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Biochemical Genetics of Blood Coagulation*

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THE CHAIRMAN of this symposium asked me to discuss the biochemical genetics of blood coagulation. I assented before I discovered, too late to express my regrets, that this was a formidable job. The literature is very large, and my own experience has been "existential" only with regard to hemophilia. By restricting myself to recent publications suspected of having or purporting to have genetic implications, I was able to limit my reading to about 300 papers. I should warn you not to expect a discussion of blood coagulation as if it were *Neurospora* metabolism. There are, however, some points of genetic interest which I shall attempt to summarize. I have tried to be judicial rather than polemical and hope that my analysis will indicate the direction for future investigations.

It might be worthwhile to outline recent developments in blood coagulation research. This sketch necessarily will be more abbreviated than that of Milstone (61), the historian of ideas in this field. Most biologists have been exposed at one time to the diagram in Figure 1 representing the steps in blood clotting. This has been known as the Morawitz theory since its enunciation in 1904 (62). During the period of the 1930's the Morawitz formulation was useful because primary emphasis was on the development of methods for assaying prothrombin and thrombin, on the concentration and purification of these proteins, and on their metabolism. This was the period when was done the classical work on the relationship of prothrombin to Vitamin K and the identification, purification and synthesis of this vitamin. This culminated, you may remember, in a Nobel prize in 1943 for Edward Doisy and Henrik Dam.

As regards the Morawitz hypothesis, a disquieting note was sounded by workers at the University of Iowa in 1939 (64). They noted that the one-stage prothrombin method gave normal results with infant's plasma while the two-stage method showed marked reduction of prothrombin. They inferred, correctly in retrospect, that circulating plasma contains a "convertibility factor" controlling the rate of transformation of the proenzyme, prothrombin, into active thrombin. They believed that in infant's blood a normal level of the "convertibility factor" was concealing a deficit of prothrombin. This suggestion opened Pandora's box. During the succeeding 15 years, at least seven distinct substances were described, in addition to the already known

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1. PROTHROMBIN + THROMBOPLASTIN + Ca IONS ⟶ THROMBIN

2. FIBRINOGEN + THROMBIN ⟶ FIBRIN

FIG. 1. The Morawitz theory of blood coagulation.

antihemophilic factor (AHF), which might be considered to fit into the category of prothrombin "convertibility factors." These discoveries are summarized in Table 1.

The first discovery was made during the war by blood clotters working independently in several countries (26, 66, 74, 105). They discovered a plasma protein which accelerates the conversion of prothrombin to thrombin. This factor is now variously known as "labile factor," "Factor V," "Accelerator globulin" and "accelerin."

In 1949 another "convertibility factor," distinct from Factor V, was discovered in serum (1). This factor acts with tissue thromboplastin to shorten the prothrombin time, and has been designated by various authors as "SPCA" (serum prothrombin conversion accelerator), "proconvertin," "Factor VII" and "stable factor." Several small families deficient in this factor have been described (51, 53, 67).

In 1952, almost simultaneously in the United States (4, 88) and Great Britain (8), was discovered yet another protein required for normal clotting. This substance was called "plasma thromboplastin component" (PTC) in America and "Christmas factor" in England. I shall use the term "Christmas factor" hereafter because the term "PTC" has been been preempted by geneticists in another connection.

Shortly after the reporting of "Christmas factor", a group in New York City (82) reported still another factor said to be required for normal clotting. They named this substance "plasma thromboplastin antecedent" or "PTA", a term dictated by their conception of the blood clotting mechanism. Pedigree study has been attempted in several families thought to lack this substance (32, 82, 84).

In 1954, some of the American discoverers of "Christmas factor" (93) reported the discovery of a possible additional plasma factor, tentatively named the "fourth plasma thromboplastin component". Also in 1954, a group of Swiss workers reported

TABLE 1. HISTORY OF THE DISCOVERY OF THE "CONVERTIBILITY FACTORS"

	"Convertibility Factors"	
Year of Discovery	Name of normal factor	Name of deficiency disease
<1940	Antihemophilic factor	Hemophilia
1947	Factor V, (accelerin, Ac-globulin, labile factor)	Factor V-deficiency, (Parahemophilia)
1949	SPCA, (Factor VII, Proconvertin, stable factor)	SPCA-deficiency
1952	Christmas factor, (PTC)	Christmas disease, (PTC-deficiency)
1953	PTA	PTA-deficiency
1954	Fourth thromboplastin component	Tetartohemophilia
1954	Factor X	Not known
1955	Hageman factor	Hageman disease
>1955	?	?

the discovery of a new clotting factor which they have called "Factor X" (30). Thus far in 1955, I am aware of the reporting of only one new blood-clotting component, the "Hageman factor" (79), but am informed by a "usually reliable source" that the announcement of another discovery is being prepared in Britain.

