Hyperglobulinemia as the Cause of Hemophilia-like Disease

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In recent years several cases of a formerly unrecognized coagulation defect have been described. This is characterized by a hemophilia-like hemorrhagic state and caused by the presence in blood of a circulating anticoagulant which is active in the first phase of coagulation and which is neither heparin nor anticephalin. The first case was reported in 1940 by Lozner, Jolliffe, and Taylor. Thereafter, more than twenty similar cases have been described. In 1950, Dreskin and Rosenthal summarized the twelve cases of the disease known to that time and recommended the name of “hemophiloid disease”. The same year, Deutsch published a monograph on the disease, including one case of his own. He suggested the name “Hemmkörper-Haemophilie”.

Singer et al., as well as other workers, have reported the presence of circulating anticoagulants antagonistic to thromboplastin, or to the formation of thromboplastin, in patients with true hemophilia following multiple transfusions, and appearing de novo or in association with various other diseases.

No clear-cut distinction can be made between these two groups. In the second group we have also placed those cases in which it was not absolutely certain, although probable, that a genuine hemophilia originally existed, as, for example, Singer’s second case. The anticoagulant in the first group has been assumed to develop as a result of immunization following blood transfusion, with the occurrence of antibodies antagonistic to antihemophilic globulin.

The first group comprises only genuine hemophilies, that is, only men, while the second includes both men and women. Singer proposes that the designation “hemophilia-like disease” should be reserved for the latter group, which seems to be heterogeneous with regard to both the chemical and the physical properties of the anticoagulant factor and to the clinical course and pathogenesis. In four cases immunization may have taken place, at least in the opinion of the authors. Thus, Dreskin and Rosenthal considered the possible cause of the clotting defect in their own case, as well as in those reported by Fant and Chargaff, to be isoimmunization in connection with pregnancy. Deutsch was of the opinion that a circulating anticoagulant directed against the platelets had developed following numerous blood transfusions in his second case, which was one of essential thrombocytopenia.

In other patients included in the second group, a demonstrable dysproteinemia was found, or was probable from clinical findings. Lozner and Taylor’s patient had generalized lymph node tuberculosis, and hyperglobulinemia was demonstrated. Other cases in this group are that of Dieter and the second case.

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of Tzanck, clinically diagnosed as pemphigus and dermatitis herpetiformis, respectively. Both these diseases are known to be associated with protein anomalies with a demonstrable increase in the gamma globulins. Deutsch's first patient also showed increased gamma globulins, and the sedimentation rate was 115 mm. per hour. The case reported by Mueller also belongs to this group. The diagnosis was bacterial endocarditis together with cryoglobulinemia and an increase in the gamma globulins.

It is open to discussion whether or not Singer's first case should be included in this group. In addition to the five cases with dysproteinemia reviewed above, a sixth case is presented in this paper.

A common feature in all the cases with dysproteinemia was a change in the serum protein with an increase in the globulin fraction. It has been previously known that such a disturbance may cause the development of circulating anticoagulants directed against the later phases of coagulation as well as against the initial one. Conley described a case of cirrhosis of the liver with a circulating antithrombin. Lüscher and Lohhart and Uehlinger reported dysproteinemias in connection with inhibition of the conversion of fibrinogen into fibrin.

On the basis of available information it is impossible to say whether, in the remaining cases within the second group, the clotting disturbance was caused by isoimmunization, protein defects, or some other factor.

A case of chronic hyperglobulinemia in a 44 year old woman is presented here, in which a probable family history of a protein disturbance was present, complicated by a circulating anticoagulant directed against the initial phase of the clotting phenomenon.

**Report of Case**

The patient, a 44 year old married woman; nullipara.

