Pathogenesis of Thrombosis: Platelet Contribution

Several different platelet agglutinating agents may share a pathway in producing white thrombi.

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The white thrombus, composed predominantly of platelets, forms at the point of initiation of most thrombi in the smallest to the largest vessels of the body. Thrombus formation can lead to infarction of tissue, yet it plays a major role in hemostasis. These facts have been known since the studies of Virchow, Eberth and Schimmelbusch, Bizzozero, and others in the latter half of the last century. Despite the advances made by the late-19th-century investigators, the field of thrombosis research stood nearly dormant for the first half of the 20th century. It was not until after World War II that the development of microcinephotography, quantitative platelet agglutination technique, phase-contrast and electron microscopy, differential centrifugation procedures, silicone-coated surfaces, and microbiochemical techniques permitted further elucidation of the role of the platelet in blood coagulation and thrombosis. Today thrombosis is a major cause of morbidity and mortality. Platelets, despite their importance in thrombosis, have received relatively little attention as compared with the general field of blood coagulation. It was with these facts in mind that the National Research Council's Subcommittee on Thrombosis and Hemorrhage organized its Conference on Pathogenesis of Thrombosis—Platelet Contribution, which was held at the National Academy of Sciences in Washington, D.C., 25 and 26 May 1961. The purpose of the conference was to achieve a critical evaluation of the current status of our knowledge of the contribution of platelets to the pathogenesis of thrombosis, with emphasis on potential areas of research.

Basically, two types of thrombi which develop in flowing blood were discussed. The first type of thrombus begins in vessels as a white thrombus composed of platelets, leukocytes, and fibrin. The thrombus may propagate along a vessel as a softer red thrombus. This type of thrombus often produces infarcts in the body locally, or it may be carried as an embolus to a distant part of the body. Some people believe that this type of thrombosis leads to the vascular lesions characteristic of atherosclerosis. Prevention of thrombosis in arteries and veins is a major goal of modern therapy. However, it is generally felt that in order to improve antithrombotic drugs, the pathogenesis of thrombosis must first be better understood.

The second general type of thrombus discussed at the conference is the so-called hemostatic thrombus. It, too, is a white thrombus. In contrast to the first type of thrombus, it performs a useful function. When injury to small vessels occurs, as from a laceration or a bruise, the hemostatic thrombus prevents excessive bleeding by forming in and around the injured vessels. Since an inability to form the hemostatic thrombus can be inimical to health and can even threaten life itself, therapeutic efforts are made to permit and promote the formation of these thrombi in patients who have inadequate hemostasis.

Methods of Study

The study of blood platelets is technically difficult. The platelet exists in vivo as a non-nucleated disk-shaped cell 2 to 3 microns in diameter, surrounded by the complex milieu of the blood plasma. These tiny formed elements may rapidly alter their size, shape, and biochemical composition in response to various environmental changes. The development of the morphologic alterations has been followed in vivo by means of microcinephotography. A study of these morphologic alterations has also been made in biopsy material. In general, the in vitro study of morphologic and biochemical platelet changes has been accomplished by the use of platelet-rich plasma or washed platelets. Both in vivo and in vitro studies appear necessary for a full understanding of so labile and complicated a formed element. Studies have been performed with the platelets of swine, rat, hamster, rabbit, dog, and man. Research has proceeded at different technical levels, ranging from detailed morphologic studies to varied biochemical analyses of the platelet and its plasmatic atmosphere. These different technical approaches appear to form a sound basis for future correlation of morphology, biochemistry, and function.

Platelet Morphology

There was general agreement at the conference that loss of endothelial integrity, formation of platelet pseudopods, and agglutination of platelets are among the first morphologically detectable changes which occur during the formation of white thrombi. Chandler used the light microscope to study these changes when platelet-rich plasma or whole blood was allowed to clot in a revolving plastic tube (the thrombogenerator). Henry used the light microscope to detect platelet aggregation which occurred after vascular injury. Berman used the phase-contrast microscope and microcinephotography to study the effect of a number of agents on the induction of platelet agglutination in the cheek pouch of the hamster. Borchgrevink reported the appearance under the light microscope of human hemostatic thrombi in skin biopsies. McKay used bacterial endotoxin to induce platelet agglutination in the rabbit and recorded the result with the light microscope. Mustard used the light microscope to show that the deposits formed on the walls of extracorporeal shunts were composed predominantly of platelets. Despite the diverse methods of inducing platelet agglutination, the early appearance of the platelet aggregates appears to be

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much the same whether these are observed with the light microscope or the phase microscope.

