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# present concepts

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*in Internal Medicine*

#2 = AD 740625  
VOL IV No 3 March 1971

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1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION	
Department of Medicine			
3. REPORT TITLE		2b. GROUP	
HEMATOLOGY SYMPOSIUM.			
Vol IV No 3, March 1971, PRESENT CONCEPTS IN INTERNAL MEDICINE* (See Item #11)			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)			
Symposium (Medical) March 1971			
5. AUTHOR(S) (First name, middle initial, last name)			
Culp, Neil W. (Guest Editor). <u>Contributors</u> : Cohen RJ, Spivack AR, McCracken JD, Logan LJ, Culp NW, Schoen RF.			
6. REPORT DATE		7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
March 1971		102 (Vol IV, pp 189-290)	263
8a. CONTRACT OR GRANT NO.		9a. ORIGINATOR'S REPORT NUMBER(S)	
N/A		N/A	
b. PROJECT NO.		9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
		N/A	
c.			
d.			
10. DISTRIBUTION STATEMENT			
Distribution of this document is unlimited.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY	
*Each issue of PRESENT CONCEPTS is a symposium in some specialty of medicine. There are 12 issues each year (per volume)		LETTERMAN GENERAL HOSPITAL, Presidio of San Francisco, Calif 94129	
13. ABSTRACT			
<p>This issue of <u>Present Concepts</u> is devoted to reviewing some of the areas in hematology which have been most strongly influenced by the advances of the past ten to fifteen years. Much of the issue deals with disorders of hemostasis, a subject that two decades ago was sometimes referred to as the "graveyard of hematology". Two contributions in this issue review important advances in our knowledge of platelet function and coagulation -- normal and pathological, and two other articles present the current thinking and findings on two of the most common hereditary abnormalities of hemostasis -- classical hemophilia and vonWillebrand's disease.</p> <p>By defining many hereditary erythrocyte defects <u>at the molecular level</u>, a rational classification of a number of hematological syndromes, (the etiologies of which were totally obscure before 1950) has been possible. More importantly, this definition of many inherited red cell abnormalities at the molecular level has served as a model for the geneticist and molecular biologist in their more basic quest for unlocking the secrets of human biology. The hemoglobinopathies and red cell G-6-PD deficiency represent classic examples of genetically determined molecular disease. G-6-PD deficiency, the most common of the recognized red cell enzyme deficiencies, is reviewed in this issue with emphasis on the spectrum of clinical manifestations encountered in affected subjects.</p> <p>The last of the six articles in the symposium is a review of the difficult subject of hypoplastic anemia.</p>			

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1 NOV 66REPLACES DD FORM 1473, 1 JAN 64, WHICH IS  
OBSOLETE FOR ARMY USE.

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Security Classification

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Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
HEMATOLOGY						
PLATELET						
HEMOSTASIS						
COAGULATION, Intravascular						
HEMOPHILIA A						
VON WILLEBRAND'S DISEASE						
G-6-PD, red cell deficiency						
ANEMIA, hypoplastic						

UNCLASSIFIED

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PRESENT CONCEPTS IN INTERNAL MEDICINE

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**"Blood is a very special juice."**

**JOHANN WOLFGANG von GOETHE (1749-1832)**  
*Faust*, Part I, Act I, Scene iv

HEMATOLOGY  
SYMPOSIUM

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**IMMUNIZATION REACTIONS**

**EXERCISE-INDUCED ASTHMA**

*and others*

## FOREWORD

The past two decades have witnessed unparalleled advances in medical science. Few fields of medicine have failed to share in this progress, but none has been more greatly influenced than the specialty of Hematology. During this period we have seen Hematology evolve from a discipline concerned principally with the number and quality of blood cells to one which has expanded to encompass biochemistry, genetics, and molecular biology as they apply to hematological disease. Progress has been greatest in the area of delineating "basic mechanisms of disease" and in advancing our understanding of the complex personalities of the red cell, the platelet, and the coagulation system.

This issue of *Present Concepts* is devoted to reviewing some of the areas in hematology which have been most strongly influenced by the advances of the past ten to fifteen years. Much of the issue deals with disorders of hemostasis, a subject that two decades ago was sometimes referred to as the "graveyard of hematology". In 1961 Tocantins said of coagulation and hemostasis: "This hardy perennial has flowered bravely in the last few years, though surrounded by predatory weeds which all but hid the brightly colored blossoms." The flowering has been even more profuse in the subsequent decade. Although far from complete, present knowledge of normal and pathological hemostasis has reached the point when a systematic approach to investigation of the patient with abnormal bleeding is possible and with reasonable assurance that the defective mechanism will be defined.

The contributions of Doctors Cohen and Spivack in this issue review important advances in our knowledge of platelet function and coagulation — normal and pathological. Doctors McCracken and Logan have brought us up to date on two of the most common hereditary abnormalities of hemostasis — classical hemophilia and vonWillebrand's disease.

By defining many hereditary erythrocyte defects *at the molecular level*, a rational classification of a number of hematological syndromes (the etiologies of which were totally obscure before 1950) has been possible. More importantly,

### *Foreword*

this definition of many inherited red cell abnormalities at the molecular level has served as a model for the geneticist and molecular biologist in their more basic quest for unlocking the secrets of human biology. The hemoglobinopathies and red cell G-6-PD deficiency represent classic examples of genetically determined molecular disease. G-6-PD deficiency, the most common of the recognized red cell enzyme deficiencies, is reviewed in this issue with emphasis on the spectrum of clinical manifestations encountered among affected subjects.

Doctor Schoen has reviewed the difficult subject of hypoplastic anemia — an etiologically diverse group of syndromes that continue to evade rational classification and specific therapy.

A number of other hematological topics might well have been included in an issue concerned with "advances" in the field. Obviously, no pretense is made of a comprehensive review. Subjects selected for this symposium reflect to some extent areas of interest of the authors. They were also judged, however, to be subjects with clinical relevance and therefore, hopefully, will be those topics of particular interest to our reading audience.

LTC NEIL W. CULP, MC  
*Guest Editor*

## THE BLOOD PLATELET Its Function and its Disorders

Richard J. Cohen, M.D.\*

One of the most outstanding developments in hematology within the past five years has been the recognition of the complex personality of the blood platelet, and the intricate and critical role this blood element plays in normal hemostasis. I would like to review with you some of our understanding of normal and abnormal platelet function and discuss some of the newer diagnostic procedures that are now available for the evaluation of suspected platelet disorders.

When a blood vessel is severed or damaged, a series of reactions is set in motion to rapidly stem the exit of blood from the vascular bed. The major event initiating this reaction is the exposure of collagen fibers which underlie the vascular endothelium. The platelet, migrating through the blood stream, is chemically attracted to these exposed fibers and the contact between collagen and platelet leads to important morphologic and biochemical changes within the platelet itself. The platelet alters its shape from a thin disc-like structure to a spiny sphere and the many granules contained within the platelet begin to disappear. At the same time, high concentrations of serotonin and adenosine diphosphate (ADP) begin to appear. Although the actual function of this serotonin is not fully known, there is some evidence to suggest that it leads to contraction of the severed vessel walls thereby reducing the size of the hole through which blood can leave the vessel. The ADP, in a poorly understood manner, serves as a potent stimulator of platelet adhesion to the vessel wall, as well as cohesion between the platelets themselves. One platelet sticking to another leads to the aggregation of many platelets. This results in the formation of a plug having the temporary ability to halt blood loss. During this stage, the platelets retain their morphologic identity and can be disaggregated to return as viable agents into the blood stream. Thrombin now begins to appear at the site of injury, stimulated at this early stage of hemostasis by

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*The Blood Platelet - Cohen*

the immediate release of tissue thromboplastin through the severed vessel walls. Thrombin causes newly arrived platelets to release rapidly their ADP and aggregate further on top of the already aggregated platelet mass. Under the influence of thrombin, in a process called viscous metamorphosis, the platelet plug now becomes a dense aggregate, irreversibly bound together with loss of identifiable individual platelet architecture. As more time passes, new thrombin becomes generated, stimulated through the biochemical events of normal intrinsic blood coagulation. Fibrinogen, floating in and about the platelet mass, is transformed by this thrombin into a network of fibrin strands -- serving effectively to reinforce the platelet mass and leading to a solid definitive plug. As a further protective device, normal platelets release the protein thrombasthenin which causes contraction of the platelet-fibrin mass and promotes additional consolidation and impermeability of the definitive hemostatic plug. In this manner, the body sufficiently seals small leaks in its vascular compartment in the normal individual.

The blood platelet possesses within its confines many amino acids, proteins and other chemical materials. The purpose of many of these is unclear. It is known that the platelet membrane supplies phospholipid necessary for effective blood coagulation. This function of the platelet has been called "platelet factor 3 activity" and further clarification of the nature of this action is required. It appears that a plasma factor is also necessary if all of these platelet reactions are to take place adequately. The absence of this factor appears responsible for many of the hemostatic abnormalities seen in von Willebrand's disease, and accordingly the plasma factor has been referred to as the "von Willebrand's factor".

The clinical result of a defect anywhere within this primary hemostatic mechanism is bleeding. The bleeding characteristically takes place in the skin or mucous membranes and produces the characteristic findings of petechiae, purpura or ecchymoses. It is also seen as a persistent ooze following trauma or surgery. The three groups of abnormalities involving the primary hemostatic mechanism that can eventuate in clinical bleeding include (1) thrombocytopenia, (a quantitative defect in platelet numbers), (2) platelet dysfunction (an acquired or congenital qualitative platelet defect), and (3) absence of the plasma factor necessary for

### *The Blood Platelet - Cohen*

normal platelet reactions to take place (the characteristic finding in von Willebrand's disease). Only the qualitative defects in platelet function will be discussed.

The terminology used to define platelet dysfunction has been, in the past, confusing and inadequate. Certainly the progress in recent years has allowed further definition of specific categories of abnormalities and it is probable that new and more useful terminology will gradually develop. Thrombocytopathy has been used to indicate a qualitative platelet defect in which platelet factor 3 activity is not available to the blood coagulation system. Conditions in which it is found as an acquired defect included myeloma, macroglobulinemia, and following extensive use of Dextran®. In these circumstances a coating of the platelet membrane by the protein or Dextran® prevents exposure of the platelet factor 3 activity to circulating coagulation proteins. In thrombopathia, platelet aggregation induced by collagen or thrombin is ineffective; however, the platelets appear to respond promptly and normally to exogenous ADP, suggesting that the disorder is due to impaired release of ADP from platelets. Thrombasthenia is viewed as a disorder in which ADP is normally released by stimuli, such as collagen and thrombin, but the platelet membrane does not react to this material and no aggregation occurs. Even the addition of normal exogenous ADP does not produce aggregation. It should be recognized that these terms, confusing and semantically inappropriate, were devised before the true intricacies of platelet physiology were appreciated.

A significant finding has been the recognition that a large number of commonly prescribed medications have the ability to interfere seriously with the collagen-platelet interaction. These include such drugs as aspirin, butazolidin, indomethacin, glyceryl guaiacolate and the vasodilator, dipyrimadole (Persantin®). Their effect produces a state of induced 'thrombopathia' where ADP release from platelets does not occur on exposure to collagen or thrombin. A state of defective primary hemostasis may then occur, and is manifested by an abnormal bleeding time and by excessive spontaneous or trauma-induced bleeding. Since the effect of

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these drugs has been shown to last as long as one week after ingestion it is possible that excessive surgical or post-operative bleeding may be the result of recent ingestion of any of these drugs. Many investigators have reasoned that the ability of these medications to impair platelet aggregation may have important therapeutic implications. The formation of a thrombus appears to depend to a large degree on platelet interaction and aggregation. Studies are currently underway throughout the world to see if the prophylactic administration of anti-aggregating agents will reduce the incidence of thrombosis in coronary artery, cerebrovascular, and peripheral vascular disease. Recent work by Murphy and Mustard has demonstrated that pretreatment with aspirin prevents the generalized Schwartzman reaction and disseminated intravascular coagulation in experimental animals. Additional reports showing that Persantin® lowered the incidence of thrombosis in prosthetic heart valves following cardiac surgery, and that intravenous aspirin produced remissions in thrombotic thrombocytopenic purpura suggests that this approach may have exciting applications. Certainly the literature over the next several years will contain much information relative to this subject.

In the evaluation of the bleeding patient in whom a primary hemostatic defect is suspected, the initial critical laboratory determination is the platelet count. The often neglected procedure of looking at the peripheral blood smear may yield quickly the diagnosis of thrombocytopenia but may also show large abnormal platelets such as those seen in several congenital platelet disorders. It should be stressed that the most important bedside test for the possible presence of an acquired or congenital platelet hemostatic defect remains the Ivy bleeding time. Through the standard skin puncture made with #11 scalpel blade, one creates an exposure of collagen to the blood platelet. By recording the time necessary for all oozing from the cut to cease, one has an excellent measure of whether or not all the hemostatic events have been able to take place adequately. Current evidence suggests that the sensitivity of the test is further enhanced by repeating the bleeding time two hours after the ingestion of 10 grains of aspirin. Whereas this drug will disturb only mildly the hemostatic sequence in normal individuals, those patients with a defect in primary hemostasis, irregardless of the cause, will develop marked

*The Blood Platelet - Cohen*

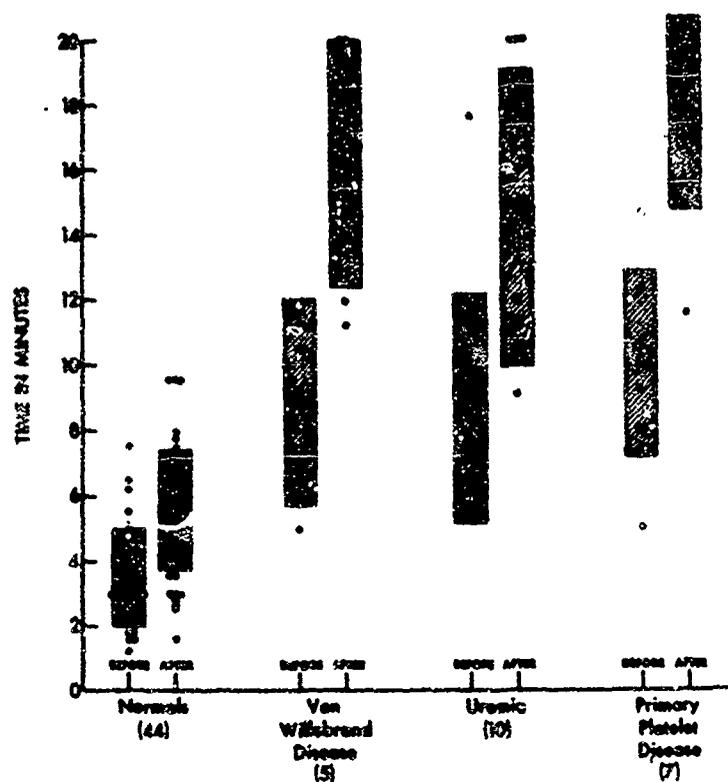


Fig 1. Bleeding time values (Ivy method) in normal and diseased states before and two hours following ingestion of 10 grains acetylsalicylic acid.

prolongation of bleeding time. Figure 1. This is helpful where the initial bleeding time is borderline or slightly elevated in a patient suspected of having a hemostatic abnormality. The Rumpel-Leede capillary fragility test, while simple enough to do, is much too variable and nonreproducible to be of significant value. Observation of clot retraction when carefully done in a 37 C waterbath gives useful information. The contractile protein, thrombasthenin, is necessary for this reaction and defective clot retraction either implies an insufficient quantity of thrombasthenin due to a low platelet count, as seen in severe thrombocytopenia, or a defect in the release of thrombasthenin as is seen in the congenital disorder, Glanzmann's thrombasthenia.

### *The Blood Platelet - Cohen*

The Salzman platelet adhesiveness test is a procedure which measures the capacity of platelets to adhere to glass beads under controlled conditions. The Salzman apparatus consists of a standard number of glass beads contained in a plastic tube. A platelet count is done before and after blood has been passed through this column of glass beads, the beads serving as a nidus to which the platelets may stick. With the loss of normal characteristics of platelet adhesion, the pre- and post-passage platelet counts are essentially similar. The major diagnostic use of this test has been as an aid in the recognition of von Willebrand's disease and of metabolic induced platelet dysfunction, such as that which occurs in uremia.

Perhaps the greatest boon to the detection of platelet dysfunction has come about with the use of the platelet aggregometer, an instrument for recording in vitro the capacity of platelets to aggregate. This technique is no longer to be considered an esoteric research procedure and should be understood and appropriately used. The apparatus is based on the principle that a light beam passing through plasma in which platelets are freely circulating will be deflected by contact with these platelets. If one then adds to this platelet rich plasma, materials known to affect platelet aggregation in vivo such as epinephrine, collagen, thrombin or ADP, aggregation of these platelets should take place in vitro if they retain normal function. As they begin to aggregate into clumps, the light beam can now pass through clear areas in the plasma without being deflected and can be recorded. This technique can then be effectively used to pinpoint the specific functional inability of a particular patient's platelets. The response of normal platelets in this in vitro system to the addition of ADP, collagen and epinephrine is illustrated in Figure 2. This is in contrast to the classic aspirin-induced platelet function abnormality that is illustrated in Figure 3. The platelet cannot apparently release its own ADP to enact the aggregating response and therefore, there is no aggregating response to collagen or epinephrine. However, the addition of exogenous ADP produces a prompt and normal response. It is provocative to think that such a response occurs in all of us after the ingestion of only one aspirin tablet and that this effect may remain present for as long as seven days after that one tablet is ingested. A similar type of defect can be seen to exist in Figure 4. The

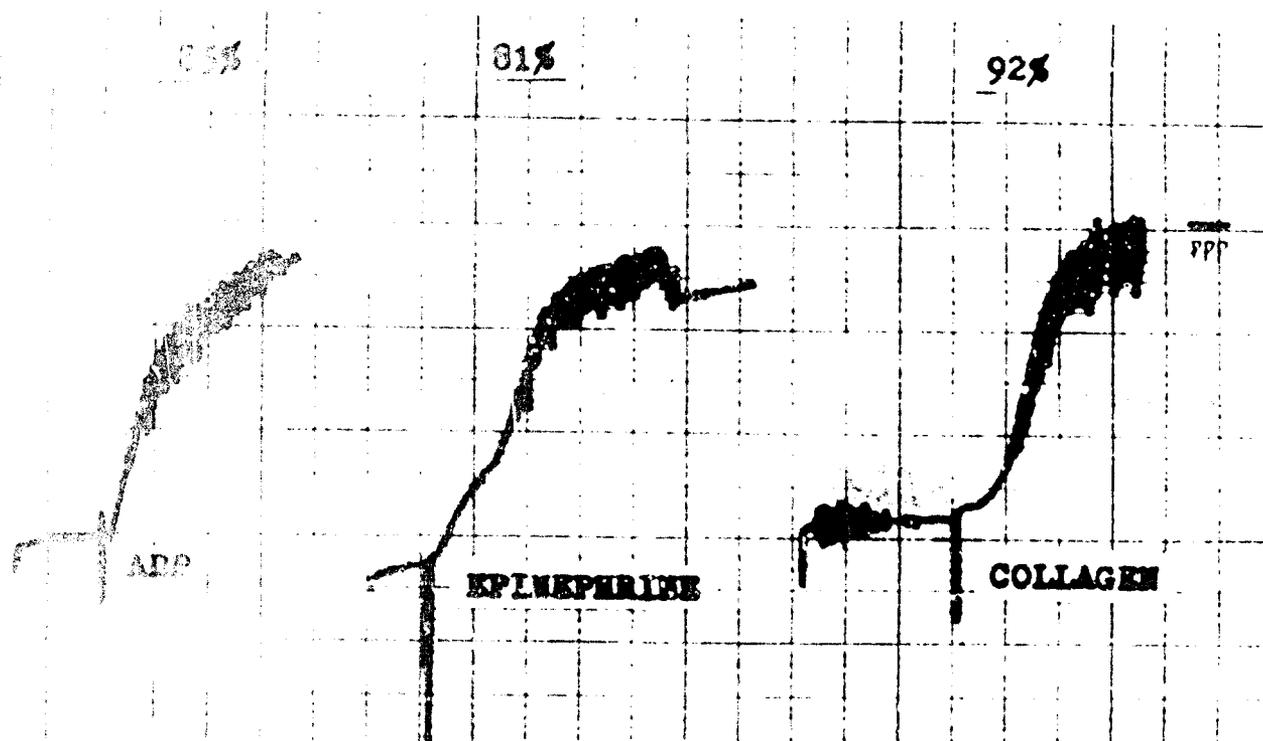
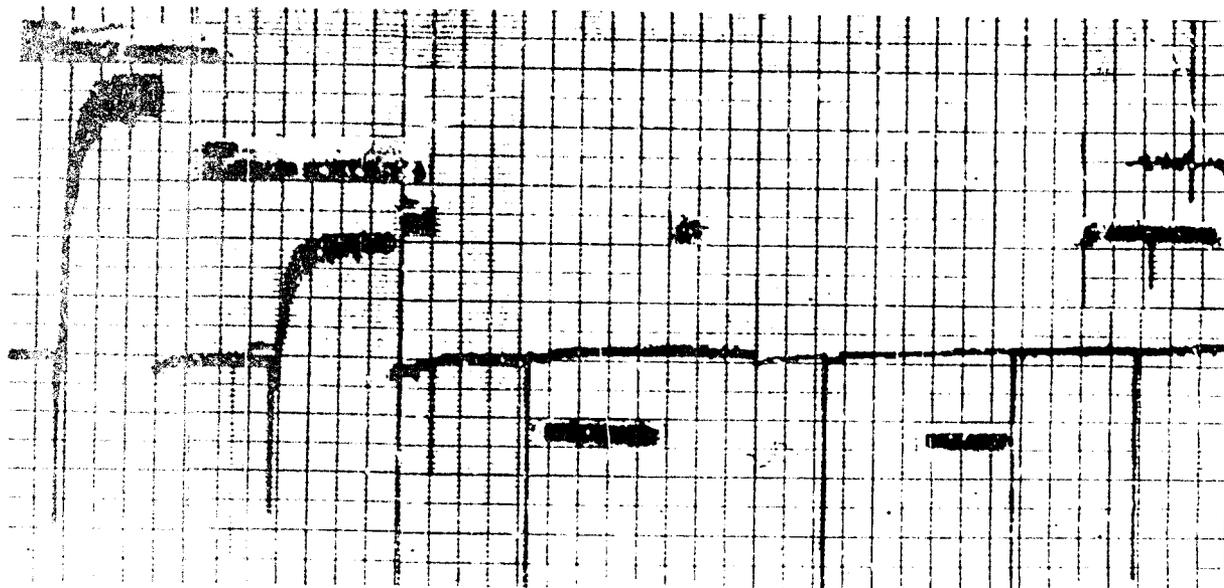


Fig. 2. (above) Aggregation of normal platelets in response to ADP, epinephrine and collagen.

