Blood Changes in Hemolytic Anemia under ACTH Therapy

By Kai Bent Hansen, M.D. and Jørgen Andersen, M.D.

During the last several years, reports have appeared pointing to the often dramatic effect of ACTH and cortisone upon the rate of blood destruction in the acquired hemolytic anemias. Together with these, however, other papers appeared telling of little or no effect in cases clinically undistinguishable from the responsive group.

Recently, one of us has published some cases of immuno-hemolytic anemia, reporting the finding of substantially elevated gamma globulin values as demonstrated by electrophoresis. The response in one case treated with ACTH was prompt and correlated well with the depression of gamma globulin values, an observation often reported in the treatment of collagen disease with ACTH. The alpha II globulin fraction was also somewhat elevated so that the whole picture showed some similarity to that of the collagen diseases. Davidson et al. have treated a case in which there was inversion of the plasma proteins with ACTH and achieved a similarly prompt effect. In Hansen's above mentioned case, re-examination five months after cessation of ACTH therapy revealed normal blood values, normalized gamma globulins and no reticulocytosis. However, the Coombs test was persistently positive and the erythrocyte sedimentation rate was considerably elevated. In another patient with a history of severe hemolytic anemia, which had apparently been stopped by splenectomy, there was a very substantial elevation of gamma globulins together with a strongly positive Coombs test.

In view of these findings it seemed of interest to investigate the action of ACTH in a case of acquired hemolytic anemia, in which no initial elevation of gamma globulins could be demonstrated.

Methods

The electrophoretic analyses were performed by means of the Antweiler micromethod. This method uses the interferometric principle with white light for the measurement of concentrations, thus yielding a high degree of accuracy. Only 0.1 cc. serum was used for each analysis, and they were repeated twice to three times in each case. The accuracy of measurement (Δ c) is about 0.0015 per cent protein. The buffer used is barbiturate buffer, pH 8.6. A difficulty in this method is the division of the albumin and alpha I globulin fractions. Since this cannot accurately be done, the relative percentages of the two fractions are given in one number covering both fractions. Normal values for a man are considered the following: albumin and alpha I globulin, 66.6 per cent (58.6 to 72.4 per cent); alpha II globulin, 7.4 per cent (4.8 to 10.2 per cent); beta globulin, 10.1 per cent (6.9 to 15.9 per cent); gamma globulin, 15.9 per cent (11.2 to 21.0 per cent).

The Coombs test was performed at the State Serum Institute, blood typing department. Rabbit serum, absorbed and cross titrated in the usual way, was used in all tests, except with albumin. This was due to the fact that up to the present time it has not been possible to obtain a completely pure preparation. All samples were examined in dilutions 1:15 and 1:64 with readings after 3 and 5 minutes. In the case of a positive reaction, titra-
tions were performed with dilutions of 1:2 to 1:250,000. At these titrations readings were also made after 3 and 5 minutes for estimation of avidity. The same rabbit serum was used for all samples. On reading the results of titrations, the strength of agglutination in the different tubes was expressed in the following way: 0 - (+) - + - (+++) - ++ - ++(+). The final result, expressed in units from 1 to 7, rested partly from the strength of agglutination (and the number of tubes) and partly from the avidity as estimated from the readings after 3 and 5 minutes.

CASE REPORT

J. H., a 76 year old male, had two previous admissions due to cardiac insufficiency. There was no family history of any blood ailment. At the previous admissions no signs of increased blood destruction had been elicited: his hemoglobin level being 12 Gm. and erythrocyte sedimentation rate 8 mm. per hour. Icterus index (Meulengracht) was 5. There was no previous history of blood transfusions.

The patient was admitted on October 17, 1951, because of angina pectoris. ECG disclosed coronary insufficiency. Examination of the blood gave no signs of increased hemolysis on admission. Reticulocytes were 0.5 per cent. He was given nitrites, digitalis and diuretics. Without further treatment, five days after admission, the patient suddenly complained of pain over the kidney regions, especially on the right side. His temperature rose from normal to 101.2. No pulmonary or abdominal disturbance was detected. After five days the temperature returned to normal.

