Spontaneous Inhibitors of Factor VIII
(Antihaemophilic Factor)

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Approximately 5 percent of haemophiliacs ultimately develop an inhibitor to Factor VIII(1) a much dreaded complication. In our four years experience of dealing with a haemophiliac population approaching 300, we have fortunately encountered only one such case.

Haemorrhagic disorders due to deficiency of Factor VIII are occasionally found in patients who have no history of classical haemophilia. Investigation reveals the presence, in the plasma, of a substance which inactivates Factor VIII. This substance is referred to as a'spontaneous, inhibitor, in contrast to the inhibitor which may be acquired by haemophiliacs(2).

Patients with spontaneous inhibitors usually have a coexistent disorder and three groups of such cases can be discerned. The first group comprises those in whom the inhibitors are associated with pregnancy or parturition (3,4,5). The second group includes those inhibitors which occur in association with various disease states such as rheumatoid arthritis, drug reactions, rheumatic heart disease, crohn's disease, ulcerative colitis, and certain neoplasms (6,7,8,9). Finally, there is the group composed of those who have no apParent disease underlying inhibitor formation. These inhibitors are generally held to be auto–immune in origin (10, 11, 12, 13).

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Allergic reations to penicillin, in particular, have been associated with spontaneous Factor VIII inhibitors in six instances (14 to 19). A seventh case is reported below, in addition to a further case where Rheumatoid Arthritis was associated with spontaneous Factor VIII inhibitor formation. In the latter case, immunological studies were carried out demonstrating the identity of the inhibitor as an IgG immunoglobulin.

Materials and Methods

Bovine Fator V, diluted serum and citrate saline were prepared as described by Biggs and Macfarlane (20).

Phospholipid was Cephaloplastin by Dade & Co.

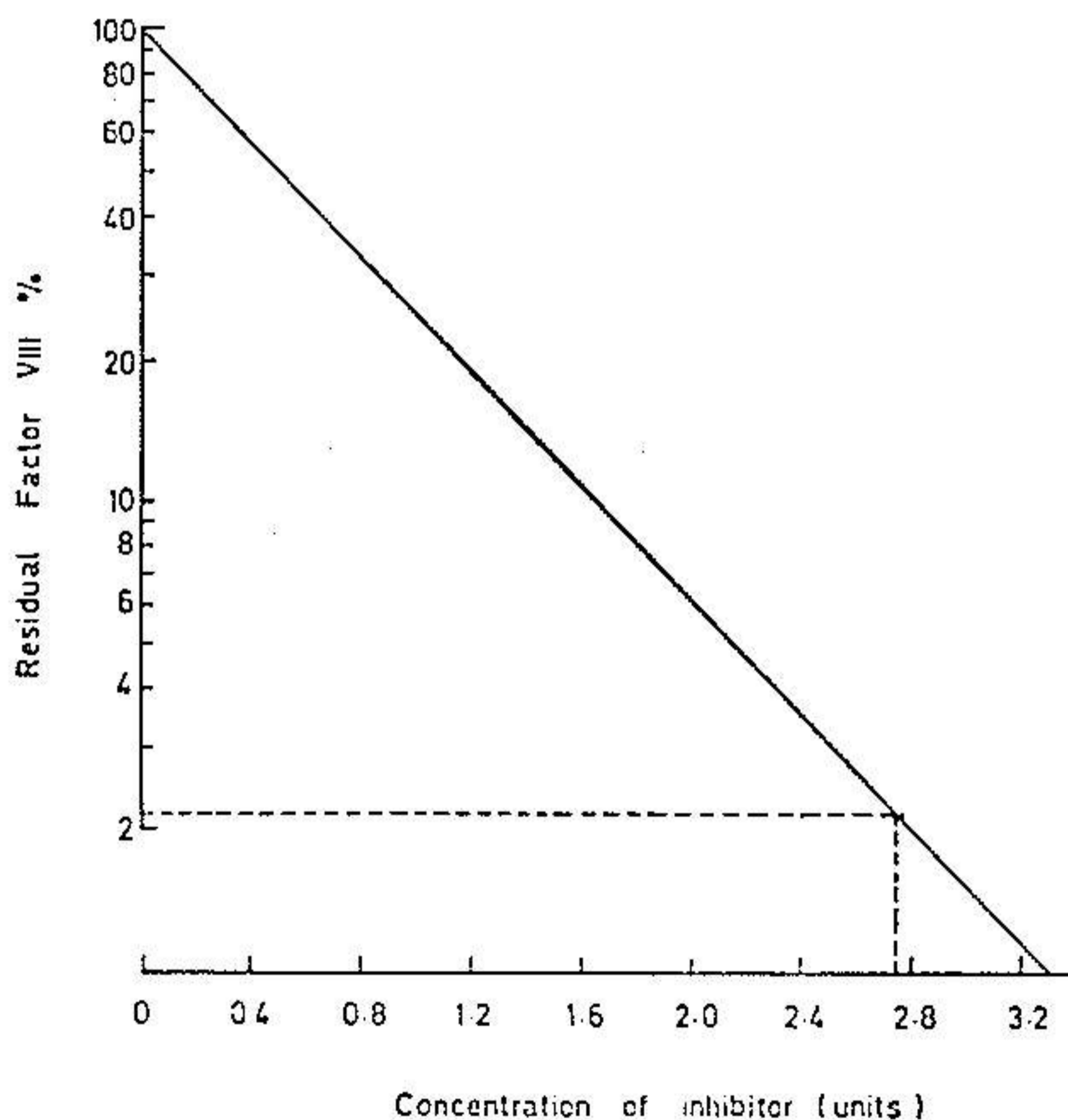Porcine AHG was supplied by Diagnostic Reagents Ltd., Oxon, England.

