as the one organized by project ASTRA (*13*).

Finally, esthetic arguments against useless outdoor lights are beginning to be appreciated. The chairman of the Los Angeles City Planning Commission actually proposed in 1972 that the Santa Monica Mountains be outfitted with many searchlights to scan the skies every night, for their dramatic effect (*14*). Public outrage was instantaneous and nearly unanimous. But aside from this isolated and somewhat bizarre incident, there is some growing feeling that a dark night sky is a nice thing; millions of urban children have never seen the Milky Way. In 1971, the board of directors of the Sierra Club adopted a policy against unnecessary outdoor lighting because it wastes energy, is esthetically unpleasant, and interferes with astronomical research. This point of view should be encouraged.

Summary

There have been major qualitative and quantitative changes in outdoor lighting technology in the last decade. The level of skylight caused by outdoor lighting systems is growing at a very high rate, about 20 percent per year nationwide. In addition, the spectral distribution of man-made light pollution may change in the next decade from one containing a few mercury lines to one containing dozens of lines and a significantly increased continuum level. Light pollution is presently damaging to some astronomical programs, and it is likely to become a major factor limiting progress in the next decade. Suitable sites in the United States for new dark sky observing facilities are very difficult to find.

Some of the increase in outdoor illumination is due to the character of national growth and development. Some is due to promotional campaigns, in which questionable arguments involving public safety are presented. There are protective measures which might be adopted by the government; these would significantly aid observational astronomy, without compromising the legitimate outdoor lighting needs of society. Observatories should establish programs to routinely monitor sky brightness as a function of position, wavelength, and time. The astronomical community should establish a mechanism by which such programs can be supported and coordinated.

References and Notes

1. A. L. Broadfoot and K. R. Kendall, *J. Geophys. Res.* **73**, 426 (1968).
2. C. W. Allen, *Astrophysical Quantities* (Athlone, London, 1955); 1 stilb = 1 lumen per square centimeter per steradian = 1.15×10^6 stars of visual magnitude 0 per square degree.
3. W. A. Baum, in *Astronomical Techniques*, W. A. Hiltner, Ed. (Univ. of Chicago Press, Chicago, 1962), p. 1.
4. Street and Highway Lighting Committee, *A Report on Street and Highway Lighting throughout the United States* (Edison Electric Institute, New York, 1970), p. 15.
5. *Census of Manufacturers* (Bureau of the Census, U.S. Department of Commerce, Washington, D.C., 1954, 1958, 1963, 1967).
6. M. F. Walker, *Proc. Astron. Soc. Pac.* **82**, 672 (1970).
7. A. B. Meinel, *Stars and Stellar Systems* (Univ. of Chicago Press, Chicago, 1960), p. 54.
8. Joint Committees of the Institute of Traffic Engineers and the Illuminating Engineering Society, *Illum. Eng. New York* **59** (No. 9), 585 (1966).
9. Street and Highway Safety Lighting Bureau, *5 Reasons and 18 Ways to Improve Your Street Lighting* (Street and Highway Safety Lighting Bureau, New York, 1969).
10. *Crime in the United States* (Uniform crime reports, Federal Bureau of Investigation, Washington, D.C., August 1971), p. 65.
11. The Steward, Kitt Peak National Observatory and Smithsonian Astrophysical Observatory, *Light Pollution: A Threat to Astronomy in Pima and Santa Cruz Counties* (Stellar Division, Kitt Peak National Observatory, Tucson, Ariz., 1971).
12. City ordinance number 3840, Tucson, Ariz., adopted 5 June 1972.
13. N. Laulainen and P. W. Hodge, Eds., *Astronomy and Air Pollution* (Project ASTRA Publ. No. 15, Univ. of Washington, Seattle, 1972).
14. Los Angeles *Times*, 17 May 1971, part II, p. 1.

15. I would like to acknowledge useful discussions with, and comments on the manuscript by L. Aller, L. Blakeley, H. Epps, A. Hoag, E. King, D. Popper, T. Riegel, R. Roosen, M. Walker, and R. Weymann.

The Genetics of Hereditary Disorders of Blood Coagulation

Functional and immunological studies provide evidence for the heterogeneity of many familial clotting disorders.

Oscar D. Ratnoff and Bruce Bennett

The dramatic nature of the symptoms of hereditary disorders of blood coagulation and the ease with which the affected tissue, circulating blood, can be studied contribute to the disproportionate interest in these rare conditions. Among the results of many published studies, a large volume of information has been generated which supplies support for principles of heredity adduced from other sources. The discovery that many of these diseases are heterogeneous in nature has overthrown our simplistic views and has raised, in the usual way, more questions than answers. The next few years should see a rapid expansion of our knowledge in this field, and the time seems appropriate for us to take stock and see just where we are.

Normal Blood Coagulation

In mammals, blood clotting results from the conversion of a soluble plasma protein, fibrinogen (factor I), into fibrin, an insoluble network of fibers. The jelly-like appearance of blood clots is due to the entrapment of cells and serum within the meshes of this network. The formation of fibrin is catalyzed physiologically by a hydrolytic plasma enzyme, thrombin, which cleaves two pairs of small polypeptides, fibrinopeptides A and B, from each fibrinogen molecule (Fig. 1). What remains, so-called fibrin monomer (*1*), then aggregates into an insoluble polymer, fibrin. The monomeric units of fibrin are further bound covalently through the action of another

Dr. Ratnoff is professor of medicine, Case Western Reserve University School of Medicine and Career Investigator of the American Heart Association, University Hospitals of Cleveland, Ohio 44106. Dr. Bennett is research fellow in medicine, and fellow of the Pink Family Foundation, Case Western Reserve School of Medicine.

plasma enzyme, fibrin-stabilizing factor (factor XIII), a transamidase bearing some functional resemblance to a similar enzyme in liver. Fibrin, formed in the presence of functional fibrin-stabilizing factor, is insoluble in such dispersing agents as 5*M* urea or 1 percent monochloroacetic acid, while that formed in its absence readily dissolves in these agents.