**Family History**

Father died of icterus at the age of 70. Mother died at 72 of malignant tumor of the parotid gland. One sister died at 30 of acute renal insufficiency. Autopsy report: septicemia, focal nephritis, lipoid nephrosis, fatty infiltration of the liver. Another sister died at the age of 44. Autopsy report: aplastic anemia in connection with cirrhosis of the liver; there was also a maximally elevated sedimentation rate, anemia, pleuritis, infiltrations in the skin, a tendency to superficial and deep hematomas, positive thymol and Takata tests, positive formalin gel, and elevated serum globulin values. She received almost two hundred blood transfusions which had only a transitory effect on her anemia and did not otherwise alter the clinical picture. No examination of the coagulation was performed, but the clinical picture conforms so closely with that seen in our patient that this sister can be assumed to have suffered from the same protein disease.

One brother had painful joints. Another brother was in good health.

**Past Medical History**

The patient had acute gangrenous appendicitis in 1923. Appendectomy was performed without complications.

**History of Present Illness**

The patient had rheumatic fever at 9 or 10 years of age. In 1937 she had pleuritis of the left lung. Mantoux test was positive at that time. Roentgenologic examination of the lungs revealed no parenchymal changes.

Since 1939 the patient had suffered from continuous fatigue, often accompanied by head-
aches. She had been able to work only intermittently. She had had bilateral pleurisy several times. There had also been recurrent transient pains in and around the joints, sometimes with transient swelling of the joints but never with lasting deformities. Deep hematomas had occurred frequently, usually after mechanical stress. Her skin often bruised easily but she had never noticed petechiae or mucosal bleeding. There was marked hemorrhage after trauma. The menstruation was normal.

The sedimentation rate was found to be elevated as early as in 1937 and had gradually increased; during the past few years the sedimentation rate had never been below 90 mm. per hour.

In 1946 hyperglobulinemia was demonstrated to be present but no clotting studies were made. A prolonged coagulation time was observed in 1950 and was then 25 to 50 minutes. All other routine clotting tests were normal. The patient's prolonged coagulation time was shown to be caused by a circulating anticoagulant. Three blood transfusions had no effect on the clotting time. The presence of an anticoagulant was observed prior to the first blood transfusion. The patient had never previously received any transfusions.

In the course of the illness, the patient's subjective symptoms remained unchanged but the clotting time gradually increased to about 120 minutes. Following recent blood transfusions, the coagulation time became still more prolonged.

**Physical Examination**

The patient was well nourished but not obese. Large hematomas were seen on the arms and legs; the joints were normal. The liver and the spleen were not palpable and there was no lymphadenopathy. There was no ascites. Roentgenographic examination of the heart revealed nothing abnormal. The electrocardiogram was normal. X-ray examination of the lungs showed healed pleuritis. Urography and x-ray of the skeleton, esophagus, and abdomen revealed nothing abnormal. Ophthalmologic examination was negative.

**Laboratory Findings**

The patient had a normochromic anemia with 63 to 70 per cent hemoglobin; erythrocyte count 3.2 to 3.6 mill, leukocyte count 3000 to 5000. Differential count of the white cells and the sternal punctate showed normal values. Serum proteins: total protein was 8 to 9 Gm. per cent (copper sulphate method and Kjeldahl's method). Fractionation of the total protein according to Kjeldahl: globulin 5.8 to 6.5 Gm. per cent. Albumin 1.6 to 3.3 per cent. Fibrinogen 0.3 to 0.37 per cent.

Formol gel in serum appeared after 8 to 15 minutes. Thromboplastin 0.5 to 0.6 extinction. Takata positive. Zine sulphate 23 units. Wassermann reaction was negative. Meinicke reaction was faintly positive. Euglobulin positive (+). Electrophoresis (Pedersen) 41 per cent. Albumin, alpha 2 + 5 per cent, beta 6 + 4 per cent, gamma 19 + 23 per cent. Ultracentrifugation 7 comp. 26 per cent. Cold agglutinins were not present. The antistreptolysin titer was negative. There was no agglutination with sensitized sheep blood corpuscles. Bilirubin in serum was not elevated. Citric acid in serum 32 µg. per ml. Alkaline phosphatases 5 to 7 units.