Figure 1. The relationship between inhibitor concentration and residual Factor VIII. One unit represents the destruction of 75 ·/. of the AHG added in the space of one hour.

Factor VIII was assayed by the two-stage method of Biggs & Macfarlane (20).

The two-stage assay of Factor VIII inhibitors was performed according to the method of Biggs and Bidwell (21). Inhibitor plasma is incubated with a Factor VIII concentrate, with an activity equivalent to three times that of normal fresh plasma. The residual Factor VIII activity of the mixture is then measured. One unit of inhibitor is defined as the amount which will inactivate 75 percent of the added Factor VIII concentrate in one hour at 37°C. The inhibitor potency, so defined, is calculated by reference to a curve relating residual Factor VIII activity to inhibitor units (Figure 1). In each case, two or more different dilutions of inhibitor plasma were selected, and the final result is expressed in terms of undiluted plasma.

Case reports

CASE 1: (A.K.), aged 71 Years, is a small–holder, owning a farm in the vicinity of Ghazvin. He was entirely well until 1967, when he developed a right-sided lobar pneumonia. He was treated with penicillin by injection, and following the third injection, exacerbation of fever accompanied by exfoliative dermatitis developed. The drug reaction gradually responded to antihistamines and topical triamcinolone. Eight days later, profuse haematuria suddenly developed and the patient noted large haematomata on the buttocks, at the sites of injection, together with wide-spread, expanding ecchymoses over the back and legs. Bleeding became more generalized still, with melaenae and profuse epistaxes. It was at this point that he was first seen and admitted to our care. The laboratory findings are summarized in Table 1.

Table 1: Blood Coagulation Studies in Case 1.

Bleeding time (IVY):   3 min.

Whole blood clotting time: Over 60 min.

Platelet count (per cu. mm.):   700,000

ADP aggregation of platelets: Normal.

Thrombin time (control = 18 sec.):   20 sec.

Prothrombin time (Quick; control = 13 sec.):   13 sec.

Patrial thromboplastin time differential: 63 sec.

Factor VIII assay: 0 percent.

Factor VIII inhibitor assay (at dilutions of 1:256 & 1:512): 530 Units-corrected for inhibitor dilution.

In that the haemoglobin level was 6 G. Per 100 ml. On admission, the patient was transfused with three units of fresh blood, but bleeding continued unabated. After it was realized that we were dealing with a spontaneous Factor VIII inhibitor, transfusion was withheld and large doses of prednisone (80 mg. per day) were administered. Signs of a bleeding diathesis gradually regressed and the patient made a complete clinical recovery, although we have been unable to obtain further samples of blood for follow -up studies.