Thrombin is not found in normal circulating blood, but generates from its precursor, prothrombin (factor II) only as the clotting process proceeds. The formation of thrombin can be brought about, under normal conditions, by one or both of two mechanisms, designated as the extrinsic and intrinsic pathways of thrombin formation (Fig. 2). When blood comes into contact with injured tissue, the latter initiates clotting via the extrinsic pathway. The active moiety of tissue, a lipoprotein called tissue thromboplastin (factor III, a seldom-used synonym) interacts with a plasma protein, factor VII, and calcium ions to form an agent that transforms another plasma protein, Stuart factor (factor X) to an active state; the enzymatic groups responsible are probably on the factor VII molecule (*2*). The thromboplastic lipoprotein contains at least two functional components, a peptidase and a mixture of phospholipids, of which phosphatidyl ethanolamine is most important. These two components are both required for the formation of a thromboplastin-factor VII complex and for the activation of Stuart factor. In its active form, Stuart factor has esterolytic and presumably specific proteolytic properties. Optimal conversion of prothrombin to thrombin by activated Stuart factor occurs only after complex formation with another plasma protein, proaccelerin (factor V), which takes place in the presence of calcium ions on phospholipid micelles provided by tissue thromboplastin itself. The product of this interaction, vaguely called the prothrombin-converting principle, splits prothrombin enzymatically into two fragments of approximately equal size, one inert and the other possessing the properties of thrombin.

Plasma, depleted of blood cells, including anuclear platelets which are needed for hemostasis in small wounds, clots when placed in glass tubes. The process through which this comes about has been designated the intrinsic pathway, although it cannot take place without exposure of plasma to substances extrinsic to plasma such as

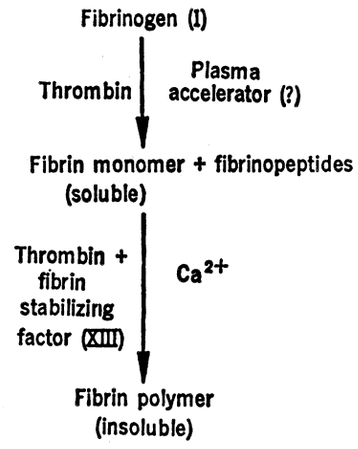

Fig. 1. The formation of fibrin in human plasma. The site of action of the plasma accelerator, if this exists, is not known. [From Ratnoff (*51*); courtesy of Lippincott, Philadelphia]

glass or other agents whose common property seems to be a negative surface charge. Glass activates a plasma protein, Hageman factor (factor XII), which then activates a plasma enzyme, plasma thromboplastin antecedent (PTA, factor XI). This, in turn, changes another protein (the list seems endless), Christmas factor (PTC, factor IX) to activated Christmas factor, a step requiring calcium ions. Activated Christmas factor interacts with antihemophilic factor (AHF, factor VIII), the protein functionally deficient in classic hemophilia, on the surface of phospholipids, but only if calcium ions are present. The product, like that formed by tissue thromboplastin and factor VII, converts Stuart factor to its active state; in all probability, the enzymatic groups required for this step are on the Christmas factor molecules. The succeeding steps in the formation of thrombin appear to be the same as those of the extrinsic pathway. The stepladder nature of the intrinsic pathway has led to its description as a waterfall (*3*) or cascade (*4*). Phospholipids required for the intrinsic pathway are supplied by the plasma itself or, when whole blood clots via this mechanism, by the lipids present in platelets.

In vitro, both the participation of AHF and of proaccelerin in clotting occurs optimally only if these clotting factors have first been altered by thrombin. Thrombin is also needed to activate fibrin-stabilizing factor. Thus, this enzyme has functions other than the conversion of fibrinogen to fibrin.

Two uncertainties among many must be recorded. First, we do not know the physiologic stimulus which activates the intrinsic pathway, although collagen (the structural protein of connective tissue, including blood vessel walls) and sebum (the oily secretion on skin) seem probable candidates. Second, a hereditary disorder has been recognized, Hageman trait, in which plasma is deficient in Hageman factor, the agent activated by glass. Although blood clotting in vitro is grossly impaired in Hageman trait, patients with this abnormality usually escape any consequences of the defect. Thus, other mechanisms must exist in vivo for activation of the intrinsic pathway. Recently, evidence has been obtained that pancreatic trypsin can bring about activation of PTA, eliminating the requirement for activated Hageman factor (*5*). Because trypsin is not present in normal blood, perhaps some other protease plays this role. The physiologic necessity for the intrinsic pathway is revealed by the major hemorrhagic disorders which accompany functional deficiencies of Christmas factor or AHF.

Plasma contains inhibitors of the activation of Hageman factor and of activated Hageman factor, activated PTA, activated Stuart factor, and thrombin. The role of these inhibitors in modulating the clotting process is still uncertain.

This short review of the normal process of blood coagulation would be incomplete without our noting that four of the protein factors involved, Christmas factor, factor VII, Stuart factor, and prothrombin, require vitamin K for their synthesis, which takes place within the parenchymal cells of the liver. Although a persuasive argument can be made that these four clotting factors are derived from a single parent molecule, most evidence suggests that they are distinctive proteins. An unsolved genetic puzzle is why these four proteins, all involved in blood coagulation, are the only proteins known whose synthesis requires vitamin K.

The Heterogeneity of Hereditary Clotting Disorders

Hereditary disorders due to abnormal functioning of each of the plasma protein clotting factors have been identified; in fact, the existence of several of the known clotting factors has been discovered as a result of the study of individuals with coagulation abnormal-

ities. In almost every case, the abnormality has been restricted to a single factor. The familial clotting disorders serve as model systems to support long-held views concerning hereditary principles.

At first, the assumption was universal that patients who shared the same spectrum of symptoms had the same biochemical defect. The first major disturbance to this view came from the demonstration by Pavlovsky (*6*) that a mixture of plasmas, obtained from two patients thought to have hemophilia, behaved like normal plasma. This startling observation led to the division of hemophiliacs into two groups. Some patients appeared to be deficient in AHF and were said to have classic hemophilia (hemophilia A), while others appeared deficient in an entirely separate protein, Christmas factor, and were said to have Christmas disease (hemophilia B, PTC deficiency). Thus, a single clinical phenotype resulted from functional protein deficiencies representing either of two different genotypes.

Soon it became apparent that even when only a single clotting factor was involved, the disorder might nonetheless be heterogeneous. Thus, in different families individuals with classic hemophilia or Christmas disease were affected to different degrees, the intensity of symptoms being in proportion to the degree of the deficiency of AHF or Christmas factor, as measured in clotting assays. That this heterogeneity might be due to the existence of multiple alleles for the defective gene was apparent from the fact that within a single family different bleeders had approximately the same degree of functional abnormality (*7*). In these two diseases, the allelic genes appeared to direct the quantity of functional clotting activity of the factors synthesized by each patient.