The sedimentation rate was 100 mm. per hour when tested on oxalated or citrated plasma. This value was reached after 15 to 20 minutes. On the other hand, there was no sedimentation of the red blood corpuscles in native blood kept in ice and silicon coated centrifuge tubes until after several hours, simultaneously with the appearance of the first fibrin threads. The red blood corpuscles then descended rapidly for 15 minutes and a clot was formed. When heparinized blood containing 5 µg. heparin per 0.1 ml. was drawn into sedimentation tubes (Westergren) immediately after venipuncture, no sedimentation occurred until after about 45 minutes when there was rapid increase to 100 mm. The blood in the clotting tube, which had been drawn at the same time, coagulated simultaneously. Thus, in this patient, there seems to be a connection between some phase in the clotting and the sedimentation of the red blood corpuscles.

**Routine Clotting Studies**

The thromboocytes were 130,000 to 200,000 (Kristenson, Fonio), calcium in serum 10.6 mg. per cent, bleeding time (Duke) 3 to 4 minutes, tourniquet test negative, fibrinogen
normal. Prothrombin, determined according to Quick, was about 80 per cent and according to Owren 80 to 100 per cent. The prothrombin consumption was markedly lowered. The coagulation time, determined according to Hedenius and Lee-White at room temperature and at 37°C, was 25 to 50 minutes (1950) and 60 to 120 minutes (1952). The exact determination of the coagulation time was complicated by the fact that the clot formation was not instantaneous. There was found a slow and initially incomplete formation of the clot as only part of the fibrinogen was transformed into fibrin. For several hours after the first signs of coagulation had appeared there was noted the formation of new fibrin threads. No signs of clot retraction could be observed either during this long drawn out process of clot formation or during the following hours when clotting seemed to be complete. After four to six hours there were, however, signs of retraction. These observations could be performed on native plasma where the erythrocytes had been centrifuged down. Addition of sufficient amounts of thrombin to the patient's plasma caused normal fibrin formation and retraction. The same stepwise formation of fibrin may be observed in the blood from hemophiliacs and in normal oxalated plasma which is brought to coagulation by the addition of repeated small amounts of thrombin.

**Demonstration of a Circulating Anticoagulant**

The patient's oxalated plasma or serum was pipeted in advance in coagulation tubes according to Hedenius or Lee-White. Normal blood was added to these tubes directly from the venipuncture cannula so that the ratio between normal blood and the patient's plasma/serum became 9:1. The results are shown in figure 1. The patient's plasma/serum lengthened, in all instances, the clotting time of normal blood. The same result was obtained when the recalcification time of normal plasma was studied instead of the coagulation time of whole blood. There was always a lengthening of the recalcification time when the patient's plasma/serum was added in various concentrations.

**Mechanism of Action of the Anticoagulant**

**Test for Antithrombin**

In order to establish whether the anticoagulant factor was antagonistic to existing thrombin, the patient's oxalated plasma was titrated with thrombin and compared with a
similar titration of normal oxalated plasma of the same age. The thrombin preparation employed was Topostasin. A series of distilled water and thrombin in increasing dilutions up to 1/1400 were prepared from a stock solution containing 300 N.I.H. thrombin per ml. In the experiment, 0.2 ml. of thrombin solution was added to 0.2 ml. of oxalated plasma, as shown in figure 2. The patient's coagulation time never exceeded that of a normal person in any thrombin dilution. The anticoagulant factor could thus not be an antithrombin.

![Figure 2](image_url)

**Fig. 2.—Comparative thrombin titration of normal plasma and patient's plasma.**

The separated gamma globulin fraction (see below), which contained the patient's anticoagulant factor, did not lengthen the thrombin time when added to normal oxalated plasma. The patient's oxalated plasma was, moreover, kept at room temperature for three days and thrombin tests were carried out at different times. The thrombin values with the patient's plasma were increased but no more so than that of the normal controls.

It is thus evident that the anticoagulant factor was not active as an antithrombin. The patient's fibrinogen could also be regarded as quite normal in the process of coagulation.

