticoagulant effect of the excess platelets, inhibiting the thromboplastin phase of coagulation.

Polycythemia vera^{114,195-197} is probably a divergent form of myeloproliferative disorder. Platelets from polycythemic blood show a shortened life-span when infused in thrombocytopenic recipients, but any hemorrhagic manifestations in the recipients remain improved for several hours after the transfusion. It is now thought that the bleeding tendency seen in some polycythemic patients is most likely due to a qualitative defect of platelets, Platelet Factor 3 deficiency. Exaggerated structural metamorphosis is also seen. Bone-marrow depressants such as radioactive P³² and urethane seem to be of some value in the control of the disease, though periodic phlebotomy is the traditional regimen.

An interesting facet of the thrombocytotic syndromes is the appearance of hyperkalemia,¹⁹⁸ due to the release of potassium from the excess number of platelets. There is no increase in platelet potassium concentration. As yet, no signs or symptoms of hyperkalemia have been reported in these patients.

QUALITATIVE DEFECTS OF PLATELETS

These diseases have long been regarded as a group of esoteric and unexplained conditions, whether primary platelet dyscrasias or secondary to other diseases. In the last few years the pathophysiology has been determined for some of them, and important inroads have been made in others.

Thrombocytopathy, a purpuric condition characterized by increased bleeding time, normal clot retraction and platelet count, and defective prothrombin consumption and platelet thromboplastin generation test, is thought to be due to an inability of the platelets to release Platelet Factor 3 upon activation of the coagulation mechanism.^{114,199-201} There is no intrinsic deficit of Platelet Factor 3, since incubation of the patient's platelets in distilled water, or their subjection to sonic oscillation, permits their normal performance in the thromboplastin generation test, but the platelets show an increased osmotic resistance. Defective aggregation but excess structural metamorphosis are seen, and the chromomere, which contains Platelet Factor 3, is not released easily. There appears to be no defect in capillary structure. Anisocytosis of the platelets has been described,

and ascribed to an abnormal cell-wall lipoprotein. The disease produces a mild to moderate hemorrhagic diathesis, and is thought to be the result of non-sex-linked simple dominant genetic transmission. It has also been reported in association with AHF deficiency, as thrombocytopathia hemophilica.²⁰¹

Thrombocytoasthenia is a disease characterized by increased bleeding time, normal platelet count and Platelet Factor 3, and decreased clot retraction.^{199,201} Morphologically, the platelets show defective pseudopod formation and adhesiveness, with lack of hyalomere spreading. It is not known whether the disease arises spontaneously, or if it is due to a simple recessive genetic factor. In association with AHF or PTC deficiency, this condition is known as thrombocytoasthenia hemophilica, but it is further distinguished from the more frequent disease by normal clot retraction.²⁰¹

A combination of these two diseases has been described as thrombocytoasthenia thrombocytopathia, manifested by increased bleeding time, decreased clot retraction and Platelet Factor 3 release, and normal platelet count.²⁰¹ Morphologically, defective aggregation, adhesion, pseudopod formation and hyalomere spreading of the platelets are seen.

Macroglobulinemia frequently presents with a hemorrhagic diathesis. This has been shown to be due to the physicochemical activity of the abnormal globulin covering the platelet and preventing the release of Platelet Factor 3.^{202,203} There is no evidence for any anti-platelet immunological activity of the abnormal globulin; its inhibitory effect appears to be directly proportional to its molecular size. Dendrites are not formed by the affected platelets, presumably due to a restraining capacity of their protein covering. Screening tests will usually reveal increased bleeding time, normal clot retraction, decreased prothrombin and prothrombin consumption, and low levels of fibrinogen; circulating anticoagulants may have to be ruled out. There is evidence that dextran may act in much the same way in massive doses.²⁰⁴

The pathogenesis of bleeding in *leukemia* is not well understood. Current evidence^{114,149,205-208} fails to demonstrate any consistent deficiencies that can cause bleed-

ing. Thrombocytopenia and deficient plasma Factor V levels are most frequently found, but they do not correlate with the hemorrhagic tendency. Platelet thromboplastic function is frequently decreased, as is serum serotonin (i.e., either platelet serotonin or platelet release of serotonin). Clinical bleeding rarely occurs at platelet levels over 50,000 per cubic millimeter, but it may, and bleeding does not always occur at even lower platelet counts. Structural metamorphosis may not occur, and platelets may be swollen and without dendrites. Thus it seems that the overall functional hemostatic activity of the platelet is involved in leukemic hemorrhage, and probably includes vascular factors as well.