How good is the evidence that these "convertibility factors" do in fact exist as separate entities? The answer depends on one's criteria. All of them fall into the category of what has been called the "trace proteins", proteins in such low concentration for example, that their absence cannot be detected by chemical or electrophoretic analysis of plasma. Most of them have not been isolated, purified, and characterized in the colloid chemistry sense. However, I think that there is fairly good physiological evidence for the existence of 7 of the 8 factors listed.

If the history of the discovery of these substances is examined, one can see that, with two exceptions (SPCA and Factor X), they have been discovered by the technic which has been so fruitful in the recent work on human hemoglobin and on human blood groups. That is to say, a "new" disease is segregated by studying exceptional cases, in this context exceptional cases of hemophilia. The implicit operational principle is that in each hereditary disease of blood clotting there is a deficiency of one and only one specific factor, an intuitive "one gene-one enzyme" hypothesis. Thus, for example, when plasma from an apparent case of hemophilia corrects the clotting defect in plasma from an established case of hemophilia, the diseases are assumed to be due to deficiencies of entirely different substances. When the exceptional plasma is found to correct the clotting defect in all other types of hemophilia-like deficient plasmas, it is generally agreed to be unique. Also, it is generally agreed that the patient providing the plasma suffers from a previously undifferentiated disease. It is then inferred that plasma of normal individuals contains a previously unrecognized

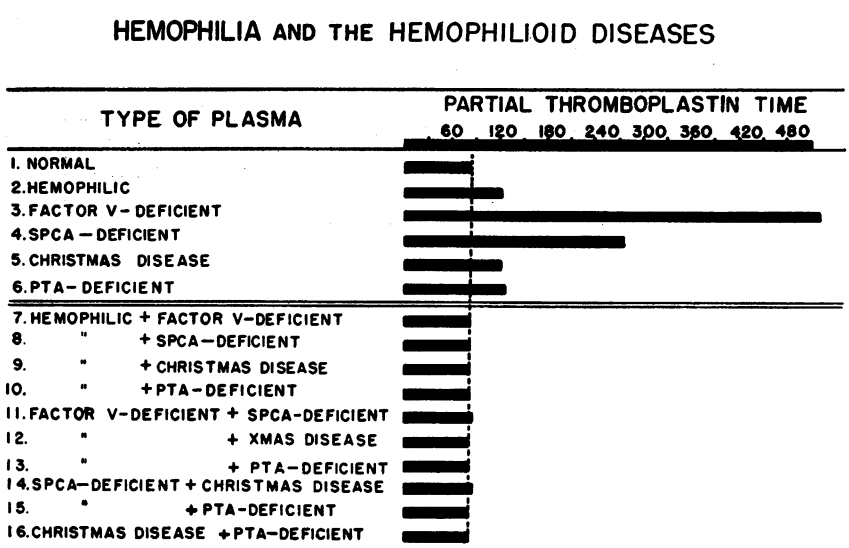

FIG. 2. A physiological experiment showing the corrective effect of each type of deficient plasma on each of the other types of deficient plasma. The broken vertical line projects the clotting time of the normal plasma for comparison.

factor which is missing in the affected person's plasma. This conclusion is conventionally followed by an attempt to isolate the newly discovered factor from normal plasma and characterize it chemically.

An example from our laboratory (Figure 2) will illustrate this general method of analysis. We managed to obtain fresh plasma simultaneously from persons having hemophilia, Factor-V deficiency, SPCA-deficiency, Christmas disease, and PTA-deficiency. All plasmas were tested by a simple clotting test developed in our laboratory which we have named the partial thromboplastin time (48). The partial thromboplastin times of all the abnormal plasmas were prolonged, Lines 2–6. However, the clotting times of the mixtures of equal parts of plasma from each pair of diseases were normal, Lines 7–16. This general type of experiment reproduced by many workers under many different sets of conditions is consistent with the current hypothesis that each disease is caused by a deficiency of a specific clotting factor. This concept has been expressed pictorially in an almost identical fashion by a Swiss investigator recently (46).