CASE 2: (R.M.), aged 61 Years, a former clerk, employed in the oil industry, who had no relevant past or family history of illness, although he had been treated for mild hypertension, at one time. When first seen, he gave a clear-cut one year history of progressively more incapacitating rheumatoid arthritis with morning stiffness and pain mainly confined to the small joints of both hands and feet. On examination of the hands there was typical "spindling" of the fingers and some wasting of the small muscles of the hands. He had received no specific treatment for his arthritis. Presentation was with the dramatic onset of a severe bleeding diathesis. Very extensive ecchymoses, heamaturia, melaena, and several large haematomata in the muscles of the thighs and back, which were unrelated to trauma. The laboratory findings are summarized in Table 2.

Table 2: Blood Coagulation Studies in Case 2.

Bleeding time (IVY):	2 min.
Whole blood clotting time:	Over 60 min.
Platelet count (per cu. mm.):	850,000
Euglobulin lysis time (normal = over 45 min.):	Over 60 min.
Fibrinogen titre (normal = over 1:128):	Over 1:256
Thrombin time (control = 11 sec.):	10.5 sec.
Prothrombin time (control = 13 sec.):	12.5 sec.
Partial thromboplastin time differential:	78 sec.
Factor VIII assay:	0%

Factor VIII inhibitor assay (at dilutions of 1:100; 1:256 & 1:512)

= 1423 units with porcine AHG–Corrected for dilution.

1372 units with human AHG concentrate– »

1200 units with bovine AHG – »

The Latex test for rheumatoid arthritis factor was strongly positive.

In view of the findings, transfusion of whole blood and plasma were deliberately withheld for fear that the anti–VIII titre would rise even further. It was hoped that treatment of the underlying condition with large doses of prednisone (100 mg. per day) alone would bring about a diminution of the inhibitor titre. Carefully washed red cells were administered to make good the progressive blood loss anaemia. Although the patient's plasma anti–VIII activity did not alter, there was considerable symptomatic improvement and bleeding ceased. He was maintained on prednisone 20 mg per day and treatment with azathioprine 50 mg. b.d. was commenced.

At follow–up 6 months later, the patient was apparently well and no bleeding had occurred in the intervening time. Symptoms of arthritis and also completely regressed and prednisone was reduced to 5 mg. b.d. The plasma Factor VIII level was still 0 percent, although the inhibitor titre had diminished to 80 Units per ml. of undiluted plasma.

We subsequently lost touch with the patient, although it was reported that he enjoyed good health and did not suffer any recrudescence of bleeding until recently, when, almost three years after he first presented, the patient suddenly became unconscious; he developed a right–sided Jacksonian seizure which soon became generalized, and he died without regaining consciousness. It was thought that a cerebral haemorrhage caused his demise, but permission for necropsy was not obtained.

Characterization of the Inhibitor in Case 2.