Fig. 3. (below) Aggregation of platelets from a subject receiving acetylsalicylic acid demonstrating a normal response to exogenous ADP but failure of aggregation in response to epinephrine and collagen.



*The Blood Platelet - Cohen*

Individual had marked thrombocytosis and all of his platelets were rendered defective by his clinical state of severe uremia. Utilizing this technique a significant number of congenital bleeding problems have been characterized and further clarified. Subsequent research methods which may eventually find their way into more routine clinical use include measurement of nucleotides released from platelets during their aggregating response, measurement of specific platelet enzyme systems, and assay of cyclic AMP. The importance of these factors is just beginning to be recognized.

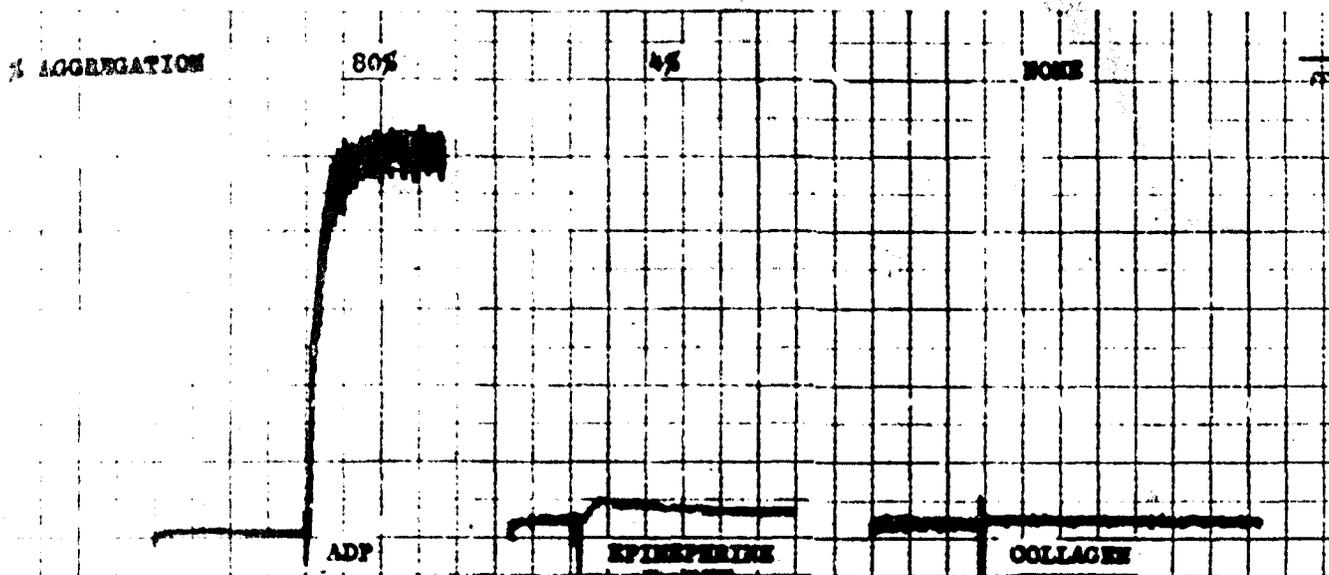


Fig. 4. Aggregation of platelets from a uremic subject demonstrating absence of aggregating response to epinephrine and collagen.

In conclusion, these advances in technique have permitted us to appreciate the multiplicity of splendid functions of the blood platelet. Certainly, more is yet to come. Whereas, until recently, the bleeding time was the only procedure available for testing for hemostatic dysfunction, these new methods have permitted more accurate categorization and more rational therapy of bleeding disorders. In addition, the recognition of the effect of commonly used drugs on platelet function has opened up a new and exciting era in therapeutics.

*The Blood Platelet - Cohen*

*Reading References*

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*It may seem curious that blood coagulation, which is after all a subsidiary and non-essential fragment of total bodily activity, should involve the multiplicity of factors illustrated, and a complexity which grows rather than resolves with increasing knowledge.*

- R.G. MACFARLANE, 1948

## HEMOSTASIS AND INTRAVASCULAR COAGULATION

### A Review

Alan R. Spivack, M.D.\*

Intravascular coagulation is now recognized as a frequently occurring entity associated with a number of diseases and in a number of clinical settings. When the syndrome is full-blown, there is little difficulty in making the diagnosis. However, all gradations of disseminated intravascular coagulation (DIC) exist /1/ and it is the recognition of this syndrome in its mild form that is most difficult. The syndrome must be recognized in its earliest stages if the physician is to be successful in its treatment. To do this, an understanding of the coagulation and fibrinolytic systems is necessary. One must also understand the various tests of coagulation and the interpretation of the data so that the proper treatment may be instituted without delay.

Normal hemostasis may be conveniently divided into three phases for study - (1) the platelet phase, (2) the fibrin formation phase, and (3) the fibrinolytic phase. Although each of these phases will be discussed separately, they are not isolated processes and probably all react simultaneously resulting in continuous hemostasis. Defects may occur in any one of the three phases. Multiphasic defects, as well as multiple defects within one phase, are also possible.

In order for blood to coagulate a number of events must occur. These criteria are as follows:

- - The coagulation mechanism must be intact.
- - A proper stimulus must be present to activate either the intrinsic or the extrinsic coagulation pathways (vide infra).

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*Hemostasis and Intravascular Coagulation - Spivack*

- - Stasis of blood is necessary to prevent the procoagulant materials from being rapidly diluted or washed away.
- - The hepatic or the reticuloendothelial clearance mechanism must be impaired in some way so that procoagulant materials are not removed from the circulation.

The last criterion need not be present for the normal response to bleeding from a traumatized vessel but it is necessary for the occurrence of pathologic coagulation such as one sees in the disseminated intravascular process. When these criteria are present coagulation may proceed either in normal response to injury or in the supercharged fashion that makes up the subject of this paper. The diagram (Figure 1) will be a basic graphic reference for each of the phases discussed.

**The Platelet Phase**

The initial stages of hemostasis are afforded by platelet function. When tissue is sufficiently traumatized so that vessels are injured and bleeding occurs a number of reactions follow. There is vasoconstriction which is initially a product of mechanical irritation and the bleeding is decreased. This reaction however is insufficient by itself to arrest hemorrhage./2,3/ Circulating platelets are then seen to adhere to the defect in the vessel, a reaction known as platelet adhesion and aggregation which seems to be mediated by exposure of circulating platelets to collagen fibers. That exposed collagenous fibers are necessary for this reaction to take place was shown by Hovig./4/ Ashford and Freiman /5/ further demonstrated that platelet adhesion to an injured area of small vessel did not take place unless the injury was sufficient to cause disruption of the endothelial layer and expose the underlying collagen fibers to the circulation. When this requirement is fulfilled, platelets swell, extend pseudopodia, degranulate and selectively release a number of products into the surrounding medium. This reaction is known as viscous metamorphosis. The products, e.g. serotonin, catecholamines,



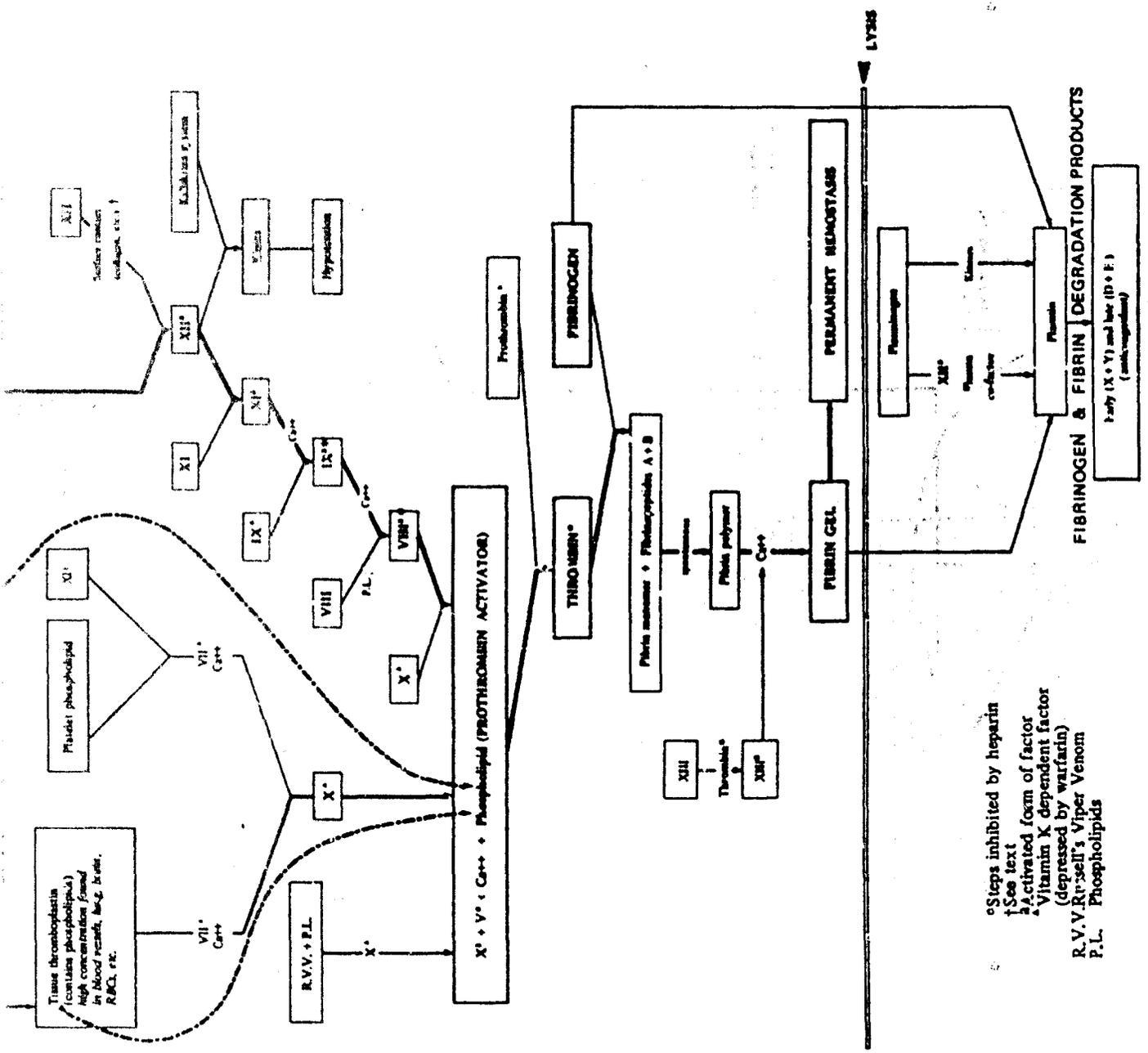
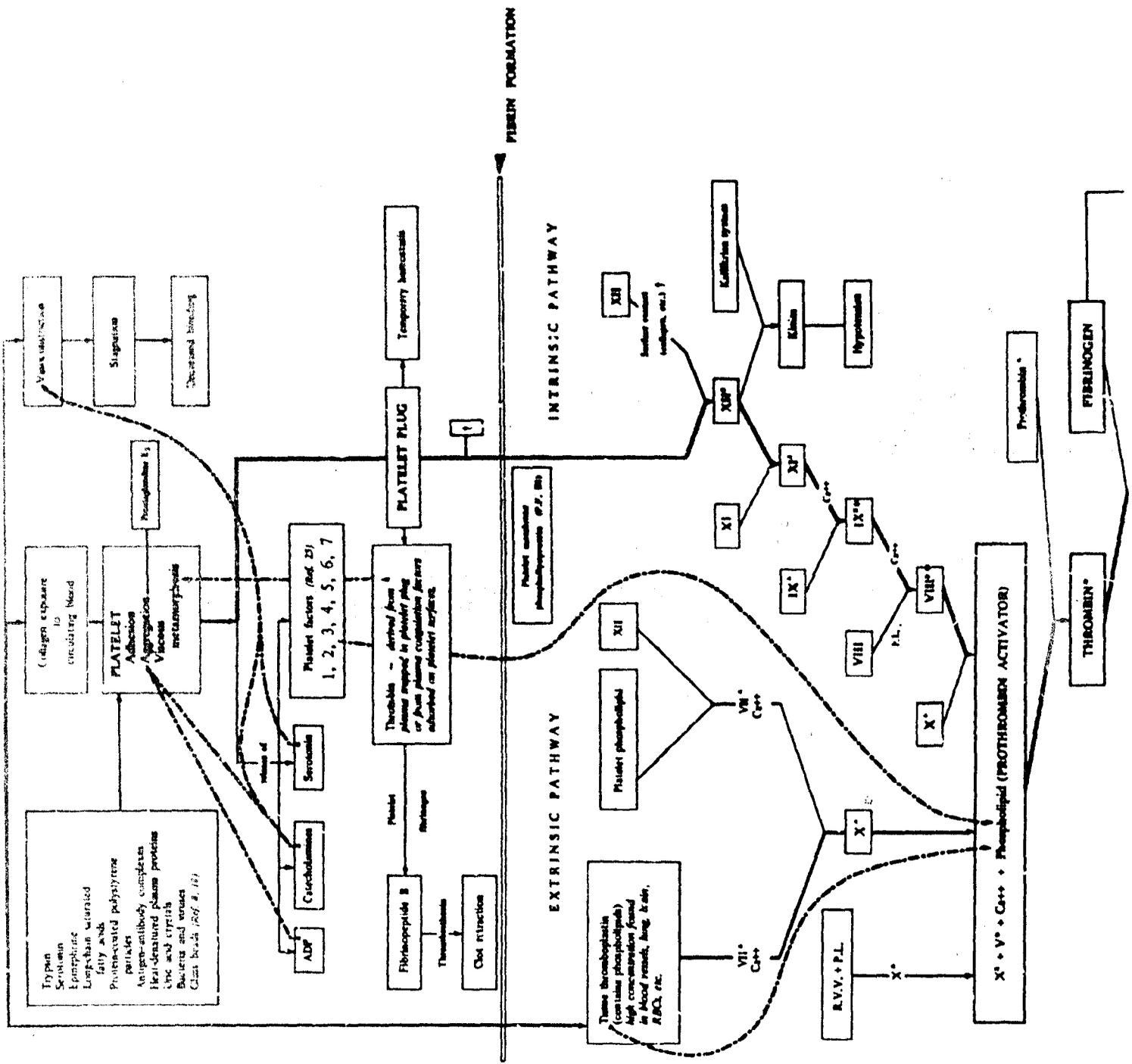


Figure 1.

Fig 1. - Spivack, Pres Con IV (3) Mar 71

TRAUMA - TISSUE INJURY



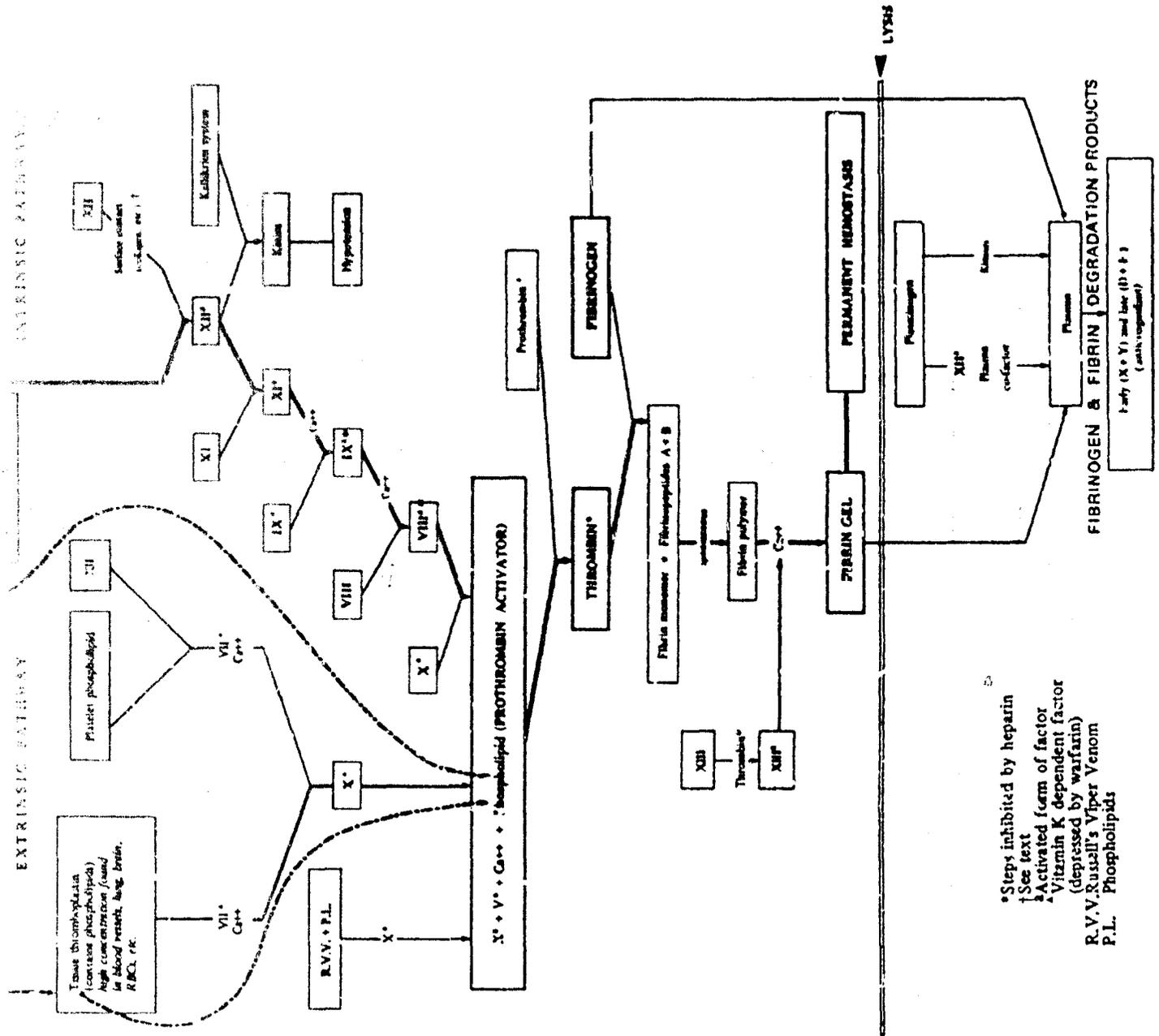


Fig 1. • Spivack. Pres Con IV (3) Mar 71

Figure 1.

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### *Hemostasis and Intravascular Coagulation - Spivack*

adenosine diphosphate (ADP), and platelet factor 4, cause further platelet aggregation mediated primarily by ADP and continued constriction of the injured blood vessel (catecholamine and serotonin). Platelet factor 4 is a highly basic, low molecular weight protein with anti-heparin activity which is also able to neutralize the anticoagulant effect of fibrinogen breakdown products./6/ The reaction of platelets to exposed collagen is reversible and requires no cofactors. ADP induced platelet aggregation is also reversible /7,8/ but requires the presence of calcium, fibrinogen and other plasma cofactors./9,10/ Although there are several hypotheses, the exact mechanism of platelet aggregation has not been fully explained./11/ Low concentrations of epinephrine, in addition to maintaining vasoconstriction and stasis, allows the ADP-platelet reactions to proceed more rapidly./12/ Serotonin release also causes platelet aggregation /8/ but its role in platelet metabolism and coagulation has not been elucidated. It does have potent vasoconstrictor ability and its function in the hemostatic process may be entirely in this capacity.

The result of the above processes is a platelet plug that fills in the vascular defect and bleeding ceases. Although this platelet plug is hemostatic, it is effective only temporarily. If it is not supported by intrinsic and extrinsic fibrin formation disintegration will occur secondary to the slightest mechanical stimulation and bleeding will again commence. This is the presently accepted explanation for the delayed bleeding or rebleeding phenomenon exhibited by injured hemophiliacs who may be asymptomatic until a few hours have elapsed after their traumatic episode./13/ In this case platelet plug formation is normally completed but the subsequent steps of fibrin formation which are necessary to solidify the friable platelet plug are defective.