Attuned to the times, we generally rewrite the two steps of the Morawitz scheme as enzymatic rather than stoichiometric reactions (Figure 3). It is clear that all of the "convertibility factors" act prior to or during the prothrombin-to-thrombin reaction and are not related to the polymerization of fibrinogen. How each individual substance reacts is less clear. At present it is believed that each of the new factors either participates in the formation of thromboplastin, or acts to accelerate the transformation of prothrombin to thrombin in some other fashion.

You will notice that I have been exceedingly naive thermodyamically in Figure 3, but rather crafty. The first reaction step has been written as though it were catalyzed by an agent which is itself produced as the result of a tenth-order reaction. Even if thromboplastin were clearly established, such a reaction would be highly improbable. It is more likely that these factors react with one another in sets of first, second, and possibly third-order reactions in some definite sequence. The result of

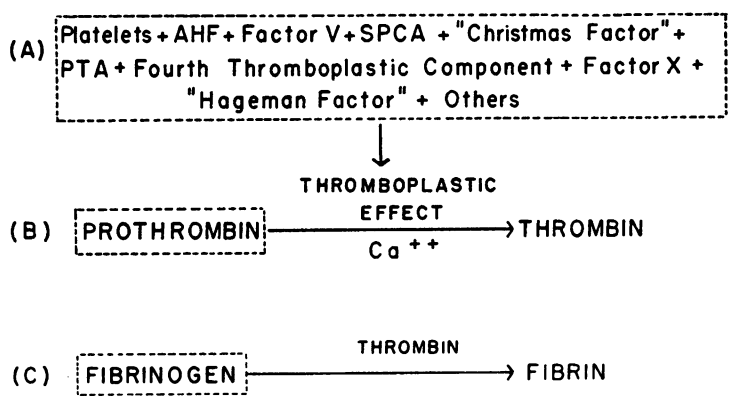

FIG. 3. A modification of the Morawitz theory of blood coagulation which takes into account the discoveries of the past 15 years. This is grossly oversimplified as it does not take into account the undoubted but presently unclear interrelationships of the "convertibility factors".

these interactions I have purposely avoided committing myself on, merely indicating a catalytic action called the "thromboplastic effect." I would opine that during the next decade blood clotters will be as fully occupied in isolating these factors and studying their interrelationships as the nuclear physicists will be with the myriad of subnuclear particles.

There are several facts which I would like to emphasize at this point.

1. The end product of this series of reactions, whatever the sequence, is fibrin. Fibrin is used in the body to stanch bleeding. Whenever fibrin formation is impaired, a hemorrhagic state may result.

2. There are basically four ways now known in which fibrin formation may become impaired:

   a. Reduction in, or physiological inactivity of, any one of the substances contributing to what I have chosen to call the "thromboplastic effect."
   b. Reduction in or physiologic inactivity of prothrombin.
   c. Absence of fibrinogen.
   d. Excess of an inhibitory substance which neutralizes the action of one of the known procoagulants. (I shall not discuss inhibitors further except to say that several types are well known.)

With these facts in mind, I think present knowledge of the genetic control of blood clotting factors may be summarized succinctly.

1. The areas on Figure 3 included within the broken rectangles and designated (A) and (C) are clearly under genetic control; there is good evidence for an hereditary deficiency of the AHF, Factor V, SPCA, Christmas factor, and fibrinogen and fair evidence for an hereditary defect of platelets, PTA, "fourth thromboplastin component" and "Hageman factor."

2. The genetic control of prothrombin synthesis is less clear. There is at present very little evidence supporting an hereditary deficiency of prothrombin, the large literature to the contrary notwithstanding. With the possible exception of Quick's cases (76), the cases of so-called prothrombin deficiency (to my knowledge) have either proved to be SPCA-deficiency, or this possibility has not been excluded. Recently, Seegers and his associates have reported evidence interpreted as showing that SPCA is an altered form of prothrombin (86, 87). If this is confirmed, it may imply that what we now know as "SPCA-deficiency" is, in reality, the hereditary defect of prothrombin which has been so elusive.