The treatment of these cases is clearly most unsatisfactory and Denson (22), has shown that spontaneous inhibitors are more active against heterologous antihaemophilic globulin of animal origin, than haemophilic inhibitors, which are most frequently monospecific (against homologous human AHG), allowing the physician to treat emergencies with some other
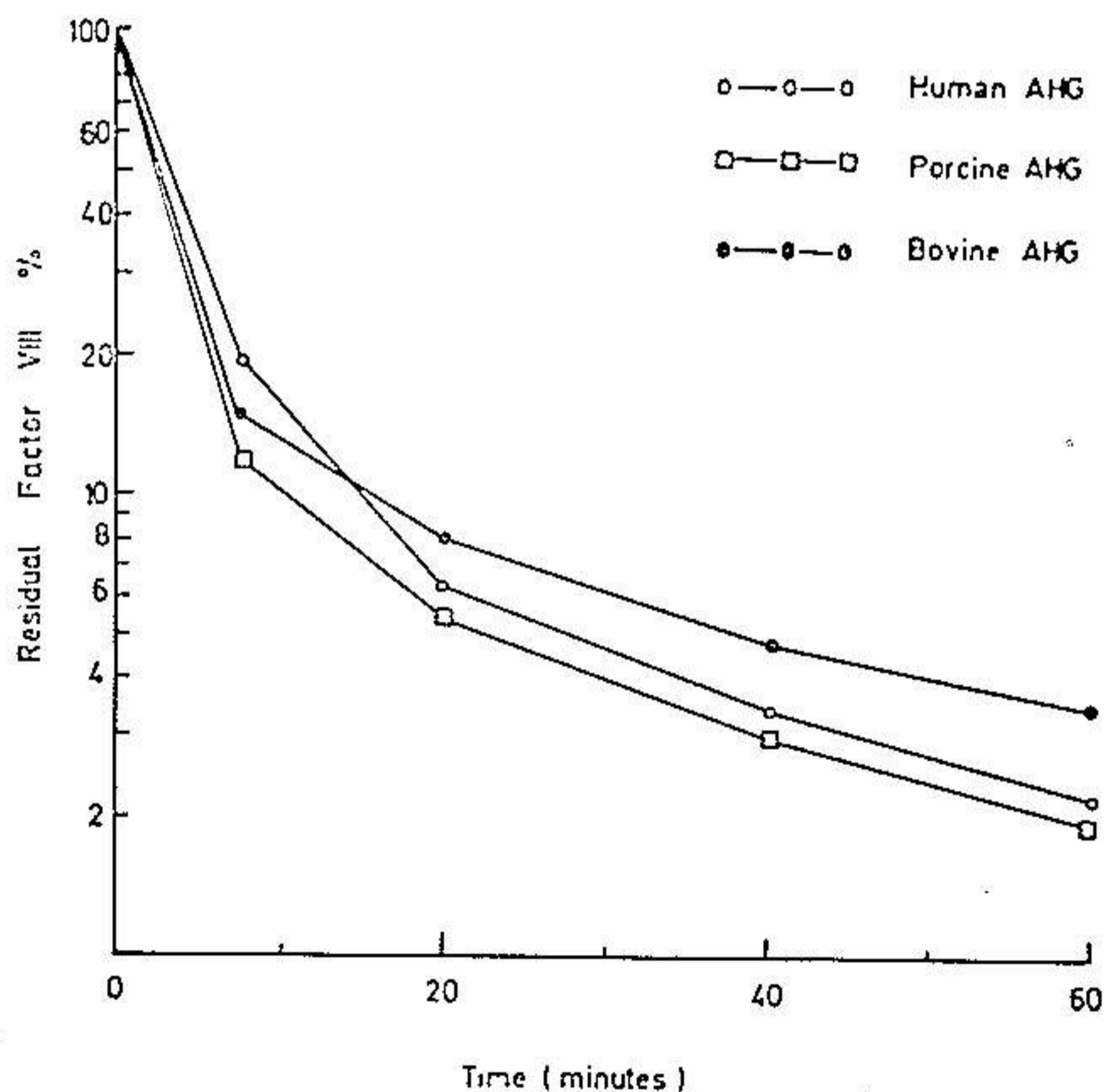

Figure 2. The progressive destruction of Human, porcine and Bovine AGH concentrates by a spontaneous inhibitor (Case 2), diluted 1:256.

form of AHG–usually of animal origin. Figure 2 shows the neutralization of porcine, bovine and human Factor VIII by the inhibitor of Case 2. which remained extremely active, even at high dilutions. Spontaneous inhibitors are capable of inactivating amounts of Factor VIII out of proportion to their degree of dilution (23).

Kinetics of Inactivation

Biggs and Bidwell (24) have shown that the amount of Factor VIII inactivated by a haemophilic inhibitor during a given time interval (one hour has been chosen as a standard time lapse), is proportional to the concentration of the inhibitor, when the inhibitor is present in excess. The relationships obtained in these cases are almost exactly linear.

When spontaneous inhibitors are incubated with Factor VIII, an entirely different pattern of inactivation is noted. Disappearance of Factor VIII follows a biphasic course which is very rapid in the initial phase of incubation and is succeeded by a gradual reduction in residual Factor VIII activity. In addition, the direct relationship between inhibitor concentration and the amount of residual Factor VIII which is characteristic of haemophilic inhibitors no longer obtains with spontaneous inhibitors. In consequence, even highly dilute solutions of inhibitor plasmas are capable of inactivating significant quantities of Factor VIII. Our findings, in support of these observations, are illustrated in Fig. 3.

