HAEMOPHILIA WITH A SPECIES SPECIFIC INHIBITOR OF FACTOR VIII

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The effectiveness of haemostasis in haemophilia is directly related to the blood level of factor VIII (antihaemophilic globulin - AHG) in the patient. In most haemophiliacs the blood factor VIII level can be raised sufficiently to control minor bleeding with intravenous fresh-frozen plasma. For more serious bleeding or major surgery factor VIII concentrates from human or animal sources may be required.

In some haemophiliacs therapy is made more difficult or impossible by the presence in the patient's blood of an inhibitor of factor VIII. Therapy in such patients is largely determined by the inhibitor titre. In the presence of a high titre it may be impossible to raise the patient's factor VIII level even with animal AHG concentrates.[1] Occasionally a high inhibitor titre to human AHG may be associated with negligible inhibitory activity against animal AHG.[2,3] We report here our observations on the management of severe gastro-intestinal haemorrhage in a haemophiliac who had an inhibitor active against human but not porcine or bovine AHG.

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MATERIALS AND METHODS

Infused plasma had been stored freshly-frozen at −20°C for up to 3 weeks. Porcine and bovine AHG concentrates were obtained from Messrs. Maw and Sons, London.

The reagents for the coagulation tests were prepared as described by Biggs and Macfarlane,[4] except that aluminium hydroxide gel from Cutter Laboratories, Berkeley, California, and celite-512 from Johns-Manville, London, were used.

Assay of Factor VIII

Factor VIII was measured by a plasma clotting-time method in which surface contact was standardized by incubation of the plasma with celite, and cephalin was used as a platelet substitute. The tests were carried out in silicone-treated tubes and the clotting mixtures consisted of:

0·1 ml. haemophilic plasma
0·1 ml. dilution of test plasma or factor VIII concentrate in citrate-saline
0·1 ml. cephalin (1/100 in saline)
0·1 ml. celite (20 mg./ml.)

The mixture was incubated at 37°C for 1 minute and 0·1 ml. of $CaCl_2$ 0·050 M added. At least 3 dilutions of each

test sample were tested, and the clotting times plotted on double logarithm paper against the dilution. The best straight line through the points was drawn and compared with a similar parallel line obtained with a standard plasma sample. A pool of 4 fresh normal plasma samples was used as a plasma standard containing 100% factor VIII. The method is essentially similar to those described by Hardisty and Macpherson[5] and Breckenridge and Ratnoff.[6]

Measurement of Inhibitor Activity

Inhibitory activity was measured by methods similar to those described by Biggs and Bidwell[7] and Breckenridge and Ratnoff.[6] Aluminium hydroxide-absorbed test plasma was incubated for one hour at 37°C in stoppered tubes with normal plasma (human AHG) or animal AHG, and the percentage of AHG destroyed was determined. The unit of inhibitor activity used was similar to that defined by Biggs and Bidwell[7] —a dilution of inhibitory plasma is said to contain 1 unit of inhibitor per ml. when the plasma destroys 75% of the initial AHG activity in 1 hour at 37°C. A human AHG concentrate was not available but a sample of the patient's plasma was assayed for inhibitory activity with the use of a human AHG concentrate by Mr. K. W. E. Denson at the Medical Research Council's Blood Coagulation Research Unit, Churchill Hospital, Oxford, England. Comparable inhibitor titres were found in Oxford and here.

CASE REPORT

The patient, a 38-year-old farmer, had 3 haemophilic brothers but no other relevant family history (Fig. 1). The carrier

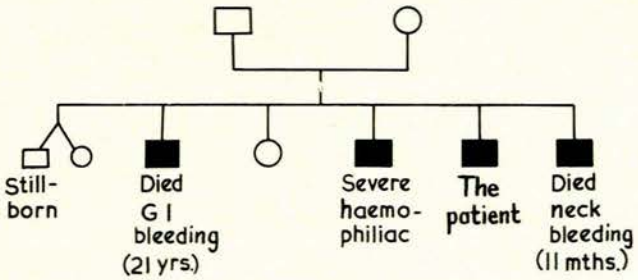

Fig. 1. The patient's family tree.

mother had an AHG level of 82%. The patient had bled from the umbilical cord soon after birth and thereafter suffered numerous haemarthroses. As a result of joint deformity and muscle contractures he had been bedridden for several years. He had experienced occasional post-cibal epigastric discomfort readily relieved by alkalis.