The study of platelet ultrastructure has further extended our knowledge of the morphologic changes that precede and follow platelet agglutination. Rodman described the initial steps in agglutination as a concentration of the granulomere particles in the center of the platelet, with subsequent appearance of pseudopods. These changes were followed by the aggregation of platelets into a mosaic. Fibrin was observed within the platelet aggregates, surrounding the aggregates, and in areas devoid of platelets. Agglutination was followed by partial disintegration of platelet membranes, with production of large aggregates with electron-opaque, granular centers surrounded by clear vesicles. This latter step probably corresponds to "fusion" of agglutinated platelets, as seen by light or phase-contrast microscopy. Cottier described the adhesion of individual platelets to endothelial cells of the irradiated rat pituitary. This early change was followed by formation of intravascular platelet aggregates which adhered primarily to bare areas of the vessel basement membrane.

It was evident as the conference progressed that the precise relationship between the observed morphologic changes and the complex biochemical interactions which initiate and maintain the processes causing platelet agglutination are largely unknown. Most studies have focused upon either the morphologic aspects or the chemical initiators of platelet agglutination. Knowledge of the interaction of physical and chemical forces during the transformation of isolated platelets to agglutinated masses of platelet material is of sufficient practical and theoretical importance to suggest that greater emphasis should be placed on correlative biochemical and morphologic studies in future research.

Platelet Agglutinating Agents

There are numerous agents which may induce platelet agglutination. Five of these agents were considered. Prothrombin and thrombocyte agglutinating factor occur normally in plasma but require activation. Adenosine diphosphate (ADP) is present in high concentration in erythrocytes and platelets. The two remaining agents, collagen fibers and endotoxin, come in contact with platelets only under pathologic conditions.

Thrombin. The induction of platelet agglutination by thrombin in the presence of certain necessary divalent cations is now a well-accepted fact. Mason reported that the concentrations of Mg^{++} and Ca^{++} necessary for the platelet agglutination reaction are well within the physiologic plasma levels of these cations. He pointed out that as little as 0.03 unit of thrombin per milliliter can induce rapid agglutination of washed platelets at physiologic concentrations in the semiquantitative macroscopic platelet agglutination test of Brinkhous *et al.* This concentration of thrombin clots fibrinogen only slowly. The realization that small amounts of thrombin, in the presence of specific cation, can induce platelet agglutination has enabled workers in this field to reinterpret many of the earlier, conflicting reports on the platelet agglutinating activity of various clotting-factor preparations. For example, the platelet agglutinating activity of crude Product I preparations is now attributed to thrombin contamination. At present it appears that, of the known coagulation factors, only thrombin has been clearly shown to induce platelet agglutination.

The mechanism by which thrombin induces platelet agglutination is as yet not clear. It is known that the platelet, even after many washings, still retains fibrinogen. Fibrinogen is a natural substrate for thrombin which, by catalyzing the splitting of fibrinopeptides from fibrinogen, permits the fibrin polymer to form. Jackson and Conley showed that mild trypsinization of platelets rendered them unagglutinable by thrombin-cation mixtures but did not prevent agglutination by antiplatelet antibodies. If the trypsinized platelets were incubated with plasma or a solution of fibrinogen, thrombin-cation mixtures would again induce agglutination of such platelets. Mason emphasized that thrombin can clot fibrinogen in the apparent absence of cations, but a significant concentration of cation is necessary for the thrombin-induced agglutination of platelets. The role of cations in platelet agglutination remains to be elucidated.

The action of thrombin on platelets produces changes in addition to simple aggregation. Zucker reported that thrombin brings about the release of serotonin and factor 3 from platelets. Thrombin causes "fusion" of agglutinated platelets, but perhaps only in a concentration higher than that required for simple clumping of platelets. It was suggested that the possible relationship between "fusion" and thrombin or fibrinogen concentration might explain some of the conflicting reports on the occurrence of this platelet alteration.

Thrombocyte agglutinating factor. This factor is a naturally occurring agent present in the plasma of several species, including man. It appears to be distinct from thrombin, but like thrombin it requires the presence of certain divalent cations for the induction of platelet agglutination. Although it may play a significant role in thrombosis and hemostasis in lower species, it does not appear to play such a role in man. It is interesting to note that thrombocyte agglutinating factor, despite its presence in human plasma, has no apparent action on human platelets but causes rapid agglutination of platelets in several other species. It may well be that its action on human platelets produces a result more subtle than gross agglutination.

Adenosine diphosphate. Adenosine diphosphate appears to be responsible for the platelet agglutinating activity of the "R factor" of Hellem, which he obtained from red blood cells. Adenosine diphosphate can induce agglutination of washed platelets as well as of platelets in platelet-rich plasma. It appears that, like thrombin, ADP requires a cation cofactor for its action on platelets. Zucker reported that ADP releases only a small part of the serotonin of platelets, that it does not release platelet factor 3, and that it does not produce "fusion" of agglutinated platelets. The mechanism by which ADP induces platelet agglutination is not known.

