The Adrenal Cortex and Hemolysis

I. Phenylhydrazine

By J. D. Feldman, M.D., M. Rachmilewitz, M.D., O. Stein and Y. Stein

Cortisone and ACTH have been shown to mitigate the hemolysis in many cases of acquired hemolytic anemia and often to arrest the disease.1, 5, 6, 9, 17, 20, 23. These hormones have also been reported to be beneficial in other types of anemia.2, 13, 14, 22, 23 Observations in animals have indicated that the adrenal cortex is to some extent associated with the maintenance of normal red cell levels in the peripheral blood21 and that the administration of ACTH increases red cell volume and hemoglobin in the intact animal.8, 10, 12, 15, 16, 19

The purpose of the present investigation was to determine the role of the adrenal cortex during a period of induced red cell destruction. Phenylhydrazine was employed as the hemolytic agent.

Materials

An inbred strain of white male rats, ranging in weight from 150 to 230 Gm., was used. Red cell counts were made from freely flowing tail vein blood taken at the same time of each day during the experimental period. The baseline level was determined by averaging the counts of two or three consecutive daily examinations and all counts agreed to within less than 5 per cent. Counts were also made each day for five days after the injection of phenylhydrazine.

Phenylhydrazine, in a concentration of 10 mg. per ml. of physiologic saline, was injected into the tail vein once to each rat in a dosage of 4 mg. per 100 Gm. of body weight. This quantity was selected because it approached the lethal amount tolerated by adrenalectomized rats and at the same time was sufficient to elicit a rapid and distinct anemia.

Three sets of experiments were performed and the rats were grouped as follows:

Group 1. A. Fourteen adrenalectomized rats were given intravenous phenylhydrazine. An additional 6 rats died between the first and fourth day after injection of the drug and were discarded. Three rats developed an anemia during the period of determination of the red cell baseline and these were also eliminated.

B. Ten sham-operated rats were given intravenous phenylhydrazine. One additional animal developed a spontaneous anemia and was excluded.

Group 2. C. Eight adrenalectomized rats were given 0.5 mg. of cortisone per 100 Gm. of body weight intraperitoneally three times daily. The cortisone was administered for two days prior to the injection of phenylhydrazine and for 5 days after.

D. Six sham-operated rats were treated with cortisone and phenylhydrazine in the same manner as described above (C).

E. Three adrenalectomized and 3 sham-operated rats received intravenous saline, 0.4

From the Department of Pathology, Medical Department “B,” and the Department of Clinical Research, of the Hebrew University-Hadassah Medical School and Hadassah Hospital, Jerusalem, Israel.

This study was supported by the Research Council of the Government of Israel and the Mr. and Mrs. Harry A. and Etta Freedman Foundation, New York.

Submitted October 4, 1952; accepted for publication October 16, 1952.

The authors wish to express their appreciation to Dr. Irby Bunding, Biochemical Research Department, The Armour Laboratories, for the generous provision of ACTH.
ml per 100 Gm. of body weight, instead of phenylhydrazine. These animals were grouped together because no significant differences between them were found.

All animals of the above groups were given 1 per cent saline for drinking water. Experiments were started fifteen to thirty-five days after operation. Control animals were examined at the same time as experimental rats.

Group 3. F. Ten intact rats were given 0.4 mg. of ACTH (in a concentration of 1 mg. per ml. of physiologic saline) four times daily intraperitoneally. The ACTH was injected for a period of 24 hours before the administration of phenylhydrazine and continued for five days after. The administration of ACTH did not alter the baseline level of red cells.

G. Six intact rats, controls for group F, were given phenylhydrazine intravenously and received no other treatment.

**Results**

Table 1 and figures 1 and 2 summarize the results of all experiments.

1. **Adrenalectomy**

   The anemia produced by phenylhydrazine was significantly greater in the adrenalectomized group (A) than in any other group (fig. 1, table 1). From the first to the fifth day after injection the red cell level was significantly lower in group A. The maximal decrease was gradually reached on the fourth day and amounted to −48 per cent of the baseline. In the control group the maximal fall also occurred on the fourth day and reached −31 per cent.

   It is seen in table 1 that the baseline red cell level of the adrenalectomized group was not significantly different from the red cell level of any other group.

2. **Cortisone**

   In the adrenalectomized group receiving cortisone (C) the anemia induced by phenylhydrazine was of the same degree as that found in the sham-operated group (D) (fig. 1, table 1). The destruction of red cells was significantly less than that observed in adrenalectomized animals which did not receive cortisone, (group A). Curves C and D in figure 1, representing the percentage fall in red cells, are almost identical. The maximal drop occurred on the third day for both groups C and D and was −25 per cent and −26 per cent respectively.