His first blood transfusion followed haematemesis and melaena at the age of 20. Since then numerous transfusions of fresh blood had been given. At 33 years of age he had a second episode of haematemesis and required transfusion. On 8 April 1964, following epigastric discomfort, he had 3 small haematemeses. Gastro-intestinal bleeding persisted and he received 46 pints of blood before admission to Groote Schuur Hospital on 23 April.

On examination he was overweight with pale mucous membranes. Deformity and limited movement of the knee, ankle, elbow and wrist joints were present. His pulse rate was 120/minute, temperature 102°F, haemoglobin 7 G/100 ml. and PCV 24%. Coagulation tests showed 0% factor VIII, 2·8 units/ml. inhibitory activity to human factor VIII, a one-stage prothrombin time of 12·4 seconds and a factor IX level of 100%.

Treatment. He was treated with an ulcer regime and transfusions of 1 - 2 litres of fresh-frozen plasma daily. Persistent gastro-intestinal bleeding necessitated daily blood transfusion to maintain his haemoglobin level; by 11 May he had received a total of 113 pints. Prednisone (60 mg./day) from 7 to 13 May had no clinical or laboratory effect. The factor VIII level was always 0%, even while plasma was being infused. On 12 May porcine AHG was administered intravenously. For the first time the patient's AHG level rose (to 83%) and simultaneously there was clinical evidence that bleeding had stopped. Three further infusions of porcine AHG were given on the 3 subsequent days. The cessation of bleeding was confirmed by a steady rise in the patient's haemoglobin and the passage of normal stools. Progress thereafter was interrupted only by a subcutaneous staphylococcal infection of the right forearm. Two barium-meal radiographs of the stomach and duodenum were normal. A record of the therapy and response of the patient is shown graphically in Fig. 2.

The Inhibitory Activity of the Patient's Plasma

The results of a screening test for inhibitory activity are shown in Table I, and inhibitor measurements in Table II. The inhibitor titre to human factor VIII varied between 1·5 and 2·8 units/ml. and throughout there was at most only trivial inhibitory activity to porcine and bovine factor VIII. The clinical significance of the inhibitory activity is demonstrated by the failure of 1·5 - 2 litres/day of human plasma to produce a measurable rise in the patient's factor VIII level, whereas porcine AHG produced a rise and fall-off rate similar to that previously observed for this factor.[5]

TABLE I. SCREENING TEST FOR INHIBITOR ACTIVITY

Test plasma	*Clotting time (secs.)* *A**	*Clotting time (secs.)* *B†*
Patient plasma	171·4	80·9
Haemophilic plasma with no inhibitor	76·0	82·7

**A* = 0·1 ml. of normal plasma and 0·1 ml. of test plasma incubated together at 37°C for one hour; 0·1 ml. of cephalin and 0·1 ml. of celite (20 mg./ml.); incubate for 1 minute; 0·1 ml. of $CaCl_2$ 0·050M.

†*B* = Normal and test plasma incubated in separate tubes at 37°C for 1 hour. Mixture made, clotted as in *A* without incubating for 1 hour.

TABLE II. TITRE OF INHIBITOR TO FACTOR VIII*

Date	*Human*	*Porcine*	*Bovine*
22 April	2·8	0·4	—
27 April	2·4	0·5	0·5 and 0
8 May	1·6	0·5	0·5
20 May	0·5	—	—

*Expressed as units/ml.

DISCUSSION

The continuing severe haemorrhage in this patient, as long as his factor VIII level was zero, and its prompt cessation when the factor VIII level was elevated, emphasizes the relation of the factor VIII level to haemostasis in haemophilia. For most bleeding episodes, infusion of fresh-frozen plasma produces prompt clinical improvement. Lack of clinical improvement may be related to an insufficient factor VIII level, either due to insufficient administered AHG, or to a specific inhibitor of factor VIII, as occurred in this patient.