In the remainder of this paper, I would like to discuss in more detail four of the blood clotting factors, the four about which genetic knowledge is most sound.

FIBRINOGEN, FIBRIN AND AFIBRINOGENEMIA

Fibrinogen is present in plasma at a higher concentration than any of the other coagulation proteins, 300–500 mg%. The work on fibrinogen is sufficiently advanced that one or another of several relatively pure preparations may be purchased at the pharmacy. Physical chemical studies of the fibrinogen molecule are consistent with a thin rod model about 600 x 50 Å, of molecular weight about 330,000 (89). Under the action of the active enzyme, thrombin, fibrinogen is polymerized to the larger mole-

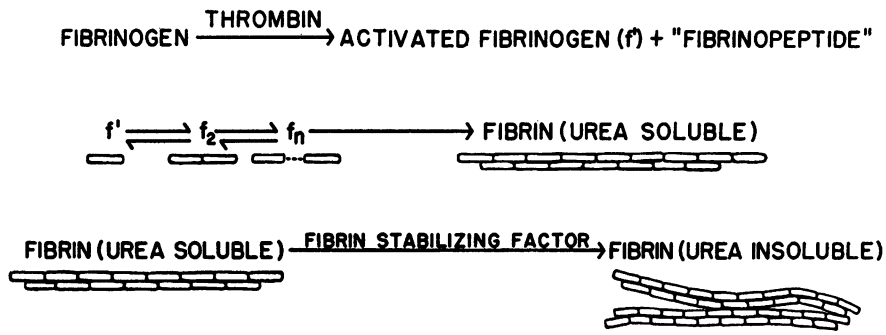

FIG. 4. The formation of fibrin from fibrinogen.

cule, fibrin, molecular weight approximately 5,000,000 or about 16 fibrinogen residues. Studies on the molecular properties of fibrinogen have been reviewed recently by Lorand (55) and by Ferry (28), and are shown schematically in Figure 4.

Thrombin appears to act on fibrinogen by decreasing the net negative charge and redistributing the remainder so that the molecules no longer repel each other. This is accomplished by splitting out from each fibrinogen molecule a negatively charged peptide of molecular weight 4000–8000 which has been named "fibrinopeptide" (55). The less negatively charged "activated fibrinogen" then forms intermediate polymers, of all sizes, reversibly, in many steps. This process terminates in the large fibrin molecule. There is evidence that the fibrin end-product is composed of fibrinogen units linked not only end-to-end but also side-to-side with overlapping ends (28).

Fibrin prepared from purified fibrinogen by the action of purified thrombin is soluble in urea. However, if fibrin forms in plasma, or if plasma (or serum) and calcium ions are added to the mixture of purified thrombin and fibrinogen, the clots become insoluble in urea. This change in solubility led to the obvious hypothesis that plasma contains a "fibrin-stabilizing factor" (55). This factor is believed to promote the formation of side linkages between fibrin molecules. In preliminary studies, the "fibrin-stabilizing factor" has been shown to be a thermolabile protein, constituting only a small fraction of total plasma protein (55).

It has been shown that the "fibrinopeptides" split out of human and bovine fibrinogen by thrombin differ in amino acid composition (55). Also, the terminal amino groups of fibrinogen molecules are different in the two species (54). However, human and bovine fibrinogen molecules are of approximately the same size and shape (89), and the fibrinogens of both species form fibrin with approximately equal speed when acted on by the thrombins of both species. The great similarities and minor differences would imply to geneticists that fertilized human and bovine ova are carrying approximately the same information regarding fibrinogen synthesis. The quite slight species differences in chemical structure appear to have no physiological significance, suggesting that fibrinogen samples from humans *without* bleeding tendencies might show similar chemical differences, perhaps genetically determined. Geneticists might anticipate also that close scrutiny would reveal mutations affecting "fibrinopeptide" and "fibrin-stabilizing factor." Studies on both substances in

human populations are possible in the near future since assay procedures for both are available (55, 56).

Congenital fibrinogen deficiency was described originally in 1920 (77). Theoretically, it is possible that the defect in this condition is not the deficient production of normal fibrinogen, but either excessive destruction or a major alteration in the fibrinogen molecule changing its physical, chemical and physiologic properties. There is evidence which allows rejection of the excessive destruction hypothesis. A fibrinogen half-life of 5.6 days has been found by feeding S^{35} labeled d-l methionine and yeast to normal humans (59), and a half-life of 3.5 days by transfusing labeled fibrinogen (102). It has been shown also that the half-life of normal fibrinogen transfused into afibrinogenemic humans is about 4 days (34, 52). Such close correspondence would not be found if the disease state resulted from excessive destruction.