In other malignancies, thrombocytopenia is occasionally seen secondary to bone-marrow invasion by the tumor cell. However, functional defects in the platelet may also be detected on rare occasions.

Uremia is occasionally accompanied by a bleeding abnormality. The hemorrhagic tendency has been ascribed and denied to many mechanisms,^{64,209-215} and probably no one individual mechanism is responsible in all cases. Roughly half of all bleeding uremic patients exhibit mild to moderate thrombocytopenia. Some of the rest show normal platelet counts but definite thrombocytopathies have been demonstrated in them, including deficiencies of Platelet Factors 3 and 4. Deficiencies in some of the plasma coagulation factors have been found in a few patients. Platelet serotonin is frequently low. Capillary fragility is occasionally observed. Clotting and bleeding times may be prolonged, but clot retraction is usually normal. No morphologic abnormalities have been described in the platelets, and thrombopoiesis appears to be normal. Platelet phosphatides are normal. In these studies no correlation between blood urea nitrogen or nonprotein nitrogen and bleeding was found, and urea was found not to be the major factor. There was some degree of correlation between bleeding and duration of anuria in acute renal failure, but not in long-standing chronic disease. In the face of this evidence, we can only accept a postulate of multiple qualitative and quantitative defects of unknown origin at this time.

Liver disease is frequently accompanied by platelet defects.^{91,209,216} Mild thrombocyto-

penia has been reported, but a defect in platelet thromboplastic activity (Platelet Factor 3?) has been found most often, usually associated with decreased plasma coagulation factors. Platelet Factor 4 has also been found decreased.

THERAPY OF PLATELET DISORDERS

The management of the diseases discussed in the previous section of this review presents some of the most challenging problems in modern medicine. It must be emphasized at the beginning that there is no standardized regimen for any of these cases, but most require subtle manipulation of existing techniques. In addition, we are gradually building up an armamentarium of new agents that show promise for the near future.

Not all of the platelet diseases are amenable to treatment by their nature. The major treatable diseases, which numerically outrank all the others, are the thrombocytopenias, and these are the main topic of the remainder of this review. The primary qualitative defects are largely untreatable, but are also frequently mild and not life-threatening. In such patients, replacement and supportive therapy are usually beneficial in hemorrhagic crises. Treatment of the underlying cause of secondary qualitative and quantitative defects will usually correct the hemorrhagic tendencies. The thrombocytoses show remission following phlebotomy.

The generally accepted critical platelet level is 50,000 per cubic millimeter; bleeding is more likely to occur at levels of less than 10,000 per cubic millimeter, and does not occur with more than 100,000 platelets per cubic millimeter in the absence of qualitative platelet or other coagulation factor defects (though a few patients undergoing major surgery will bleed with 150,000 platelets per cubic millimeter, largely due to associated plasma protein defects).²¹⁷ Clinically, we should like to maintain levels of at least about 100,000 platelets per cubic millimeter.

Bone-marrow transfusion has become the treatment of choice for irradiation injury syndromes^{106,218} in the absence of other disease, and isologous grafts appear to perform the entire hematopoietic function for a month or so until the patient's own marrow recovers. Autologous, homologous and isologous bone-marrow transfusions have been used

with varying success in producing temporary remission of leukemia following whole-body x-irradiation.²¹⁹⁻²²² The technic has also been used successfully in aplastic anemia²²³ and secondary thrombocytopenia of unknown origin.²²⁴

Whole-blood transfusion is useful in the management of mild qualitative and quantitative deficiencies of platelets. As noted earlier, it must be fresh in order to supply appropriate amounts of platelets. Five per cent of donor platelets are lost by collection alone into acid-citrate-dextrose solution in glass bottles or plastic bags, followed by a 2 to 3 per cent loss daily during storage for twenty-one days.²²⁵ No morphological changes were noted. However, despite this slow loss of the platelets themselves, the remaining platelets show a survival time inversely proportional to the length of storage of the blood,^{226,227} as determined by their ability to circulate in the recipient's bloodstream.