* Supplied through the courtesy of Hoffmann-LaRoche Inc.
Test for Heparin

Titrations with protamine and toluidine blue were carried out under varying experimental conditions. Protamine sulphate or toluidine blue in quantities of between 1 to 300 \( \mu \text{g} \) per 0.2 ml. of blood or plasma was added to native blood and to oxalated plasma. There was no shortening of the coagulation time.

Test for Antiprothrombin

In repeated determinations according to Quick the prothrombin content was about 80 per cent and according to Owren 80 to 100 per cent of the normal value. There was no quantitative agreement between the slight decrease in the normal amount of prothrombin and the patient's lengthened coagulation time. Dicoumarol plasma was not affected more than normal plasma by the patient's plasma in recalcification tests; the patient's plasma could, in the presence of undiluted thromboplastin, reduce the lengthened prothrombin time of Dicoumarol plasma according to Quick. These findings all contradict the supposition that the anticoagulant factor should be antagonistic to the prothrombin.

Test for Antiproaccelerin

The patient's almost normal prothrombin time determined according to Quick and the fact that the effect of the anticoagulant was the same when tested on both fresh and stored oxalated plasma determined according to Quick or according to Owren on Seitz filtered bovine oxalated plasma do not support the assumption that the anticoagulant factor is directed against the proaccelerin.

Test for Antithromboplastin

The anticoagulant factor is thus not directed against the prothrombin complex, nor is it an antithrombin. The transformation of fibrinogen to fibrin is normal. The point of attack of the factor must, therefore—granted that the previous observations are correct—he the initial phase of the coagulation, that is to say, it must be directed either against the existing thromboplastin or against one of its prestages. In agreement with this theory, the patient's coagulation time was normalized on the addition of thromboplastin or Russell Viper venom (Stypven, Burroughs, Wellcome, and Co.) to the coagulation tube. At the same time the prothrombin consumption increased so that serum from blood which had been allowed to clot together with thromboplastin showed normal consumption. In the same way, the patient's clot retraction became normalized.

The relationship between the anticoagulant factor and the thromboplastin can be studied more exactly on oxalated plasma. In a determination of the prothrombin content according to Quick, the patient's plasma showed only a slight increase of the coagulation time compared to normal plasma (corresponding to a prothrombin content of 80 per cent). When the thromboplastin was diluted, the difference increased more and more, reaching its maximum when no thromboplastin was added, i.e., in the determination of the recalcification time. As pointed out also by Singer,\(^2\) this was to be expected if the anticoagulant factor was antagonistic to thromboplastin or its formation. The same situation occurred on dilution of the thromboplastin in hemophilia. In this condition the coagulation time became normal on the addition of undiluted thromboplastin, and the more the thromboplastin was diluted the greater was the difference from normal plasma. The curve for thromboplastin titration of Dicoumarol plasma ran an entirely different course—lying always above but parallel to the curve for normal plasma.

The experiments were carried out on human brain thromboplastin according to Owren, but control tests made on rabbit brain and Stypven yielded similar results.

Since the coagulation time of the patient's plasma upon the addition of diluted thromboplastin is clearly different from normal plasma, it is possible to demonstrate the occurrence of the anticoagulant factor in plasma also without employing the difficult and lengthy re-
calcification technic. The experiments were carried out as follows:

\[ 0.2 \text{ oxpl.} + 0.2 \text{T/100} + 0.2 \text{ oxpl.}/3 + 0.2 \text{Ca 30 mM} = \text{x sec.} \]

normal oxalated plasma Thromboplatin patient's oxalated plasma
or
or
patient's oxalated plasma normal oxalated plasma

By using the patient's plasma as the base in the coagulation system and adding diluted normal plasma, it was possible to see whether the patient's plasma became normalized by normal plasma.