The possibility of an altered molecule cannot be excluded with the same confidence, since the methods for identifying and measuring the quantity of fibrinogen in plasma depend on its molecular characteristics. The quantity of fibrinogen, 300–500 mg%. is sufficiently small that an abnormal molecular species might be overlooked in the 7,000 mg% pool of plasma proteins. However, since the cases described appear to lack fibrinogen as tested by the three technics of thrombin action, electrophoretic analysis and immunochemical precipitation, it is reasonable to assume that the affected persons simply synthesize little or no fibrinogen.

There are 29 cases of congenital afibrinogenemia in the literature[1], exclusive of Risak's cases (80) which are dubious. Those reported prior to 1954 were well summarized by Frick and McQuarrie (33), and the cases reported since are included in the bibliography (3, 52, 73, 100). Of the patients described, 21 were males and 8 females. In 9 of the 25 matings, the parents were first cousins. The high degree of consanguinity suggesting a recessive gene, it is instructive to look at the data pertaining to carriers. Fibrinogen determinations are available on 17 parents. In only 4 parents was the fibrinogen below 200 mg% and in only one below 100 mg%. Clearly, most heterozygotes are normal overlaps. Furthermore, none of the authors report the variation in normal populations or the internal variation of the fibrinogen methods used. Even more fundamental, it is not clear in any of the studies that precautions were taken to bypass the action of the fibrinolytic enzyme which can cause erroneously low fibrinogen values. It is my opinion that no one has yet clearly shown that the carrier state in this condition can be detected with confidence.

HEMOPHILIA AND THE ANTIHEMOPHILIC FACTOR (AHF)

Classic hemophilia was probably the first hemorrhagic disease recognized, and it is likely that all hemorrhagic diseases at one time were considered cases of hemophilia. There is little doubt, for example, that in Andreasson's inclusive survey in Denmark (5) examples of the other "hemophilioid diseases" (deficiencies of the other "convertibility factors") were included amongst his hemophiliacs. With the diagnostic methods he used, this was inevitable.

Some experimental work was done on hemophilia during the 1930's. It was shown

[1] The actual number of cases is probably less. Several have been reported more than once.

that normal plasma contains a substance, isoelectrically precipitated at pH 5.3, which shortens the clotting time of hemophilic blood both *in vitro* and *in vivo* (68). However, this so-called "antihemophilic globulin" was never satisfactorily purified, and work on it ceased about 1940 to be taken up again only quite recently (7, 104). A significant break-through in the pathogenesis of hemophilia was made in 1939 with the demonstration by Brinkhous (13) that the slow clotting in this disease was due to slow transformation of prothrombin to thrombin. This worker also demonstrated later the essentiality of platelets for coagulation, and that a plasma factor absent in hemophilia reacts with platelets before blood clotting begins (14).

Competing with the concept that hemophilia is a deficiency disease has been the hypothesis that the bleeding tendency results from excess of an inhibitor, anticephalin. A summary of the supporting data for this view was published several years ago (99). Only recently has it been shown that the anticephalin hypothesis is probably erroneous (39, 40), removing this objection to the deficiency hypothesis.

Hemophilia in dogs (29)—severe, sex-linked, and identical with the human disease—has been studied in our laboratory since 1947 (35). The first female indubitably homozygous for hemophilia was a dog (15), and human homozygotes have been seen rarely (43, 60a). Since the breeding of female hemophilic dogs became routine, it has been possible to mate them with hemophilic males, producing as many as 8–10 hemophiliacs in a litter. Two moderately accurate methods for determining the AHF content of various fluids were developed using the hemophilic dogs (37, 48). With these technics, the titre of AHF in normal animals has been shown to have wide species variations (16, 94). The use of dogs for experiments on the AHF has resulted in the discovery of many facts about this factor which have not been ascertainable about the other "convertibility factors". It was discovered in normal dog blood that the antihemophilic factor disappears during coagulation (36). There is also evidence suggesting that the antihemophilic factor participates in a reaction with "Christmas factor" and platelets producing thromboplastic activity (9). This reaction is the basis for the thromboplastin generation test (10), a new blood clotting method well adapted to large-scale clinical studies. A reaction between AHF and platelets has been suggested also by the simultaneous decrease in both elements after cold injury to soft tissues or injection of thromboplastin (18).