The next phase of platelet hemostasis is mediated by the action of thrombin. Minute quantities of thrombin, probably derived from plasma trapped within the platelet plug or derived from clotting factors adsorbed on platelets /6/, cause consolidation of the platelet plug. In this phase platelet aggregates are made impermeable by the contraction of the platelet protein thrombosthenin./14/ This protein is similar in many respects to actomyosin found in skeletal muscle. It is speculated that thrombosthenin and fibrinopeptide B derived from the action of thrombin on platelet fibrinogen are responsible for clot retraction commonly observed in vitro as a crude test of platelet

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function./15,28/ Thrombin also induces viscous metamorphosis of platelets /16/ during which amines, nucleotides, potassium and amino acids are released./8/ Further ADP is released which acts synergistically with thrombin to perpetuate platelet aggregation./17/ This action of thrombin occurs without systemic fibrinogen proteolysis because only small amounts of thrombin are required to aggregate platelets, an amount less than that required to convert and polymerize fibrinogen./8/ The thrombin contained within the platelet plug is not accessible to the systemic circulation.

Collagen also has the ability to cause viscous metamorphosis. This reaction is limited to the platelets at the periphery of the plug in contact with collagen fibers whereas thrombin affects the entire aggregate./16/

In addition to ADP, collagen, and thrombin, a large number of other substances are able to elicit a similar platelet response. Figure 1. After reading Zucker's enumeration /18/ of these "other substances", one is immediately impressed by the host of stimuli to which the platelet is vulnerable. The relationship of prostaglandin  $E_2$  to the regulation of platelet aggregation is currently under study./19/ These findings have set the stage for a large amount of work on the platelet-aggregation reaction and on the various pharmacologic agents which are inhibitory./20-22/

Various platelet factors perhaps serve as a bridge between the platelet phase of coagulation and fibrin formation. Platelet factor 3, the best-known platelet factor, has the ability to act in the intrinsic coagulation pathway (vide infra)./8/ The existence of this factor as a distinct biochemical entity has been questioned. Marcus /8/ proposes that platelet factor 3 represents a unique platelet function mediated through phospholipoproteins contained, for the most part, in platelet membranes. Platelet membrane lipoprotein, therefore, will be subsequently referred to as platelet factor 3. This factor acts in the intrinsic coagulation pathway at two points. First, it aids in the conversion of Factor VIII to its active form and secondly, (along with calcium, Factor X and Factor V) platelet factor 3 forms the complex known as prothrombin activator (vide infra)./6/ Biggs et al /23/ have recognized another platelet factor that is active in the extrinsic system where platelet factor 3 is inert. They found that clotting times were shortened when incubated platelets

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were washed and added to coagulation mixtures of normal plasma and plasmas deficient in Factors XII, XI, IX, and VIII. This effect was less marked when the same platelets were added to plasmas deficient in Factors V, VII, and X. Apparently small amounts of Factor XII are necessary for this reaction to proceed but no other intrinsic factors are needed. They therefore concluded that platelets had the ability to elaborate a tissue factor similar to tissue thromboplastin that could initiate coagulation entirely through the extrinsic pathway. The significance of platelet tissue factor is minor because it is well known that Factor XII deficiency is not associated with a spontaneous bleeding tendency./24/

The other platelet factors have been enumerated by Bowie et al /25/ who recognize seven platelet factors. Platelet factor 1 has been shown to be coagulation Factor V. Platelet factor 2 and 4 are low molecular weight proteins. The former has been observed to shorten the time for fibrinogen conversion by thrombin. Platelet factor 4 is an antiheparin which also inhibits the action of fibrinogen breakdown products./6/ In addition to these factors, virtually all of the coagulation factors are adsorbed on the surface of the platelet. Fibrinogen, Factors VIII, XI, XII, and XIII are tightly bound, while the vitamin-K-dependent factors II, VII, IX, and X are easily dissociated by washing./6/

Platelets temporarily arrest hemorrhage and platelet function sets the stage for fibrin formation with resultant permanent hemostasis.

### *The Fibrin Formation Phase*

In 1957 Waaler /26/ offered a convenient way of studying the coagulation system. This concept employs two systems which usually, but not necessarily, react simultaneously. The common end point is prothrombin activator. Figure 1. The extrinsic and intrinsic pathways are discussed in detail in an excellent review by Williams./27/ The extrinsic system is dependent on tissue juices or tissue thromboplastin which is present in many tissues but particularly in blood vessel walls, lung, and brain. Calcium, Factor VII and tissue thromboplastin form a complex /29/ with the ability to convert Factor X to its active form. Activated Factor X, Factor V, calcium, and phospholipid (collectively referred to as prothrombin activator) will support the conversion of prothrombin to thrombin.

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The intrinsic system involves a number of reactions commonly referred to as the cascade or waterfall system of coagulation. This system is initiated by activation of Factor XII. A number of in vitro substances have the ability to activate this factor but the mechanisms involved, at the present time, are only postulated./30,31/ Skin /32/, ellagic acid /33,34/ and other electronegative, wetttable surfaces such as glass, diatomaceous earths, micelles of long chain fatty acids and collagen fibers /7,35,36/ all have this ability. To facilitate activation, Factor XII plus other coagulation factors are bound to platelets and Factor XII is activated by collagen contact when platelets adhere to these fibers exposed to circulating blood. Activated Factor XII enzymatically converts Factor XI to its active form./37/ In addition, activated Factor XII promotes the formation of kinins via activation of the kallikrein system./38/ Kinins are potent hypotensive agents with marked effect on splanchnic circulation. Activated Factor XII plus a plasma cofactor has recently been shown to convert plasminogen to plasmin./39/ Although a considerable amount is known about Factor XII, our knowledge is incomplete. For instance, Factor XII deficiency is not associated with a spontaneous hemorrhagic diathesis /24/ and how this factor is bypassed in intrinsic coagulation is still a matter of speculation.

Once activated, Factor XII enzymatically converts Factor XI to its active form./37/ Activated Factor XI activates Factor IX./40,41/ This reaction is calcium-dependent and is inhibited by heparin in low concentration./40/ If activated Factor IX is mixed with solutions of partially purified Factor VIII in the presence of calcium and phospholipid, coagulant activity evolves. Phospholipids are required /41-43/ and thrombin, in trace amounts, potentiates this reaction./44/ Heparin is a potent inhibitor of this step./42/ Factor VIII, when enzymatically activated by activated Factor IX, is able to convert Factor X to its active form in the presence of calcium as a cofactor./43,45,46/ When Factor X is activated it forms a complex with Factor V, calcium and phospholipid (prothrombin activator) and it is able to proteolytically split prothrombin to thrombin. Thrombin proteolytically digests fibrinogen and forms soluble fibrin monomer, plus two classes of peptides./47/ Fibrin monomer spontaneously polymerizes through hydrogen bonding to form fibrin polymer which is insoluble in isotonic media./48/ Fibrin stabilizing factor (Factor XIII) /49,50/ converted to its active form by thrombin converts fibrin polymer into a hemostatically competent

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fibrin gel which is insoluble in five molar urea. Heparin, in the presence of heparin cofactor, (antithrombin II) /51,52/ a plasma alpha globulin, is inhibitory to thrombin and thus prevents the thrombin induced fibrinogen reaction and the conversion of fibrin stabilizing factor to its active form./50/ The intrinsic and extrinsic pathways following activation of Factor X are identical. Figure 1. In the intrinsic system, the phospholipid is of platelet origin (platelet factor 3). Phospholipids acting in the extrinsic system are present as microsomal particles in tissue thromboplastin and biochemically function identically to platelet factor 3./27/ Russell's viper venom, an agent used for assaying Factor X and for evaluating platelet function, has the ability to activate Factor X directly without utilizing Factor VII./54/ When using this agent for estimating procoagulant activities, phospholipid must be added /27/ because it is not present in the venom and tissue thromboplastin is not used in the reaction mixture.

Factor VII is unique to the extrinsic system while Factors XII, XI, IX, and VIII are utilized only in the intrinsic system. Factors V, X, and II function in both systems as a final common pathway to the conversion of fibrinogen to fibrin gel.

Table I summarizes the coagulation factors.

TABLE I  
COAGULATION FACTORS

FACTORS Number	Name	PLACE OF SYNTHESIS	PLASMA HALF-LIFE	PRESENT IN BANK BLOOD
I	Fibrinogen	Liver	3-6 days	yes
II	Prothrombin	Liver	2½ days	yes
III	Tissue thromboplastin	...	...	...
IV	Calcium	...	...	...
V	Proaccelerin	Liver	Few hours	no
VI	...	...	...	...
VII	Proconvertin	Liver	5 hours	yes
VIII	Antihemophilic Factor or Globulin (AHF or AHG)	Reticuloendo- thelial system	6-12 hours	no
IX	Plasma Thromboplastin Component (PTC)	Liver	20-30 hours	yes
X	Stuart-Prower	Liver	2-3 days	yes
XI	Plasma Thromboplastin Antecedent (PTA)	?	60 hours*	yes
XII	Hageman Factor	?	?	yes
XIII	Fibrin Stabilizing Factor (FSF)	?	?	yes

\* According to Nossel HL et al: *Proc Soc Exp Biol Med* 115:896-897, 1964

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The commonly used tests to evaluate the various steps of the coagulation mechanism consist of the prothrombin time (PT) the partial thromboplastin time (PTT) and the thrombin time (TT).

The PT tests the extrinsic system and employs the addition of tissue extract and calcium to undiluted plasma./55/ This test primarily reflects the presence of Factors VII, X, and V and when prolonged any one or a combination of these factors may be deficient or an anticoagulant may be present. Also fibrinogen must be present in a concentration of at least 100mg/100cc.

The PTT evaluates the intrinsic system employing a mixture of kaolin (for surface activation) /56/ and cephalin (phospholipid brain extract),/57/ calcium and undiluted plasma. Factors XII, XI, IX, VIII, X and V are reflected and prolongation may result from deficiencies of any one or more of these factors. Prolongation of the PTT, like the PT, may be due to an anticoagulant or a fibrinogen concentration of less than 100mg/100cc.

The integrity of the fibrinogen to fibrin reaction is measured by the thrombin time. In this test thrombin is added to undiluted plasma. The thrombin time is the most sensitive test for hypofibrinogenemia or the presence of a circulating anticoagulant directed against thrombin or fibrinogen such as heparin or fibrinogen breakdown products./58/

Prothrombin deficiency may not be reflected by a prolonged PT or PTT because these tests are relatively insensitive to this factor. To be certain of prothrombin concentration an assay specific for factor II must be done.

When any of these coagulation tests are prolonged one must determine whether or not a deficiency state as opposed to the presence of a circulating anticoagulant exists. This can simply be done by mixing equal volumes of patient plasma with known normal plasma and repeating the abnormal test(s). If a deficiency state is present, the abnormal test should correct on mixing whereas the test would remain prolonged in the presence of a circulating anticoagulant.

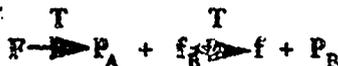
Tests of platelet function consist of the platelet count, the primary and secondary bleeding times, in vivo and in vitro

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tests of platelet adhesiveness, platelet aggregation, clot retraction, and evaluation of platelet factor 3 activity. These tests are discussed by Marcus /8/ and Ginsberg and Astor /6/.

#### The Fibrinolytic Phase

From the time thrombin is formed, the conversion of fibrinogen to fibrin proceeds at a rapid rate. The first step in thrombin induced proteolysis of fibrinogen results in the formation of fibrinopeptide A ( $P_A$ ) plus fibrinogen deprived of  $f_B$ .  $P_A$  plus  $f_B$  are further degraded by thrombin producing fibrin monomer ( $f$ ) plus fibrinopeptide B ( $P_B$ ). This reaction can be schematically diagrammed as follows. /59/



$F$  = fibrinogen;  $T$  = thrombin;  $f_B$  = fibrinogen deprived of fibrinopeptide A;  $f$  = fibrin monomer;  $P_A$  and  $P_B$  are fibrinopeptides.

The first breakdown product formed is  $f_B$  and it is able to polymerize and clot without further thrombin action. The recognition of this early degradation product in the circulation may provide useful information that the patient is developing intravascular coagulation. /59/

Ten milliliters of blood contain sufficient thrombin to clot 2,500 ml of plasma in ten seconds if all the prothrombin were converted. /60/ Thrombin therefore must be inhibited lest a small insignificant injury produce widespread devastating intravascular coagulation. A number of checks and balances exist in the sequence of events. Leykin et al /61/ showed that the perfused rabbit liver is able to remove activated Factor X from the circulation, thereby inactivating the final common pathway before thrombin formation occurs. The liver also has the ability to clear activated Factors XI and IX. /62/ In addition to the liver which inactivates, and a rapid circulation which dilutes and washes away the procoagulant material, there are a number of circulating antithrombins. As discussed by Soulier /63/ there are six antithrombins whose mechanisms of thrombin inactivation include adsorption and neutralization; the former is a rapid process while the latter takes place over a protracted period of time. /60/

There exists still another potent enzyme system which interferes with the fibrinogen-fibrin conversion which produces anticoagulant byproducts. Plasminogen, a circulating plasma globulin, is activated by highly specific proteolytic enzymes called plasminogen activators or kinases. The kinases probably open the arginyl-valine bond in plasminogen and expose the active enzyme center of plasmin./64/ Kinases are found in trace amounts in all body fluids but sharply increase when there is increased fibrinolytic activity in the blood. They are also found in urine (urokinase) and in most body tissues; and they are especially concentrated in lysosomal granules and in vascular endothelial cells./65/ Plasminogen activation also occurs in the presence of activated Factor XII plus a plasma cofactor./39/ The product of plasminogen activation is plasmin, a proteolytic enzyme similar to trypsin, capable of hydrolyzing a number of plasma proteins including coagulation Factors V and VIII./65/ Its action on fibrinogen and fibrin results in a number of breakdown products that differ from those produced by thrombin. The first reaction of fibrinogen subjected to plasmin is the release of two groups of polypeptides./66/ The first group are small polypeptides, soluble in trichloroacetic acid, and comprise about 30 percent of the total nitrogen derived from fibrinogen./66/ The second group are high molecular weight (HMW) fragments accounting for 70 percent of the total fibrinogen nitrogen./66/ It is these HMW fragments that have considerable importance in the hemorrhagic diathesis produced by fibrinogen breakdown products. Under the influence of plasmin the HMW fragments form intermediates known as X and Y./67/ Further proteolysis occurs and fragments D and E are formed which are resistant to further plasmin action./68/ The proteolytic action of plasmin on fibrin results in similar end-products to those resulting from its action on fibrinogen, yielding fragments Y, D and E /59/ but no X fragment.

As noted by Kowalski /59/ in his excellent review of fibrinogen derivatives, fragments X and Y and fragments D and E are referred to as the "early" and "late" fibrinogen degradation products. Fragment X and fibrinogen have a similar molecular weight and electrophoretic mobility. Fragment X is clottable by thrombin but clots at a much slower rate than fibrinogen and probably results in a defective clot structure. Fragments Y, D, and E are unable to clot and are potent inhibitors of the clotting process. When using the thrombin time to compare the anticoagulant affects of the fibrinogen degradation products, one finds that the early fibrinogen degradation products (X and Y) cause greater prolongation than the late

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products (D and E). Presumably this is because the early products have two anticoagulant capabilities. (1) They compete with fibrinogen for available thrombin and (2) they form insoluble non-clottable complexes with fibrin monomer. Although fragments D and E are less potent in prolonging thrombin time, their presence at the time of fibrin polymerization results in a grossly abnormal fibrin gel. The mechanism of action of fragment E is unknown although it is thought to inhibit the fibrinogen-fibrin conversion. Only a slight prolongation of the thrombin time is obtained when this factor is tested in a purified state.

Fibrinogen degradation products (FDP) are also able to inhibit platelet function. The early FDP are more effective but significant inhibition is also afforded by the late products. Platelet adhesion to glass, aggregation, and release of adenine nucleotides are all impaired./67,69-72/

There are various inhibitors circulating which inactivate the plasminogen system. Plasma contains potent antiplasmins in sufficient concentration to inactivate ten times the available plasmin. The active factor is an alpha-1-globulin probably identical with alpha-1-antitrypsin. It has both immediate and protracted activity./65/ The antiplasmins are particularly important since the proteolytic activity of plasmin is not specific for fibrinogen but is capable of digesting other plasma proteins as well./64/

Plasminogen in vivo probably exists as a two-phase system. (1) The circulating fraction accounts for fibrinogenolysis when activated, and rapid inactivation by circulating antiplasmin occurs lest the anticoagulant properties of the degradation products cause a generalized bleeding state. (2) Fibrinolysis is afforded by plasminogen activation occurring in the interstices of a thrombus. When fibrin gel forms, plasminogen is trapped within the network. Diffusion or incorporation of activator into the thrombus produces a localized plasmin deposit within the clot where the only substrate available for proteolysis is fibrin. When the thrombus finally breaks down, the unused plasmin is rapidly inactivated by circulating antiplasmins./64/

The net result of the two-phase plasminogen system is that no plasmin is present in the circulation but a potential for widespread activation exists. This can occur systemically, as in fibrinogenolysis, or locally which results in thrombus dissolution by fibrinolysis./64/

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### **DISSEMINATED INTRAVASCULAR COAGULATION (DIC)**

In order to recognize the syndrome of DIC the physician must have a high index of suspicion, be aware of the various settings in which it occurs, and be able to interpret the coagulation laboratory data.

When blood clots in vitro, Factors I, II, V, and VIII and platelets are consumed.<sup>/73/</sup> This results in prolongation of the PT, PTT, and TT. Specific factor assays will show the amount of depression of the involved factors. When these laboratory results are obtained and the clinical setting is proper, there is, of course, no problem in making the diagnosis. The situation, however, is rarely so clear cut. In the early stages of the syndrome, factor levels may not be significantly changed from baseline values. The first clinical manifestation may be ecchymoses resulting from consumption of platelets. When the syndrome is acute, the patient may deteriorate rapidly as evidenced by progressive bruising, purpura, oozing from venipuncture sites and mucous membrane surfaces, as well as by developing thrombotic lesions. At this point the PT and PTT are almost invariably prolonged as a result of utilization of coagulation factors. Catastrophic hemorrhage may occur, particularly in obstetrical patients, but usually the bleeding is in the same patterns as described for other patients.<sup>/1/</sup> In the subacute form of the syndrome laboratory values may be only slightly abnormal. Diagnosis in such instances will require the performance of more selective laboratory tests, such as the euglobulin lysis time, the evaluation of fibrinogen breakdown products and circulating plasminogen levels. (The euglobulin lysis time <sup>/74,75/</sup> is a somewhat crude test grossly measuring the amount of circulating plasminogen activator. If increased levels are present, more plasminogen is activated and the clot lysis time shortens.)

The most informative test is the measurement of FDP by one or more methods. Thomas et al <sup>/76/</sup> studied four commonly used methods for evaluating fibrinogen degradation products. No single test was 100 percent reliable. The Fi test <sup>/77,78/</sup> and the staphylococcal clumping test (SCT) <sup>/79/</sup> were primarily sensitive to the early degradation products while the tanned red cell hemagglutination inhibition immunoassay (TRCHII) <sup>/78,80/</sup> was sensitive to both early and late fibrinogen degradation products. In DIC with markedly elevated levels of fibrinogen degradation products all four tests were positive and there was

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excellent correlation between the F1 test, SCT, and TRCHII. The immunodiffusion was positive in only 70 percent of the patients however. /76/ The simplest test and many times a most informative one is the examination of the blood smear for evidence of a microangiopathic hemolytic anemia /81/ where fragmented cells, helmet cells, burr cells, and microspherocytes may be present in great abundance. Presumably the mechanism for this is trauma to the red blood cells as they pass through a microcirculation clogged with fibrin deposits.

*The Clinical Setting*

DIC occurs in a variety of clinical settings. Bachmann /82/ has utilized a classification consisting of three categories based on the causative pathophysiologic mechanisms.

Bachmann's first category consists of those diseases in which blood flow is markedly reduced and stasis results. The prototype of this category is the giant hemangioma (Kasabach-Meritt syndrome). In the enlarged tortuous hemangiomatous vessels blood flow is markedly reduced and slow activation of the intrinsic pathway occurs even in the presence of a normal vascular endothelium. Activated coagulation factors are not released to the systemic circulation to be cleared by the liver and the reticuloendothelial system. /83/ Primarily via intrinsic coagulation fibrin is deposited in the hemangioma. In response to this localized coagulation, fibrinolysis is stimulated with resultant fibrinogen degradation products. Factors I, II, V, VIII and platelets are continually utilized and plasminogen levels are decreased. When utilization of these factors exceeds the rate of production, a deficiency state results. Bleeding may develop and be further potentiated by the anticoagulant properties of the fibrinogen degradation products. Disseminated intravascular coagulation also occurs by the above mechanism in patients in severe heart failure with marked edema (pulmonary, peripheral, or both) and in patients at bed rest for any reason (especially in the elderly).