It has been suggested by Breckenridge and Ratnoff that the Factor VIII inhibitor is an example of enzymic induction (25). They have held that antigen–antibody reactions occur almost instantaneously and are not temperature dependent. More recently, Denson (26) has clearly demonstrated these two characteristics in the case of antibodies (specific antisera deliberately produced in laboratory animals) to Factor VIII, Factor VII, Factor V and Factor X). The inhibitor in our patient has also been shown
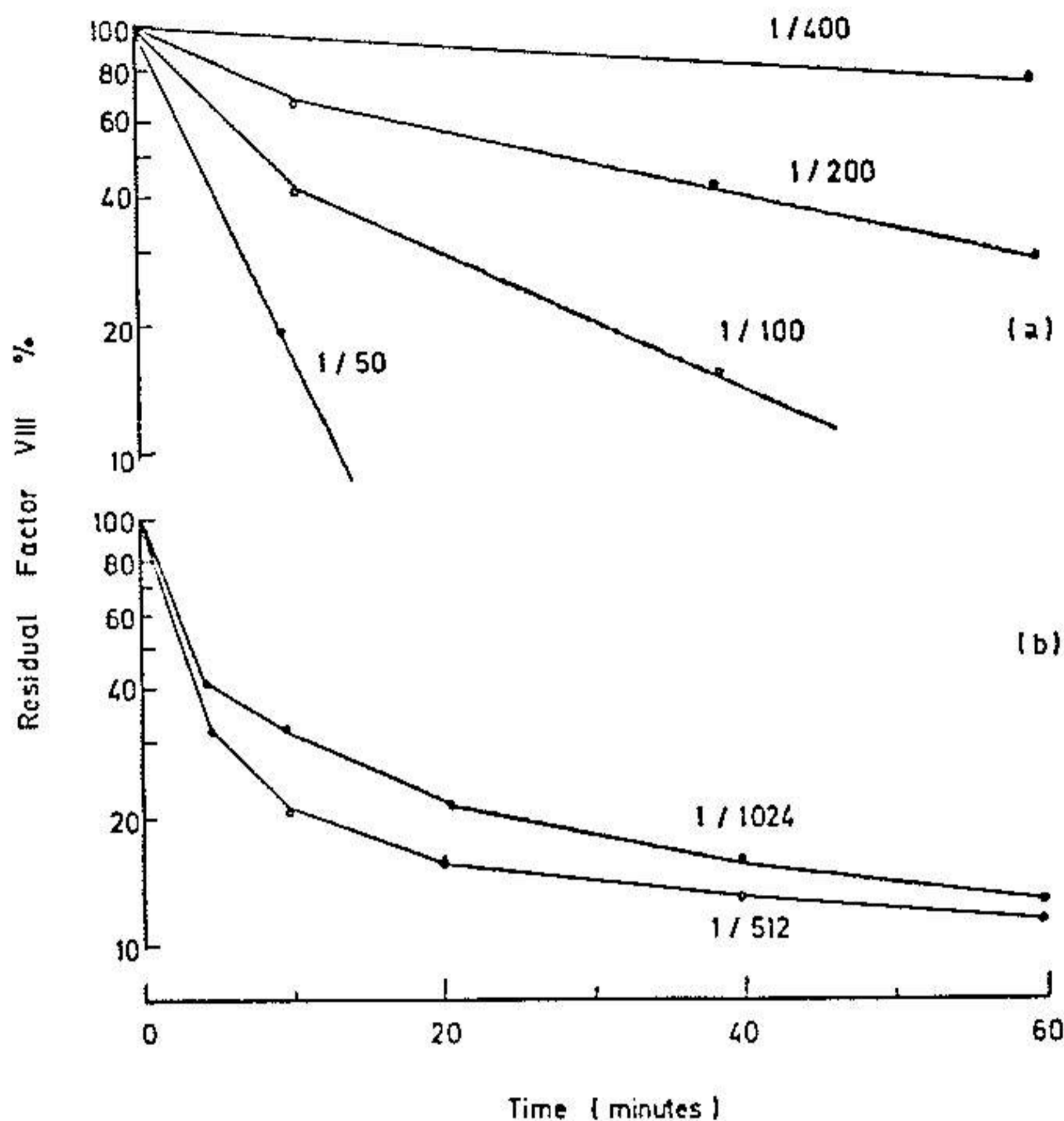
Figure 3. Inactivation of Factor VIII by Haemophilic and spontaneous inhibitors.