It has been shown that severe liver damage and dicoumarolization do not affect the AHF level (37, 71). It has been shown also that AHF remains at a normal level during life in dogs given a lethal dose of X-irradiation (70). These data seem to eliminate the liver and lymphoid tissue as sites of formation of this substance. Splenectomy has been shown both in dogs and humans not to affect the clinical course of hemophilia, nor alter the level of AHF in normal dogs (41). Pancreatectomy also seems not to affect AHF (71).

Recently it has been found that d-l ethionine depresses AHF levels in rats. Severe deterioration of the bone marrow cells was noted in these animals also. It was provisionally concluded that the elusive site of AHF synthesis had been located in the reticulo-endothelial cells (71). While this is a logical inference from the evidence, it may not be the correct one. It seems to me that there is an alternate explanation of this important observation which is equally likely. It is probable that d-l ethionine

depresses AHF by competitively inhibiting incorporation of methionine into the AHF molecule and blocking AHF production. It is possible that reticulo-endothelial deterioration and AHF depression are merely coincidental. Protein synthesis is high in the marrow, and this may make these cells particularly susceptible to destruction when adequate methionine is not available. It is interesting that in these experiments the liver cells also showed severe deterioration, because the liver is also the site of large scale protein synthesis. It is possible that the difficulty in pin-pointing the site of synthesis of AHF arises because AHF is produced in many parts of the body. This possibility would be consistent with the fact that production has been interfered with only once and then by an agent which affects all cells.

The turnover rate of the AHF has been determined recently by transfusing both normal and hemophilic dogs with plasma and plasma fractions (49). An extraordinarily short half-life, 2 hours, has been found in both canine genotypes. The agreement of the turnover data in the two dog types is consistent with the conventional impression that the hemophilia gene acts by decreasing the production of AHF rather than by producing an inhibitor which neutralizes AHF. More recently, preliminary data have suggested a half-life of about 4 hours in hemophilic humans (19), a matter of great therapeutic importance.

Normal plasma is being fractionated for AHF in many laboratories. In our laboratory canine AHF has been purified 100 times (104). In England a similarly potent bovine AHF has been obtained (7) and is being used for intravenous therapy in humans (57). In this connection it is to be hoped that bovine AHF proves to be no more antigenic to humans than bovine thrombin (98).

The clinical severity of hemophilia varies widely. Even in inbred hemophilic dogs, all transmitting the same hemophilia gene, there is considerable variation (35). Very mild human hemophilia, diagnosed only by a quantitative AHF assay, has been described in detail in one pedigree (38). This pedigree and the observation that the antihemophilic activity of plasma from normal persons varies widely has led to the postulation of an allelomorphic series of genes at the hemophilia locus (38). The difficulties with this hypothesis are exemplified by the observations shown in Table

TABLE 2. COMPARISON OF ANTIHEMOPHILIC FACTOR LEVELS OF HETEROZYGOTES AND HEMIZYGOTES FOR THE TWO TYPES OF HEMOPHILIA

Type of Hemophilia	Level of Antihemophilic Factor (% of control)					
	Heterozygous females			Hemizygous males		
	No.	Mean	Sum of squares	No.	Mean	Std. dev.
Mild hemophilia						
Type "A"	4	70%	588	6	19%	5%
Type "B"	6	42%	294			
Classic hemophilia	4	116%	15,553	—	<5%	—

"t" test of the differences in group means of the three types of heterozygotes.

1. Mild hemophilia Type "A" v. Mild hemophilia Type "B": $t = 4.285$, 8 d.f., $.01 > P$.
2. Mild hemophilia Type "A" v. Classic hemophilia: $t = 1.254$, 6 d.f., $.3 > P > .2$.
3. Mild hemophilia Type "B" v. Classic hemophilia: $t = 2.576$, 8 d.f., $.05 > P > .02$.