In the second category are the conditions in which thromboplastic substances are released into the circulation. This includes a great many conditions as thromboplastin is present in varying concentrations in virtually all body tissues. The

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highest percentage of this group are obstetric patients with abruptio placenta, a retained dead fetus, or amniotic fluid emboli. Acute intravascular hemolysis secondary to incompatible blood transfusion, drowning, infections, etc. may also be associated with DIC presumably from thromboplastin released from the lysed red blood cell. Massive tissue injury, burns, transplant rejection and surgery all release thromboplastin into the circulation. In transplant rejections antigen-antibody complexes have been shown to cause the platelet contraction-release phenomenon./18/

DIC associated with surgery probably has a higher frequency than has been realized. Lung surgery and surgery associated with extracorporeal circulation are prime situations for the occurrence of DIC. The high concentration of thromboplastin in lung tissue, the extensive foreign surface contact, and tissue trauma associated with operations requiring extracorporeal circulation allow for massive generation of prothrombin activator via both intrinsic and extrinsic pathways. Neoplastic diseases are frequently associated with thromboembolic complications. In Merskeys series of patients with DIC one-third of the cases had cancer./1/ The most frequent diagnoses were carcinoma of the prostate and promyelocytic leukemia. Cancer of the lung, breast, stomach, pancreas, cervix, and colon have all been implicated.

The third category of Bachmann consists of those conditions in which there are multiple reasons for activation of coagulation mechanisms. The infectious diseases fall into this category and there are extensive reviews covering this subject./84-91/ Gram-positive (pneumococcus, meningococcus), Gram-negative (*Escherichia coli*, *pseudomonas*, *klebsiella*, *proteus*), *rickettsiae* (Rocky Mountain spotted fever), viral diseases, and parasites (*Plasmodium falciparum*) all may initiate DIC through variable mechanisms. The Gram-negative organisms have particular importance because of the potent endotoxins released. Perhaps the most common mechanism of DIC initiation in this category is via endothelial damage and platelet aggregation. Other diseases also falling into this category are glomerulonephritis, purpura fulminans, thrombotic thrombocytopenic purpura, and the hemolytic-uremia syndrome.

The association of shock and DIC has been extensively reviewed by Hardaway./85.92.93/ Shock, from whatever cause, is a state associated with inadequate tissue perfusion and slowing of

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capillary blood flow resulting in acidosis and stasis. These conditions are sufficient to initiate coagulation via the intrinsic pathway. Usually there is associated vascular endothelial damage, tissue necrosis or trauma resulting in activation of the platelet phase as well as the extrinsic coagulation mechanism. Particularly important is inadequate perfusion of the liver because activated clotting factors cannot be cleared from the circulation and the liver dependent factors (including the vitamin K dependent factors) are produced at suboptimum rates. This sets a situation for DIC and the bleeding associated with consumption of coagulation factors is abetted by the decreased circulating levels of the factors synthesized in the liver.

If the syndrome of DIC is allowed to progress, a number of organs are particularly damaged. Severely affected are the kidneys where renal cortical necrosis may result. Hemorrhagic adrenal necrosis, periportal hepatic necrosis and postpartum pituitary necrosis are common findings. In addition, fibrin deposits are frequently present in the lungs, spleen, heart, GI tract and brain. /94,95/

Renal cortical necrosis most commonly occurs in association with pregnancy and is identical to that produced in the generalized Schwartzman reaction (GSR) of experimental animals. The similarity of the lesions of the GSR to that of DIC makes the GSR an excellent experimental model for studying the pathogenesis and treatment of the latter syndrome. /94/

**PRIMARY FIBRINOLYSIS**

Fibrinolysis occurs, for the most part, as a secondary manifestation of DIC. Primary fibrinolysis is a rare condition /1/ but there are a few clinical situations in which this syndrome may exist without antecedent intravascular coagulation. Sherry /65/ describes three situations in which this may occur. (1) If inordinate amounts of plasminogen activator are admitted to the circulation, the antiplasmins are overwhelmed and fibrinogenolysis occurs which produces a bleeding syndrome clinically indistinguishable from DIC. Prostatic tissue is rich

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in urokinase and carcinoma of the prostate or prostatic surgery may allow the release of considerable activator material. Severe anoxia, shock and extensive surgery, especially lung and cardiac bypass surgery, have also been implicated as causing excessive activator release.

(2) Cirrhosis and other hepatic diseases impair the ability of the body to inactivate or clear activator substances and significant hyperplasmaemia results. (3) Activation of plasminogen may occur when proteolytic enzymes capable of fibrinogen degradation appear in the circulation. In some leukemias increased cell turnover is associated with the liberation of intracellular proteases in excessive quantities. The diagnosis of this condition may be difficult because the laboratory data are similar to those seen in DIC. The condition is likely when there is marked reduction in plasma plasminogen levels, marked fibrinolytic activity as evidenced by the markedly shortened euglobulin lysis time, and only a modest reduction in Factors V and VIII./65/

**TREATMENT  
for DIC, and PRIMARY FIBRINOLYSIS**

Successful treatment of the patient with DIC necessitates removal of the initiating cause, the use of agents to terminate the accelerated coagulation process, replacement of depleted coagulation factors and platelets, and prevention of further plasminogen activation. No single agent exists that will effectively meet all of these requirements. In some instances merely the early removal of the coagulation stimulus (such as, the retained dead fetus or abruptio placenta) will stop the process because plasma checks and balances had not been overwhelmed. Usually, this is not the case and definitive treatment is required.

The use of fresh whole blood is recommended for its ability to replenish the consumed coagulation factors including Factor V, Factor VIII and platelets which are not present in bank blood. If fresh whole blood is not available, platelet concentrates and fresh frozen plasma are suitable substitutes. Transfusions alone may occasionally be able to arrest the hemorrhage if the syndrome is mild, but usually heparin must be added lest the transfusion merely adds "fuel to the fire".

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Heparin is effective in treating DIC. Effectiveness of heparin therapy is demonstrated by a return to normal of the labile coagulation factors, by arrest of hemorrhage, and by improvement in the clinical status of the patient./1,96/ Heparin will ameliorate the coagulopathy, but, as suggested by several studies /97-102/, it may not increase the chances for survival of patients in septic shock. The study by Corrigan and Jordan /97/ showed that of the 26 children with septic shock, 24 had associated coagulation defects and were treated with heparin. Fourteen (58 percent) of the patients treated with heparin did not survive despite the significant improvement of coagulation parameters before death in three of the fatal cases. Therefore, treatment of the coagulopathy alone is insufficient to improve survival statistics. Failure to correct irreversible shock will lead to death in spite of adequate therapy of the associated coagulopathy.

Methods of heparinization are as many as there are authors who write about them. The pros and cons of one method as opposed to another will not be discussed. I use a modification of the method of Stamm./103/ The initial heparin dose is usually 10,000 units by intravenous push. Subsequent dosages are adjusted to prolong the baseline clotting time  $2\frac{1}{2}$  times when measured four hours after injection. Initially heparin is given on a six-hour schedule, but four and two-hour schedules are sometimes required when the coagulation process is severe. I have no experience with the constant infusion method although it has been reported to be effective./1/

If there is evidence of hyperplasminemia, as reflected by a shortened euglobulin lysis time, and plasma plasminogen levels are low, epsilon amino caproic acid (EACA) or trasylol may be indicated. These agents are potent inhibitors of plasminogen conversion and are the agents of choice when primary fibrinolysis is present. One must be certain of the diagnosis lest this agent cause acute renal failure secondary to disseminated intraglomerular coagulation. /96/

The coumarin-group of anticoagulants, in common therapeutic dosages, do not have the antithrombotic capability to halt DIC./20/ The primary value of this group of anticoagulants is maintenance of long-term anticoagulation after an acute process (such as, pulmonary embolism or thrombophlebitis) has subsided.

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In this review of normal hemostasis and disseminated intravascular coagulation, an attempt has been made to present the coagulation scheme in a lucid fashion. Indeed it is complicated, and many questions remain unanswered in our present state of knowledge. The syndrome of DIC is being recognized with increasing frequency as physicians become more familiar with the clinical settings in which it may occur. A patho-physiologic classification of DIC has been reviewed and a brief guideline for therapy presented. It is hoped that the bibliography will serve as a ready reference for those desiring more detailed knowledge of the mechanisms of hemostasis and the syndrome of disseminated intravascular coagulation.

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If the least scratch is made on the skin as mortal a hemorrhage will eventually ensue as if the largest wound was inflicted. The divided parts, in some instances, have had the appearance of uniting. . . when, generally about a week from the injury, an hemorrhage takes place from the whole surface of the wound, and continues several days; . . the strength and spirits of the person become rapidly prostrate; the countenance assumes a pale and ghostly appearance; the pulse loses its force, and is increased in frequency; and death, from mere debility, then soon closes the scene.

It is a surprising circumstance that the males only are subject to this strange affection, and that all of them are not liable to it. . . Although the females are exempt, they are capable of transmitting it their male children. . .

Various remedies have been employed to restrain the hemorrhage — the bark, astringents used topically and internally, strong styptics, opiates, and, in fact, all those means that experience has found serviceable have been tried in vain.

— JOHN C. OTTO, M.D., 1803  
*On Hemophilia*

## PRESENT CONCEPTS IN THE MANAGEMENT OF HEMOPHILIA A

MAJ Joseph D. McCracken, MC

Hemophilia A is one of the oldest hematologic problems known to mankind. The etiology of this condition, its diagnosis and treatment baffled physicians for many centuries. Only in the last decade have truly great advances been made. Today it is possible to detect easily even mild cases of hemophilia and to treat effectively such patients so that they may live reasonably normal lives.

The genetics of hemophilia (factor VIII deficiency) are a classic example of x-linked recessive inheritance. There also appears to be two variations of the presentation of this condition — severe and mild. Abnormalities of the same degree are transmitted in the same family, i.e. severe hemophiliacs give rise to severe, and mild hemophiliacs to mild hemophilia in subsequent generations.

Severe deficiency states in classical hemophilia A are those in which factor VIII levels are usually not detectable. These people present with spontaneous hemorrhage in the form of hematomas, hemarthroses, or hematuria; and bleeding after any type of trauma or minor surgery is potentially serious.

A mild deficiency of factor VIII (a plasma level of 2-10 percent of normal) is rarely associated with spontaneous hemorrhage and may become apparent clinically only after surgery or trauma when such patients may have severe difficulties with hemostasis.

Detection of hemophilia A has advanced considerably with the introduction of the partial thromboplastin time (PTT). Traditionally, factor VIII deficiency was suspected when a patient presented with a normal bleeding time and a prolonged clotting time. The prothrombin time does not measure factor VIII, is not dependent on it, and is normal in hemophilia.

### *Hemophilia A - McCracken*

The PTT in normal plasma is 45-85 sec, in mild hemophilia 85-115 sec, and for severe hemophilia greater than 120 sec. /1/ If one uses the kaolin activated PTT, mild hemophilia would be suspected in the 45-60 second range, and severe hemophilia will usually result in values over 60 seconds. By adding various dilutions of either normal plasma or low factor VIII plasma from a severe hemophiliac one can perform a quantitative assay to determine the percent of factor VIII present in the patient's plasma.

The stability of factor VIII in vitro depends on a variety of conditions including the anticoagulant used for blood collection, the rapidity with which the plasma is separated and frozen, and the temperature at which it is stored. /2/ Factor VIII activity is preserved best with acid citrate dextrose (ACD) and plasma should be separated from the red cells and stored without delay. During the first 18 hours of storage at 4°C, 20-40 percent of the original factor VIII activity is lost. Also the process of freezing and thawing plasma may be associated with a 20 percent loss of factor VIII. However, once plasma is frozen, factor VIII remains relatively stable for periods of several months provided the material is kept at 20 C or below.

### *Factor VIII Concentrates*

Before the development of effective factor VIII concentrates, the large amounts of fresh plasma necessary to maintain therapeutic levels of factor VIII for prolonged periods invariably led to vascular overload. However, in recent years several methods have evolved to produce factor VIII concentrates. There are four principal factor VIII concentrates available and TABLE I gives a basic description.

Hemofil is a new, high potency glycine precipitated factor VIII concentrate, from 100 to 400 times purified and can be administered to patients in solutions 100 times more concentrated than plasma. /3/ The product appears stable and causes no immediate untoward side reactions. The plasma factor VIII levels of patients with classical hemophilia can be normalized with small volumes of the glycine precipitated material given by syringe. Thus, its principal merits are the lowering of amounts of volume necessary to obtain therapeutic levels, and being able to store it in refrigeration rather than freezing. /4/

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TABLE I  
FACTOR VIII CONCENTRATES\*

PRODUCT	AHF UNITS		COST per AHF unit (cents)	STORAGE REQUIREMENT
	per package	per ml		
Hemofil® (Hyländ)	230 725	33.0 28.0	13 13	Refrigerate
Fibro-AHF® (Merck)	75	0.75	23	Refrigerate
Antihemophilic Factor (AHF) (Courtland)	200	8.0	20	Room temperature **
Cryoprecipitate	100 to 150	4 to 6	15†	Freeze

\*Blood banks prepare cryoprecipitate rich in factor VIII by centrifugation of plasma. Besides Hemofil®, two other factor VIII concentrates are available commercially. The above table from *The Medical Letter on Drugs and Therapeutics* 11:95, 1969, listed the sources of the concentrates with their cost and other data.

\*\*Can be stored at room temperature for almost six months.  
†Price in New York City; prices vary throughout the country.

Fibro AHF is an ethanol precipitate and contains a considerable amount of fibrinogen from which factor VIII is difficult to separate. Because it is prepared from pooled plasma there is a considerable risk of serum hepatitis. It is also somewhat expensive. Hemolysis appears to be a recognized complication of Fibro AHF with a positive Coombs' test, splenomegaly, and increased osmotic fragility. /5/ Possibly this is related to its high fibrinogen content, because hemolysis is seen less often with the other preparations lower in fibrinogen content.

Antihemophilic factor (Courtland) is a lympholized preparation of cryoprecipitate from pooled plasma which is the only product which may be stored at room temperature up to six months. Again serum hepatitis is a risk when using this product.

Cryoprecipitate was prepared in 1964 by a simple method of separating factor VIII from plasma by cold or "cryo" precipitation. This technique is feasible because much of the factor VIII remains associated with a fibrinogen precipitate that forms when frozen plasma is gradually thawed at 5 C. This material is harvested and then frozen until used. Using this method most large blood banks can easily produce cryoprecipitate. Thus it can be prepared inexpensively. The risk of serum hepatitis is decreased as the material is harvested from a few select units of plasma rather than pooled plasma. The most serious shortcoming is its variable factor VIII

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content - the average yield being 68 percent and varying from 20 to 90 percent. /6/

**Treatment of Factor VIII Deficiency**

Dosages of these various agents depend on their standardization to determine the number of antihemophilic factor (AHF) units per milliliter. One unit of factor VIII is defined as the amount of factor VIII in one milliliter of normal plasma.

To calculate the amount of factor VIII necessary to obtain an 80 percent level of factor VIII in a 70 kg patient with severe hemophilia whose hematocrit is 45 percent may be done by the following formula: /7/

$$\begin{aligned}
 \text{Blood Volume} &= (70 \text{ kg}) (8\%) = 5600 \text{ ml} \\
 \text{Plasma Volume} &= (100 \text{ minus hematocrit}) \times \text{Blood volume} \\
 &= 55\% \times 5600 \text{ ml} \\
 &= 3080 \text{ ml} \quad (100\%) \\
 \text{80\% level} &= 3080 \text{ ml} \times 80\% \\
 &= 2464 \text{ units}
 \end{aligned}$$

Thus approximately 2500 units of factor VIII are required. Therefore, the initial dosages of the various factor VIII concentrates would be as follows:

$$\begin{aligned}
 \text{Hemofil®} (33 \text{ AHF u/ml}) &= 75 \text{ ml} \\
 \text{Fibro-AHF} (0.75 \text{ AHF u/ml}) &= 3300 \text{ ml} \\
 \text{AHF - Courtland} (8 \text{ AHF u/ml}) &= 300 \text{ ml} \\
 \text{Cryoprecipitate} (6 \text{ AHF u/ml}) &= 400 \text{ ml}
 \end{aligned}$$

Hemofil® is the product which meets with much praise and is rapidly becoming the standard preparation used because of its high concentration per unit volume. In vivo studies have been made using this product in which dosages of 12, 24, and 50 units factor VIII per kilogram body weight resulted in immediate levels of 32, 65 and 100 percent of factor VIII respectively. /8/ Thus if one wishes to avoid these somewhat cumbersome calculations, one can predict an 80 percent level of factor VIII by giving 40 units per kilogram of body weight.

The initial dosage of factor VIII given for hemostasis, however, is just the beginning step of therapy. One must

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carefully monitor the patient's factor VIII levels and give additional treatment to maintain an adequate level of factor VIII for hemostasis. For example, if a postinfusion yield of 80 percent of factor VIII is achieved in a nonbleeding patient, it can be predicted that approximately 50 percent of this level will disappear in eight hours. The further disappearance of factor VIII proceeds with the biologic half-life of approximately 14 hours. It can further be predicted that in an additional 14 hours (22 hours post-infusion) the patient's factor VIII level should reach approximately 20 percent. Thus if one were attempting to maintain a patient's factor VIII level greater than 20 percent, a second infusion would be necessary 22 hours after the first.

However, several circumstances may alter this ideal theoretical situation. In a patient who is actively bleeding, factor VIII will be consumed at a more rapid rate proportional to the amount of bleeding. Thus, in the actively bleeding patient, frequent levels of factor VIII must be monitored and performed every 4 to 6 hours depending on the individual. /9/

The importance of closely monitoring the patient's factor VIII level is stressed. Patients given calculated dosages of factor VIII to high levels may have persistent bleeding and the laboratory reports low factor VIII levels. This is caused by a factor VIII inhibitor which markedly accelerates the disappearance of factor VIII from the plasma. Factor VIII inhibitors are more common in patients with severe factor VIII deficiency and usually become manifest after exposure to multiple transfusions. This finding is not rare — in a larger series 16 of 77 patients (20.8 percent) had levels of factor VIII inhibitor appearing 4-5 days after infusion of factor VIII and peaking at 10-14 days. /10/

**SPECIAL PROBLEMS IN TREATMENT OF HEMOPHILIA****Surgical Management**

Before taking the patient to surgery the patient should

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be given enough factor VIII to raise the level to 75 to 100 percent. Adequate hemostasis can be maintained as long as the factor VIII level is kept above the 20 percent level. Frequent determinations of factor VIII levels are important and maintenance therapy to keep the factor VIII level greater than 20 percent is imperative in the immediate post-operative period. Maintenance therapy then may be given every 12 hours after checking factor VIII levels over the next several postoperative days. Patient should be continued for 10-14 days depending on the severity of the surgery. One must be alert for the presence of an AHF inhibitor after the first several days of therapy as mentioned previously. Using the products available even neurosurgical procedures <sup>/11/</sup> and major orthopedic surgery such as hip arthroplasty <sup>/12/</sup> can be accomplished without excessive bleeding.

### **Management of Hematomas and Hemarthroses**

For simple hematomas of soft tissues an initial dose to raise AHF to greater than 50 percent should be given and a local ice pack applied. If the hematoma is extensive and enlarging — maintenance therapy every 12 hours for three days should be given.\* For hemarthrosis the patient should receive initial treatment plus maintenance for three days as above with an ice pack and bed rest. Many authors believe that aspiration of the hemarthrosis should be done in addition to prevent serious arthropathy. <sup>/13/</sup> Intra-articular injections of steroid have also been tried to reduce the incidence of arthropathy following hemarthrosis. <sup>/14/</sup> When joint deformity is caused by formation of hemophilia pseudotumors (destructive lesions due to subperiosteal hemorrhage) local radiotherapy may be used to help improve joint function. <sup>/15/</sup>

### **Dental Procedures**

A similar regimen to that of the surgical procedure should be followed when an extraction is necessary with extra attempts made for complete hemostasis. Maintenance treatment every 12 hours for 10 days is indicated.

\*Personal communication, R.F. Schoen, MD, Letterman General Hospital, 1971

*Hemophilia A - McCracken***FUTURE CONCEPTS**

Currently experimental prophylaxis of severe hemophilia A has been attempted with Hemofil® (glycine precipitated AHF). /16,17/ Perhaps with further sophistication of AHF concentrates, the hemophiliac may be able to control effectively his disease similar to the diabetic.

Reports of splenic transplantation in animals with resultant "cure" of hemophilia is another avenue being explored with much controversy. /18/ Perhaps this may represent another means of control of this dreaded disease.

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## VON WILLEBRAND'S DISEASE

LTC Laurence J. Logan, MC

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For years the name "von Willebrand's disease" served as a 'wastebasket' diagnosis for ill-defined bleeding disorders. Recently, use of this eponym has been narrowed to a much more precisely delineated hemostatic disorder. Historical reviews /1,2/ demonstrate the previous diversity of opinions as to what constituted "von Willebrand's disease". The many synonyms used in the earlier literature -- e.g. hereditary capillary purpura /3/, vascular hemophilia /4/, pseudohemophilia /5/, Jürgens-von Willebrand thrombocytopathy /6/ -- attest to the differing concepts of etiology and to the confusion which surrounded this disorder. von Willebrand's disease is now understood to be a vertically transmitted hereditary disease in which there is a double hemostatic defect: 1. a defect in primary hemostatic plug formation due to deficiency of a plasma factor\* 2. a defect in the coagulation phase of hemostasis manifested by depressed levels of factor VIII.

The following discussion will include the clinical features of the disease; a brief review of normal primary hemostatic plug formation with attention to the defect manifest in patients with von Willebrand's disease; laboratory tests which aid in diagnosis; and problems encountered in differential diagnosis.

