Researches on a Circulating Anticoagulant in a Hemophiliac

II. Effect of Administration of ACTH and Cortisone

By S. van Creveld, M.D., P. G. Hoorweg, M.D.
and M. M. P. Paulssen, Ph.D.

In a previous communication we described a hemophiliac whose blood showed the presence of a circulating anticoagulant. This had probably developed as a result of the many blood transfusions he had received over a period of years. At the same time antibodies against the Duffy antigen could be demonstrated in the patient's serum. The presence of these antibodies appeared to explain the occurrence of so many reactions in the form of chills after transfusions. When new donors were selected of O, Rh positive and Duffy negative types the patients experienced no more post-transfusion chills. We concluded that there was no connection between the presence of these antibodies and the circulating anticoagulant.

The patient was severely handicapped by the presence of this anticoagulant because the clotting time of his blood could not be sufficiently decreased by a blood transfusion. We thus investigated the possibility of modifying the activity of the anticoagulant by ACTH or cortisone and at the same time studied the influence of such treatment on the Duffy antibodies. The following considerations guided us: in the first place there was an antibody (the anticoagulant) which acted upon the antigen (the so-called antihemophilic factor). In the second place, the anticoagulant was very probably in the gamma globulin fraction, i.e., that protein fraction which is generally considered to be the carrier of antibodies.

As it is known that ACTH and cortisone have an influence upon processes connected with the antigen-antibody interrelationships, a trial treatment with these substances was the obvious course to take. Before commencing this treatment we investigated the inhibiting effect of the patient's plasma on the clotting of normal blood repeatedly, over a period of one year. For this purpose the patient's blood was obtained by venipuncture and collected in a siliconized centrifuge tube. It was centrifuged for a period of 20 minutes at 4500 r.p.m.; 0.5 and 0.2 ml. of the supernatant plasma were directly added to two tubes each containing 1 ml. of freshly drawn normal blood. The clotting time of this mixture was also estimated when saline instead of plasma was added in the same quantities.

It was noticed during these investigations, that the prolongation of clotting time in the later months was less marked than previously. In our early studies...
the addition of 0.5 ml. of the patient's plasma prolonged the clotting time from 12 to 75, while in later investigations we obtained a prolongation from 12 to 28 to 35 minutes. This represented a less important retardation of clotting, which might be related to the fact that in this period the patient obtained fewer blood transfusions than before.

1. Influence of ACTH on the circulating anticoagulant

On November 9, 1951 the influence of the patient's platelet-deficient plasma upon the clotting time of normal blood was investigated. The result is given in figure 1. For the first three days the patient received one intramuscular injection of 5 I.U. ACTH daily; during the following three days two injections of 5 I.U. ACTH were given daily. On November 12 the anticoagulant action of the patient's plasma on normal blood was estimated after the second injection of ACTH, and again on November 15, 3 hours after a further injection of 5 I.U. ACTH. These relatively small quantities of ACTH did not decrease the activity of the anticoagulant materially. From November 20, for a period of three days, the patient received ACTH in a dosage of 20 I.U. twice daily. On November 23, at 9:30 a.m. a further dose of 20 I.U. was given, and at 4 p.m. the plasma inhibitory effect was once again estimated. A marked decrease in anticoagulant
activity of the patient’s plasma was found, and continued for two days later in the same degree. Beginning on November 27, the patient received another three day course of ACTH (20 I.U. twice daily) and on November 30, at 9:15 a.m., the clotting inhibition was practically absent. ACTH was then withheld, and the retarding activity of plasma on the clotting of normal blood was repeatedly studied. The anticoagulant again slowly gained activity (fig. 1). The interrupted line in this figure shows the limits between which the clotting time varied at 37 C. when, instead of 0.5 ml. of the patient’s plasma 0.5 ml. of NaCl 0.9 per cent solution was added to 1 ml. normal blood.

2. Influence of Cortisone on the Circulating Anticoagulant

On January 2, 1952, immediately before cortisone administration, the anticoagulant action of the platelet-poor plasma of the patient was:

<table>
<thead>
<tr>
<th>Clotting time at 37 C.</th>
<th>Clotting time at 37 C.</th>
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</thead>
<tbody>
<tr>
<td>0.5 ml. patient’s plasma 1.0 ml. normal blood</td>
<td>12 min</td>
</tr>
<tr>
<td>0.2 ml. patient’s plasma 1.0 ml. normal blood</td>
<td>11 min</td>
</tr>
</tbody>
</table>

From January 21 to January 28, 25 mg. cortisone were given thrice daily, half an hour before meals. For the following week 25 mg. cortisone were given four times daily and for a few more days in the dosage shown in fig. 2. The anticoagulant action of the platelet poor plasma decreased steadily and reached the lowest value on February 4. On that day the patient’s plasma did not prolong the clotting time of normal blood.

The last dose of cortisone was given on February 23. Estimations of the anticoagulant activity on February 28 and March 13 again showed a distinct increase (see fig. 2). On March 6 the following results were obtained:

<table>
<thead>
<tr>
<th>Clotting time at 37 C.</th>
<th>Clotting time at 37 C.</th>
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<tbody>
<tr>
<td>0.5 ml. patient’s plasma 1.0 ml. normal blood</td>
<td>clotting time at 37 C., 19 min</td>
</tr>
<tr>
<td>0.2 ml. patient’s plasma 1.0 ml. normal blood</td>
<td>clotting time at 37 C., 15 min</td>
</tr>
<tr>
<td>0.5 ml. saline 0.9% 1.0 ml. normal blood</td>
<td>clotting time at 37 C., 12 min</td>
</tr>
<tr>
<td>0.2 ml. saline 0.9% 1.0 ml. normal blood</td>
<td>clotting time at 37 C., 12 min</td>
</tr>
</tbody>
</table>

It is our opinion that the administration of ACTH and cortisone in the dosage mentioned markedly—although temporarily—reduced the activity of the circulating anticoagulant.