![Graph showing coagulation time in seconds for different plasma types](image)

**Fig. 3.**—Thromboplastin titration of normal plasma, hemophiliac plasma, Dicoumarol plasma, and patient's plasma.

The results are given in figure 4. The coagulation times of normal plasma plus normal plasma/3 are given on the abscissa in figure 4A and the times of the patient's plasma plus the patient's plasma/3 on the abscissa in figure 4B. Values for normal plasma plus the patient's plasma/3 are given on the ordinate in figure 4A and the patient's plasma plus normal plasma/3 on the ordinate in figure 4B. It is evident from curve A that the patient's plasma prolonged the coagulation time of the normal plasma in all the experiments. In curve B the coagulation times lie round the bisectrix. This means that the coagulation time of the patient's plasma is not reduced following the addition of normal plasma. When, in the above experiment, hemophiliac plasma was substituted for normal plasma, the clotting time of the former was lengthened more by the addition of the patient's plasma than normal plasma was. The hemophiliac plasma was not normalized by the patient's plasma.

Experiments were done to neutralize the anticoagulant factor by means of antihemophiliac globulin. The prothrombin time according to Quick became normalized and the recalcification time also became normal. Moreover, antihemophiliac globulin inactivated the globulin
fraction isolated by paper electrophoresis which contained the patient's anticoagulant factor (see below).

The patient's plasma was not studied with regard to the presence of precipitins against antihemophilic globulin.

**Chemical and Physical Qualities of the Patient's Anticoagulant**

Experiments were carried out to isolate the anticoagulant factor and to determine its chemical characteristics. When the proteins in the plasma were precipitated, the prolonging effect could no longer be demonstrated. The anticoagulant substance could therefore be assumed to consist of a protein or to be associated with such a compound. In order to determine in which protein fraction the anticoagulant substance was to be found, a separation of plasma and serum protein components was carried out, both by salting out with ammonium sulphate and by paper electrophoresis. On salt precipitation a potent

![Diagram](image)

**Fig. 4.**—(A) Comparison between coagulation time of normal plasma (abscissa) and normal plasma + patient's plasma (ordinate). (B) Comparison between coagulation time of patient's plasma (abscissa) and patient's plasma + normal plasma (ordinate).

Determinations of clotting time were carried out with addition of thromboplastin in dilution 1/100 and optimal calcium concentration.

A retarding factor was obtained from the pseudoglobulin fraction with the same action as the anticoagulant in the patient's plasma. The albumin fraction and the euglobulin fraction did not cause any prolongation of the coagulation time. Paper electrophoresis was carried out according to a somewhat modified Cramer-Tiselius method. The electrophoresis diagram showed a pronounced increase in the gamma globulins. On testing the eluate from the albumin fraction and from the globulin fractions the patient's anticoagulant was found in the gamma globulin fraction. The alpha or beta globulin fractions or the albumin fraction did not give any lengthening of the coagulation time. Similar elutions were made with normal serum but did not have inhibitory effect on the coagulation.

Trypsin Merck was added both to the pseudoglobulin fraction and to the gamma globulin fraction obtained by electrophoresis. The lengthening factor was still present when tested in a fresh condition; following 24 to 48 hours' incubation at 37 C. it could no longer be demonstrated.

The anticoagulant factor was not dialysable; after dialysis for 48 hours against both sodium chloride and running tap water the prolonging effect was still present. The thermostability of the anticoagulant factor was examined both in plasma and in the separated gamma globulin fraction. Following heating at
56°C for 30 minutes the factor retained its activity; on continued heating the retarding effect was successively reduced and at 70°C the factor was no longer active.

Another characteristic of the retarding factor was the only slight reduction in activity following prolonged storage (three weeks at 17°C).

The extractability of the anticoagulant factor with lipoid solvents was tested on the patient’s plasma and on her gamma globulin fraction in solution by shaking with ether (2 to 3 volumes of ether added to 1 volume of plasma or gamma globulin and shaken for 20 minutes). After separation of the ether phase the factor remained unchanged. Experiments were also carried out with chloroform; the factor disappeared but could not be recovered back from the chloroform phase; the chloroform seemed to denature the factor.

The patient’s anticoagulant was not adsorbed by barium sulphate and tricalcium phosphate; it was still active after Seitz filtration of the plasma.

From these experiments the conclusion was drawn that the patient’s anticoagulant factor was a gamma globulin. Because of her basic illness, the patient had a pathologic increase in the gamma globulins, and it is evident that one of these pathologic gamma globulins acted as an anticoagulant.