### CLINICAL FEATURES

Although laboratory tests disclose defects in two different phases of the hemostatic process, i.e. impaired hemostatic plug formation and depression of a coagulation factor (factor VIII), the bleeding problems suffered by patients with von Willebrand's disease are most often

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\*The plasma factor necessary for primary hemostatic plug formation which is deficient in patients with von Willebrand's disease has been called the "anti-bleeding factor", "platelet adhesiveness plasma factor (PAPF)", etc. The term "anti-von Willebrand's factor" will be used herein to avoid confusion with factor VIII.

### *von Willebrand's Disease - Logan*

attributable to the former. Thus a typical history from a patient with von Willebrand's disease may include frequent or severe epistaxis (often tending to abate in adulthood), prolonged and excessive bleeding from minor cuts, increased bruisability (but usually without the huge ecchymoses seen in disorders of coagulation -- e.g. hemophilia), excessive bleeding of the "immediate" type after dental extractions and minor surgical procedures, and in females, menorrhagia. The severity of symptoms varies considerably from patient to patient and among afflicted members of a kindred. Some patients experience moderately severe bleeding problems whereas other patients, if they recognize an increased bleeding tendency at all, regard it as merely a nuisance.

Depression of factor VIII levels, a major laboratory feature of the disease is usually of slight to moderate degree. If factor VIII levels are below 35%, however, retarded coagulation may contribute to the impairment of hemostasis. /7/ Although distinctly uncommon one must be aware that factor VIII levels may be severely depressed in von Willebrand's disease and result in the same bleeding problems which afflict the hemophilic, including hemarthroses. /8/

### **PRIMARY HEMOSTATIC PLUG FORMATION**

Injured tissue exposed to blood clotting components initiates the 'first line of defense' in hemostasis: formation of the primary hemostatic plug. The integral role of the platelet in this earliest stage of hemostasis is discussed elsewhere in this issue in detail (*viz. pp 191-199*). The major steps leading to formation of the primary hemostatic plug are summarized in Figure 1.

The hallmark of impaired hemostatic plug formation is prolongation of the bleeding time (See Figure 2). For years many investigators (including von Willebrand) regarded the bleeding disorder bearing his name as an intrinsic platelet abnormality. /6/ The findings on which this assumption was based have been refuted. /11, 12, 13/ As will be discussed, none of the currently available tests of platelet function provides good evidence for an intrinsic platelet defect in von Willebrand's disease. Defective primary hemostatic plug formation in patients with von Willebrand's disease is evinced by the nature of the predominant bleeding symptoms which are of the "mucosal" type, prolongation of the bleeding time, and

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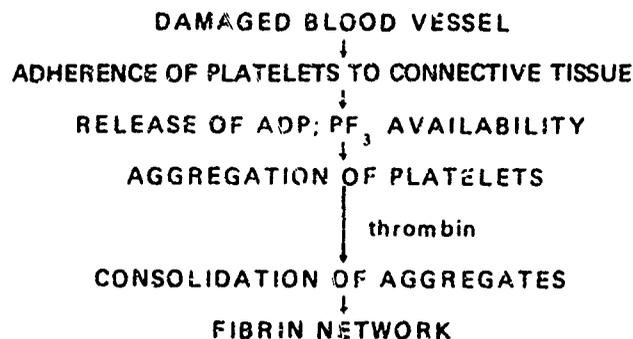


Fig. 1 Platelets adhere to collagen in the subendothelial connective tissue /9/ and a complex interaction results in the release of adenosine diphosphate (ADP) from a metabolically inert pool in the platelet. /10/ This ADP induces adherence of platelets to one another -- i.e. aggregation -- producing a loose, semipermeable, reversible plug. At this stage the platelet exerts its role in intrinsic coagulation by rendering available the lipid activity which is referred to as "platelet factor 3" (PF<sub>3</sub>). Upon generation of minute amounts of thrombin on the platelet surface the platelets change shape, the aggregates coalesce, and the plug becomes impermeable and irreversible. Perimetric fibrin formation in and around the primary plug occurs due to activation of the coagulation phase of hemostasis and the clot is complete.

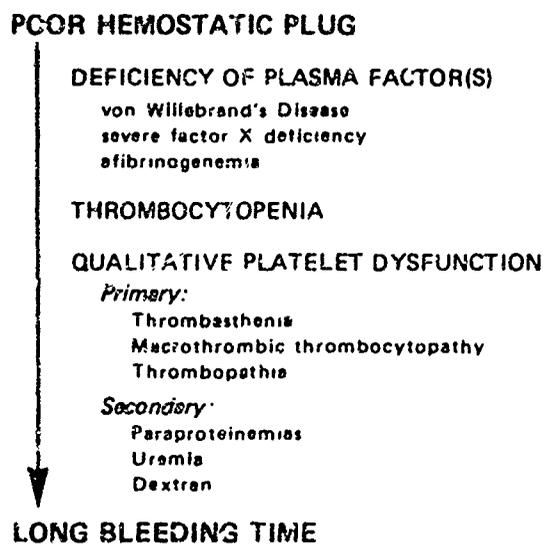


Fig. 2. A prolonged bleeding time reflects impaired hemostatic plug formation from any cause. Hemostatic plug formation is normal in most defects of the coagulation phase of hemostasis (e.g. the hemophilias). Afibrinogenemia, very severe factor X deficiency, and von Willebrand's disease are conditions in which hemostatic plug formation is impaired due to an absence or a deficiency of a plasma factor.

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impaired adhesion of platelets to glass. It is appropriate to stress that the refined definition of what set of abnormal findings constitutes 'von Willebrand's disease' has not provided clarification of the basic mechanism whereby effective hemostasis is impaired in this condition. The precise role of the anti-von Willebrand factor in normal primary hemostatic plug formation remains unknown.

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**LABORATORY TESTS: INTERPRETATION AND LIMITATIONS****1. Tests of primary hemostatic plug formation**

a. The primary bleeding time: Failure to demonstrate prolongation of the bleeding time after several determinations makes difficult (without necessarily ruling out) a diagnosis of von Willebrand's disease. /7/ I favor the standardized Ivy bleeding time by the template method /14/ in which an incision of 9mm length and 1mm depth is made on the forearm with a blood pressure cuff inflated to 40mm Hg. This superb modification of the Ivy bleeding time insures reproducibility from determination to determination and minimized differences obtained by different testers. The Dike time has been shown to lack the sensitivity of the Ivy method in detecting von Willebrand's disease. /15/ Nonetheless, certain distinguished hematologists adhere to use of the Dike method and are doing have not missed cases of hemostatic plug abnormalities. /16/ This fact may well reflect the extensive, personal experience of such individuals with the Dike bleeding time. In the hands of the average person who performs bleeding times, however, the template Ivy method is almost certain to be more sensitive and accurate.

Use of acetylsalicylic acid to 'uncover' occult von Willebrand's disease has been advocated. /17/ Aspirin has been shown to interfere with the release of platelet ADP /18/ thereby tending to lengthen the bleeding time. The mean bleeding time of a normal population by the standardized Ivy method is increased from five to nine and one-half minutes two hours after ingestion of 1 Gm of aspirin. /14/ In von Willebrand's disease where an aspirin induced defect in ADP release may result in a "double defect" in primary hemostasis or in certain platelet disorders in which ADP release is already impaired, the bleeding time may be more patently abnormal after administration of aspirin.

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b. Platelet counts, platelet morphology on the blood smear, and clot retraction are normal in von Willebrand's disease. Normalcy of these findings helps to exclude other causes of poor hemostatic plug formation -- e.g. thrombocytopenia, certain intrinsic platelet disorders in which alteration of platelet morphology may be present /17, 20/, and thrombasthenia of Glanzmann in which clot retraction is impaired. /21/

c. Platelets from patients with von Willebrand's disease adhere normally to connective tissue (collagen fibers) /22/ and release ADP in normal amounts. /23/

d. In vitro studies of platelet aggregation in response to exogenous ADP, to dilute connective tissue suspension (collagen), to epinephrine, and to weak thrombin are characteristically normal in von Willebrand's disease. One study suggested that the addition of dilute ADP to platelet rich plasma from patients with von Willebrand's disease produced normal initial aggregation followed by rapid disaggregation. /24/ Others have not found specific aggregation abnormalities in von Willebrand's disease. /23, 25/ (We have occasionally seen rapid disaggregation of platelets in samples from normal individuals when dilute ADP was used, especially when the platelet rich plasma platelet count was lower than usual. /26/)

Various abnormal patterns of platelet aggregation when properly interpreted may indicate the presence of a platelet disorder.

e. "Availability" of platelet factor 3 upon exposure of platelet rich plasma to kaolin /27,23/ is normal in von Willebrand's disease. /23/ This test may yield abnormal results in various platelet defects, primary or acquired.

f. The modified Salzman glass adhesiveness test: Disorders of primary hemostatic plug formation tend to be associated with relatively poor adherence of platelets to glass surfaces. Presumably, this lowered adhesivity to glass reflects decreased platelet "stickiness" which may contribute somehow to impaired primary hemostasis. At least five different methods of assessing platelet-glass interactions have been developed /28/ the most promising of which is Salzman's test /27/ Blood is drawn from the vein into a glass bead column and platelet counts before and after passage through the column are compared.

Normally, 25-60% of platelets adhere to glass beads. From its inception numerous technical problems attended the use of the Salzman test and reports attempting to evaluate its use as an aid in the detection of von Willebrand's disease were conflicting. It was noted, for example, that results were not reproducible with different batches of glass beads /30/, that the rate of flow through the column was critical /23/ etc. With scrupulous attention to technique many of the initial problems have been surmounted and it does now appear that a modification of this test is useful in separating patients with von Willebrand's disease from normal individuals. In a representative series 15 percent of normal subjects had low adhesiveness whereas 79 percent of patients with von Willebrand's disease had abnormally low results. /31/ Despite this overlap, the test is a useful adjunct diagnosing von Willebrand's disease, when used in conjunction with other findings (e.g. family history suggesting autosomal dominant transmission of the bleeding disorder, prolongation of the bleeding time, lowered factor VIII levels, etc.). Unfortunately, the experience of several investigators has been that when the bleeding times and/or factor VIII levels are borderline, glass adhesiveness tends also to be borderline. /7, 25/ Since the test may yield different results at different times /25/ it should be done more than once when possible.

In patients with von Willebrand's disease impaired platelet-glass interaction is the only other laboratory abnormality besides prolongation of the bleeding time which reflects a disorder of primary plug formation. Of particular interest is the fact that in this disease the abnormal adhesion of platelets to glass can be corrected by in vivo administration of plasma /22/ or in vitro by drawing the patient's blood into normal plasma before passage through the glass bead column. /30, 32/ This finding provides one of many pieces of evidence that impaired primary hemostasis in von Willebrand's disease is due to deficiency of a plasma factor -- the anti-von Willebrand factor. However, the relevance of faulty glass-platelet interaction to pathophysiologic mechanisms whereby formation of the hemostatic plug is impaired in von Willebrand's disease is not yet understood.

## 2. The coagulation system (tests of factor VIII activity)

a. The activated partial thromboplastin time /32/ reflects the combined activity of all the coagulation factors

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which participate in the so-called "intrinsic" system of coagulation. Thus depression of factor VIII may cause prolongation of the PTT. Appropriate "correction" studies (e.g. normalization of the prolonged PTT upon addition of barium sulfate adsorbed plasma versus aged serum) would support (but not confirm) that a depressed factor VIII level accounted for a prolonged PTT. Unfortunately, such "correction" studies are least accurate in those cases in which factor VIII levels are only slightly to moderately reduced.

b. Assay of factor VIII activity by a one-stage /34/ or a two-stage /35/ method is essential in any suspected case of von Willebrand's disease. Results in von Willebrand's disease are not uncommonly borderline. It is important to remember that in this disease, factor VIII levels increase in response to the same stimuli which increase factor VIII levels in normal individuals - e.g. stress, late pregnancy, etc. Often the initial sample is collected during a stressful period and yields a normal value in an individual who, if tested under "baseline" conditions would show a lowered value.

c. Response of factor VIII levels to transfusion with various preparations: A fascinating observation, first reported by Nilsson et al, /11/ is that transfusion of normal plasma, serum or Cohn fraction I to patients with von Willebrand's disease alters factor VIII in a very different manner from that seen in patients with hemophilia A. Shortly after infusion of one of these preparations individuals with von Willebrand's disease demonstrate an increase in factor VIII level to values greater than can be accounted for by the amount of administered factor VIII. Furthermore, the factor VIII level rather than dropping progressively in accordance with its known half-life of about 10 hours, continues to rise over the following 24-48 hours. Even more dramatically, plasma from patients with hemophilia A (i.e. factor VIII deficient plasma) when administered to a patient with von Willebrand's disease produces this same pattern in factor VIII levels. /36/

By contrast, administration of factor VIII containing preparations to patients with hemophilia A results in elevation of this factor to a value which would be expected based on the amount given. Peak values are immediate and a predictable curvilinear decay follows corresponding to factor VIII half-life. Therefore, the response of lowered factor VIII levels to transfusion of factor VIII



containing materials may distinguish von Willebrand's disease from hemophilia A. /7/ Careful attention must be given to the considerable risk of hepatitis which attends the use of these preparations before employing transfusion studies for purely diagnostic purposes.

When spontaneous bleeding is uncontrolled by simple measures or when a person with a hemostatic defect requires a procedure which is likely to cause undue bleeding there is therapeutic importance in knowing whether depressed factor VIII levels are due to hemophilia A or to von Willebrand's disease for two reasons. Firstly, it is clear from the above that factor VIII levels in patients with von Willebrand's disease can be raised and sustained fairly easily with a transfusion requirement considerably less than is needed in patients with hemophilia A. Secondly, the anti-von Willebrand factor which normalizes the prolonged bleeding time and impaired platelet adhesiveness to glass is present in some but not all preparations which contain factor VIII. Fresh or fresh-frozen plasma and the Blömbäck I-C fraction contain the anti-von Willebrand factor. /36/ Fibrinogen concentrates, /37/ stored plasma, and Cohn fraction I concentrates do not. /38/ Currently available commercial factor VIII concentrates do not appear to contain the anti-von Willebrand factor. Fortunately, however, cryoprecipitate prepared as described by Pool /39/ is rich not only in factor VIII but in the anti-von Willebrand factor. /40/ Evidence indicates that the anti-bleeding factor is highly labile and may be lost if the concentrate is exposed to glass surfaces. /41/ Accordingly, when cryoprecipitate is prepared primarily for its anti-von Willebrand factor content, rapid processing of single units at a time at cold temperatures in an all-plastic system is desirable.

d. Immunologic techniques of diagnosis: Recently, Feinstein et al /42, 43/ and two other groups /44, 45/ independently demonstrated that by employing naturally occurring factor VIII antibody 90-95 percent of patients with hemophilia A had no detectable factor VIII antigenic material in their plasma, whereas in 5-10 percent of patients with hemophilia A there was immunologic evidence of a factor VIII molecule, although it was ineffective in the coagulation system. /42/ Even more recently, Ratnoff's group /46/ using heterologous factor VIII antibody demonstrated material antigenically resembling factor VIII in each of 10 patients with hemophilia A in

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quantities comparable to that found in normal individuals. Of great interest was the discovery that in seven cases of von Willebrand's disease the quantity of immunologically detectable factor VIII was directly equivalent to the degree of deficiency seen in the usual coagulation assay systems which measure factor VIII activity. This highly important observation may provide an effective *in vitro* method of establishing whether depressed factor VIII levels in a given patient are due to hemophilia A or to von Willebrand's disease.

### **DIFFICULT PROBLEMS IN DIFFERENTIAL DIAGNOSIS**

The classic case of von Willebrand's disease is not difficult to diagnose. Unfortunately, not all patients with this disease present with a clear-cut family history indicative of autosomal dominance, a distinctly prolonged bleeding time, unequivocal impairment of platelet adhesion to glass and abnormally low factor VIII. The foregoing discussion suggests the two major challenges in differential diagnosis which the physician may face in attempting to establish that a patient has von Willebrand's disease: 1. To eliminate insofar as possible a platelet disorder as the cause for poor primary hemostatic plug formation. 2. To establish that low factor VIII levels are not due to hemophilia (or the carrier state).

**Platelet Disorders:** Every attempt must be made to eliminate an intrinsic platelet defect as the cause of a long bleeding time in each suspected case of von Willebrand's disease because of the therapeutic implications. Platelet concentrator, effective controlling bleeding in intrinsic platelet disorders are without benefit in von Willebrand's disease. Cryoprecipitate, the treatment of choice in von Willebrand's disease is of no value to the patient with intrinsic platelet dysfunction. Tests which indicate platelet dysfunction have been mentioned and are discussed in detail elsewhere in this issue. (viz. pp 191-199 ) One must remember that prolongation of the bleeding time and defective platelet adhesion to glass are common to both platelet disorders and to von Willebrand's disease.

**von Willebrand's disease or hemophilia A?** A moderate to moderately-severe bleeding disorder in a patient with low factor VIII

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levels, an equivocal or unavailable family history and bleeding times which are within or toward the upper limits of normal necessitates discerning between von Willebrand's disease and hemophilia A. This problem in differential diagnosis is not limited to males, since in accordance with the Lyon-Boutler hypothesis /47,48/ carriers of hemophilia may have very low levels of factor VIII activity as the result of random inactivation of a greater number of the 'normal' X chromosomes than of the 'hemophilia-bearing' X chromosomes. The Salzman test may aid in the proper diagnosis of such cases since adherence of platelets to glass is not impaired in hemophilia. Transfusion studies with serial determinations of factor VIII levels may be necessary. Possibly the most helpful test to establish whether a low factor VIII level is due to hemophilia A or von Willebrand's disease may prove to be the immunologic method described above. It is too early to assess the ultimate role of this technique as a diagnostic tool. The differing approach to therapy based on whether the patient has von Willebrand's disease or hemophilia A has been discussed.

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**CONCLUDING REMARKS**

It is doubtful if one who reviews what we know about von Willebrand's disease can escape the many intriguing and unanswered questions which pose themselves. As a great Army physician said: "We must be unencumbered by . . . pompous notions that we physicians have the right to define disease. If (the physician) concludes that disease "X" cannot be present because a certain sign or lab test is not present, he limits his agility. He certainly can't discover something new!" /49/ We are now able to define what clinical and laboratory features entitle us to use the diagnostic term "von Willebrand's disease" but we have not thereby clarified the disease. Recent progress in the understanding of platelet disorders has given us some insight into what von Willebrand's disease is not. Our definition allows better "sorting" of patients, permits selection of more appropriate therapy etc. but we are left with many questions (e.g. At what stage of primary hemostasis and how does a deficiency of anti-von Willebrand factor exert its deleterious effect(s)? Transfusion studies clearly demonstrate that unlike the patient with hemophilia A, patients with von Willebrand's disease

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have the capacity to synthesize factor VIII. Does the anti-von Willebrand factor provide this stimulus? Or does yet another factor, distinct from the anti-von Willebrand factor provide that stimulus? Is more than one gene (perhaps closely linked) defective in "classic" von Willebrand's disease and if so, does the inheritance of but one of the genes account for what we glibly refer to as "variable expressivity" among members of a kindred? Where is the site of production of anti-von Willebrand factor? etc. etc.) Finally, there remain a significant number of patients with a mild to moderate bleeding disorder and prolonged bleeding times whose family histories do not support von Willebrand's disease or whose factor VIII levels are consistently normal or high but in whom no evidence for a platelet disorder can be detected by presently available tests. Whether such patients are part of a "spectrum" of von Willebrand's disease (50%), are victims of intrinsic platelet dysfunction too subtle for detection by our tests, or represent examples of a disorder (or disorders) unrelated either to von Willebrand's disease or intrinsic platelet dysfunction remains to be seen.

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## RED CELL G-6-PD DEFICIENCY Its Clinical Spectrum

LTC Neil W. Culp, MC

Genetically determined deficiency in the red cell enzyme glucose-6-phosphate dehydrogenase (G-6-PD) represents one of the most common, clinically significant, inherited abnormalities of man. In excess of 100 million individuals on a world-wide basis are affected. /1/ The clinical significance of this hereditary biochemical lesion was clearly established when it was demonstrated to be the underlying defect in subjects with "primaquine sensitive hemolytic anemia." Today, however, the import of this most prevalent of the known enzymatic defects of the red cell is not limited to those relatively few physicians who dispense antimalarial drugs, but it has considerable significance to every physician charged with treating patients. Not only is there a growing list of commonly employed drugs which possess the potential of precipitating hemolysis in G-6-PD deficient subjects, but recognized with increasing frequency are episodes of hemolysis which are precipitated by infectious and metabolic diseases when affected subjects acquire them. In addition, some individuals with G-6-PD deficiency manifest chronic hemolytic anemia in the absence of drug exposure or complicating illness and such subjects are a significant percentage of those patients diagnosed as having congenital nonspherocytic hemolytic disease (CNHD).

It is the purpose of this paper to review the spectrum of clinical manifestations now recognized to occur among those subjects with genetically determined abnormalities in erythrocyte G-6-PD.

### Historical and Genetic Features

Although favism (now known to be conditioned by a deficiency of red cell G-6-PD) was recognized more than 2,000 years ago, it was not until the introduction of the first antimalarial 8-aminoquinoline compound, primaquine (1926) that a clear relationship between drug exposure and hemolytic anemia in selected recipients was appreciated. /2/ It was almost 30 years later, when a group

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of Negro volunteer subjects were given the newer drug primaquine that sensitivity to the drug was demonstrated to be an intrinsic property of the red cell./3/ A series of subsequent investigations culminated in 1956 with the recognition that the abnormality in red cell metabolism responsible for primaquine sensitivity was a deficiency of the enzyme G-6-PD./4/ For the first time the adverse effect of a drug was defined at the molecular level.