At this point the patient became icteric; the reticulocytes rose rapidly to 15 per cent and a bone marrow examination revealed a hyperplastic marrow with markedly increased erythropoiesis, which was entirely normoblastic. There were immature cells of the erythroid series in the peripheral blood. Spherocytes were observed in fixed smears. Since overstained cells may resemble spherocytes, our impression was based only on fields in which there were many cells showing the normal central area of relative translucency. The WBC count and the thrombocyte count were normal, as was the differential count. The erythrocyte sedimentation rate was greatly elevated (123 mm. per hour), as was the icterus index (15). There was an excess of urobilin in the urine, but no bile pigment. The Coombs test was positive.

Electrophoresis disclosed quantitative serum protein values well within normal except for a decidedly depressed alpha II globulin fraction. The albumin and alpha I fraction were high normal. Fibrinogen values were greatly increased. During the ensuing period the red cell count and the hemoglobin value decreased steadily (to 1.5 million per cu. mm. and 47 per cent) while reticulocytes remained at a high level. The spleen, however, was not readily palpable, though doubtlessly enlarged as seen by x-ray examination. Two blood transfusions were given without abnormal reactions. At this point the patient demanded release for urgent private reasons.

Upon readmission three weeks later, the patient was still moderately icteric. Blood examination showed hemoglobin values around 6.8 Gm., red cells 1.4 million per cu. mm., sedimentation rate 126 mm. per hour, reticulocytes 10 to 13 per cent, icterus index 15 and osmotic fragility considerably increased (hemolysis beginning at above 0.80 per cent sodium chloride, complete at 0.42 per cent sodium chloride). The Coombs test was 3 units (see fig. 1). The bone marrow
picture was essentially unchanged and the white blood cell and thrombocyte counts remained normal. Color index 1.26 and volumetric index 1.30.

Electrophoresis now showed all values well within normal limits (see table 1). The percentage of gamma globulin had risen slightly, but was still within normal limits.

ACTH was given over a ten day period. The dose was 20 mg. every 6 hours. Simultaneously potassium chloride and diuretics were given and the patient was closely followed. No unfavorable effects were elicited. The results are depicted in figure 1.

Analysis of figure 1 shows the usual decrease in fibrinogen values together with an abrupt decrease in erythrocyte sedimentation rate during ACTH treatment. There is general agreement that the plasma fibrinogen level is the main factor influencing the rate of erythrocyte sedimentation, though some authors have reported cases in which the gamma globulins seemed of major importance.

### Table 1—The Variations in Serum Proteins before, during and after ACTH Treatment. When Treatment Was Started, the Patient Had Been Ill for More Than Two Months

<table>
<thead>
<tr>
<th>Date</th>
<th>Albumine and Alpha 1 %</th>
<th>Globulins</th>
<th>Gamma %</th>
<th>Total Protein %</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Alpha II %</td>
<td>Beta %</td>
<td>Total Protein %</td>
</tr>
<tr>
<td>1/10</td>
<td>72.5</td>
<td>1.8</td>
<td>10.3</td>
<td>15.4</td>
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<td>6.3</td>
<td>8.3</td>
<td>23.3</td>
</tr>
<tr>
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<td>68.8</td>
<td>5.8</td>
<td>7.7</td>
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<td>3.8</td>
<td>8.4</td>
<td>16.5</td>
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<tr>
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<td>5.8</td>
<td>8.1</td>
<td>13.3</td>
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<tr>
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<td>66.5</td>
<td>11.1</td>
<td>8.4</td>
<td>14.0</td>
</tr>
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<td>62.6</td>
<td>8.7</td>
<td>11.1</td>
<td>17.0</td>
</tr>
<tr>
<td>3/5</td>
<td>60.7</td>
<td>4.7</td>
<td>9.2</td>
<td>25.4</td>
</tr>
</tbody>
</table>

A rise was seen in red cell count of about 0.6 million per cu. mm. Coincidentally the reticulocytes reached a peak of 173 per 1000 cells at the middle of the period of ACTH treatment following an initial fall and ending in a return to pre-treatment values in spite of continued administration of ACTH. The rise in number of circulating red cells did not appear very significant and does not rival the substantial increase seen in cases responding well to treatment. The potency of the ACTH preparation was evidenced by prompt disappearance of circulating eosinophils. Objections may be raised that the period of ACTH therapy was too short, but the condition of the patient did not permit any protracted administration of ACTH.