It is perhaps even more exciting that heterogeneity has been established by discovering that the disorder in synthesis of clotting factors may occur in qualitatively different forms. Until recently, the dominant view was that patients with hereditary disorders of blood coagulation were unable to synthesize normal amounts of the clotting factor whose deficiency was recognized by functional tests. The equally logical possibility, that the abnormality might be due to synthesis of a defective variant of the normal clotting factor, was first proposed by Fantl, Sawers, and Marr (*8*) in a study of Christmas disease. Although little attention was paid to these pioneer studies at the time they were conducted, a bewildering number of examples have now been found in which patients synthesize an abnormal form of the supposedly missing clotting factor.

In most instances, the abnormal variant has been discovered by finding, in the plasma of a patient with a hereditary hemorrhagic disorder, antigenic material related to the clotting factor whose functional activity is deficient. Such antigens are recognized by a specific antiserum. A patient whose plasma contains antigenic material is said to be CRM+ (that is, the plasma contains antigenically cross-reacting material), while a patient whose plasma contains no demonstrable antigenic material is said to be CRM−. This division is an oversimplification. In some instances, such as in classic hemophilia, heterologous antiserum demonstrates the presence of antigens in every patient, while homologous antiserum detects antigens in only a small proportion of bleeders. Presumably, homologous antiserum reacts with a much more limited portion of the aberrant protein than heterologous antiserum, which recognizes not only the aberrant portion of the molecule but also species differences.

Heterogeneity has been recognized in others ways. Thus, patients with hereditary disorders of fibrinogen may be unable to synthesize any substance recognizable as fibrinogen by chemical or physical as well as immunologic means, while other patients may have essentially normal amounts of functionally abnormal protein in their plasma. Among the latter patients, said to have dysfibrinogenemia, affected individuals in different families may have qualitatively distinguishable defects, as if different parts of the fibrinogen molecule were aberrant. Still another type of heterogeneity has been observed in Hageman trait, the hereditary deficiency of Hageman factor. In most individuals, the disorder has been inherited in a classic autosomal recessive manner. In one family, however, a severe deficiency of Hageman factor has apparently been inherited as an autosomal dominant trait with variable expressivity (*9*). Thus, the phenotype, Hageman trait, may result from two genotypic abnormalities.

We have made the assumption that a patient whose plasma contains no detectable CRM is unable to synthesize the missing clotting factor. Perhaps, instead, such a patient synthesizes a variant protein that is so rapidly catabolized that no appreciable amount can be detected. Alternatively, a variant protein may be present but not detected by the methods available. Similarly, the presence of aberrant protein in CRM+ patients may arise in several different ways. Perhaps an error in DNA coding results in the substitution of an amino acid in a polypeptide chain, a situation analogous to that in the hemoglobinopathies.

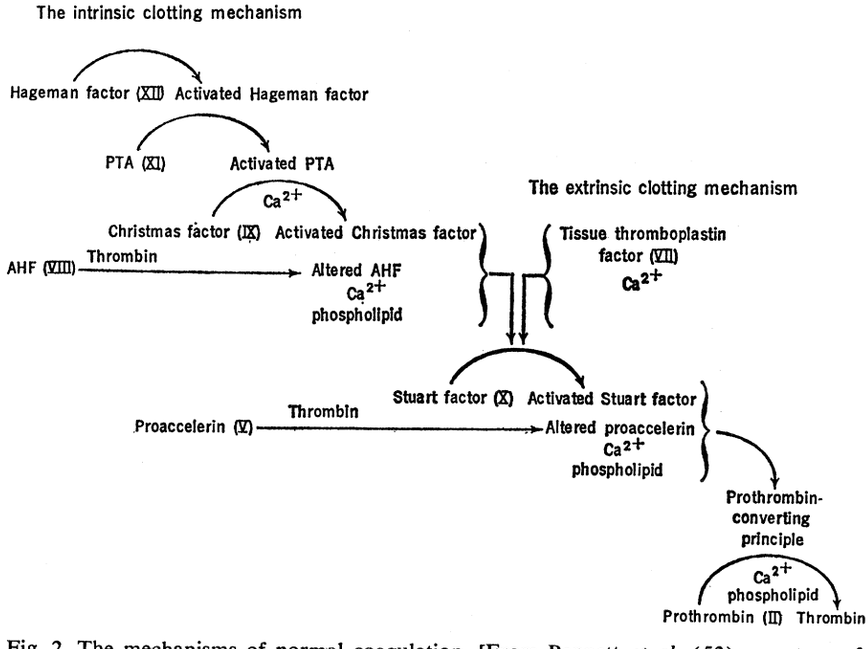

Fig. 2. The mechanisms of normal coagulation. [From Bennett *et al.* (*52*); courtesy of *Medical Clinics of North America*]

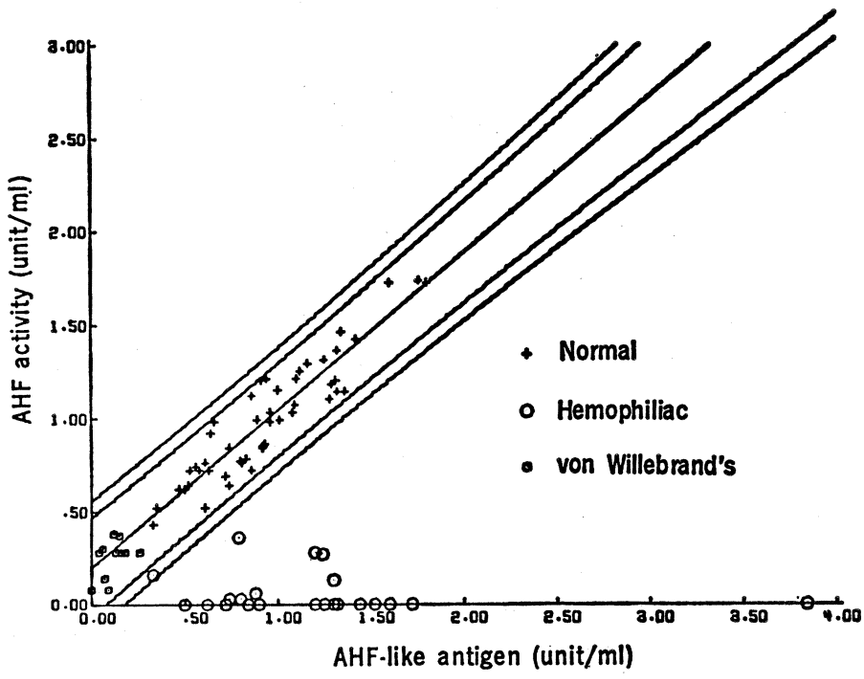

Fig. 3. The relationship of AHF activity to AHF-like antigen in normal individuals, hemophiliacs, and individuals with von Willebrand's disease. AHF-like antigen was measured by quantitative immunoelectrophoresis. The center line is the regression line for normal individuals; the outermost lines represent the 99 percent confidence belt; and the other two lines, the 95 percent confidence belt. One unit of AHF activity or antigen is the amount present in 1 milliliter of pooled normal plasma. [From Zimmerman *et al.*(*24*); courtesy of *Journal of Clinical Investigation*]