It soon became apparent that G-6-PD deficiency was not a single defect, but rather a heterogeneous group of disorders due to numerous mutations affecting the G-6-PD molecule. This is analogous to the multiple mutations affecting the structure of the hemoglobin molecule which result in the group of conditions known as hemoglobinopathies. To date more than 50 distinct genetic variants of the G-6-PD molecule have been recognized./5,6,7,8,9/ This number undoubtedly represents only a fraction of the variants yet to be identified. Each variant appears to be explained by a single amino acid substitution in the structure of the G-6-PD molecule, which is also true of the hemoglobinopathies./10/ Some of these variants are functionally adequate while the structural alteration of other variants results in a severe impairment of enzyme activity.

The multiple biochemical variants of the G-6-PD molecule are inherited as sex-linked traits; that is, the gene is carried on the X chromosome. Male hemizygotes and female homozygotes manifest full expression of the defect while female heterozygotes are variable in the degree of enzyme deficiency expressed. Affected females possess a mosaic of erythrocytes, some with normal enzyme activity and some with deficient enzyme activity. The ratio of red cells with normal activity to those with deficient enzyme activity is variable and thus the degree of susceptibility to hemolysis expressed in any given individual is unpredictable. This has been explained on the basis of the X inactivation hypothesis which proposes that during the early embryogenesis one of the two X chromosomes in each cell of the female becomes inactive and remains inactive throughout all subsequent cell divisions during the life of the individual./11/ Stem cells destined to develop into mature circulating red cells may in the heterozygote possess either the functional X chromosome with its gene for normal G-6-PD activity or the X chromosome with the mutant gene for G-6-PD deficiency.

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The distribution of G-6-PD deficiency is world-wide. Extensive population surveys have to date, however, been performed only in selected groups. Table I /12/ lists the approximate frequency of the genetic defect in some of the ethnic groups studied.

TABLE I  
INCIDENCE OF G-6-PD DEFICIENCY IN VARIOUS RACES\*

RACES	INCIDENCE (%)
CAUCASIANS	
Northern Europeans	0.1
Sardinians	3-30
Greeks	2-5
Italians (northern)	2-5
NEGROES	
American (USA)	10
Nigerian	10
Congolese	15-20
Tanganyikans	15-30
Bantu	2
JEWS	
European	0.2
Iraqi	25
Turkish	5
Kurdish	60
ASIAN	
Chinese	2
Japanese	0
Filipinos	12

\*Adapted from Frankerd.

*Clin Pharmacol Ther* 21:73-103,1969

THE ROLE OF G-6-PD IN RED CELL METABOLISM

The human erythrocyte is designed to circulate in the peripheral blood for approximately 120 days. The mature red cell

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is metabolically and nutritionally restricted because it has lost much of the metabolic machinery characteristic of nucleated cells. After the reticulocyte stage, protein synthesis does not occur. Then the mature red cell is dependent upon a limited supply of crucial enzymatic proteins which must serve it unreplenished to the point of cell destruction. Certain of these enzymes are absolutely essential to the maintenance of cellular integrity and are critical for the protection of hemoglobin from chemical denaturation. With progressive erythrocyte aging, there is a gradual attrition of some enzymatic proteins so that a point in time is reached (normally about 120 days) when the metabolic machinery dependent upon these enzymes is unable to supply the energy requirements necessary for the cell to survive. It is removed then from the circulation. If the red cell is released into the circulation with an inadequate supply of one of these crucial enzymes or if the biological half-life or the kinetic properties of the enzyme are abnormal, the life span of the red cell is reduced and, by definition, hemolysis ex

The Embden-Myerhof anaerobic pathway of glycolysis (Figure 1) which converts glucose to lactate, normally accounts for 90 percent of the energy production in the mature red cell. The high energy phosphate compounds resulting from this pathway are necessary for maintenance of the high intracellular osmotic pressure characteristic of the intact red cell. /13/ Until recently, the hexose-monophosphate (HMP) shunt (pentose phosphate pathway) was generally considered to play a role of limited importance in erythrocyte metabolism because under usual circumstances only + 10 percent of glucose is metabolized via this pathway. It is now apparent, however, that the contribution of this latter pathway varies with conditions of intracellular  $PO_2$  and pH, and under conditions of oxidative stress to the red cell, this pathway is greatly stimulated. This enhanced metabolism of glucose through the HMP shunt during periods of oxidative stress to the red cell is related to the critical role played by reduced glutathione (GSH) in the protection of hemoglobin and the red cell membrane from oxidative damage. /13/ GSH is generated solely via the HMP shunt.

G-6-PD occupies the critical site of entry for glucose into the HMP shunt. Figure 1. Generation of NADPH, and in turn GSH, is dependent upon the reaction catalyzed by G-6-PD and the step which follows. Availability of NADP appears to be the factor limiting the rate of glucose utilization by this pathway under usual circumstances. /14/ In the individual with deficient G-6-PD activity, however, the limiting factor may be

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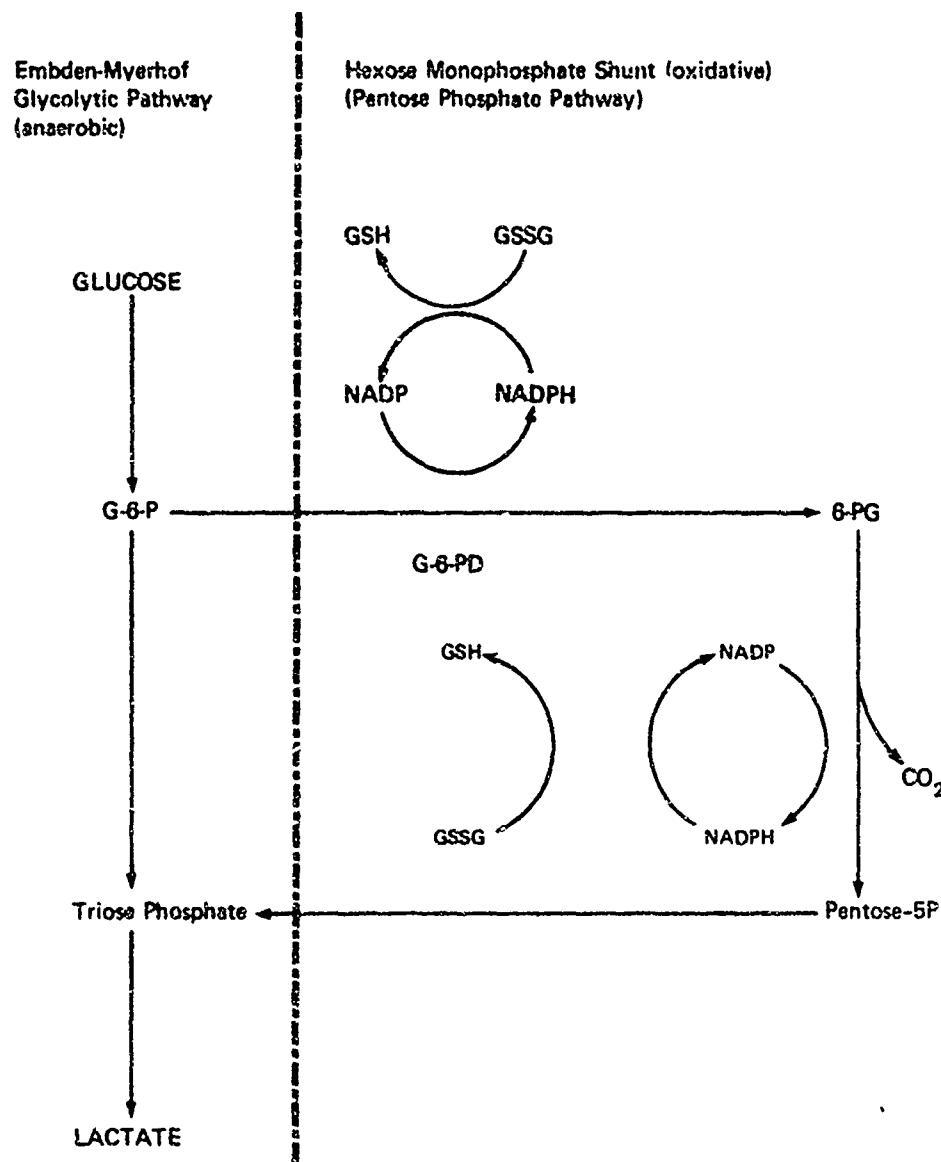


Fig. 1. Simplified diagram of pathways for erythrocyte energy production.

**Legend:** G-6-P = glucose-6-phosphate  
 G-6-PD = glucose-6-phosphate dehydrogenase  
 6-PG = 6-phosphogluconate  
 NADP = nicotinamide adenine dinucleotide phosphate (TPN)  
 NADPH = reduced nicotinamide adenine dinucleotide phosphate (TPNH)  
 GSSG = oxidized glutathione  
 GSH = reduced glutathione

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the impaired enzyme system during periods where metabolic demands exceed its functional capability. The fundamental pathophysiology in G-6-PD deficient red cells then seems to be an inability to meet increased demands for NADPH and the consequent failure to maintain sufficient concentrations of GSH which is necessary to protect the cell from oxidative damage. In support of the hypothesis that GSH plays a critical protective role in face of oxidative stress to the red cell, are the observations that either a primary deficiency of GSSG or a deficiency in the enzyme glutathione reductase which catalyzes the conversion of GSSG to GSH, may be associated with hemolysis precipitated by those agents responsible for hemolysis in G-6-PD deficient subjects./15/

**CLINICAL FEATURES**

Subjects with genetically determined abnormalities in the G-6-PD molecule may for clinical purposes be categorized into three general groups: (a) those with no clinical symptoms; (b) those experiencing symptomatic hemolysis on exposure to certain oxidant drugs or associated with infectious disease, and (c) those with evidence of chronic hemolytic disease of the congenital nonspherocytic type (CNHD).

Some of the variants of the G-6-PD molecule, like the genetic variants of the hemoglobin molecule (i.e. hemoglobinopathies), are primarily of interest to the geneticist because they are associated with no apparent clinical abnormality. The most commonly encountered variant in the United States has been termed G-6-PD(A) and is found in approximately 22 percent of the North American Negro population. It is distinguished from the normal enzyme, G-6-PD(B), by a more rapid electrophoretic mobility characteristic of the former. G-6-PD(A) is functionally adequate and subjects with this variant have no symptoms. An electrophoretically similar but chromatographically distinguishable enzyme, G-6-PD(A-), is found in 10 to 12 percent of the Negro population and is associated with deficient activity and clinical symptoms of hemolysis in settings where the red cell is subjected to oxidative stress, such as, that imposed by ingestion of certain drugs. G-6-PD (Mediterranean) is the deficient enzyme variant occurring among Sardinians, Greeks, Sephardic Jews, and perhaps other Mediterranean groups, which is associated

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with a more severe form of drug induced hemolysis, favism, and occasionally with chronic hemolytic disease in the absence of stress. A number of distinct abnormal variants of the G-6-PD molecule have been demonstrated in subjects with CNHD due to G-6-PD deficiency./16/

The degree of impairment in enzyme activity characteristic of each of the recognized variants is correlated with the absence or presence of hemolytic manifestations. In general those variants in which red cell enzyme activity is greater than 30 percent of normal are unaccompanied by problems of hemolysis. Below this range of activity however the severity or persistence of hemolysis is less well correlated. Assays of enzyme activity in unselected circulating red cells may be misleading as only the older cells may be enzyme deficient, and assays may fail to reflect abnormalities in enzyme stability or kinetic alterations of enzyme function. Better correlation is seen when consideration is given to these additional factors./15/

With this appreciation of the genetic heterogeneity among subjects with G-6-PD deficiency, some clinically important distinctions between the two most common groups of deficient individuals encountered in the United States, G-6-PD(A-) and G-6-PD (Mediterranean), deserves emphasis.

#### MANIFESTATIONS IN SUBJECTS WITH G-6-PD<sup>A-</sup> (Negro-type Deficiency)

Although minimal shortening of the red cell survival has been reported in some Negro males with G-6-PD(A-) in the absence of any apparent precipitating stress, such individuals are generally clinically and hematologically normal except during the administration of certain drugs or associated with systemic illness./17/ The typical course of hemolysis in a deficient Negro male hemizygote is depicted graphically in Figure 2. With the administration of 30 mg of primaquine base daily, the <sup>51</sup>Cr red cell survival time begins to shorten almost immediately. During this period Heinz bodies (precipitated denatured hemoglobin within the red cell) may be demonstrated by supravital staining. Within 48 to 72 hours, evidence of intravascular hemolysis becomes apparent with a falling hematocrit and frequently hemoglobinuria. The period of acute hemolysis lasts for 8 to 10 days and is followed by a period of hematological recovery which occurs in spite of continued drug exposure at a constant dose. The subsequent phase of apparent resistance

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or equilibrium represents a state of mild but fully compensated hemolysis. Since an increase in the dose of primaquine to 60 mg a day would precipitate a second acute hemolytic phase, the "resistance" of the circulating red cells to further hemolysis is relative and not absolute./18/ The self-limited nature of the acute hemolytic episode is explained by the now appreciated fact that susceptibility to a given dose of a hemolytic drug is a function of red cell age; older red cells being preferentially destroyed./19/ The age related susceptibility is due to the increased rate of enzyme denaturation characteristic of the Negro G-6-PD(A-) variant. The calculated in vivo half-life of this (A-) enzyme is approximately 13 days as compared with the normal enzyme which has a half-life of 62 days./20/ The reticulocytes and the relatively young erythrocytes in such deficient Negro subjects possess normal activity whereas the older cells are grossly G-6-PD deficient.

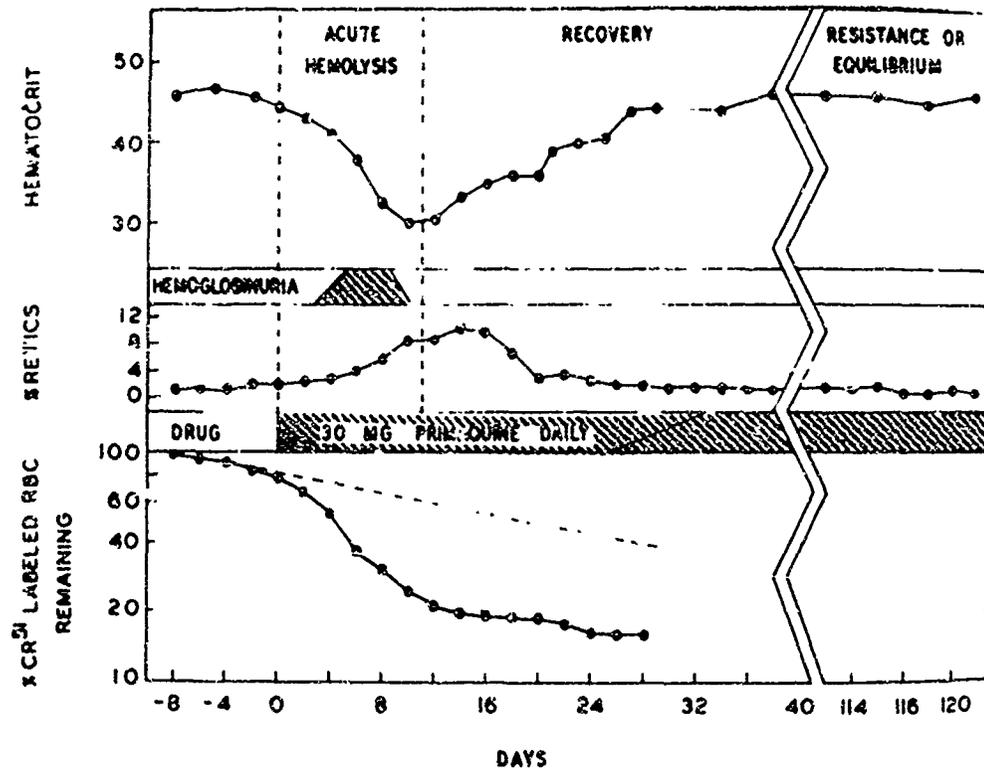


Fig 2 Typical course of hemolysis induced in hemizygous male Negroes by daily administration of 30 mg primaquine base. From *Bull WHO* 22 625, 1960

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MANIFESTATIONS IN SUBJECTS WITH G-6-PD <sup>Mediterranean</sup> ENZYME VARIANT

In contrast to the self-limited course of acute hemolysis and the relative resistance to continued drug exposure which characterizes the Negro type deficiency, Caucasian subjects with the Mediterranean enzyme variant behave differently. Although minimal shortening of the red cell survival in the absence of any oxidative stress has been reported in individuals possessing the Mediterranean enzyme variant, the majority of such individuals, like deficient Negro subjects, are clinically and hematologically normal. [21/ Red cell G-6-PD activity in subjects with the Mediterranean enzyme variant is, however, generally less than found among affected Negroes and hemolytic episodes are therefore more severe. Figure 3 [22/ illustrates the course of hemolysis in a male individual with the Mediterranean variant who received 30 mg of primaquine base daily.

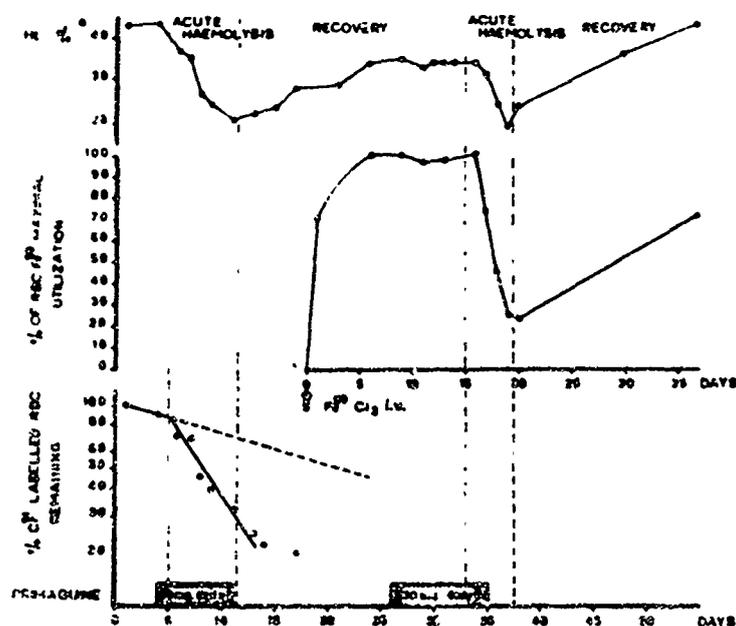


Fig. 3. In a month's period, two severe hemolytic crises were caused in a mutant Sardinian male by two course of primaquine (30 mg daily). In the second hemolytic episode a young population (10-16 days) of  $^{59}\text{Fe}$ -tagged red blood cells was rapidly destroyed. [22] Reproduced with permission.

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The acute hemolytic phase continued until the drug was withdrawn at a point when more than half the red cell mass had undergone hemolysis. Radioactive iron ( $^{59}\text{Fe}$ ) was administered to label the newly formed red cells which were to replete the circulating red cell mass over the succeeding 14 days. A second course of primaquine in the same dosage was followed initially by several days of hemolysis sufficient to offset the increased rate of red cell production, and finally, sufficiently severe hemolysis resulted in a precipitous fall in the hematocrit and again necessitated discontinuation of the drug. One important observation is that the newly formed red cells, labeled with  $^{59}\text{Fe}$  and therefore less than 14 days of age, were susceptible to the hemolytic effects of the drug, which confirmed that even the very young cells were severely enzyme deficient in this subject with the Mediterranean variant. No phase of relative resistance to continued drug exposure existed (which was characteristic of the deficient Negro subject).

The rate of G-6-PD synthesis in red cell precursors from deficient Mediterranean and Negro subjects is probably normal, however, the biological half-life, or the rate of decay in enzyme activity, is much more rapid in the Mediterranean variants and even the reticulocytes emerge from the bone marrow already deficient in G-6-PD activity. Whereas the reticulocytes from enzyme deficient Negro subjects possess normal G-6-PD activity and it is only after circulating for several weeks that the red cell enzyme activity prematurely decreases to a point where the cells are sufficiently susceptible to hemolize when subjected to oxidant stress./20/

The examples illustrated in Figure 2 and 3 represent generalizations regarding the severity of expected hemolysis among subjects with these two enzyme variants. Considerable individual variation may exist. The weekly administration of 45 mg of primaquine base and 300 mg of chloroquine base (the combination presently employed as malaria chemoprophylaxis for US troops in Republic of Vietnam) generally produces mild hemolysis which is completely compensated within a few weeks in the majority of enzyme deficient Negro males./23/ Occasionally however, such an individual without any additional drug therapy or apparent complicating illness will develop symptomatic hemolysis following initial ingestion of a single chloroquine-primaquine tablet./24/ Weekly chloroquine-primaquine administration results in hemolysis of unpredictable severity among

### *Red Cell G-6-PD Deficiency - Culp*

Caucasian males with the Mediterranean enzyme variant and generally the results are sufficiently severe so that this regimen should not be employed in such subjects./25/

#### ASIAN GROUPS

Asian ethnic groups with G-6-PD deficiency have not been as well studied as Negro and Caucasian groups. The most common variant found among Chinese is probably the variant termed G-6-PD(Canton). This variant has functional enzyme characteristics as well as clinical expression similar to that of the Mediterranean variant. /26/ Both are associated with an increased incidence of severe neonatal jaundice, favism, and drug-induced hemolytic episodes.