TREATMENT

Therapy of patients with a factor VIII inhibitor is largely determined by the inhibitor titre. When the titre is 1 u./ml. or less, large doses of concentrated antihaemophilic globulin may elevate the circulating factor VIII level sufficiently to achieve haemostasis.[1,7] In this patient the inhibitor exhibited species specificity against human AHG, a phenomenon recorded only rarely before.[2,3] It was therefore possible to use a type of AHG not affected by the inhibitor.

Patients with an inhibitor have also been treated by exchange transfusion[3] or steroid administration. Steroids or corticotrophin have seldom been effective in treating circulating factor VIII inhibitor,[1,9-13] but about one-third of patients so treated (11 of 32) have been reported as responding.[9,12-17] The improvement in these cases is not

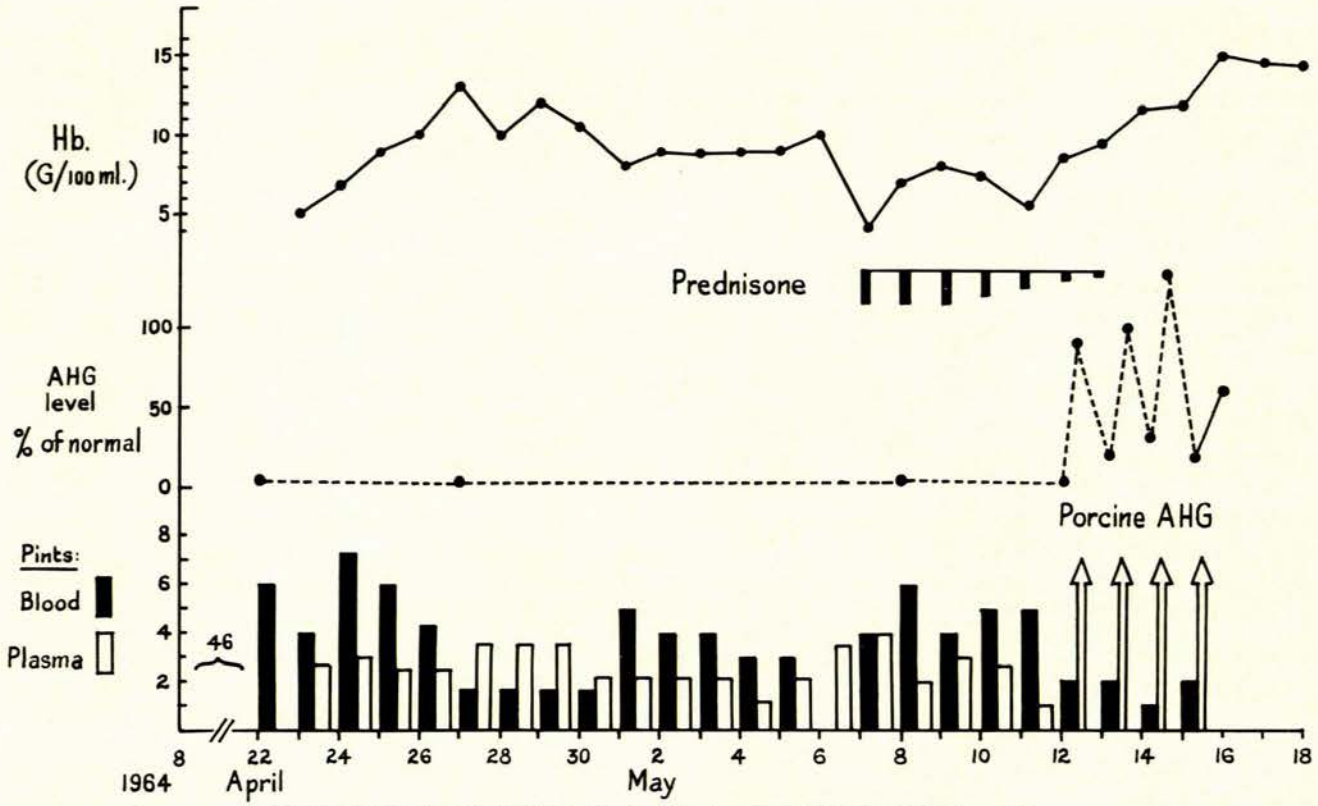

Fig. 2. Record of the therapy, factor VIII level and haemoglobin in the patient described