An almost infinite variety of amino acid substitutions can be imagined, but this area has hardly been explored. In other instances, perhaps the patient synthesizes an incomplete form of the clotting factor, or fails to carry out excision of a portion of a parent molecule. Conceivably, too, the patient may synthesize an additional inhibitory fragment which, attached to an otherwise normal clotting factor, keeps it from functioning normally.

We will now discuss briefly some specific examples of hemorrhagic disorders to illustrate this heterogeneity. Many other examples have been described in a recent review (*10*).

Disorders of Fibrinogen

Congenital afibrinogenemia is an autosomal recessive disorder in which affected individuals lack all but traces of any protein recognizable as fibrinogen by chemical, physical, or immunologic means (*11*). As befits an extremely rare autosomal recessive disease, the patients' parents were consanguineous in 31 of 50 families. The heterozygous state is usually not detectable, although occasionally parents or sibs have had an intermediate deficiency of fibrinogen. As might be expected, the plasma of patients with congenital afibrinogenemia does not clot upon the addition of thrombin. It is conceivable that patients with congenital afibrinogenemia synthesize a variant protein which so differs from normal fibrinogen that our present techniques cannot detect it. Alternatively, a variant might be synthesized which is so rapidly catabolized that no appreciable amount remains in the plasma. Congenital afibrinogenemia, then, is the prototype of a CRM− state.

Congenital dysfibrinogenemia, in contrast, is clearly a CRM+ condition. In studies that began with those of Ingram (*12*) and Imperato and Dettori (*13*), a number of individuals have been identified whose plasma contains normal, or almost normal, amounts of a variety of fibrinogen which behaves abnormally upon the addition of thrombin. At least 22 families have been described in which the fibrinogen of affected individuals clots abnormally slowly upon the addition of thrombin. In most cases, the abnormal fibrinogens have been distinguishable on the basis of electrophoretic or chromatographic behavior of the abnormal protein, its content of carbohydrate (a normal constituent of fibrinogen) or its functional characteristics (Table 1). In some cases, the functional defect in fibrinogen has been a failure to release fibrinopeptides at a normal rate upon the addition of thrombin. In others, fibrinopeptide release has proceeded at a normal rate, but aggregation of the fibrin monomers to form a visible clot has been delayed. In a family under study, the abnormal protein, fibrinogen Cleveland II, releases its fibrinopeptides abnormally slowly, and the fibrin monomers then aggregate at a slower than normal rate, so that both of these functional abnormalities are present.

Patients with these defective fibrinogens have had relatively few symptoms. A few have had a mild bleeding tendency, and three individuals have had postoperative disruption of wounds, as if the fibrin which formed was incapable of sealing the wound edges. Paradoxically, a few patients have had repeated episodes of thrombosis. In all affected families, dysfibrinogenemia has been identified in both sexes and in succeeding generations, as if the trait were inherited in an autosomal dominant manner. In agreement with this, in some affected individuals both normal and abnormal fibrinogens have been demonstrated in plasma, but studies of this phenomenon have been incomplete.

The abnormal fibrinogens immediately bring to mind the apparently innumerable variants of hemoglobin. Only in fibrinogen Detroit has it been possible to demonstrate an abnormality in protein structure; in this disorder, a serine residue is substituted for arginine No. 19 in the amino terminal part of the α (A) chain (*14*). Differences in carbohydrate composition have been described in some abnormal fibrinogens; whether these changes are secondary to alterations in the polypeptide chains is not yet known.

As might be anticipated, families have been found in which the abnormal fibrinogen exhibits functional defects other than delayed response to the addition of thrombin. In one interesting family, Egeberg (*15*) observed the association between frequent thrombotic episodes and the presence of a variant of fibrinogen which clotted unusually rapidly upon addition of thrombin. In another family studied by Hampton (*16*), the fibrinogen of affected individuals was incapable of forming normal covalent cross-links when treated with fibrin-stabilizing fac-

tor; the defect was inherited as an X chromosome–linked trait. Presumably, as more individuals are studied, fibrinogens will be found which behave in still other abnormal ways. The dysfibrinogenemias, then, exemplify disorders of clotting in which patients synthesize functionally defective variants of a normal clotting factor.

Fibrin-Stabilizing Factor Deficiency

In the hereditary deficiency of fibrin-stabilizing factor, the fibrin of clotted blood is of poor tensile strength and dissolves readily in agents such as *5M* urea or 1 percent monochloroacetic acid, because the covalent bonding induced by this enzyme is missing. Patients with this disorder, first described by Duckert *et al.* (*17*), have a severe bleeding tendency, manifested within a few days after birth by bleeding from the umbilicus, and all too often terminating in hemorrhage of the central nervous system. Two groups of patients have been distinguished (*18*). In families in which females are affected, the disorder appears to be inherited as a rare autosomal recessive trait, parental consanguinity being recognized in about half of the families. In other families, only males have been affected, and consanguinity is much less frequent. The possibility that this bizarre distribution of consanguinity has occurred by chance is less than 0.01, supporting the view that fibrin-stabilizing factor "deficiency" is the phenotypic expression of at least two genotypes. No evidence has yet been obtained that inheritance in families in which males alone are affected is by way of the X chromosome.

The heterogeneity of fibrin-stabilizing factor deficiency is manifest by other observations. In most cases, regardless of the mode of inheritance, the plasma of affected individuals contains antigens cross-reacting with rabbit antiserum directed against fibrin-stabilizing factor, albeit in somewhat reduced amounts (*19*). In at least one case, however, no antigenic material was detected (*20*).