#### AGENTS PRECIPITATING HEMOLYSIS IN G-6-PD DEFICIENT SUBJECTS

##### Drugs

Antimalarial drugs, and especially primaquine, represent the best studied group of agents known to precipitate hemolysis in G-6-PD deficient subjects. The increasing number of drugs including many commonly used by physicians practicing medicine in temperate climates are now known to have hemolytic potential in affected subjects. Table II lists most of the clinically important drugs by their common or trade name and the daily dosage reported to have been associated with hemolysis in either Negro or Caucasian subjects. Most case reports and studies are based on observations in deficient American Negro subjects and in many instances it has only been assumed, but not necessarily established, that the dosage of any drug resulting in hemolysis in these subjects would be even more hemolytic if administered to deficient Caucasian subjects. As noted in Table II, a few drugs have been reported to precipitate hemolysis only in Caucasian subjects.

It must be emphasized that the spectrum of drugs which possess hemolytic potential for G-6-PD deficient erythrocytes is to date incompletely defined. There are several reasons for this. First, insufficient observations have been made among the less commonly encountered deficient Caucasian subjects to predict if drugs other than those listed may produce hemolysis

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TABLE II  
 DRUGS PRECIPITATING HEMOLYSIS IN G-6-PD DEFICIENT SUBJECTS\*

DRUG	DAILY DOSAGE
<i>Drugs frequently resulting in significant hemolysis</i>	
Acetanilid	3.6 gm
Azulfidine®	6.0 gm
Dapsone® (DDS)	25 mg
Furacin®	..
Furadantin®	400 mg
Furaltadone ( Altafur®)	1.0 gm
Kynex®	2.0 gm
Neosalvarsan	600 mg
Primaquine	30 mg
Promizole	...
Quinocide	...
Sulfanilamide	3.6 gm
Sulfapyridine	4.0 gm
Tricofuron®	400 mg
<i>Drugs only rarely producing significant hemolysis under normal conditions</i>	
Ascorbic acid	1.5 gm
†Aspirin	3.6 gm
Aspirin	10-12.0 gm
†Chloramphenicol	1.0 gm
Chloroquine	300 mg
Diasone	300 mg
Gantrisin®	300 mg
Phenacetin	3.6 gm
†Quinidine	800 mg

\*Data compiled from Beutler E: Drug-induced hemolytic anemia *Phan.icol Rev* 21:73-103, 1969

†Reported in Caucasians only

### *Red Cell G-6-PD Deficiency - Culp*

selectively in such individuals. Secondly, clinical reports of hemolytic anemia developing during drug therapy have generally been ascribed to the drug alone when the illness itself may have induced hemolysis in G-6-PD deficient subjects. Lastly, the number of subjects studied with any single agent is usually by necessity small and individual differences in drug metabolism as well as variable degrees of impairment of enzyme activity among deficient subjects may significantly influence the response to drug administration./27/ In vitro observations have revealed that some parent drugs are inactive in producing abnormalities in G-6-PD deficient red cells, whereas metabolites of these drugs may be capable of inducing severe membrane damage in G-6-PD deficient cells./28/ It has recently been demonstrated that gentisate, a known metabolite of aminosalicyclic acid, plays a major role in aspirin-induced hemolysis in G-6-PD deficient Caucasian subjects. It acts not only as an oxidizing agent but apparently as a direct inhibitor of enzyme activity as well. This latter function is variable from one deficient subject to another and may explain to some extent the unpredictable affect of this drug in enzyme deficient subjects./29/

### *Hemolytic Agents Other than Drugs*

It is now recognized that hemolytic episodes may occur in G-6-PD deficient subjects under conditions other than drug exposure. Bacterial infection,/30,31,32/ viral hepatitis,/30,33,34,35/ viral upper respiratory infections,/30/ chickenpox,/36/ diabetic ketoacidosis,/37/ and nephritis /30/ have all been reported to be associated with hemolysis in G-6-PD deficient subjects. The pathogenesis of hemolysis associated with infection or metabolic abnormalities has not been clearly defined. It has been postulated that the "oxidant drugs" in patients with hepatic and renal insufficiency and perhaps other metabolic abnormalities are those metabolic products that accumulate as a result of organ dysfunction. In addition, azotemia is known to be associated with reduced levels of GSH in normal erythrocytes; a factor that would further increase the susceptibility of G-6-PD deficient cells to hemolysis./38/ The role of altered blood pH in systemic illness and its effect on metabolism through the hexomonophosphate content is not known.

Some interesting in vitro observations made recently may help to clarify the mechanism by which viral infections induce hemolysis of G-6-PD deficient erythrocytes./39/ Figure 4./29/

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depicts values for autohemolysis in the presence of influenza A virus with both normal and G-6-PD deficient erythrocytes. In the absence of virus both normal and G-6-PD deficient cells undergo less than two percent hemolysis in 24 hours. With concentrations of virus greater than  $10^3$  increased autohemolysis of G-6-PD deficient red cells occurs. This could be prevented by prior heat inactivation or antibody neutralization of the virus. To establish that increased autohemolysis was related to metabolic impairment resulting from the presence of the virus particles, metabolic activity of the HMP shunt in normals and G-6-PD deficient red cells was evaluated. Since the only locus of decarboxilation of glucose in the mature red cell is the HMP shunt (Figure 1),  $CO_2$  production by the erythrocyte is directly proportional to the activity of the shunt. In the presence of significant concentrations of live virus particles normal red cells increased markedly the activity of their HMP shunt which suggested that the virus represented an oxidative stress. These cells however tolerated the oxidative stress because of their ability to increase GSH production through their intact shunt. In contrast, G-6-PD deficient cells exhibited little or no increase in  $CO_2$  production reflecting an inability to increase activity of their HMP shunt due to deficiency of the enzyme./39/

The frequency with which illness in itself precipitates hemolytic episodes among G-6-PD deficient subjects has not been well studied, but is possibly greater than has been appreciated. One retrospective study /30/ evaluated 139 episodes of recognized hemolysis among 63 Negro and Caucasian patients with G-6-PD deficiency who were admitted to a general hospital for a variety of indications. Sixty percent of the observed hemolytic episodes were apparently related to concurrent illness alone -- most frequently, to bacterial or viral infections. The severity of hemolysis varied from minimal to extremely severe and the duration of the observed episodes was transient in some, while in others several weeks were required for recovery. Thirty-six percent of the patients with documented hemolytic episodes were female, which emphasized that clinically significant hemolysis may frequently occur in heterozygotes. In another recent study /31/ of 206 consecutive hospital admissions of Negro patients, 47 had acute infections and 18 (38 percent of the group) developed anemia with hematocrits less than 30 percent. Although the incidence of G-6-PD deficiency among the entire group was only 16 percent, 67 percent of the anemic-infected patients were found to be G-6-PD deficient. Although it was not possible to establish the relative role of infection per se as opposed to

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drug therapy as precipitating factors among this group, a relationship between anemia and acute infection in G-6-PD deficient Negro subjects was strongly suggested.

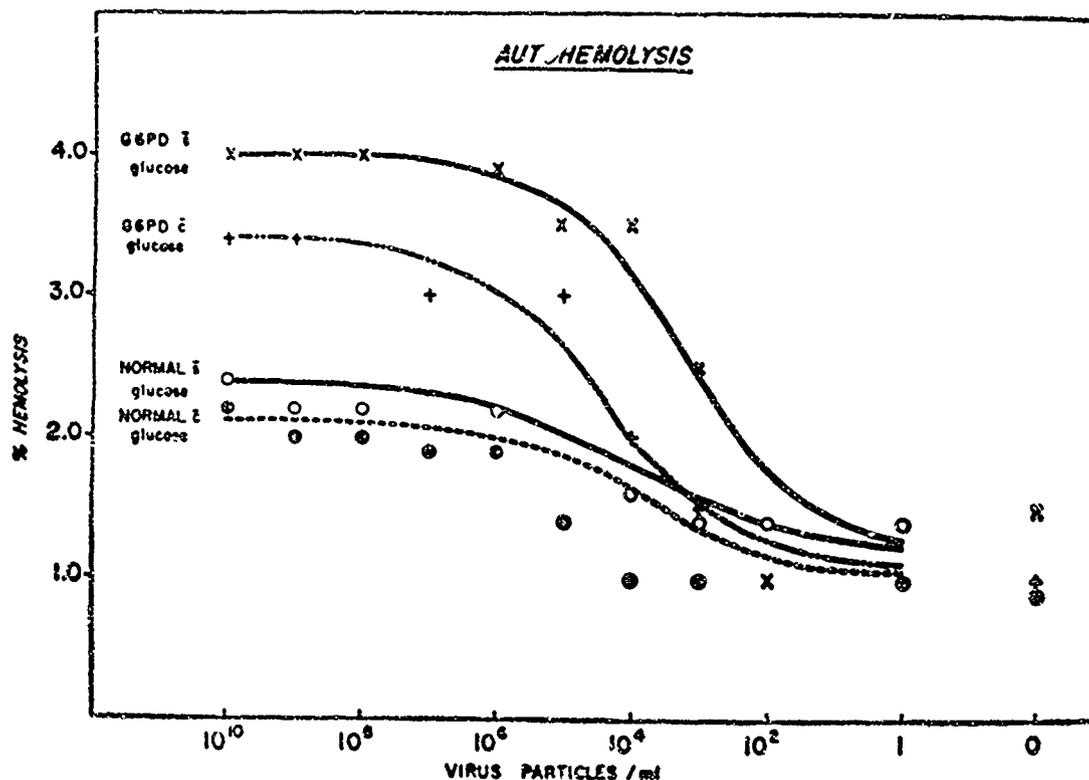


Fig 4. Autohemolysis, in the presence of influenza-A virus, in erythrocytes either normal or deficient in G-6-PD. [39] *Reproduced with permission.*

Clinically, the more commonly encountered situation is one in which both illness and effects of drug administration are present. There is evidence that additive hemolytic effects result when a susceptible individual is exposed to more than one agent simultaneously. [23,30] Asymptomatic affected Negro males serving in Vietnam and receiving weekly chloroquine-primaquine for a period of several months were not infrequently observed to develop significant intravascular hemolysis with hemoglobinuria when hospitalized for an intercurrent febrile illness. [24] Figure 5 illustrates the course in such a patient receiving weekly chloroquine-primaquine prophylaxis. [23] He was non-anemic but with manifestations of inapparent compensated hemolysis prior to the development of a febrile illness of

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undetermined etiology. The result was brisk hemolysis. This patient received aspirin (less than 2 gm/day) during his febrile course. Normally this drug in such a dosage would not have precipitated hemolysis in this individual, however, the combined effect of illness, small doses of salicylates, and continued chloroquine-primaquine administration resulted in a significant hemolytic episode.

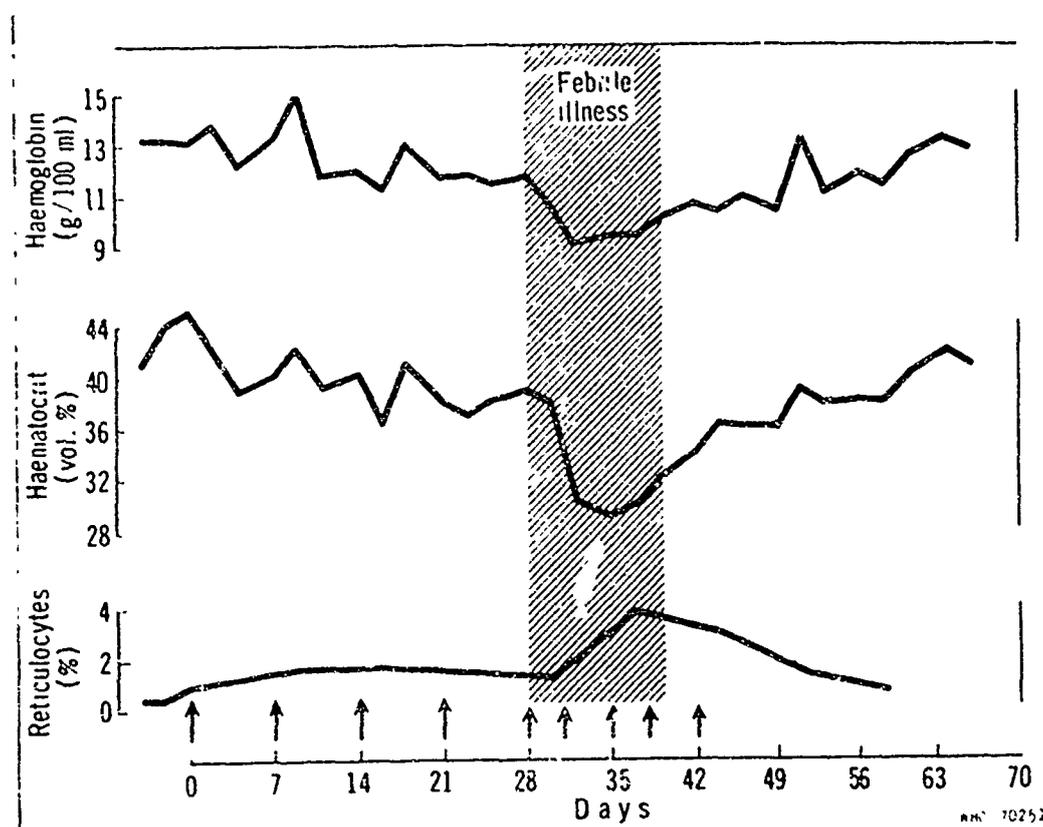


Fig. 5. Hemolytic effects of an intercurrent febrile illness (etiology not established), salicylate administration and chloroquine-primaquine administration in a Negro male with G-6-PD deficiency. ↑ = administration of 300 mg chloroquine and 45 mg primaquine. [23]

There are several situations in which unrecognized G-6-PD deficiency may complicate the manifestations of an associated underlying illness or in which G-6-PD deficiency may masquerade as another disease state. One of these is homozygous sickle cell disease in which chronic hemolysis and painful crises due to intravascular sickling are expected features of the disease, but in which acute hemolytic crises seldom occur. A recent

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evaluation of 8 subjects with documented sickle cell anemia who did experience acute episodes of increased red cell destruction, revealed that 7 of the 8 individuals had associated G-6-PD deficiency which was probably the predisposing factor for brisk intravascular hemolysis rather than the hemoglobinopathy itself./40/

The incidence of severe neonatal hyperbilirubinemia among infants with either the Mediterranean or the Canton enzyme variants is significantly increased when compared with normal infants of the same ethnic group. Although hemolysis is suspected of playing a significant role in the pathogenesis of this syndrome, the pathophysiology is not completely defined at the present time./41,42/

Transfusion of G-6-PD deficient red cells to recipients receiving oxidant drugs may result in intravascular hemolysis which mimics an incompatible hemolytic transfusion reaction. A fatal outcome has been noted in one instance where G-6-PD deficient blood from a Caucasian subject was transfused to a recipient receiving Dapsone® (DDS) for the treatment of leprosy./43/

### CONGENITAL NONSPHEROCYTIC HEMOLYTIC DISEASE (CNHD)

Patients with chronic hemolytic anemia in whom no specific diagnosis can be readily established by such routine tests as examination of the blood smear, osmotic fragility, hemoglobin electrophoresis, or the Coombs' antiglobulin reaction, have in the past been classified in a heterogeneous group under the diagnostic term "congenital nonspherocytic hemolytic disease" (CNHD). The basis for the diagnosis lies in the results of the standard autohemolysis test of Dacie, et al./44/ Over the past decade an increasing number of such patients have been found to possess deficiencies of specific erythrocyte enzymes essential to metabolic processes of the red cell. Although to date 14 distinct red cell enzyme deficiencies have been reported to be associated with hemolytic disorders, G-6-PD deficiency represents the most frequently encountered example./45/ It has been estimated that two percent of subjects with G-6-PD deficiency manifest chronic hemolytic disease in the absence of drug challenge or precipitating acute illness./46/

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The genetic variants of the G-6-PD molecule identified among patients with CNHD are characterized by marked *in vivo* instability of the enzyme, severe impairment in activity, or unfavorable alterations in the kinetic properties of the mutant enzyme, so that functionally the HMP shunt is virtually inactive./16/ Such affected individuals have been Caucasians and Orientals rather than Negroes. Since the role of the shunt seems to be primarily one of production of GSH for the protection of hemoglobin and the red cell membrane from oxidative injury, the mechanism of hemolysis in these subjects in whom no readily apparent oxidative stress can be identified remains less than completely defined.

Clinically such patients manifest chronic hemolysis but anemia is generally mild and in some the hemolysis is compensated and the patients are non-anemic. The diagnosis is usually made in childhood but may go unrecognized until adult life. Hemolysis may be aggravated by any of the factors that precipitate hemolytic episodes in other G-6-PD deficient subjects. Splenectomy and corticosteroids have no therapeutic efficacy.

*Laboratory Diagnosis*

Although spectrophotometric assays of erythrocyte G-6-PD activity are performed in many laboratories, the procedure is time-consuming, requires technical skill, and is not necessary for detection of most cases of G-6-PD deficiency. A number of screening procedures have been developed, most of which depend upon the generation of NADPH from NADP which is a function of the reaction catalyzed by G-6-PD. Figure 1. The tests are based upon the ability of generated NADPH to reduce visible dyes as brilliant cresyl blue, methylene blue, or compounds as methemoglobin. Of these simple screening procedures, the most sensitive are apparently the methemoglobin reduction test/47/ and the ascorbate test,/48/ although the latter lacks specificity./49/

Although these screening procedures are sufficiently sensitive to detect the affected male hemizygote under usual circumstances, detection of female heterozygotes with their varying proportion of normal and enzyme deficient cells presents a greater problem. At best, approximately 80 percent of heterozygotes are recognized by the methemoglobin reduction test./50/

It is important for physicians to recognize that affected Negro subjects who have recently experienced a significant hemolytic episode may at the time of post-episode evaluation possess

### *Red Cell G-6-PD Deficiency - Culp*

normal or near normal enzyme activities because the enzyme deficient cells have been destroyed and replaced by reticulocytes with relatively high activities. The recognition of G-6-PD deficiency is an even more difficult problem in the female heterozygote who has undergone hemolysis. Postponing the identification of the deficiency until a time when the disproportionate number of young cells have aged sufficiently to manifest it, is impractical. Delayed diagnosis may result in failure to withdraw the offending agent, or may lead to the institution of inappropriate therapeutic measures. A simple technique has recently been described which serves to increase the likelihood of detection during or immediately following a hemolytic episode.<sup>/51/</sup> This technique is based on selective measurement of G-6-PD activity in the most aged, and therefore the enzyme-deficient, red cells. Since density of erythrocytes increases with cell age, simple centrifugation results in separation of a population of the most enzyme deficient cells which then may be selectively studied for G-6-PD activity.

Diagnosis in subjects with genetic variants of G-6-PD other than the A-type usually presents little difficulty because recognition of subjects with the Mediterranean and Canton variants is usually possible even during a hemolytic crisis because they have a more severe enzyme deficiency which is less dependent upon cell age for expression.

### *CONCLUDING REMARKS*

Erythrocyte G-6-PD deficiency is a common hereditary abnormality of world-wide distribution. Genetic heterogeneity underlies the broad spectrum of manifestations encountered among affected subjects. A striking analogy to the hemoglobinopathies is apparent. Biochemically a single amino acid substitution in the protein molecule represents the structural abnormality which characterizes the genetic variants of both hemoglobin and G-6-PD. In each there are variants unassociated with any functional impairment, those associated with episodic symptomatology, and some which result in chronic hemolytic disease. On the molecular level our understanding of the hemoglobinopathies and G-6-PD deficiency far surpasses that of most

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other diseases, yet knowledge concerning the pathogenesis of hemolysis and more importantly, what to do about it, is limited. Careful clinical observations have, however, provided a basis for rational management of such patients and have obviated many of the needless therapeutic gestures employed in the past. The future undoubtedly holds knowledge which will permit a more direct therapeutic approach; i.e. one aimed at assisting the red cell to functionally circumvent its structurally altered hemoglobin or enzymatic protein.

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*While it may be a great personal satisfaction to make a discovery in the laboratory, we do not save people's lives in the laboratory. People's lives are saved where people are.*

- GEORGE N. PAPANICOLAOU

## HYPOPLASTIC ANEMIA

MAJ Roland F. Schoen, Jr., MC

The concept of hypoplastic anemia as we know it today has been long in evolving because of mystery of its pathogenesis and unpredictability of its clinical course. Furthermore, the findings in the bone marrow and peripheral blood have been variable, and not always easily reconciled.

The original view of "aplastic anemia" was that of a fulminant disease. Peripheral pancytopenia was a reflection of markedly or totally reduced erythroid, granulocytic, and megakaryocytic cells in the marrow. Resultant infection and hemorrhage supervened early. However, other peripheral pancytopenic states or normochromic normocytic anemias with reticulocytopenia were included later in the syndrome. Therefore, some anemias of chronic infection (with normocellular or slightly hypocellular marrows) and refractory normoblastic anemia (an iron-loading, probably premalignant condition with hyperplastic marrow) were included.