   It is of interest to note that the red cell level of sham-operated rats receiving cortisone was higher than that of sham-operated or non-operated rats (groups B and G) without cortisone. This difference, however, was not statistically significant.

   The administration of saline to adrenalectomized and sham-operated rats (group E) caused no significant changes in the red cell level.

3. **ACTH**

   In the group of rats receiving ACTH (F) the anemia produced by phenylhydrazine was slight and was significantly less than that found in its control group (G) (fig. 2, table 1). Curve F in figure 2 is almost a straight line from the first to the fifth day and the percentage decrease of red cells did not exceed 10 per cent. Comparison of curves B and G of figures 1 and 2 and of the data in table 1 shows that the operation per se had no influence upon the destruction of red cells by phenylhydrazine.
TABLE 1—Absolute Red Cell Counts and Standard Errors

Summary of red cell data after intravenous injection of phenylhydrazine and saline

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats</th>
<th>Baseline 10^6</th>
<th>1 Day 10^6</th>
<th>2 Day 10^6</th>
<th>3 Day 10^6</th>
<th>4 Day 10^6</th>
<th>5 Day 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. adrenalectomy</td>
<td>14</td>
<td>9.81 ± .14</td>
<td>6.01 ± .35</td>
<td>6.10 ± .36</td>
<td>5.51 ± .33</td>
<td>5.09 ± .27</td>
<td>5.40 ± .22</td>
</tr>
<tr>
<td>B. sham-operated</td>
<td>10</td>
<td>9.79 ± .13</td>
<td>7.96 ± .31</td>
<td>7.20 ± .31</td>
<td>7.06 ± .17</td>
<td>6.76 ± .24</td>
<td>7.12 ± .17</td>
</tr>
<tr>
<td>C. adrenalectomy cortisone</td>
<td>8</td>
<td>9.67 ± .17</td>
<td>8.01 ± .22</td>
<td>7.46 ± .14</td>
<td>7.11 ± .22</td>
<td>7.20 ± .17</td>
<td>7.17 ± .35</td>
</tr>
<tr>
<td>D. sham-operated cortisone</td>
<td>6</td>
<td>9.89 ± .15</td>
<td>7.92 ± .19</td>
<td>7.71 ± .14</td>
<td>7.38 ± .07</td>
<td>7.51 ± .17</td>
<td>7.60 ± .21</td>
</tr>
<tr>
<td>F. ACTH</td>
<td>10</td>
<td>9.66 ± .06</td>
<td>8.99 ± .19</td>
<td>8.81 ± .20</td>
<td>8.90 ± .20</td>
<td>8.74 ± .14*</td>
<td>8.81 ± .20</td>
</tr>
<tr>
<td>G. control for F</td>
<td>6</td>
<td>9.77 ± .06</td>
<td>7.16 ± .40</td>
<td>7.20 ± .19</td>
<td>7.20 ± .19</td>
<td>6.91 ± .29</td>
<td>7.13 ± .29</td>
</tr>
</tbody>
</table>

Italicized figures of Group A are significantly different (P less than 0.01) from figures in the same columns of remaining groups. Italicized figures of Group F are significantly different from figures in the same columns of all groups except E.

* Significantly different from corresponding figure in Group E.
FIG. 1.—Percentage decrease in red cells after intravenous injection of phenylhydrazine. Group A, adrenalectomized; group B, sham-operated; group C, adrenalectomized plus cortisone; group D, sham-operated plus cortisone; group E, sham-operated and adrenalectomized after intravenous injection of saline.

FIG. 2.—Percentage decrease in red cells after intravenous injection of phenylhydrazine. Group F, ACTH; group G, controls for group F.

COMMENT

The results obtained in this investigation showed clearly that in adrenalectomized rats the anemia produced by phenylhydrazine was significantly greater than in intact animals. Administration of cortisone to adrenalectomized rats
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prevented this increased destruction and maintained a red cell level comparable with the level found in control animals. Under the conditions of the present experiments ACTH was more effective than cortisone (compare groups D and F) and almost completely prevented the red cell destruction induced by phenylhydrazine.