The role of ADP in thrombosis was discussed extensively. The release of ADP from injured red blood cells may explain the episodes of diffuse thrombosis associated with incompatible blood transfusion reactions. However, certain other hemolytic disorders do not appear to be associated with widespread thrombosis. Platelets contain appreciable quantities of ADP, and it has been suggested that the platelets themselves may liberate ADP, which then brings about their agglutination.

Collagen. Collagen fibers have been reported to furnish foci for the initiation of platelet agglutination. These fibers are often in close proximity to the endothelium of blood vessels. Hugues has reported observing masses of agglutinated platelets centered about

perivascular collagen fibers. Zucker, referring to the studies of Hugues, reported that when purified collagen fibers were added to citrated platelet-rich plasma, platelet aggregates rapidly appeared along the surfaces of the fibers. In this case platelet serotonin and factor 3 were not released, but "fusion" of the platelet aggregates occurred. Collagen fibers induce only weak agglutination of washed platelets. It is not known whether a cation is necessary for these reactions. Polybrene, a synthetic polysaccharide macromolecule, closely resembles collagen fibers in its action on platelets.

Endotoxins. McKay and Des Prez each described the platelet agglutinating activity of bacterial endotoxin. These two investigators used different endotoxin preparations. The induction of platelet agglutination by endotoxins is well illustrated by the generalized Shwartzman reaction, in which the intravenous injection of endotoxin into a previously sensitized recipient produces white thrombi primarily in the lungs, liver, and spleen but not in the kidney. If a second injection of endotoxin is given, thrombi are then formed in the kidney. McKay reported that injection of endotoxin was followed by a fall in platelet, leukocyte, and fibrinogen levels and a decrease in whole-blood clotting time. Apparently endotoxin in some way initiates in vivo thrombin production, with subsequent agglutination of platelets and thrombus formation. Special comment was made concerning the work of Lee and others, who have shown that if the sensitized recipient's reticuloendothelial system is first blocked by injection of denatured albumin, Thorotrast, or cortisone, the first injection of endotoxin produces thrombi in the kidney as well as in the lung, liver, and spleen. This was interpreted by various participants at the conference to mean that the reticuloendothelial system may play an important role in the prevention of intravascular thrombosis.

How does endotoxin initiate thrombus formation? There was no agreement on this question, but the possibility that the induction of platelet agglutination by endotoxin is mediated through thrombin formation was discussed. Des Prez showed that the addition of endotoxin alone to platelet-rich plasma produces platelet agglutination with subsequent "fusion," and release of platelet serotonin and factor 3. The same changes in platelet-rich plasma can be initiated by the ad-

dition of endotoxin-antibody complexes. A better understanding of the mechanism of action of endotoxin may be attained through future tests with plasmas deficient in antibodies to endotoxin. It appears that platelets, or some other source of thromboplastin, are necessary for the coagulant action of endotoxin. However, a direct action of endotoxin on platelets was not demonstrated. The importance of understanding these problems is apparent, since syndromes similar to the generalized Shwartzman reaction can be induced by vitamin E deficiency in pregnancy, by certain diets, or by injection of such diverse agents as tissue thromboplastin, placenta, trypsin, some snake venoms, and various types of organic and inorganic particulate matter.

Prevention of Thrombosis

The prevention of thrombosis has long been the dream of clinician and research worker alike. There is ample proof that coumarin derivatives lower the plasma levels of prothrombin and certain other procoagulants. Heparin not only inhibits the generation of thrombin but blocks the action of thrombin on fibrinogen. Recently, numerous questions concerning the efficacy of coumarin and heparin therapy in the prevention of thrombosis have prompted investigators to study the effects of these agents on platelet agglutination. Both Berman and Borchgrevink reported that neither coumarin agents nor heparin, in the usual therapeutic dose, prevent the formation of hemostatic thrombi. On the other hand, Mustard reported that heparin, but not coumarin derivatives, prevents the formation of thrombi in extracorporeal shunts.

Mason and Brinkhous reported that coumarin derivatives, when given in doses within the usual clinical dose range, do not abolish the in vitro generation of platelet agglutinating activity of plasma. Such hypoprothrombinemic plasma can still generate sufficient thrombin to induce rapid platelet agglutination. On the other hand, heparin over a wide range of concentrations not only inhibited the generation of thrombin in plasma but also blocked the agglutination of platelets by preformed thrombin. Zucker reported that heparin has little effect on the agglutination of platelets induced by collagen fibers. Perhaps a more thorough understanding of the sequence of reactions

leading to platelet agglutination and thrombus formation will permit the development of new anticoagulants. It was suggested that anticoagulants might be found with sufficient specificity of action to inhibit pathologic platelet agglutination but leave physiologic agglutination mechanisms intact.