Today the term "hypoplastic" rather than "aplastic" is the qualifier of this anemic syndrome because, as with most syndromes, a spectrum of severity is encountered. Diverse etiologies are recognized, and more are postulated. The common features include peripheral anemia (usually normocytic or slightly macrocytic) reticulocytopenia, and reduced erythroid precursors in the bone marrow. White cells and platelet numbers may or may not be reduced peripherally or in the marrow.

### *Classification*

In many cases the etiology is not apparent and hence remains "idiopathic". A toxic substance should be sought out because many of these cases probably have some toxic exposure which is responsible for the blood picture. It is imperative

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to remove this substance from the patient's environment. Other causes must also be considered; namely, pure red cell aplasia, hyporegenerative crises, uremia, Franconi's syndrome and malignant conditions.

## TOXIC EXPOSURE

These may be divided into two general groups. First there is exposure to those agents which regularly produce hypoplasia after sufficient exposure, whose effects are more or less predictable, and whose effect may be demonstrated in experimental animals/1/; and secondly, there are exposures (of which most drug reactions are a part) which are only occasionally and unpredictably associated with hypoplasia and are not necessarily dose-related. The agents of both groups seldom produce a selective red cell hypoplasia, but are accompanied by reduced granulocytes and platelets in a general hypoplastic reaction. Many drugs selectively depress either the granulocytic or platelet precursors alone, and are not considered here.

## Predictable dose related hypoplasia

Ionizing radiation. Excessive doses can lead to severe and even fatal aplastic anemia, such as the cases which have been seen after nuclear explosions and industrial accidents. At lower doses, this is a reversible phenomenon, provided the victim receives exogenous blood products until regeneration of marrow has occurred. At higher dose levels, probably above 2000r of total body irradiation, regeneration will not occur. Knospe et al/2/ have demonstrated this aspect in rats and think that regeneration does not occur because the marrow adventitia has been destroyed.

The type of irradiation is an important factor. Alpha and beta particles are highly damaging, but since they have a penetrating effect of several millimeters, they are harmful to the sensitive hematopoietic tissues only if injected systemically. It is the gamma particles and fast neutrons which can penetrate the entire body and cause immediate widespread damage. The mechanism of this damage is obscure, but includes damage to enzymes containing sulfhydryl groups, and the creation of free radicals in the cell which react readily with proteins and nucleic acids./1/

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In therapeutic radiation, marrow damage occurs only in the field of exposure, and is reversible in a time course proportional to the dose given. Localized radiation, however, may produce mild marrow depression at sites distant from the radiation field (abscopal effect). This may be reflected peripherally by reduced numbers of white cells and platelets. Radiation given to a patient with much prior chemotherapy produces much more marrow depression, supposedly because of prior marrow sensitization.

Animal studies suggest that lymphocytic and erythrocytic precursors are more sensitive to radiation than are megakaryocytes and granulocytes./1/ However, radiation-induced anemia is much less of a problem functionally than leukopenia and thrombocytopenia.

Cancer chemotherapeutic agents. Most of the chemotherapeutic agents for malignant disease have marrow depressive effects. Indeed, it is the reaction of this highly sensitive tissue that often limits and may even preclude administration of a full tumoricidal dose. Again, anemia is not as significant functionally as leukopenia and thrombocytopenia.

Benzene and its derivatives./3,5/ This toxic depression usually occurs in industrial workers with chronic exposure to this widely used solvent. Control measures have aimed largely at keeping the concentration of the vapor below a critical level, because it is usually absorbed by inhalation in a poorly ventilated room.

Classic pancytopenia is the most severe form, and rarely even then is the marrow completely aplastic. However, many more subtle forms of toxicity exist. The earliest sign is probably mild hemolytic anemia with a tendency toward macrocytosis (increased reticulocytes) and a shortened red cell survival. Increased serum bilirubin has been described in many cases. Some cases of benzene poisoning have resulted in extra medullary hematopoiesis and splenomegaly, but these are rare. A significant number of the chronic benzene-induced hypoplastic states have evolved to acute myelogenous leukemia.

Trinitrotoluene (TNT) has produced hypoplasia, but more often it produces a dermatitis and gastritis. Benzene hexachloride (lindane) is a popular insecticide and has been implicated as a causative agent in hypoplastic disease.

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## Agents Unpredictably producing hypoplasia

The list is an ever-expanding one, and only the more common examples will be considered.

Chloramphenicol. Until recently, most cases of acquired hypoplastic anemia in which a toxic exposure was elicited historically have been caused by chloramphenicol. The reaction was first recognized in the early 1950s but because of concomitant drug therapy in many instances, the early reports were not heeded, and the peak incidence of new cases of hypoplastic anemia (83) occurred in 1959./6/ The incidence has declined ever since.

The risk of acquiring hypoplasia from the drug has been estimated by comparing reported cases to drug sales. It is estimated that there is one in every 60,000 to 500,000 patient-exposures. A variable predominance in females has been reported (a ratio as high as 1.6 to 1.0) especially in the premenopausal group, as well as an increased incidence in Caucasians in the northern European countries. In 75 percent of the cases, all three blood cell lines were depressed and marrow hypoplasia was found. In 50 percent of the cases evidence of "reaction" was found within 30 days of the last dose, in 22 percent during treatment, and in 10 percent after 130 days had elapsed./1/ Overall mortality rate was 50 percent; half of these deaths occurred within 50 days of onset. Favorable prognostic variables were (1) fewer blood lines depressed, (2) non-Caucasian race, (3) development of reaction during therapy or shortly thereafter, and (4) large daily dose of drug.

The marrow was truly hypocellular in only two-thirds of the cases and the remaining one-third had normocellular (or rarely hypercellular) marrows with or without a specific depression of one of the cell lines./6/ The normocellular marrows only rarely (18 percent) presented with pancytopenia. Most of the patients ( ) 80 percent) with aplastic marrows died (all of these within 18 months) primarily of cerebral or gastrointestinal hemorrhage. The presenting sign was usually purpura which occurred after therapy had stopped. Those cases of aplastic anemia occurring during therapy usually presented with anemia, were normocellular in the marrow, and often recovered in several months, but sometimes as long as a year after presentation.

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The two types of chloramphenicol toxicity are summarized in Table I./6/

TABLE I  
CHLORAPHENICOL TOXICITY

Bone Marrow Cellularity	TYPES OF TOXICITY	
	Normocellular	Hypoplasia or Aplastic
PERIPHERAL BLOOD	Anemia with/without Leukopenia or Thrombocytopenia	Pancytopenia
DRUG DOSAGE	Usually large and given in a short period of time	No particular relationship to dose, frequently intermittent
TIME RELATION (of Toxicity to last dose)	Concurrent	2 to 20 weeks later
MOST COMMON PRE- SENTING SIGN	Anemia, pallor	Purpura, hemorrhage, or both
OUTCOME	Usually nonfatal	Usually fatal

Human studies were carried out in the fifties in an attempt to delineate the dynamics of the "dose related" lesion. Rubin et al found plasma iron was elevated with high carrier saturation, a delay in the disappearance of  $^{59}\text{Fe}$  from the plasma, and failure of  $^{59}\text{Fe}$  to appear in circulating red cells for at least eight days. Definite marrow depression occurred in two percent and borderline depression in six percent of 50 healthy volunteers. Saidi et al /9/ have demonstrated striking morphologic changes in the marrow of patients receiving therapeutic doses of chloramphenicol. Large vacuoles are seen in the primitive erythroblasts, associated with anemia and reticulocytopenia, and have been considered the classic erythropoietic lesion. Wallerstein /10/ suggests that the vacuolar change is part of the pharmacologic effect, occurring in anyone taking the drug, and is reversible. Chloramphenicol given with  $\text{B}_{12}$  to patients with pernicious anemia did not inhibit the correction of megaloblastosis, so the chloramphenicol lesion is apparently not involved with nucleic acid metabolism./9/ The vacuolization is not a unique lesion;

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it is also seen in acute alcoholism./11/ The appearance of such vacuoles does not warn of impending marrow hypoplasia.

No clear explanation has been forthcoming to describe the mechanism of hypoplastic disease with chloramphenicol. Careful monitoring of drug therapy cannot prevent the disease from developing later; the best method of prevention seems to be highly judicious use of the drug clinically.

Organic arsenicals (arsenoaminobenzols used in early syphilis therapy) occasionally produced a hypoplastic syndrome but this type is not seen now. Inorganic arsenic in sufficient dose will always produce marrow depression; arsenite salts do not./1/

Anticonvulsants, such as methylphenylhydantoin (Mesantoin<sup>®</sup>), trimethadione (Tridione<sup>®</sup>), and carbamazepine (Tegretol<sup>®</sup>), have produced the syndrome. Diphenylhydantoin (Dilantin<sup>®</sup>) has been reported in one case./12/

Phenylbutazone usually produces leukopenia as a side-effect, but may produce erythroid hypoplasia.

Gold compounds, used in treatment of rheumatoid arthritis, may produce both leukopenia and hypoplastic anemia and are usually reversible on cessation of treatment.

### *Treatment*

The most important specific therapeutic measure is the identification of any causative toxin, removing it from the patient and his environment for all time. In over one-half of cases, toxic exposure is not discernible, which suggests a toxin is not causative, or continues occultly, perhaps, to insult the marrow because of uninterrupted exposure. Etiologically, these remain "idiopathic".

Supportive measures, including blood transfusions for anemia, platelet fractions for thrombocytopenia, and vigorous antibiotic treatment for infections permitted by granulocytopenia, are important. These are not without hazard, because transfusion reactions and sensitization occur. Cirrhosis and liver failure may develop from transfusional hemosiderosis./13/

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Not to abandon attempts at therapy is the primary theme. Multiple hematinics have been tried, such as pyrioxine, folic acid, vitamin B<sub>12</sub>, liver extract, and a trial of these is probably warranted only to prove that they will not be a beneficial alternative to repeated transfusion. Some spontaneous remissions have occurred many months after onset, and so vigorous support is justified. Splenectomy /14,15/ has appeared to be helpful in some cases when other measures have failed, and may be justified on an empiric basis rather than theoretic basis. Cortisone has been tried with some success; Sanchez-Medal, et al /16/ had a 12 percent response rate.

The most promising agents are testosterone and its derivatives, especially oxymethalone. Sanchez-Medal, et al /21/ had 50 percent response rate to this latter drug, even when, in a few cases, testosterone had failed. In 69 cases, 33 positive responses were seen, 22 of which have been apparently permanent. Therapy was initially undertaken for two months, but eight relapses occurred on discontinuing therapy. Seven of the eight responded to re-induction with oxymethalone. Improvement was noted in those who responded in two weeks to six months. Oxymethalone can produce obstructive jaundice (as a 17-methylated testosterone) but is not often a cause for stopping therapy. Its mechanism of action is unclear, but decreased <sup>59</sup>Fe plasma clearance and decreased red cell <sup>59</sup>Fe utilization have normalized on therapy. /17/ Erythropoietin values are increased in such patients, and it is postulated that androgens may work synergistically with erythropoietin to stimulate erythropoiesis.

### Pure Red Cell Aplasia

In contrast to acquired hypoplastic anemia, there is a group of syndromes (1) in which the red cell precursors are the only deficient cells in the marrow, (2) which occur independent of any toxic exposure, (3) which occur in certain age groups, (4) which have a particular therapeutic approach.

### Pure Red Cell Aplasia (PRCA) in Adults

This acquired condition has been given many names, including primary red cell aplasia, erythroblastopenia, idiopathic aplastic anemia, selective red cell aplasia, erythroblastic hypoplasia, chronic hypoplastic anemia, essential erythroblastopenia, primary refractory anemia.

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The Mayo Clinic reviewed 16 cases seen in 1951-61 and established the following diagnostic criteria./18/

- - Severe normocytic normochromic anemia requiring transfusion.
- - Reticulocyte count of 0.0 to 0.2 percent.
- - Normocellular marrow without red cell precursors (as distinguished from refractory normoblastic anemia).
- - Normal liver, spleen and lymph nodes (before transfusional hemosiderosis)

In their series 10 percent developed hemolysis. The average patient was male (2:1) and middle-aged. Ten percent developed leukemia at a later time. Some toxins have been suspected, and in other settings this syndrome (PRCA) has been seen in kwashiorkor (which responds to riboflavin) and in one case with carcinoma of the bronchus./18/

Finkel, et al/19/ have described three patients in whom cortisone has been dramatically effective, and this has been noted by others. Its mode of action is speculative, but recognized to be antireoplastic (in lymphoproliferative syndromes) and immunosuppressive in other settings. Antibodies have been demonstrated against erythroblasts and against erthropoietin. /20/ Cyclophosphamide and 6-mercaptopurin have also been used with success, suggesting inhibition of a cell that had been producing something toxic and suppressive to erythroblasts./21,22/ Therefore, cortisone and cytotoxic drugs have theoretic bases for use in PRCA.

Pure red cell aplasia in adults has also been seen coincidentally with benign thymoma, non-invasive spindle-cell type./23/ In about 15 percent of these myasthenia gravis is also present. The anemia may precede recognition of the tumor but usually occurs with it. In one case, anemia occurred three years after the removal of the tumor. Thymectomy may completely, partially, or fail to correct the anemia. Cortisone was beneficial in two cases only after thymectomy and splenectomy. Testosterone has occasionally been helpful. The

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mechanism of the anemia in combination with thymoma is obscure. An autoimmune basis has been postulated but not demonstrated.

### *Pure Red Cell Aplasia in Children*

Congenital hypoplastic anemia was described as a syndrome in 1938 by Blackfan and Diamond and bears their name. It refers to an abnormality characterized by normocytic, normochromic anemia, reduced red cell precursors in the marrow, but normal leukocytes and platelets and found in young children. The usual name applied is "congenital hypoplastic anemia,"<sup>/29/</sup> as opposed to "congenital pancytopenia" (Fanconi's anemia) discussed page 286. Other synonyms for this syndrome are hyporegenerative anemia, erythrophthisis, idiopathic hypoplastic anemia, stationary hypoplastic anemia, chronic erythroblastopenia, erythrodysgenetic anemia, chronic erythrocytic hypoplasia, pure red cell agenesis, erythrogenesis imperfecta.

These children may not be anemic at birth, or do not show sufficient pallor to entertain such a diagnosis, and so the true congenital nature is controversial. There is a slight predisposition for males, Caucasians, and one-third of cases have been products of complicated pregnancies. Most do not have other congenital anomalies like the Fanconi patients. The median age of diagnosis is one month; average is six months. Hepatomegaly has been described.

The natural course of the disease has changed. Previously 15 percent had spontaneous remissions with decreasing transfusion requirements. The usual problem of hemosiderosis cirrhosis, growth retardation, osteoporosis, bone age retardation and pubertal failure were seen after five years of transfusions. Cortisone has been of some benefit, and it has recently been appreciated that if the disease is treated within the first few months of diagnosis, 90 percent will respond, whereas after a year with the disease the response rate is much less.<sup>/25/</sup> Maintenance usually requires 5.0-7.5 mg prednisone daily, with rise in hematocrit each time a "reduced" dose of corticosteroids is raised. The mechanism of action for cortisone remains unclear.

The disease has been seen to occur more than once in five families.<sup>/24/</sup> No chromosomal aberrations have been described, in contrast to those with Fanconi's anemia. Abnormal amounts

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of anthranilic acid have been described in the urine of patients/26/ but a definitive metabolic error has not been found.

*Hyporegenerative Crisis*

There are probably many acute illnesses and stresses which universally cause temporary marrow arrest./27/ It is transient and passes unnoticed in individuals whose blood cells have a normal life span. In patients with hemolytic anemia, where red cells may live only a fraction of the normal 120 days, temporary marrow arrest may lead to severe anemia. The marrow normally works much below full capacity, implying that it can produce several times the usual number of cells per unit of time. Therefore it can compensate without anemia developing when red cells live only 12 days in some settings. Certainly this increased rate cannot be sustained indefinitely. In acute hemolytic anemia, reticulocyte rise may be delayed peripherally and the bone marrow depleted of red cell precursors. This is due to initial rapid emptying of reserves and a two to four day lag in marrow erythroid response.

Patients with chronic hemolytic anemia, such as sickle cell disease, and hereditary spherocytosis are most prone to hyporegenerative crises./28/ It cannot be prevented in the former, but splenectomy prevents it in the latter. (The cellular defect persists, but accelerated destruction ceases.) Chronic hemolytic disease may predispose to a megaloblastic aggravation of marrow failure because of a relative folate deficiency.

*Treatment*

Treatment of hyporegenerative crisis is supportive with transfusions. Occasionally platelet and leukocyte lines are also affected but do not require exogenous support. Spontaneous recovery occurs in seven to ten days, with sequential leukocytosis, normoblastosis, reticulocytosis and rise in hemoglobin level.

Anticipation of such events prevents being caught unaware. It can be prevented only by altering the length of life of the red cell, as splenectomy in hereditary spherocytosis, or possibly in elliptocytosis.

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## Uremia

Anemia is observed in most types of renal insufficiency. In mild azotemic states anemia may be a reflection of the disease causing the renal insufficiency, not of the kidney failure per se. Only when the blood urea nitrogen (BUN) is greater than 80-100 mg/100cc may erythroid hypoplasia be seen. White cells and platelets are usually present in normal numbers.

The anemia is usually normocytic, but occasionally somewhat hypochromic. Burr cells may be present. Red blood cell survival is shortened, most likely from an extracorporeal factor (uremic inhibitor?)/29/ Reticulocytes are usually reduced; the anemia usually stabilizes when the hematocrit is about 20 percent. Ferrokinetic studies have shown plasma iron clearance time is increased, plasma iron transport rate is decreased or unchanged and red cell utilization of iron is decreased. The iron may be shunted to storage in the reticulo-endothelial system and unavailable for hemoglobin synthesis. Iron may be lost through gastrointestinal bleeding, loss of transferrin in nephrosis, and, more recently, loss through disposable dialysis coils./30/

The kidney has also been cited as the main source of erythropoietin, a hormone stimulating red blood cell production. In advanced renal disease the level of this hormone is reduced, and exogenous erythropoietin stimulates the uremic marrow imperfectly, suggesting the presence of some additional repressor or inhibitor. Recently an inhibitor to erythropoietin has been isolated from the kidney (and other tissues) but it may not play a physiologic role./29/

Chronic dialysis may improve the anemia after several months. The kidney does not, supposedly, produce more erythropoietin, but improvement may occur from removal of a marrow suppressant, removal of erythropoietin inhibitor, removal of a depressant on erythropoietin production, or increased erythropoietin production from extrarenal sites. Eschbach\* has recently shown that the anemia of renal failure may be benefited by androgens, specifically fluoxymestron (Halotestin®). One to six months is required for treatment; there appears no relationship to status of kidney function. It is effective in anephric

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patients. Concomitant iron deficiency prevents its action. It rarely raises the hematocrit above 30 percent.

**Fanconi Syndrome and Other Possible Pre-malignant States**

This section raises the intriguing notion that hypoplastic disease from seemingly diverse etiologies can terminate with malignant transformation to acute leukemia. Indeed, such a diagnosis should be entertained in any differential diagnosis of hypoplastic anemia./31/ There is one case report/32/ in which chloramphenicol-induced hypoplasia, treated for seven years with androgens, terminated in acute leukemia. Several cases of leukemia following benzene induced hypoplasia have been described./4/ Many of the Japanese survivors of the atom bomb blasts were hypoplastic as a population and developed leukemia in much higher numbers at a later date than unaffected populations./33/ Paroxysmal nocturnal hemoglobinemia (PNH) has been associated with aplastic crises and acute leukemia has developed in a few patients./34/

Fanconi's syndrome, or constitutional aplastic anemia (constitutional pancytopenia), is a rare anemia of childhood. It develops usually between age four and twelve years and is rare under age two./35/ Patients are usually hyperpigmented, have small stature, and have other congenital anomalies, such as microcephaly, hypogenitalism, strabismus, extra digits, renal anomalies, and mental retardation. Bone marrow examination may initially be hypercellular/36/, but later shows depression of one or more cell lines. Chromosome studies show a normal number with a specific type of aberration termed endoreduplication./1/ Chromatid exchanges and chromatid breaks have also been seen. These abnormal chromosomal patterns have been seen in "normal" family members of the proband./37/ Patients with Fanconi's anemia and their family members have an increased incidence of leukemia./5/ The chromosomes of patients with Fanconi's anemia and those of the normal family members are also more susceptible to malignant transformation by SV 40 virus in vitro./44/

Patients with Fanconi's disease usually do not survive more than three years from diagnosis, although a few have reached adulthood. Splenectomy has been helpful occasionally in relieving thrombocytopenia. Cortisone and androgens have not been useful.

*Hypoplastic Anemia - Schoen**Conclusion*

The diagnosis of hypoplastic disease raises four important points: Is more than the red cell line involved? If so, and the stigmata of Fanconi's syndrome are not apparent, then the presence of an exogenous toxin must be suspected and its identification and elimination are paramount.

Can a specific therapeutic agent be applied early to reverse the disease? Is this a pre-malignant lesion? Even if such is suspected, no therapy can be directed toward a malignancy until it is manifest, and other therapies are considered in a normal order.

If no specific therapeutic agents are applicable, many non-specific remedies must be tried, a spontaneous remission hoped for, and transfusions given for support.

Patience, a hematologic potpourri of therapies, and hope constitute the milestones on the journey toward control, if not cure, of hypoplastic anemia.

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#### Acknowledgement

Mrs. Seraphine Thiebaut, secretary to the Guest Editor, typed the major portion of the final copy of this symposium.