Disorders of Antihemophilic Factor

Three distinct hereditary diseases have been described in which functional AHF is deficient in plasma, namely, classic hemophilia, von Willebrand's disease, and combined deficiencies of AHF and proaccelerin (factor V).

Patek and Stetson (*21*) demonstrated that the clotting defect of plasma of patients with classic hemophilia could be corrected in vitro and in vivo by the addition of a fraction of normal plasma, while the analogous fraction of hemophilic plasma was without effect. At first, the prevalent view was that patients with classic hemophilia were unable to synthesize the missing agent, AHF (factor VIII). In 1957, Shanberge and Gore (*22*) reported, on the contrary, that the plasma of patients with classic hemophilia contained material which neutralized the capacity of specific rabbit antiserum to inhibit normal AHF. This view, that patients with hemophilia were CRM+ for AHF, did not attract the attention it deserved, in part because investigators had great difficulty in confirming the reported observations.

When much more highly purified preparations of AHF became available, Zimmerman, Powell, and Ratnoff (*23, 24*) prepared antiserum in albino rabbits which inactivated AHF in normal plasma, as demonstrated in functional tests of blood clotting. The inhibitory properties of this antiserum were blocked by prior absorption with normal plasma. To our surprise, the plasma of hemophiliacs was equally efficient in absorbing the antiserum, confirming Shanberge and Gore's (*22*) view. Functional tests are cumbersome, but fortunately the antiserum could be made monospecific by appropriate absorption; it then formed a single line of precipitation upon immunoelectrophoresis against plasma or crude AHF-rich fractions of plasma. Utilizing Laurell's (*25*) technique for semiquantitative immunoelectrophoresis, we demonstrated that a linear relationship existed between the concentration of functional AHF and the amount of antigen reacting with specific antiserum (Fig. 3). In hemophilia, the concentration of AHF was decreased in func-

Table 1. Some distinguishing features in congenital dysfibrinogenemia [adapted from Ratnoff (*10*)]. Downward arrows indicate a decrease in concentration and upward arrows an increase.

Fibrinogen	Immunoelectrophoresis*	Chromatography†	Carbohydrate	Effect of fibrinogen on normal plasma	Thromboelastogram	Effect of calcium on defect
			Disordered release of fibrinopeptides			
Baltimore	Anodal	Abnormal	Hexose normal	None	Abnormal	Correction
Bethesda	Anodal	Normal	Sialic acid normal	Inhibition		
Giessen		Normal				
			Disordered aggregation of fibrin monomers			
Zurich I‡	Normal	Normal	Hexose ↓	Inhibition	Normal	Correction
Zurich II	Normal			Inhibition		
Detroit	Normal		Hexose ↓	Inhibition	Abnormal	None
Paris II‡	Normal	Normal ?		Inhibition	Slightly abnormal	Correction
Wiesbaden	Normal			Inhibition		
Cleveland‡	Cathodal			Inhibition	Abnormal	Correction
Amsterdam §	Cathodal			Slight inhibition	Normal	
Nancy	Anodal	Abnormal	Sialic acid ↑	Slight inhibition		
St. Louis	Normal	Normal	Hexose normal	Slight inhibition		Partial correction
Montreal			Sialic acid normal	Inhibition		
Bethesda II	Anodal	Normal	Normal	Inhibition		None
Paris I‡	Anodal			Inhibition	Abnormal	Correction
Vancouver	Anodal			None		
Louvain‡	Anodal‖			Inhibition		
Metz	Abnormal			None		None
Troyes						Correction

* Migration of abnormal component at *p*H 8.6 relative to normal fibrinogen. † On columns of diethylaminoethyl cellulose. ‡ Fibrinogens distinguished from fibrinogen Detroit by the presence of a normal fibrinogen A chain. § Defect observed only if α-2 globulin is present. ‖ Distinguished from fibrinogen Baltimore upon immunoelectrophoresis at *p*H 7.4.

tional tests, but the concentration of antigens related to AHF was normal. Similar results have been reported from other laboratories (*26, 27*).

The nature of the antigenic material in hemophilic plasma is not yet clear. It could be an aberrant, nonfunctional form of AHF, in which, perhaps, substitution of an amino acid occurred. Alternatively, it could be an incompletely synthesized AHF, or a combination of AHF with some inhibitory fragment which normal individuals do not produce or can separate from the parent molecule. Antiserum prepared against antigen-rich fractions of hemophilic plasma inactivates AHF (*28, 29*), but this experiment does not distinguish among the three possibilities. When fractions of normal plasma, rich in AHF, are transfused into patients with classic hemophilia, the concentration of functional AHF rises immediately to the predicted level, and then rapidly disappears from plasma, the time for half of the AHF to disappear being about 12 hours (*30*). The rate of disappearance of the infused antigenic material is much slower, the half-disappearance time being 24 to 48 hours (*31*). Experiments we describe later in this article make it seem unlikely that the longer survival of antigenic material is due to the presence of denatured AHF in the material transfused. It is possible that the infused material contains a mixture of functional AHF and a precursor of this substance which the hemophiliac is unable to utilize.

Classic hemophilia, then, is a disorder in which patients synthesize a functionally abnormal form of AHF. The defect, however, is not the same in all patients. The severity of hemophilia varies from family to family, suggesting that multiple alleles may exist for the X chromosomal gene determining synthesis of AHF (*7*). Moreover, different cases of hemophilia can be distinguished by their behavior toward human antibodies directed against AHF, found in the plasma of perhaps 10 percent of severe hemophiliacs and certain nonhemophilic individuals. These human antibodies, or "circulating anticoagulants," inhibit the clot-promoting properties of AHF. Hoyer and Breckenridge (*32*) described six patients, members of five unrelated families, whose plasma contained material which neutralized human antibodies to AHF. In contrast, the plasma of 28 patients with classic hemophilia, members of 25 families, did not neutralize the human antibodies. In this way, these workers distinguished two groups of hemophilic patients, depending on whether their plasmas neutralized circulating anticoagulants. It is interesting that goat antiserum directed against AHF distinguishes the same two groups of patients (*33*) in contrast to the rabbit antiserum used by Zimmerman, Powell, and Ratnoff (*23*). Human and goat antiserums evidently react with a much more restricted part of the AHF molecule than rabbit antiserum.