At the time of the reticulocyte peak it was also noted that the gamma globulin values reached a peak, after which return to pre-treatment values took place within the period of ACTH therapy. This finding of an increase of gamma globulins, probably initiated after beginning of ACTH therapy, is interesting if it can be confirmed in more extensive materials. Since the patient's hemolytic syndrome began two months previous to therapy, there should be no reason to assume any significant increase in gamma globulins occurring spontaneously between the two initial electrophoretic tracings and those run after beginning
of therapy. The gamma globulin increase does not comply with the results obtained in the treatment of collagen disease but it must be noted that these conditions are accompanied by an initial gamma globulin elevation. The finding of the gamma globulin peak is of special interest when seen in connection with the

![Graph](image)

Fig. 1—The relationship between blood findings before, during and after ACTH treatment. For further details see text. The chart only covers sixty days of the patient’s illness, which had begun about one month previously.

The fact that the Coombs test became negative at the same moment. This coincidence might give way to much speculation (for instance, the rather mechanical concept that the gamma globulin increase is a reflection of the liberation of the abnormal coating of the red cells), but this will have to wait until the finding has been confirmed. The albumin (and alpha I globulin) fraction followed the usual pattern,
showing depression in the presence of high globulins, while the alpha and beta globulin fractions showed some fluctuations, which may be seen in table 1. During ACTH treatment there was but slight decrease of the icterus index. Another interesting observation was that the Coombs test increased together with decreasing gamma globulin values immediately following the peak of gamma globulins.

**Discussion**

In one of the cases reported by Dameshek et al., ACTH had only slight effect. This might, however, be due to lowering of dose because of excessive fluid retention. Recently, Clearkin published a case in which ACTH seemed to have no effect upon hemolysis, while in another case there was marked improvement. In the latter case there was also a positive Wassermann-Kahn test, seemingly unaffected with treatment with penicillin. In the case of Davidson et al. and in one case of Hansen this test was also found to be positive, though no signs of syphilitic infection could be elicited. It may be supposed that in connection with elevated and abnormal gamma globulins there is a strong anticomplementary power in the serum, inhibiting the Wassermann-Kahn reaction. The Coombs test is a valuable aid in the differentiation between hemolytic anemias of the acquired and congenital types, and in this respect it seems very reliable. It generally stays positive in cases of acquired hemolytic anemia, clinically cured by ACTH. In the report by Gardner et al. decrease of titer has been shown during ACTH treatment. In the present case it was amazing to see that the Coombs test became negative during treatment, but was positive at once on cessation of treatment. However, as stressed by Rosenfield, the rabbit serum should be absorbed and cross titrated before it is used for the Coombs test to avoid antialbumin antibodies and thereby possible nonspecific reactions, if albumin is absorbed to the erythrocytes as suggested by Moolten and Clark.

The development of the disease during the hospital stay of our patient is also very interesting. Apparently his hemolytic process started off with a febrile reaction, suggesting an infectious process. In view of this, the theory suggested by Moolten and Clark as to the etiology of some anemias in the group, deserves attention. These authors have shown the possibility of virus infection initiating intravascular hemolysis, thus altering the structure of the erythrocyte so as to make it antigenic. This would fit the demonstration of elevated gamma globulins in the cases of Hansen and might also explain the occurrence of acquired hemolytic anemia in conditions of lowered bodily resistance such as the leukemias, lymphosarcoma, etc.

**Summary**

A case has been reported of a 76 year old man, who, while in the hospital, developed a rather severe acquired, hemolytic anemia with positive Coombs test. Electrophoresis showed serum protein values within normal limits. ACTH was given for ten days and the reaction followed by electrophoretic tracings. A rather strange elevation of gamma globulins was seen during treatment. Simultaneously the Coombs test became negative. Later in the period of treatment, gamma globulins decreased to normal level and the Coombs test again became
positive. These findings and the possibility of viral infection as an etiologic factor in hemolytic anemia are discussed.

REFERENCES

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