Hemophilia: The Mechanism of Development and Action of an Anticoagulant Found in Two Cases[1]

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In this preliminary report the nature of an anticoagulant found in the circulating blood of two hemophiliacs is presented. One of these patients (W. P.) was previously reported by Lawrence and Johnson (*1*). A second case was studied by Munro and Jones (*4*), who felt that the anticoagulant appeared as a result of repeated transfusions. Later analysis of the blood of this patient (*3, 5*) revealed the anticoagulant to be associated with the gamma globulin fraction of blood.

TABLE 1

DEMONSTRATION OF ANTICOAGULANT EFFECT OF BLOOD OF PATIENTS W. P. AND D. M. ON NORMAL BLOOD

Tube No.		1	2	3	4	5	6	7	8	9	10
Patient's blood (cc.)		0.0	0.0	0.0	0.4	0.8	1.2	1.6	2.0	2.0	2.0
Normal blood (cc.)		2.0	2.0	2.0	1.6	1.2	0.8	0.4	0.0	0.0	0.0
Clotting time (min.)											
Patient	Date										
*W. P	9/ 9/46	8	8	8	7	8	8	8	70	71	68
W. P.	4/24/47	6	6	7	8	10	16	36	60	52	60
W. P.	4/29/47	7	7	6	10	12	18	40	58	56	58
D. M.	4/22/47	8	6	6	14	15	16	17	75	73	75
D. M.	4/23/47	6	6	6	9	14	15	16	100	98	95

* This test was performed 8 months after the last transfusion.

The two cases included in this report are typical, proven hemophiliacs, each having had the disease since childhood. It had been observed that, at various periods when hemorrhage was severe, each would become refractory to treatment, continuing to bleed with elevated coagulation times in spite of repeated transfusions. At such a time an anticoagulant was found in the circulating blood of W. P. by Lawrence and Johnson (*1*). However, in the interim between hemorrhagic episodes both patients were relatively asymptomatic. During such a period W. P. was tested for the presence of an anticoagulant, and none was found.

At the time of admission to this hospital, both patients were bleeding severely, W. P. from the gastrointestinal tract and D. M. from the genitourinary tract. Each was immediately given whole fresh blood, unfortunately before an anticoagulant test was performed. However, the coagulation times were followed at ½-hour intervals. W. P. was found to show no response at all, either symptomatically or as measured by the coagulation time. D. M. responded transiently to the first transfusion, the coagulation time falling from 60 to 15 minutes, but rising again over a 3-hour period. Subsequent transfusions resulted in no improvement either clinically or by laboratory findings. In fact, in the case of D. M. it was noted that, after two transfusions of whole fresh blood and two injections of one vial each of antihemophilic globulin (Fraction I of Cohn–Squibb), the coagulation time had risen from an average of 60 minutes to 120 minutes.

Following these observations, both patients were tested for the presence of a circulating anticoagulant as follows:

After first injecting 5 cc. of saline, 10 cc. of blood were removed at exactly the same moment and rate from the patient and a normal subject, respectively. The two samples were then mixed in varying amounts in clean, graduated, 15-cc. centrifuge tubes which had been rinsed with saline. The results (Table 1) were found repeatedly and indicated definitely the presence of a circulating anticoagulant which had the ability to prolong the coagulation time of normal blood. Control tests performed with the blood of two ordinary hemophiliacs gave negative results, the clotting time being normal in all tubes in which any normal blood was present. The anticoagulant could also be demonstrated by mixing plasma from the patients with normal plasma in varying amounts.

An attempt was then made to determine which phase of coagulation was inhibited by this anticoagulant. The prothrombin concentration and conversion rate were normal for both patients, thus eliminating the presence of anti-prothrombin. Antithromboplastin, as described by Tocantins (*7*), was not present because, even though the blood was handled in such a way as to destroy the antithromboplastin which he described, the anticoagulant effect was still present. Also, tests using thromboplastin serially diluted to the point where its action on normal plasma was delayed failed to show any greater delay in prothrombin conversion when the plasma of either patient was used. Antithrombic activity was not found to be present since, when a known amount of thrombin (2 units) was added to the plasma of the patients (0.1 cc.), clotting occurred in the normal time. Antifibrinogen was not present since the final fibrin clot which formed in these cases was normal in its volume, retraction, elasticity, and strength. Therefore, it was evident that the anticoagulant was not antagonistic to any one of the components of the classical theory of coagulation.

Electrophoretic fractionation of the plasma of W. P. was performed by Eric Alling. Of the four fractions separated in this manner, one (fraction #1) contained only gamma globulin, and another (fraction #4) contained all components except gamma globulin. Each of these fractions was then tested for its anticoagulant activity. It was found that all fractions except fraction #4 inhibited the coagulation of normal plasma. Therefore, the anticoagulant was associated with gamma globulin. This finding is similar to that of Munro (*5*).