Our studies of hemophilia have provided a useful way to identify female carriers of the disease. Geneticists have long been puzzled by the fact that the concentration of enzymes whose synthesis is directed by genes on the X chromosome is the same in males and females, although the former have only one X chromosome in each somatic cell, and the latter, two. One current hypothesis to explain this observation suggests that at an early stage of ontogeny, one of the X chromosomes in each female somatic cell is activated (or inactivated) so that these cells, and their descendants, have only one functional X chromosome (*34*). Inactivation occurs at random, so that in some cells the X chromosome remaining functional is of paternal, and in others, of maternal origin. Carriers of hemophilia have one X chromosome derived from the normal parent, and one from the parent carrying the defective gene. As one would anticipate, the concentration of functional AHF in the plasma of carriers is, on the average, about 50 percent of normal, since only half of a carrier's functional X chromosomes would be derived from her normal parent. The variation in functional AHF concentration in the plasma of both carriers and normal women is great, so that only about one-fourth of carriers can be identified solely by this measurement (*35, 36*). On the other hand, since patients with hemophilia synthesize normal amounts of AHF-like protein, this is also true of carriers. By determining the ratio of functional AHF to antigenic AHF, it should be possible to distinguish the carrier state (*26, 36, 37*) because approximately half the AHF-producing cells of the carrier should manufacture protein which, though nonfunctional, remains immunologically detectable. Indeed, among 42 proved carriers (that is, daughters of hemophiliacs, mothers of two hemophiliacs, or mothers of a single hemophiliac who had other relatives with the disease), 40 (95 percent) could be identified because they had relatively less functional than antigenic AHF (*29, 36*). Proof of the validity of this technique comes from a study of daughters of carriers, half of whom should themselves be carriers. Among 19 daughters of carriers whom we tested, 9 (47 percent) had the laboratory characteristics of the carrier state, the predicted result (*36*).

About one-third of patients with classic hemophilia have no family history of the disorder. Presumably, in some cases the abnormal gene responsible for the defect has passed through several generations unidentified; few of us know our family medical histories beyond three generations. But in other cases, perhaps the disease arises as the result of fresh mutation. Our data support this possibility. In contrast to the results obtained in proved carriers, only 13 of 19 mothers (68 percent) of patients with isolated cases of hemophilia have been identified as carriers (*29, 36*). Perhaps some of the other six cases arose as the result of mutation in the ovum from which the patient arose.

A second type of disturbed AHF synthesis occurs in von Willebrand's disease, a hemorrhagic disorder inherited as an autosomal dominant trait. The bleeding tendency is ordinarily mild, although hemorrhage, particularly from the gastrointestinal tract, may prove fatal. The bleeding time—that is, the length of time elapsing until bleeding stops from a deliberately incised wound—is usually prolonged, and the normal adhesiveness of platelets to glass is often reduced, two abnormalities not found in classic hemophilia.

When patients with von Willebrand's disease are transfused with normal plasma or AHF-rich fractions of plasma, the concentration of functional AHF gradually rises above that which can be accounted for by the amount infused, reaches a maximum in perhaps 6 to 8 hours, and then falls at a much slower rate than in patients with classical hemophilia (*38*). A similar result follows transfusion with hemophilic plasma, although this is, of course, deficient in functional AHF (*39*). These observations imply that normal and hemophilic plasmas contain an agent stimulating the synthesis of AHF-like material in patients with von Willlebrand's disease.

Zimmerman *et al.* (*23*) observed that in von Willebrand's disease the

concentration of AHF, as measured in functional assays, was proportional to the concentration of AHF-like antigenic material, as if in this disorder there was a true deficiency of AHF. When normal plasma, or an AHF-rich fraction of normal plasma, was transfused into patients with von Willebrand's disease, the concentration of AHF-like antigenic materials increased immediately after infusion, but then decreased rapidly (*40*) (Fig. 4). Put another way, the secondary increase in functional AHF which followed transfusion in von Willebrand's disease was not associated with a proportional increase in AHF-like antigen, as if the "new" AHF-like agent generated after transfusion was antigenically distinct from normal AHF in which clot-promoting activity and antigen appear closely associated. No explanation for this phenomenon is yet available. Perhaps the high molecular weight AHF detected in normal and hemophilic plasma by physical or immunologic means is a composite of a functional component and a second fraction which bears the antigenic sites. If this is so, the newly synthesized material in the transfused patient with von Willebrand's disease might contain the functional component alone. It is interesting that Owen and Wagner (*45*) have demonstrated that the subunits of normal canine AHF, separated by the addition of detergents or salts, retain their functional activity. Another possibility is that a new form of AHF is generated in the transfused patient. In a most challenging series of experiments, Barrow and Graham and their co-workers (*42*) have demonstrated that AHF-like properties can be induced by succinylation of serum albumin, a protein not ordinarily thought of as having clot-promoting properties. The significance of this startling observation is not yet clear.

None of these experiments attacks the question of why patients with von Willebrand's disease have a long bleeding time. Early experiments indicated this defect was due to the deficiency of a plasma factor distinct from AHF (*39*). Recently, Bouma *et al.* (*28*) raised the possibility that this second factor is related in some way to AHF itself. These workers demonstrated that purified AHF corrected the defective adhesion of platelets to glass observed in von Willebrand's disease, and that antiserum against either purified AHF or the corresponding fraction of hemophilic plasma reduced adhesiveness of normal platelets to glass. The situation is probably more complicated. During pregnancy, the titer of AHF rises in patients with von Willebrand's disease. Recently, we studied a pregnant woman with von Willebrand's disease in whom the concentrations of AHF procoagulant and antigen had risen to normal levels, but the bleeding time remained abnormally long (*29*). Thus, the defect in the bleeding time cannot yet be explained by the data.

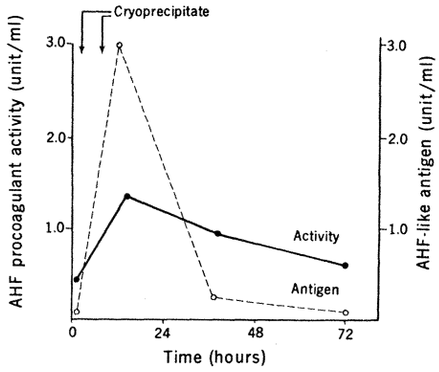

Fig. 4. Changes in levels of AHF procoagulant activity (continuous line) and AHF-like antigen (interrupted line) after infusion of cryoprecipitate. The patient was a 6-year-old child (weight, 20 kilograms) who sustained an injury to her eye with severe intraocular bleeding. As initial treatment, seven bags of cryoprecipitate were infused on each of the two occasions indicated by the arrows (one bag of cryoprecipitate is derived from 225 milliliters of plasma). [From Bennett *et al.* (*40*); courtesy of *Journal of Clinical Investigation*]

The information we have reviewed suggests that the synthesis of AHF is determined by at least two genes, one of which is carried on an X chromosome, and the other on an autosomal chromosome. A few patients have been observed in whom a combined deficiency of AHF and proaccelerin (factor V) has been detected. In these families, the disorder has been found in both sexes, has been limited to one generation, and has been associated with a high frequency of parental consanguinity (*43*). This remarkable syndrome suggests that a third gene may be involved in the synthesis of AHF, regulating a step also needed for the synthesis of proaccelerin.