usually accompanied by a simultaneous measurable fall in inhibitor titre, but there are instances in which the clinical response coincides with laboratory demonstration of decreased circulating inhibitor; even in the latter the factor VIII level may only rise at a later date.[13] There is an impression that therapeutic response to steroids is least when the inhibitor appears in classical haemophilia and on the rare occasions when no other predisposing disorder is present (see *Mechanisms of Inhibition*), beneficial response being more frequently encountered when the inhibitor has appeared postpartum or in a patient with systemic disease.[13] Chemical immuno-suppressive therapy has also been suggested.[18] Besides the therapeutic significance, several implications of the specificity of the inhibitor deserve emphasis.

Inhibitor Tests

It is very likely that inhibitory activity is commonly specific for human AHG, and if so, human AHG should be used in screening tests for inhibitory activity. The procedure of choice may be the test described by Biggs and Bidwell,[7] with human AHG used in place of bovine. If a human AHG concentrate is not available, a test similar to that described in Table I should be sufficient to exclude or establish the presence of inhibitory activity. This test is similar to those described by Margolius *et al.*[9] and Breckenridge and Ratnoff.[6]

Inhibitor Incidence

The specificity of the inhibitor may explain the variable incidence found by different groups of careful workers. Margolius, Jackson and Ratnoff[9] found inhibitory activity to human factor VIII in 18 of 84 haemophiliacs, whereas Biggs and Macfarlane[4] found inhibitory activity to animal AHG in only 6 of 300 patients. However, different diagnostic techniques may also contribute to the differences in incidence.

Nature of Inhibitor

There has for some time been controversy about the nature of the inhibitor. The arguments in favour of the antibody hypothesis have been recently marshalled by Leitner *et al.*,[18,19] and those in favour of the enzyme hypothesis by Breckenridge and Ratnoff.[6] The whole subject is well reviewed by Margolius *et al.*[9] Briefly, the circumstances under which the inhibitor occurs and its gammaglobulin nature suggest an immune phenomenon, despite failure to demonstrate the inhibitor unequivocally by precipitation, agglutination and complement-fixation techniques. On the other hand, the rate of factor VIII inactivation by the inhibitor is dependent on time, temperature, pH and substrate concentration, suggesting an enzyme reaction. The occurrence, as in this case, of species specificity resembles immune rather than enzyme behaviour, but may reflect the properties of AHG rather than of the inhibitor.

Mechanisms of Inhibition

In discussing the action of the inhibitor it may be helpful to consider the different types of inhibitory mechanism.*

*Although inhibitors have been classified in many ways such as those of Margolius *et al.*,[9] Gobbi,[11] Lee and Raccuglia,[14] and Sise *et al.*,[12] a classification based on the type of mechanism has certain advantages and is used here.

TABLE III. TENTATIVE SCHEME OF THE BLOOD COAGULATION PROCESS

Foreign surface + Hageman F. (XII)	⟶	Activated Hageman F. (XIIa)
XIIa + PTA (XI)	⟶	Act. PTA (XIa)
XIa + Christmas F. (IX)	Ca++ ⟶	Act. Christmas F. (IXa)
IXa + antihaemophilic F. (VIII)	Phospholipid, Ca++ ⟶	Act. antihaemophilic F. (VIIIa)
VIIIa + Stuart-Prower F. (X)	Ca++ ⟶	Act. Stuart-Prower F. (Xa)
Xa + factor V	Phospholipid ⟶	Act. factor V (Va)
Va + prothrombin (II)	⟶	Thrombin (IIa)
IIa (thrombin) + fibrinogen (I)	⟶	Soluble fibrin (Ia)
Ia (fibrin) + fibrin-stabilizing-factor (XIII)	Ca++ ⟶	Insoluble fibrin

F = factor; a = activated; PTA = plasma thromboplastin antecedent; phospholipid(?) = platelets.