**DISCUSSION**

The investigation showed the presence in our patient of a hemophilia-like disease, caused by a circulating anticoagulant directed against the initial stages of the coagulation, probably against the antihemophilic globulin. The patient suffered from a marked dysproteinemia and this must be considered the primary disease to which the coagulation disturbance was a secondary phenomenon. The cause of the dysproteinemia was not clear. The protein disturbance was possibly familial. The clinical course and the other findings did not suggest a primary cirrhosis of the liver. Nor was there any reason to assume a systemic disease, myeloma, or hypernephroma.

The prolonged course of illness and the absence of skin affections seemed to rule out lupus erythematosus disseminatus which the patient’s condition otherwise resembled in many respects. In all probability she had a genuine protein disturbance, previously described by Waldenström which he calls “essential hyperglobulinemia”. The progressive course of the disease and the lethal outcome of the sister’s illness may indicate a poor ultimate prognosis.

Singer found that “whereas the immunologic hypothesis offers an attractive explanation of the development of a circulating anticoagulant in hemophilia, the pathogenesis of the hemophilia-like disease remains obscure.” In the case of hemophilia-like disease reported by us, the coagulation disturbance was secondary to the primary disease of the patient, which was associated with dysproteinemia. One of the pathologic protein substances, a gamma globulin, acted as an anticoagulant. On studying the cases of hemophilia-like disease previously published, it was found that, in at least five of these, certain pathologic changes in the blood protein were observed. Although the etiologic connection between the protein disturbance and the anticoagulant substance was not as clearly demonstrated in these cases as in ours, it seems, nevertheless, safe to assume that pathologically altered blood protein can in some way act as an anti-
coagulant substance, even in the earliest phase of the coagulation. The pathologic protein substances probably combine with the antihemophilic globulin so that the latter cannot be utilized in the coagulation. A parallel may perhaps be found in the anticomplimentary effect of the gamma globulin fraction often found in hyperglobulinemia.

In order to investigate whether the pathologically altered blood protein fraction acts as an anticoagulant perhaps more often than hitherto observed, a number of patients suffering from various protein disturbances, were examined: two cases of myeloma, two of purpura hyperglobulinemica, seven of cirrhosis of the liver with a maximally elevated sedimentation rate and lowered prothrombin index, one of lupus erythematosus disseminatus, five of pathologically elevated sedimentation rate in connection with hyperglobulinemia, and two of cryoglobulinemia. In none of these cases was any lengthening of the coagulation time observed. It seems, therefore, to be a rare occurrence for pathologically changed globulins to act as anticoagulants, even in patients with serious protein disturbances in the plasma.

In other cases of hemophilia-like disease, the authors have assumed the cause to be an isoimmunization process, caused by pregnancy or by repeated blood transfusions. In our patient, who is a nullipara, there is no reason to assume an isoimmunization process and her coagulation disturbance was observed before she received any blood transfusion. If the isoimmunization hypothesis is correct, there must be various pathogeneses to the hemophilia-like diseases. This is confirmed by those cases where there is nothing to support the theory of an isoimmunization process and where there is no observable pathologic blood protein.

SUMMARY

A case is reported of a probably familial hyperglobulinemia in a 44 year old woman with a circulating anticoagulant, antagonistic to the initial phase of the coagulation, most likely to the antihemophilic globulin. The anticoagulant factor was separated by means of salt precipitation and by electrophoresis. It was shown to be identical with one of the patient’s pathologic gamma globulins. There is no reason to suppose an isoimmunization process. The coagulation defect is therefore assumed to be a secondary phenomenon to the patient’s probably familial protein disturbance. Dysproteinemia has been observed in hemophilia-like diseases several times but the etiologic connection between the coagulation anomaly and the protein disease has not been previously established. On the other hand, even serious dysproteinemias seem rarely to be complicated by a circulating anticoagulant.

A connection between the sedimentation rate and the coagulation of the blood was further shown to be present in our patient.

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Hyperglobulinemia as the Cause of Hemophilia-like Disease

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