Christmas Disease

Christmas disease, the hereditary functional deficiency of Christmas factor (PTC, factor IX), is clinically indistinguishable from classic hemophilia and, like this disorder, is inherited as an X chromosome–linked defect. The locus for Christmas factor on the X chromosome appears to be widely separated from that for the synthesis of AHF (*44*).

When Christmas disease was first described, the assumption was made that patients with this defect were unable to synthesize normal amounts of Christmas factor. The situation, however, appears to be analogous to that in classic hemophilia. Meyer and her colleagues (*45*) found that the plasma of 21 of 22 patients with Christmas disease contained antigenic material which neutralized a rabbit antiserum directed against Christmas factor. Thus, the great majority of patients seem to be CRM+, synthesizing a nonfunctional variant of Christmas factor. The patients can be divided into at least three subgroups, based in part upon their behavior toward human antibodies against Christmas factor, found in a small number of patients with Christmas disease. As early as 1956, Fantl *et al.* (*8*) distinguished two forms of Christmas disease. The plasma of one of their patients, like normal plasma, contained an agent which neutralized the effects of human antibody, as if it contained cross-reacting antigenic material. No such neutralizing activity was found in the plasma of two other patients. Among a larger group of patients, Roberts and his associates (*46*) found that cross-reacting material, detected by human antibodies effective against Christmas factor, were present in 16 percent of affected kindred individuals. In a small number of patients with cross-reacting material, a second functional abnormality is detected. The cross-reacting material appears to have the property of inhibiting the extrinsic pathway of coagulation, although normal Christmas factor is thought not to participate in this chain of reactions (*47*). Yet another variant of Christmas disease has been reported by Veltkamp *et al.* (*48*).

These several studies, then, demonstrate that Christmas disease is the phenotype for a variety of genetic traits. The great majority of patients with Christmas disease synthesize a nonfunctional variant of Christmas factor. Among these patients, only a small number can be detected by reactions with human antibodies and, in turn, these patients form a heterogeneous

group. Presumably, in the latter patients, the defect is located near an active site on the Christmas factor molecule.

Other Disorders of Blood Coagulation

We will not attempt to review the literature concerning heterogeneity among other hereditary defects of blood coagulation. Heterogeneity has been demonstrated among patients with functional deficiencies of prothrombin, Stuart factor, and factor VII, by both immunologic and functional tests (*10*). Heterogeneity has also been demonstrated, entirely on the basis of patterns of inheritance, among patients with functional deficiencies of Hageman factor (*9*). No evidence has yet been reported concerning heterogeneity of deficiencies of proaccelerin (parahemophilia) or PTA, but if experience is a guide, these disorders too will be found to be divisible into subgroups.

Discussion

We have reviewed a number of examples of heterogeneity among the hereditary disorders of blood coagulation. Two general types of abnormality have been described, those in which the patient's plasma contains normal or near normal amounts of a nonfunctional variant of the factor whose deficiency is detected in tests of clotting function, and those in which the plasma appears to be truly deficient. In other contexts, these two states have been referred to as allotypy (the synthesis of an abnormal protein) and eniotypy (failure to synthesize a protein) (*49*). It is a safe assumption that normal clotting factors may vary widely in their amino acid composition, as long as the significant functional groups are unaffected. When the heterogeneity affects functional groups, impaired hemostatic function may result. Thus, it is not surprising to find that among CRM+ patients with coagulative disorders, heterogeneity detected by a variety of techniques is commonplace. These considerations have several important pragmatic consequences. As has been pointed out, some patients with hemophilia and Christmas disease develop antibodies against the functionally deficient factors. These antibodies appear almost exclusively in patients who have been transfused. Perhaps in such patients the transfused material differs in a subtle way from their own nonfunctional clotting factors. Since only a small number of patients develop antibodies, it is likely that there are antigenic differences among the CRM+ materials found in bleeders. Perhaps, too, normal individuals differ significantly in the antigenic nature of their clotting factors. If this is the case, it may become important to learn how to type normal AHF and Christmas factor, so that type-specific preparations can be given to patients. We have all looked forward to the time when an appropriate normal tissue might be transplanted to cure the hereditary hemorrhagic disorders. These hopes seem dimmed by the discovery of cross-reacting antigenic material in the plasmas of patients with classic hemophilia and Christmas disease. The concentration of clotting factors is clearly closely regulated, so that the amount of antigenic material present both in normal individuals and those with classic hemophilia and Christmas disease is kept within narrow limits. If the rate of synthesis of clotting factors is controlled by the amount of the protein in the plasma rather than the degree of clot-promoting activity, tissues transplanted into patients who produce nonfunctional proteins may receive little stimulus to produce the active clotting factor. The gain to the patient in terms of clot-promoting activity under these circumstances will be small, even if the new factors and the transplanted tissues are not rejected by his various immunologic defenses. It is true that hepatic transplantation has appeared to induce synthesis of AHF in hemophilic dogs (*50*), but these animals, unlike human patients with classic hemophilia, have no demonstrable cross-reacting AHF-like antigens in their plasma.