The different mechanisms can most easily be described by reference to a scheme of the coagulation process based on those recently proposed by Macfarlane[20] and Davie and Ratnoff[21] (Table III). In this scheme the clotting process is envisaged as consisting of a series of reactions in which each clotting factor is transformed to an activated form which has enzymatic activity and each newly-formed enzyme reacts with its specific substrate (an inactive clotting factor) transforming it to the activated enzymatic form. Based on this scheme inhibitors may be divided into 3 groups.

Group 1. Inhibitors impeding a reaction between an activated product and an inactive clotting factor. This group of inhibitors acts by impeding a reaction between an activated product of coagulation and an inactive clotting factor. Examples are heparin, which inhibits the thrombin-fibrinogen reaction[22] and the reaction between activated factor XI and factor IX[23,24] (Table IV); and an inhibitor found in certain cases of systemic lupus erythematosus, which inhibits the reactions of activated factor X with factor V[25] and of activated factor IX with factor VIII.[26]

TABLE IV. THE ACTION OF INHIBITORS WHICH IMPEDE A STAGE IN THE COAGULATION PROCESS (GROUP I)

XIa + Ca + IX → IXa
↑
Heparin

Thrombin + Fibrinogen → Fibrin
↑
Heparin

Heparin impedes the reaction without destroying the inactive clotting factor or its active product.

Group 2. Inhibitors destroying the activated form of the clotting factor. This group of inhibitors occurs in normal blood and acts by destroying the activated form of the clotting factor. Examples are antithrombin III[27] which progressively inactivates thrombin but not prothrombin and anti-XIa, which inhibits activated but not precursor factor XI[28-30] (Table V).

TABLE V. THE ACTION OF INHIBITORS WHICH DESTROY THE ACTIVATED INTERMEDIATE PRODUCTS OF COAGULATION (GROUP II)

Foreign surface + XII → XIIa
XIIa + XI → XIa ← Anti-XIa
Prothrombin activator + Prothrombin → Thrombin ← Anti-thrombin III

These are physiological inhibitors and do not destroy the inactive clotting factors.

Group 3. Inhibitors destroying the precursor clotting factors. Inhibitors belonging to this group destroy the activity of the inactive form of the clotting factor, the commonest being the factor VIII inhibitor occurring in haemophilia (Table VI). Cases of circulating inhibitor with the same clinical and laboratory features are well documented in patients not having classical haemophilia: the majority of these comprise women after childbirth,[9,12,13,31] of which most occur in the first year postpartum; others may complicate systemic disorders like systemic lupus erythematosus,[4] rheumatoid arthritis,[9,12] penicillin reactions,[9,12] and infections;[15] while occasionally the inhibitor develops in an apparently healthy person.[9-12,13] In other hereditary coagulation deficiencies, inhibitors have been described against factors V, VII, IX and XI.[9]

TABLE VI. THE ACTION OF INHIBITORS WHICH DESTROY THE COAGULATION FACTOR IN ITS INACTIVE STATE (GROUP III)

AHG inhibitor
↓
IXa + VIII → VIIIa

The factor VIII inhibitor belongs to this group.

The mechanism of action of many coagulation inhibitors has not been elucidated and it is not clear into which group or groups the dysproteinaemic[9,11,13] cases belong.

SUMMARY

1. The occurrence of severe gastro-intestinal haemorrhage in a haemophiliac is described.

2. Large doses of freshly-frozen plasma failed to control the bleeding or raise the patient's factor VIII level. The patient's blood contained an inhibitor active against human AHG and only slightly against animal AHG. Administration of porcine AHG raised the patient's factor VIII concentration to normal levels and promptly stopped the bleeding.

The implications of the inhibitor's specificity are discussed, and the different types of coagulation inhibitor separated into 3 groups:

(a) *Group 1,* consisting of inhibitors which impede a reaction between the activated form of a clotting factor and its substrate (the inactive form of another clotting factor).

(b) *Group 2,* which includes the inhibitors present in normal blood, which destroy only the activated products or intermediate products of coagulation and not the inactive clotting factors, and

(c) *Group 3,* which includes those inhibitors which destroy the activity of the precursor coagulation factors.

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