a) The amount of Factor VIII inactivated by an inhibitor of Haemophilic origin (After Green, D. - 19), is proportional to the concentration of inhibitor.

b) When a spontaneous inhibitor (our Case 2), is incubated with Factor VIII, there is a rapid initial phase of inactivation, followed by a more gradual destruction. Even highly dilute solutions of inhibitor plasma are able to inactivate large quantities of Factor VIII.

to have a progressive action and to be temperature dependent (Fig. 4), which further supports the view that it is an antibody.

Immunological Identification of the Inhibitor

The inhibitor of Case 2 was purified and concentrated twenty-five fold according to Leitner et al. (27). After initial absorption of coagulant proteins from the patient's plasma with barium sulphate, purification was carried out by elution from a DEAE-cellulose column (3 cm. by 35 cm.).
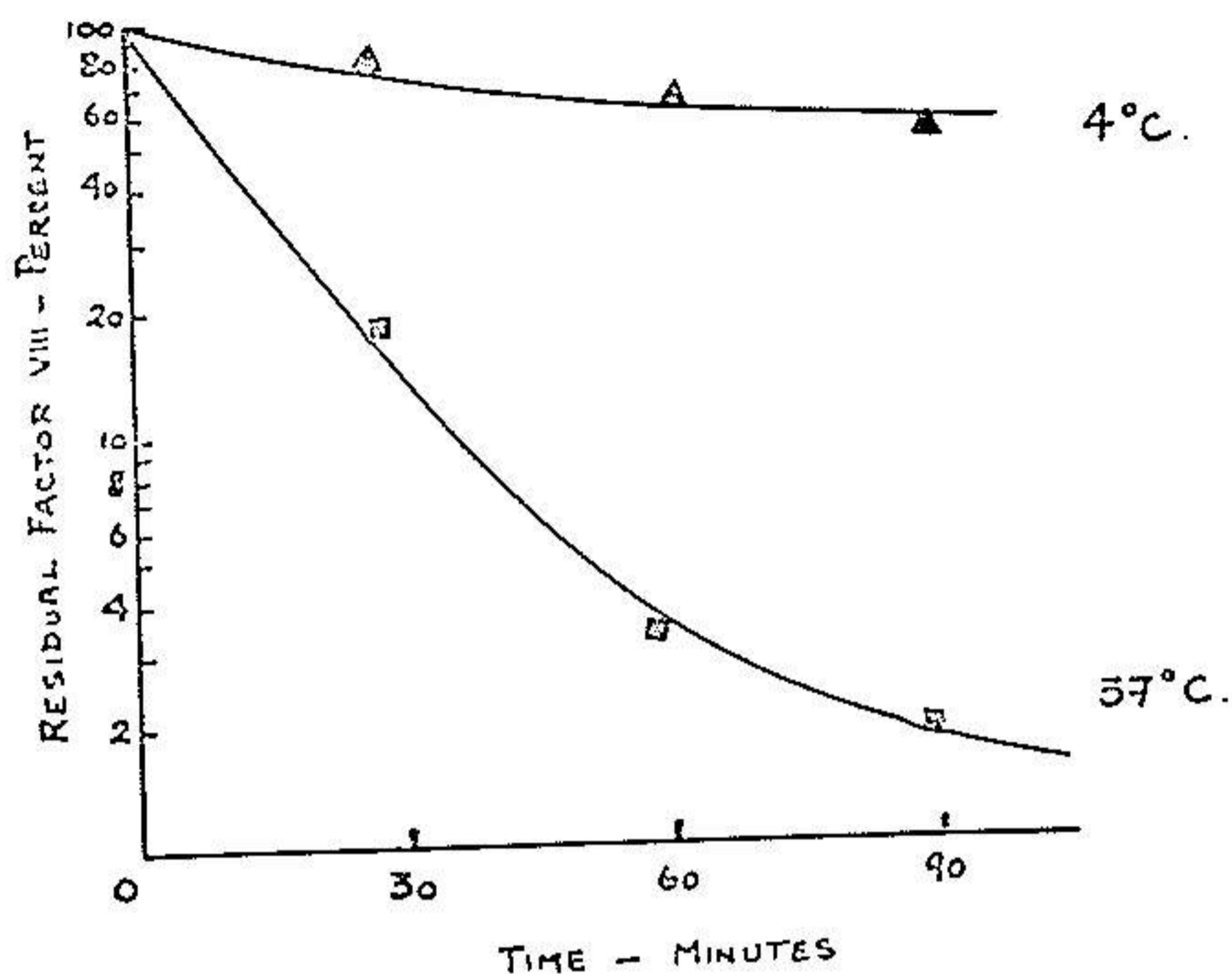
Figure 4. Time course showing the reduced Factor VIII inhibitor activity in the cold (4° C.).