References and Notes

1. This classic nomenclature is confusing, because fibrinogen is a dimeric protein made up of three pairs of polypeptide chains, designated α (A), β (B), and γ. Fibrinopeptides A and B are separated from the α (A) and β (B) chains, respectively, so that fibrin "monomer" is really a dimer of α, β, and γ chains.
2. F. A. Pitlick, quoted by Y. Nemerson and F. A. Pitlick, *Progr. Hemost. Thromb.* **1**, 39 (1972).
3. E. W. Davie and O. D. Ratnoff, *Science* **145**, 1310 (1964).
4. R. G. Macfarlane, *Nature* **202**, 498 (1964).
5. K. D. Wuepper, *Fed. Proc.* **31**, 624 (abstr.) (1972); H. Saito, O. D. Ratnoff, J. S. Marshall, J. Pensky, *J. Clin. Invest.*, in press.
6. A. Pavlovsky, *Blood* **2**, 185 (1947).
7. J. B. Graham, W. W. McLendon, K. M. Brinkhous, *Amer. J. Med. Sci.* **225**, 46 (1953).
8. P. Fantl, R. J. Sawers, A. G. Marr, *Australas. Ann. Med.* **5**, 163 (1956).
9. B. Bennett, O. D. Ratnoff, J. B. Holt, H. R. Roberts, *Blood* **40**, 412 (1972).
10. O. D. Ratnoff, *Progr. Hemost. Thromb.* **1**, 39 (1972).
11. F. Rabe and E. Salomon, *Deut. Arch. Klin. Med.* **132**, 240 (1920).
12. G. I. C. Ingram, *J. Clin. Pathol.* **8**, 318 (1955).
13. C. Imperato and A. G. Dettori, *Helv. Paediat. Acta* **13**, 380 (1958).
14. M. Blömback, B. Blömback, E. F. Mammen *et al.*, *Nature* **218**, 134 (1968).
15. O. Egeberg, *Thromb. Diath. Haemorrh.* **17**, 176 (1967).
16. J. W. Hampton, *J. Lab. Clin. Med.* **72**, 882 (1968).
17. F. Duckert, E. Jung, D. H. Shmerling, *Thromb. Diath. Haemorrh.* **5**, 179 (1961).
18. O. D. Ratnoff and A. G. Steinberg, *Lancet* **1968-I**, 25 (1968); *Ann. N.Y. Acad. Sci.* **202**, 186 (1972).
19. F. Duckert, *Nouv. Rev. Fr. Hematol.* **10**, 685 (1970).
20. T. H. Kiesselbach and R. H. Wagner, *Amer. J. Physiol.* **211**, 1472 (1966).
21. A. J. Patek, Jr., and R. J. Stetson, *J. Clin. Invest.* **15**, 531 (1936).
22. J. N. Shanberge and I. Gore, *J. Lab. Clin. Med.* **50**, 954 (1957).
23. T. S. Zimmerman, O. D. Ratnoff, A. E. Powell, *J. Clin. Invest.* **49**, 106a (1970).
24. ———, *ibid.* **50**, 244 (1971).
25. C. Laurell, *Anal. Biochem.* **15**, 45 (1966).
26. E. Bennett and E. R. Huehns, *Lancet* **1970-II**, 956 (1970).
27. D. P. Stites, E. J. Hershgold, J. D. Perlman, H. H. Fudenberg, *Science* **171**, 196 (1971); D. Meyer, J.-M. Lavergne, M.-J. Larrieu, F. Josso, *Thromb. Res.* **1**, 183 (1972); L. W. Hoyer, *Int. Congr. Soc. Thromb. Haemost.* **3**, 102 (abstr.) (1972); C. R. M. Prentice and C. D. Forbes, *ibid.*, p. 138.
28. B. N. Bouma, Y. Wiegerinck, J. J. Sixma, J. A. van Mourik, I. A. Moctar, *Nature New Biol.* **236**, 104 (1972).
29. B. Bennett and O. D. Ratnoff, unpublished observations.
30. J. G. Pool and J. Robinson, *Brit. J. Haematol.* **5**, 17 (1959).
31. B. Bennett and O. D. Ratnoff, *J. Clin. Invest.* **51**, 2593 (1972).
32. L. Hoyer and R. T. Breckenridge, *Blood* **32**, 962 (1968).
33. H. R. Gralnick, personal communication.
34. E. Beutler, in *Mechanisms of Anemia*, I. M. Weinstein and E. Beutler, Eds. (McGraw-Hill, New York, 1962); M. F. Lyon, *Annu. Rev. Genet.* **2**, 31 (1968); S. Ohno, *ibid.* **3**, 495 (1969).
35. S. I. Rapaport, M. J. Patch, F. J. Moore, *J. Clin. Invest.* **39**, 1619 (1960).
36. T. S. Zimmerman, O. D. Ratnoff, A. S. Littell, *ibid.* **50**, 255 (1971); B. Bennett and O. D. Ratnoff, *N. Engl. J. Med.* **288**, 342 (1973).
37. E. Bennett, *Clin. Sci. London* **38**, 118 (1970).
38. P. Cornu, M.-J. Larrieu, J. Caen, J. Bernard, *Brit. J. Haematol.* **9**, 189 (1963); R. Biggs and J. M. Matthews, *ibid.*, p. 203.
39. I. M. Nilsson, M. Blömback, I. von Francken, *Acta Med. Scand.* **159**, 35 (1957).
40. B. Bennett, O. D. Ratnoff, J. Levin, *J. Clin. Invest.* **51**, 2597 (1972).
41. W. G. Owen and R. H. Wagner, *Fed. Proc.* **31**, 262 (abstr.) (1972).
42. E. Barrow, R. H. Lester, A. M. Johnson, J. B. Graham, *ibid.*, p. 262 (abstr.).
43. J. Oeri, M. Matter, H. Isenschmid, F. Koller, *Mod. Probl. Paediat.* **1**, 575 (1954); *Ann. Paed. Suppl.* **58**.
44. K. Berg and A. Bearn, *Trans. Ass. Amer. Physicians* **79**, 165 (1966).
45. D. Meyer, E. Bidwell, M. Larrieu, *J. Clin. Pathol.* **25**, 433 (1972).
46. H. R. Roberts, J. E. Grizzle, W. D. McLester, G. D. Penick, *J. Clin. Invest.* **47**, 360 (1968).
47. C. Hougie and J. J. Twomey, *Lancet* **1967-I**, 698 (1967).
48. J. J. Veltkamp, J. Meilof, H. G. Remmelts, D. van der Vlerk, E. A. Loeliger, *Scand. J. Haematol.* **7**, 82 (1970).
49. B. Cinader, S. Dubiski, A. C. Wardlaw, *Nature* **210**, 1291 (1966).
50. W. P. Webster, C. F. Zukoski, P. Hutchin, R. L. Reddick, S. R. Mandez, G. D. Penick, *Amer. J. Physiol.* **220**, 1147 (1971).
51. O. D. Ratnoff, in *Pathophysiology*, E. D. Frohlich, Ed. (Lippincott, Philadelphia, 1972).
52. B. Bennett and O. D. Ratnoff, *Med. Clin. N. Amer.* **56**, 95 (1972).

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