2. It has not been possible to explain by the multiple allele hypothesis why approximately half the women carrying one gene for mild hemophilia and a normal allele have significantly lower plasma AHF levels than women carrying a normal gene and one gene for severe hemophilia. Incidentally, there are no overlaps between the Type "B" and classic heterozygotes. Another group of workers facing a similar problem recently in another connection, have theorized that more than one locus is concerned with AHF production (78). This is certainly reasonable when one considers the probable number of steps in the synthesis of a protein molecule. However, more information will be required about the action of the hemophilia gene before this paradox will be understood.

It should be emphasized that it has been only half the carriers in one pedigree of mild hemophilia who have been categorized with certainty by the AHF assay. The mistaken inclusion of carriers for the "hemophilioid diseases", some of which are dominantly inherited, with hemophilia carriers has confused the problem of carrier detection in earlier studies (5, 91). In several recent attempts at detection of carriers for classic hemophilia (10, 38, 78) normal overlaps have uniformly prevented carrier identification.

FACTOR V (LABILE FACTOR, AC-GLOBULIN) AND PARAHEMOPHILIA

Factor V, the first "convertibility factor" described, other than the AHF, was discovered independently in four widely separated laboratories during the communications breakdown of World War II. The four discoveries were made, interestingly, in three quite different contexts. In America, Quick deduced, after several preliminary attempts (74, 75), that the restorative effect of fresh, prothrombin-free plasma on aged, apparently prothrombin-deficient plasma, was due to an unidentified plasma factor. The easy deterioration of this unidentified factor in the refrigerator suggested to him the name "labile factor."

Owren, elucidating the clotting defect of a female "hemophiliac" in Norway (66), discovered that a plasma factor, distinct from the AHF, would correct his patient's clotting anomaly. He named this substance Factor V (Factors I–IV being his shorthand designation for thromboplastin, calcium ions, prothrombin and fibrinogen). He named the deficiency disease "parahemophilia" because of its clinical similarity to hemophilia.

Ware, Guest and Seegers in the United States (105) discovered that prothrombin was progressively more difficult to convert to thrombin as it was purified. They discovered that prothrombin-free plasma and serum contained a substance which promoted the conversion of purified prothrombin to thrombin. They showed further that the degree of acceleration was proportional to the amount of the new factor added to the system, but that the final yield of thrombin (within limits) was independent of the concentration of this newly discovered substance. They named the factor "accelerator-globulin", because they believed it to be a catalyst rather than a direct participant in the prothrombin-to-thrombin reaction.

The Australians, Fantl and Nance, also discovered the same accelerator by studying the activation of purified prothrombin (26), coming to essentially the same conclusions as Ware, Guest and Seegers.

Factor V has been shown repeatedly to deteriorate on storage. The rate of this deterioration is more rapid as the temperature increases, is more rapid in oxalated than citrated plasma, is more rapid as platelets increase in number, and is more rapid in ordinary glass containers than siliconized ones when platelets are present (24). Preliminary purification suggests that Factor V is a thermolabile globulin (66) which decreases during clotting (95). The coumarin drugs have little effect on Factor V production (25, 63). The turnover rate has not been ascertained, but the half-life is certainly less than 3 days (65). Chloroform intoxication causes a reduction in plasma Factor V (97), but splenectomy does not (41). These experiments suggest that this globulin is fabricated chiefly in the liver, and that little if any is produced in the spleen. When Factor V is congenitally deficient, administration of neither Vitamin K (2), protamine sulfate (12) nor aminophylline (12) will reverse this state, although aminophylline causes an increase of Factor V in normal dogs (60).

The corresponding deficiency disease, parahemophilia, resembles hemophilia clinically. Of the 18 cases reported (2, 11, 12, 23, 31, 44, 65, 67, 85, 96, 103) not more than 4 were sporadic, the remainder being familial. Ten patients were males, eight females. Recent study of a large consanguineous Afrikaner kindred using a semi-quantitative assay procedure suggests that those clinically affected are homozygous for the mutant gene (44). Using this assay technic, all parents and all children of clinically affected persons were found to have a significant reduction of Factor V. In the critical sibship, the distribution of siblings was—3 affected: 4 partially affected: 2 normal—consistent with a recessive gene. It is interesting to note that the action of the single parahemophilia gene, a clinical recessive, is detectable. This emphasizes the thesis that even a recessive gene has its effect, and implies in this instance that population surveys for Factor V deficiency are possible if one uses the proper assay procedure. Ordinary one-stage prothrombin tests are clearly not suitable for this purpose. Clinical cases of parahemophilia are certainly rarer than cases of hemophilia or Christmas disease. However, if affected individuals are actually homozygous, the Hardy-Weinberg Law would lead geneticists to wonder whether the gene frequency may not prove to be equal to or greater than that of hemophilia and Christmas disease.