In an effort to study the platelet defects which develop during routine blood banking storage of whole blood anticoagulated with acid-citrate-dextrose and stored in plastic bags, with the idea of salvaging the platelets remaining after twenty-one days of storage at 4° C., the author has measured the major platelet factors and some metabolic aspects of fresh and stored platelets.⁴³ Platelet Factors 1 and 2 were severely diminished after twenty-one days; Platelet Factors 3 and 4 were normal; clot retraction decreased gradually until its complete disappearance in fourteen to twenty-one days; aerobic and anaerobic metabolism by the platelets was not significantly reduced at the end of the storage period. These results, along with those of others,²²⁸ indicate that from all parameters that can be studied conveniently, platelets in blood stored for more than one day should be effective in the control of hemostasis, or at least some aspects of it.

Platelet transfusions have been the subject of much study in recent years. They are desirable largely in the hope of avoiding over-transfusion with whole blood in patients whose major defect is primary or secondary thrombocytopenia.²²⁹ Fresh platelets, collected in acid-citrate-dextrose, separated by differential centrifugation from blood of compatible blood groups, and resuspended in the parent plasma, are given for many thrombo-

cytopenic emergencies. However, this method is cumbersome for most blood banks, and a method of preserving separated platelets alone is in great demand. It must be remembered that even fresh platelet transfusions are not always useful, as in idiopathic thrombocytopenic purpura, in which the infused platelets are destroyed soon after infusion. In these patients, platelet transfusion is valuable immediately after splenectomy.²³⁰ Refractoriness may develop to multiple platelet transfusions, with decreased platelet survival and perhaps development of antiplatelet substances in some cases.^{230,231} In one series, no platelet antibodies could be demonstrated after multiple hemostatically effective fresh platelet transfusions.²³²

Platelet preparations are usually evaluated by their ability to depress the appearance of red blood cells in the lymph of the cannulated thoracic duct of x-irradiated dogs,²³³ by measurement of their life-span,^{234,235} or by various in vitro technics for studying individual clotting factors. Of those platelet preparations which have been studied, the following have showed some indication of usefulness, but none has been used extensively or is likely to be in the near future: collected in plastic bags and EDTA,^{234,236} frozen in parent plasma,²³⁷ and frozen in EDTA and/or glycerol.^{235,238} Lyophilized (freeze-dried) platelet material,²³⁹⁻²⁴¹ and platelets preserved in gelatin at 4° C.,²⁴² have shown most promise clinically. A cephalin complex has been reported to produce favorable changes in patients with idiopathic thrombocytopenic purpura.²⁴³ Exchange transfusion may be of value in congenital thrombocytopenic purpura with platelet antibodies, manifested in infants.²⁴⁴

The *management of idiopathic thrombocytopenic purpura* is only occasionally difficult. Each patient requires individually tailored therapy, but if properly thought out and executed permanent remissions are frequently obtained.

Platelet transfusions, including whole-blood transfusions, may be useful in acute hemorrhagic crises of idiopathic thrombocytopenic purpura, and in preparation for surgery, but they are wasted in long-term treatment, for the reasons discussed above. They are most valuable in the amegakaryocytic thrombocytopenias.²⁴⁵

Splenectomy alone produces complete remission in 60 to 90 per cent of cases of idiopathic thrombocytopenic purpura in most large series,^{147,246-248} and is more likely to be efficacious if the patient has previously showed some response to steroids,²⁴⁸ though in these same series steroid therapy alone produced few complete remissions. The steroids most likely to be clinically useful are cortisone and prednisone, along with ACTH.²⁴⁹⁻²⁵² Care must be taken with prednisone, since in high doses it shows a thrombocytopenic effect.¹⁵⁵ The clinical effects of these drugs are usually due, however, to improvement of vascular fragility, and not to any direct effect on platelets. Steroids are definitely indicated in preparation for splenectomy, and may be useful in moderate nonsurgical cases. It should be remembered that children and adolescents are likely to undergo spontaneous remission, and should be followed carefully for six to twelve months before drastic treatment is instituted.²⁴⁶

Aplastic anemia has recently been treated successfully with testosterone in some cases.^{253,254} Even without complete remissions, the hormone permits the maintenance of satisfactory platelet levels. Previously, the only useful therapy has been repeated blood transfusions.

SUMMARY

The recent literature of the normal and abnormal structure and function of the platelet has been reviewed, with emphasis on the pathophysiology of the platelet disorders. There are still many questions to be answered. One of the most pressing is the nature of the antibody reaction in idiopathic thrombocytopenic purpura; though the evidence suggests that an isoantibody to a platelet-foreign substance complex is responsible, other forms must be ruled out. The role of serotonin in platelet physiology remains to be elucidated, and practical methods of platelet preservation and transfusion remain undiscovered at this time.

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