There was one final subject of discussion: What causes platelets to adhere to one another when agglutination occurs? There are indications that several of the agents known to induce platelet agglutination may mediate their action through thrombin. The action of other agents, such as ADP, thrombocyte agglutinating factor, and collagen fibers, cannot at present be explained in this manner. Indeed, it has been suggested that thrombin merely causes the release of ADP from platelets and that only then does platelet agglutination occur. Waugh proposed several possible mechanisms or models for platelet agglutination. In one model a platelet agglutinating agent such as thrombin would act to produce hiatuses in the platelet membrane by removing a single molecular species. This would render the membrane unstable, and molecules from the interior of the platelet would be attracted to these hiatuses. These molecules, since they differ from the molecular species originally present, would be unable to stabilize the membrane and would simply continue to congregate at the membrane surface. Eventually this process would lead to the formation of pseudopods, one of the earliest morphologic changes observed in the agglutination process. In another model a platelet agglutinating agent would act upon the platelet membrane either to remove or to rearrange structurally certain molecules. In this manner new electrical charges would be uncovered which could participate in binding platelets together. It is conceivable that these two processes operate simultaneously. Other molecules, released perhaps from the interior of the platelet, along with cations from the plasma, could form the bridges which would link adjacent membranes together. At present all of this is only educated speculation, but herein lies the challenge.

Note

1. Special presentations at the conference were made by H. J. Berman, C. F. Borchgrevink, K. M. Brinkhous, R. K. Cannan, A. B. Chandler, H. Cottier, R. M. Des Prez, R. L. Henry, D. P. Jackson, R. G. Mason, Jr., D. G. McKay, J. F. Mustard, N. F. Rodman, Jr., and M. B. Zucker.

Forthcoming Events

December

11–14. American **Documentation** Inst., Hollywood-by-the-Sea, Fla. (J. B. Kaiser, 1718 N St., NW, Washington 6)

12–14. American Soc. of **Agricultural Engineers**, Chicago, Ill. (J. L. Butt, P.O. Box 229, St. Joseph, Mich.)

17–20. International **Arms Control**, symp., Ann Arbor, Mich. (IACS, P.O. Box 1106, Ann Arbor)

17–21. University **Physics Teaching** Curricula, Laboratory Experiments, and Equipment in UNESCO member states, comparative survey, Paris, France. (UNESCO, Place de Fontenoy, Paris 7°)

26–31. American Assoc. for the **Advancement of Science**, annual, Philadelphia, Pa. (R. L. Taylor, AAAS, 1515 Massachusetts Ave., NW, Washington 5)

The following 40 organizations will meet in conjunction with the AAAS annual meeting in Philadelphia:

Academy of **Psychoanalysis**. (A. H. Rifkin, 125 E. 65 St., New York 21)

American Assoc. of **Clinical Chemists**. (P. Paubionsky, Abington Memorial Hospital, Abington, Pa.)

American **Astronautical** Soc. (J. G. Stephenson, Airborne Instruments Laboratory, Walt Whitman Rd., Melville, L.I., N.Y.)

American **Economic** Assoc. (H. F. Williamson, AEA, Northwestern Univ., Evanston, Ill.)

American **Geophysical** Union. (W. E. Smith, AGU, 1515 Massachusetts Ave., NW, Washington 5)

American **Meteorological** Soc. (F. Sergent, II, Dept. of Physiology, Univ. of Illinois, Urbana)

American **Nature Study** Soc. (J. A. Gustafson, Route #1, Homer, N.Y.)

American **Physiological** Soc. (R. E. Smith, School of Medicine, Univ. of California, Los Angeles)

American **Political Science** Assoc. (E. M. Kirkpatrick, APSA, 1726 Massachusetts Ave., NW, Washington, D.C.)

American **Psychiatric** Assoc. (M. Greenblatt, Massachusetts Mental Health Center, Boston)

American **Rocket** Soc. (B. Chifos, ARS, 500 Fifth Ave., New York 36)

American Soc. of **Criminology**. (J. Chwast, New York Inst. of Criminology, 115–117 W. 42 St., New York 36)

American Soc. of **Naturalists**. (W. K. Baker, Dept. of Zoology, Univ. of Chicago, Chicago 37, Ill.)

American Soc. of **Zoologists**. (R. L. Watterson, Dept. of Zoology, Univ. of Illinois, Urbana)

American **Sociological** Assoc. (B. Clark, Center for the Study of Higher Education, 2747 Bancroft Way, Berkeley 4, Calif.)

Association of American **Geographers**. (B. Burton, 213-1500 Arlington Blvd., Arlington, Va.)

Association for **Computing** Machinery. (B. Gilchrist, IBM Research Laboratory, Post Office Box 218, Yorktown Heights, N.Y.)

Biomedical **Information-Processing** Organization. (R. S. Ledley, Natl. Biomedical Research Foundation, Silver Spring, Md.)