The increased destruction of red cells which occurred in the adrenalectomized rats receiving phenylhydrazine was not the result of an additive anemia consequent to adrenalectomy. A fall in red cells of between 12 to 25 per cent has been reported to occur following adrenalectomy alone. In the present study, fifteen to thirty-five days after removal of the adrenals, the rats had a red cell level which did not differ from control groups. In several animals of group A, red cell counts were made before adrenalectomy and no fall was found following the operation. Three rats out of 23, however, were discarded from this group because of a decrease in red cells during the period of determination of the baseline.

It was also unlikely that the effects of phenylhydrazine in adrenalectomized animals could be attributed to blood volume changes. Blood volume was not determined in these rats but White and Dougherty reported that there were no changes in blood volume in rats following adrenalectomy. Frost and Talmage have also shown that in the adrenalectomized rat fluid shifts affected the cell but did not alter extracellular volumes. Other investigators have stated that hemoconcentration occurred in species other than the rat following adrenalectomy (cf. references of Gordon and White). If hemoconcentration did occur, the changes in red cell levels reported here would be even more significant.

To explain the results of the present investigation it could be postulated that the adrenal cortex influences (1) the integrity of the red cell, either directly or indirectly by alterations of the plasma; (2) red cell breakdow’n by the reticuloendothelial system; or (3) red cell production in bone marrow and extramedullary sites.

Recent observations suggest that the adrenal cortex may affect the structure of the red cell or its surrounding milieu. Gardner et al. and Rachmilewitz, Riss and Ehrenfeld have reported that the administration of ACTH to patients with acquired hemolytic anemia caused a decrease of mechanical and osmotic red cell fragility in some instances. In the present experiments it was possible that cortisone or ACTH increased the resistance of the red cell to phenylhydrazine destruction. Gordon, Piliero and Landau, on the other hand, have demonstrated decreased fragility of red cells from adrenalectomized rats in hypotonic saline solution. This decreased fragility was transient and disappeared approximately four weeks after operation. In the adrenalectomized animals receiving phenylhydrazine, the anemia produced was significantly greater than in controls. This apparent contradiction of results is still unsolved.

Dameshek, Rosenthal and Schwartz and Best, Limarzi and Poncher have postulated that in acquired hemolytic anemia adrenocortical hormones may alter the milieu of the red cell, i.e., suppress antibody production or adsorption of hemolytic proteins on the red cell surface, and thus prevent hemolysis. With phenylhydrazine destruction of red cells there is no question of immune bodies...
or adsorption of proteins, yet cortisone and ACTH significantly alleviated the anemia caused by this chemical.

Gordon and Katsh\(^1\) and Cartwright et al.\(^2\) have reported impairment of the reticulo-endothelial system following adrenalectomy. Spain, Molomut and Haber\(^3\) described a decreased uptake of carbon particles by the reticulo-endothelial system in cortisone treated mice. The present experiments have not resolved this contradiction of results but it was observed that the average spleen weight of adrenalectomized rats receiving phenylhydrazine was significantly less than the spleen weights of control, cortisone and ACTH treated rats receiving phenylhydrazine. The possibility was considered that the phenylhydrazine-injured red cells were not readily broken down by the spleen or the reticulo-endothelial system in general in adrenalectomized animals. As a result the iron and proteins of the damaged cells were not available for synthesis of new cells. Cruz, Hahn and Bale\(^4\) have shown that the products of red cells destroyed by acetylphenylhydrazine are immediately utilized for the production of new cells.

The administration of ACTH causes increased red cell production in man and experimental animals according to Garcia et al.\(^5\) and to Hudson, Herdan and Yoffey.\(^6\) Conversely, hypofunction or extirpation of the adrenals results in bone marrow hypoplasia.\(^7\) In the light of the present study it might be assumed that the adrenalectomized rat was unable to replace quickly the red cells destroyed by phenylhydrazine, and further that the administration of cortisone or ACTH increased not only the production but also the delivery of red cells to the peripheral blood. However, in view of the facts that significant differences in the degree of anemia was observed as early as 24 hours after injection of phenylhydrazine and that reticulocytosis was prominent in both adrenalectomized and intact rats, it was considered unlikely that cortisone and ACTH effected such rapid bone marrow responses.

**SUMMARY**

The intravenous administration of phenylhydrazine caused a significantly greater destruction of red cells in adrenalectomized rats than in controls. Cortisone given to adrenalectomized rats prevented this increased destruction and maintained red cells at a level comparable to controls. ACTH almost completely prevented phenylhydrazine anemia in intact rats and the red cell level was significantly higher than that in intact rats which did not receive ACTH.

**REFERENCES**

4. Cruz, W. O., Hahn, P. F. and Bale, W. F.: Hemoglobin radioactive iron liberated by
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