Since the anticoagulant was a gamma globulin and since it appeared only after repeated transfusions of whole blood or injections of antihemophilic globulin, it was thought conceivable that the anticoagulant might be the result of an "immunization" of the patient with some substance present in normal blood or Fraction I of Cohn but lacking in the patient's blood. Consequently, precipitin tests were performed using the sera of the two patients against serial dilutions of Fraction I of Cohn, containing a known amount of protein and adjusted to a pH of 7.0. There were positive precipitins in the sera of both patients, W. P. being positive at 1/320 and D. M. at 1/160. Control tests using normal sera, sera from nonhemophilic patients who had received frequent trans-

[1] This document is based on work performed under Contract No. W-7401-eng-49 for the Atomic Energy Project at the University of Rochester.

fusions, and sera of ordinary hemophiliacs were negative. Control antigens of purified gamma globulin, human fibrinogen, and albumin gave negative results. These titers were obtained repeatedly, no change being noted throughout the hospital stay, in spite of repeated transfusions.

The anticoagulant was then shown to inhibit directly antihemophilic globulin. Taylor, *et al.* (*6*) have shown the *in vitro* effect of antihemophilic globulin in lowering the clotting time of hemophiliac patients. This could be repeated by us in the case of ordinary hemophiliacs who responded to blood or plasma in the usual fashion. However, in the two patients presented no effect was noted. Also, if the antihemophilic globulin was first incubated with the proper amount of serum from each of these patients, its acceleratory action on the clotting time of ordinary hemophilic blood was lost. If the globulin was incubated with the same amount of normal or ordinary hemophilic serum, the activity of the globulin when added to the blood of an ordinary hemophiliac was unimpaired.

It was evident from these results that the antihemophilic globulin, which alone had such a marked acceleratory effect on ordinary hemophiliac blood, was in some way tied up by the sera of these two patients. In view of the demonstration of definite precipitins in their sera it was felt that the sera probably inhibited or tied up the antihemophilic globulin by means of an antigen-antibody reaction.

On the basis of the evidence presented the following hypothesis was formed to explain the presence of the anticoagulant in these two patients. Each was deficient in, or lacked, the substance known as antihemophilic globulin in his blood—a substance shown to be essential for the coagulation of blood in the normal time. Just where this globulin enters into the coagulation mechanism is unknown, but it would seem that it is necessary for liberation of thromboplastin from platelets in the first stage of clotting. When this globulin is given intravenously to ordinary hemophiliacs, either in the form of fresh blood or plasma or antihemophilic globulin contained in Fraction I of Cohn, it causes a marked acceleration of coagulation (*2*). In these two cases repeated injections of this globulin are thought to have resulted in the formation of antibodies against this globulin. These antibodies, of course, have the ability to inhibit any globulin which is later given. Therefore, these patients became refractory to further transfusion or injection of Fraction I. No beneficial effect resulted from further injections since the active globulin factor which was being given, and which is necessary for normal coagulation, was immediately rendered ineffective by the circulating antibodies. Likewise, the circulating antibodies would exert an anticoagulant effect when the patients' blood was mixed with normal blood *in vitro* by inhibiting the globulin substance present in normal blood.

The explanation proposed for the development of a refractory state to transfusion in these two hemophiliacs, based upon an immunologic response to injections of a globulin fraction deficient or lacking in their blood, may also be the underlying factor in the refractory phase manifested by many hemophiliacs. The appearance of specific antibodies which inhibit antihemophilic globulin, and thus delay the coagulation of normal blood, may depend upon several factors which are not yet clear. Whether or not a complete absence of this globulin from the blood of the hemophiliac receiving transfusions or injections of the globulin is mandatory for the development of these antibodies is not known. Perhaps there are varying degrees of hemophilia accompanied by varying degrees of deficiency of this globulin and varying ability to respond to injections of the globulin. In some of those who have a marked lack, or perhaps a complete absence, of the globulin in their blood the response would be "isoimmunization" with the development of antibodies against the injected globulin. These cases would show a refractory state to further injections of globulin, and their blood would demonstrate anticoagulant activity when added to normal blood.

Further investigation is to be carried on to determine if a mechanism such as that described is more generally applicable to hemophiliacs who become refractory to treatment.

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A Homogeneous Emulsion of Fat, Protein, and Glucose for Intravenous Administration

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Several investigators (*3*) have reported success in the preparation of various types of fat emulsions, some of which were well tolerated on intravenous injection into animals. Also, experimental evidence has been produced to show that such fat administered intravenously was properly metabolized for energy utilization (*2*) and was not lost in either urine or feces. Intravenous studies of emulsions combining the three primary foodstuffs were attempted only once (*1*), and, had this procedure been routinely practical, it would have constituted another advance in surgical parenteral nutrition.

Egg lecithin, soybean phosphatides, and other chemical agents have been used as stabilizers in the emulsification and homogenization of fat. Such emulsions, although well tolerated in some cases, have not been uniformly dependable for intravenous injection because of varying degrees of toxicity believed to be due to the chemical complexity of the stabilizing agent. Hydrophilic colloids, both acid and alkaline, were used in the stabilization of fat but on biological investigation were discarded because of the frequency of fatal embolism after injection of emulsions prepared in such manner.

In this laboratory two satisfactory types of fat emulsion have been prepared. The first type was an emulsion of coconut oil and serum albumin which, on homogenization, yielded a highly stabile preparation well tolerated intravenously. The

[1] The cooperation of Profs. Mulholland and Co Tui is gratefully acknowledged.