3. The Influence of Transfusions of Blood and Plasma

At a time when the anticoagulant activity of the patient’s plasma was no longer evident or reduced in vitro after the administration of ACTH or cortisone, it appeared probable that blood or plasma transfusions would decrease the clot-
ting time more than previously. This was supported by the finding that small quantities of normal plasma had nearly the same influence on the clotting time of the patient as on the blood of a hemophiliac without a circulating anticoagulant. We have found, along with others, that in patients with a circulating anticoagulant, blood and plasma transfusions gave no reduction or only a slight reduction of the clotting time. Only Frommeyer et al. found that transfusions of large quantities of blood temporarily reduced the clotting time to normal.

These investigators explained that the surplus of the administrated antihemophilic factor could not be totally inactivated by the anticoagulant. We were unable to confirm this finding in our patient before the administration of ACTH and cortisone. On June 19, 0.5 liter of the patient's blood was replaced by 1 liter of citrated blood. The clotting time at 37 C. was 7 hours before transfusion. The transfusion took 2 hours. One hour after the end of the transfusion the clotting time was 2 1/2 hours; 2 hours later it was 4 hours and 24 hours later, 4 hours. At the time when the anticoagulant action on normal blood had disappeared due to the action of cortisone, we gave the patient a transfusion of 80 ml. of heparinized plasma. Three and eleven days later he received a further 160 ml. heparinized plasma at each transfusion and a further 500 ml. fresh citrated plasma on the seventeenth day.

We think we are justified in concluding from these results that cortisone, in
the doses used, had a favorable influence on the effect of a transfusion. This influence, however, was not such that the clotting time became normal.

We consider the effect of the transfusion during the administration of cortisone to be due to the cortisone action for the following reasons. Three weeks after the last dose of cortisone was given, when the anticoagulant activity had again risen markedly (see fig. 2), the patient was given another transfusion of 160 ml. heparinized plasma. The clotting time was 240 minutes before transfusion, 90

Table 1.—Results of Transfusions before and after ACTH and Cortisone Therapy

<table>
<thead>
<tr>
<th>Clotting time in minutes</th>
<th>Before administration of ACTH and cortisone</th>
<th>During administration of cortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Previous to transfusion</td>
<td>After 1 hour</td>
</tr>
<tr>
<td>Transfusion of 80 ml. heparinized plasma</td>
<td>360</td>
<td>180</td>
</tr>
<tr>
<td>Transfusion of 160 ml. heparinized plasma</td>
<td>360</td>
<td>165</td>
</tr>
<tr>
<td>Transfusion of 500 ml. citrated plasma</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 liter citrated blood</td>
<td>420</td>
<td>150</td>
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Fig. 3.—Titers of the Duffy antibodies during cortisone administration

minutes, 1 hour after transfusion and longer than 120 minutes 2 hours after transfusion. Thus at both times we found a much longer clotting time than after the transfusion given during cortisone therapy.

Finally, the clotting time during the periods when ACTH and cortisone were not given was markedly longer than during treatment. The reduction of the anticoagulant appeared also to produce a reduction of the prolonged clotting time.
4. Influence of ACTH and Cortisone on the Duffy Antibodies*

The influence of the administration of ACTH was not significant as far as the Duffy antibodies are concerned. During ACTH therapy the titer decreased 1:32 to 1:8. In the following month the titer was not estimated.

When cortisone was given (see fig. 3) the titer was 1:16. At one point the antibodies could not be demonstrated after the dose of cortisone had been raised to 100 mg. per day. Two days later, however, the antibodies were present with a titer of 1:4, while the dose of cortisone remained the same. On March 3, 1952, 9 days after the termination of cortisone treatment, the titer was 1:2. On March 8 it was 1:8.

In 1950 the titer of the antibodies decreased spontaneously to 1:2. The above mentioned decrease therefore cannot unconditionally be attributed to cortisone. A relation between the Duffy antibodies and the circulating anticoagulant could not be conclusively demonstrated. It appears probable that there were two different antibodies present in this patient. Antibodies against the Duffy factor originate in the same way in hemophiliacs as in persons who do not suffer from this disease.

SUMMARY

The influence of the administration of ACTH and cortisone on a circulating anticoagulant appearing after repeated blood transfusions in a hemophiliac was studied. It was found that the activity of the anticoagulant was diminished to a considerable degree, even though temporarily, and the clotting time was reduced by plasma transfusions more than prior to the administration of ACTH and cortisone. Antibodies to the Duffy antigen were also present together with the circulating anticoagulant, but their activity was not clearly affected by the administration of ACTH and cortisone.

REFERENCES


* We gratefully acknowledge the assistance of Miss M. van der Hart from the Central Laboratory of the Blood Transfusion Service of the Dutch Red Cross at Amsterdam (Head Dr. J. J. van Loghem, Jr.) in this part of the investigation.
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