CHRISTMAS FACTOR AND CHRISTMAS DISEASE

This disease was probably discovered and reported with the wrong interpretation by Pavlovsky in 1947 (69). He noted that the plasmas of two hemophiliacs, indistinguishable by his tests, were mutually corrective. The next reports were almost simultaneous in 1952; one by a group in New York City (88), and the other by a group in San Francisco (4). The Californians named the disease "PTC-deficiency", the term in common use today in the United States. Our British collegues published their studies several months later, during Christmas week 1952 (8). The highly improbable coincidence of having a patient with an unusual disease and the surname Christmas and publishing during Christmas week tickled the public fancy and the term "PTC" has never made headway outside the United States.

Eighteen papers reporting 69 undoubted cases of "Christmas disease" have been published since 1951, (4, 6, 8, 21, 22, 27, 32, 42, 50, 58, 72, 78, 81, 83, 88, 90, 92,

TABLE 3. FREQUENCY OF HEMOPHILIA AND CHRISTMAS DISEASE

Total cases surveyed and reclassified	= 247
Hemophilia	= 207
Christmas disease	= 40
Frequency of hemophilia in Danish males (Andreasson's survey)	= 4.45×10^{-4}
Adjusted frequencies for Danish males	
Hemophilia	= 3.73×10^{-4}
Christmas disease	= 7.16×10^{-5}

101). All cases show essentially the same features. A hemorrhagic disease resembling mild to moderately severe hemophilia clinically, is shown not to be hemophilia by study of the clotting anomaly. Where good pedigrees are available, a sex-linked mode of transmission is noted. The condition is not as completely recessive as hemophilia. For example, four of the seven mothers who have been tested adequately, have shown a clear diminution of Christmas factor (17, 78, 83). The disease is easily diagnosed with the partial thromboplastin time (48), the thromboplastin generation test (10), or the classical method, recalcification of mixtures of the unknown plasma with plasma samples from the other hemophilioid diseases. Recently it has been shown that the coumarin drugs depress "Christmas factor" production (90).

A fairly good estimate of the frequency can be given (Table 3). In nine different laboratories, from Switzerland to Australia (8, 27, 32, 47, 58, 81, 83, 92, 101) workers have surveyed the available "hemophiliacs", reclassifying 247 of them as classic hemophilia or Christmas disease. Of these, 207 were deficient in AHF, 40 in Christmas factor. Thus, 16.1% or approximately 1:6 of all "hemophiliacs" were in fact suffering from Christmas disease. This implies that Andreasson's estimate of the frequency of hemophilia in Danish males probably should be refined by reducing it from 4.45×10^{-4} to 3.73×10^{-4} before the next attempt at calculation of the mutation rate. It also allows the estimation of the frequency of Christmas disease in Danish males as about 7.16×10^{-5}.

A final bit of irony. It has been shown recently (45) that one of the large hemophilia pedigrees in the collection of Bulloch and Fildes (20), the Tenna kindred, is in reality Christmas disease. This might have been anticipated, as Christmas disease is much less severe than classic hemophilia on the average. One wonders whether the same thing is not true also of the Mampel (20) and some of the other large and famous hemophilia kindreds.

SUMMARY AND CONCLUSIONS

An attempt has been made to analyze recent developments in blood coagulation using genetic categories. From the standpoint of biochemical genetics, reliable data are scant, but a small beginning has been made with fibrinogen and the antihemophilic factor. The lack of progress along genetic lines in other areas may be attributed to:

1. the rarity of the newly discovered hemophilioid diseases,

2. concentration by most workers on identifying and subclassifying these diseases,
3. intense interest in the biochemical properties of the newly described factors and their roles in clotting, and
4. apparent failure to recognize the importance of genetic studies. This last opinion is implied by the failure to develop assay procedures sufficiently reliable for family studies, and improper application of such methods as are available. Specifically, one is impressed by the exhaustive studies on probands and almost complete neglect of relatives.

In my opinion, significant genetic advances will be made rather quickly when the workers in the field appreciate the possibilities.

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