Five ml. samples were collected at a rate of 20 ml. per hour. Maximum inhibitor activity, corresponoing with a protein peak (E 280 millimicrons == 4.0), which was virtually free of albumin and IgM immunoglobulin, consisted essentially of IgG and IgA immunoglobulins (by immunoelectrophoresis). After final concentration of the inhibitor by ultrafiltration, the fraction was incubated with rabbit IgG antiserum (Hyland Loboratories). This resulted in complete neutralization of inhibitor activity, confirming beyond doubt, the inhibitor's identity as an IgG immunoglobulin.

Recently, the immunological characterization of these Factor VIII inhibitors has been carried further, and in a review of the literature on the subject by Feinstein et al. (28), it is confirmed that the overwhelming majority of these spontaneous inhibitors are immunoglobulins that are homogeneous in regard to immunoglobulin class (IgG) and light-chain type, by the above-mentioned method of immune neutralization. Thus the majority were

of the kappa 2 gamma 2-type, and only two inhibitors, out of the 26 studied, had lambda lightchain tyes of determinants.

Discussion

Two Factor VIII inhibitors of spontaneous origin have been shown to be IgG immunoglobulins. This finding is in keeping with the observations of others (29, 30). The appearance of the first inhibitor was precipitated by penicillin allergy, and the second was associated with rheumatoid arthritis. Green (31), reported a case of penicillin induced Factor VIII inhibitor formation, experimentally demonstrating that the inhibitor was not specifically related to penicillin, but was more likely to be the product of the allergic response itself. The observed predeliction for inhibitors to Factor VIII to appear in association with allergic or hypersensitive states-rheumtoid arthritis asthma allergic reactions to sulphanilamide, arsenic and horse serum (32, 33), suggests that it is this multifactorially induced allergic state itself which is the final common pathway to inhibitor formation.

Treatment is beset with difficulties; drug treatment with adrenal steroids has occasionally been successful (34, 35), but success is often only partial, as in our case, and failures are frequent (36, 37). Immunosuppressive drugs may be dangerous in those who are already bleeding, and D-penicillamine or penicillin, which are known to reduce plasma antibody globulin when used in high concentration, are more likely to be effective in those cases where the inhibitor is a macroglobulin or a myeloma protein (38;39, 40) .

Plasma fraction therapy, in high doses, is effective in those with low-titre antibodies, the very active inhibitors being refractory to all forms of specific treatment.

Where the disorder underlying the altered immune state can be elimi-

nated, this is the most rational approach to treatment. For instance, withdrawal of penicillin resulted in the disappearance of inhibitor activity in almost all the cases on record, including our own Case 1.

Acknowledgement

Résumé

Environ 5% des hemophiles produisent éventuellement un anticoagulant circulant inhibiteur du facteur VIII.

Il est cependant connu qu'un inhibiteur spontané, inactivateur du facteur VIII, survient rarement chez les sujets non hémophiles, au cours d'une maladie auto-immune au lieu d'un état allergique.

Nous présentons deux cas de diathèses hémorrhagiques aigües dû à l'apparition d'anticoagulants circulant contre le Facteur VIII, associés chez l'un des patients à une réaction allergique à la penicilline et chez le second à un arthritisme rheumatoïd.

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