# Increase in Circulating Red Cell Volume of Normal Rats after Treatment with Hydrocortisone or Corticosterone. (23787)

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The humoral regulation of red cell production has been the subject of several recent investigations(1,2,3,4,5). However, there is very little direct evidence that the adrenocortical hormones are capable of exerting a significant influence on the hematocrit, red cell count, hemoglobin or red cell volume.

The present studies were undertaken to determine the effects of prolonged daily injections of hydrocortisone and corticosterone on the blood of the normal rat. This was deemed of fundamental interest because of previous reports showing that injections of a purified ACTH preparation in normal mice(6) and rats(7) caused an elevation in hematocrit and total red cell volume.

*Materials and methods.* Male albino rats of the Wistar (Purdue) strain were studied in 6 groups: Group (1) 6 normal controls, (2) 8 rats given hydrocortisone at a dose of 0.5 mg/kg/day, (3) 10 rats at a dose of 2 mg hydrocortisone/kg, (4) 8 rats at 5.0 mg hydrocortisone/kg, (5) 10 rats at 5 mg corticosterone/kg, and (6) 8 rats at 10 mg corticosterone/kg. The Hydrocortisone Acetate† or Corticosterone† was suspended in 0.9% NaCl solution and injected subcutaneously daily (6 days/week) at various sites under the skin of the back. Blood was obtained for erythrocyte, hemoglobin and hematocrit determinations by anesthetizing the rat with ether and clipping the tail. Erythrocyte counts were done with U.S. certified blood pipettes and the improved Neubauer counting chamber. Hematocrit determinations were made with Van Allen hematocrit tubes (1.6% aqueous sodium oxalate as diluent), spun 30 minutes at 3000 rpm with radius of 17 cm. Hemoglobin determinations were made on Klett-Summerson colorimeter by the acid hematin method(8). Rats were weighed at the time of hematologic studies. Erythrocyte blood volume was determined with $P^{32}$ tagged erythrocytes using the method of Hevesy(9) as modified by Berlin(10). Each animal was anesthetized with ether and the tagged cells were injected into a vein, exposed by incision on inner aspect of thigh and allowed to mix for 10 minutes, then a blood sample was removed *via* heart puncture for counting. P values were determined by using Fisher's "t" test for statistical significance.

*Results.* The data are presented in Table I. Hematocrit, erythrocyte, hemoglobin and total red cell volume values at 60 days were significantly elevated for all dosages of hydrocortisone and corticosterone. P values of less than .01 were obtained for all 60 days values except the hematocrit for the 0.5 mg dose of hydrocortisone which was 0.1 and the hemoglobin and hematocrit for the 5 mg dose of hydrocortisone which were 0.02 and 0.07 respectively. There was a decrease in mean corpuscular volume upon treatment with either hydrocortisone or corticosterone. Therefore, hematocrits and total red cell volumes were not elevated to the same degree as red cell counts.

The most striking results were obtained with daily injections of hydrocortisone at 2 mg/kg. After 60 days of treatment, the erythrocyte count had increased from 9.5 to 13.9 million cells/cu mm; the hematocrit, from 43.5 to 52.1%; the hemoglobin, from 16.2 to 18.0 g/100 cc; and red cell volume was elevated from 1.96 to 3.2 cc/100 g body weight. The mean corpuscular hemoglobin decreased along with mean corpuscular volume. The mean corpuscular hemoglobin concentration remained essentially normal throughout treatment. Ninety day erythrocyte counts, hematocrit and red cell volumes for the 2 mg hydrocortisone dosage were

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TABLE I. Effects of Hydrocortisone and Corticosterone on Blood Picture of Normal Adult Male Rats.

| Day | No. rats | Body wt, g | RBC, millions/mm³ | Hemato-crit, % | Hemoglobin, g/100 cc | M.C.V., $\mu^3$ | M.C.H., $\mu\mu g$ | M.C.H. conc., % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Normal controls—no treatment | | | | | |
| 0 | 6 | 251.8 ± 35 | 8.8 ± .45 | 44.0 ± 1.9 | 15.9 ± .21 | 50.0 ± 2.7 | 18.1 ± .88 | 36.2 ± 1.5 | |
| 10 | 6 | 277.8 ± 20 | 9.7 ± .51 | 44.6 ± 1.9 | 16.4 ± .40 | 45.9 ± 2.9 | 16.9 ± .78 | 36.8 ± 2.0 | |
| 34 | 6 | 330.8 ± 11 | 9.2 ± .77 | 45.5 ± 4.0 | 16.6 ± 2.0 | 49.6 ± 6.7 | 18.1 ± 2.9 | 36.5 ± 1.9 | |
| 40 | 6 | 340.2 ± 13 | 9.5 ± 1.47 | 46.3 ± 3.7 | 16.2 ± 1.0 | 49.3 ± 6.2 | 17.3 ± 2.3 | 35.1 ± 1.8 | |
| 50 | 6 | 347.7 ± 17 | 9.3 ± 1.25 | 44.0 ± 1.8 | 15.8 ± .84 | 48.1 ± 7.8 | 17.3 ± 3.3 | 35.9 ± 2.2 | |
| 60 | 6 | 373.0 ± 17 | 9.7 ± .79 | 45.2 ± 2.3 | 16.0 ± .55 | 46.7 ± 2.7 | 16.7 ± 1.3 | 35.5 ± 1.8 | |
| 76 | 5 | 390.6 ± 28 | 9.6 ± .79 | 44.8 ± 1.5 | 16.1 ± .75 | 47.0 ± 5.6 | 16.8 ± 1.8 | 35.9 ± 1.6 | * |
| 90 | 5 | 398.2 ± 21 | 8.5 ± 1.3 | 44.8 ± 3.3 | 15.7 ± .46 | 53.7 ±11.2 | 18.8 ± 3.2 | 35.2 ± 2.2 | † |
| | | | | Corticosterone—5 mg/kg/day | | | | | |
| 0 | 10 | 319.0 ± 19 | 9.7 ± .92 | 44.4 ± 2.1 | 16.0 ± .70 | 46.1 ± 4.6 | 16.7 ± 2.0 | 35.9 ± 1.4 | |
| 10 | 9 | 316.6 ± 23 | 10.8 ± 1.7 | 44.0 ± 3.3 | 16.5 ± 1.8 | 41.3 ± 5.2 | 15.5 ± 2.6 | 37.5 ± 2.3 | |
| 20 | 8 | 333.1 ± 24 | 11.9 ± 1.0 | 46.3 ± 2.6 | 16.5 ± 1.1 | 39.0 ± 3.2 | 13.9 ± 1.2 | 35.8 ± .84 | |
| 40 | 8 | 360.4 ± 35 | 10.4 ± .36 | 42.3 ± 1.0 | 16.9 ± .64 | 40.6 ± 1.5 | 16.3 ± .96 | 40.1 ± 2.1 | |
| 60 | 8 | 376.4 ± 37 | 12.3 ± 1.2 | 49.0 ± 2.6 | 17.5 ± .53 | 39.8 ± 6.0 | 14.2 ± 1.4 | 35.8 ± 1.3 | ‡ |
| | | | | Corticosterone—10 mg/kg/day | | | | | |
| 0 | 8 | 360.8 ± 38 | 9.1 ± .83 | 42.3 ± 2.3 | 15.7 ± .79 | 46.6 ± 4.6 | 17.3 ± 2.2 | 37.2 ± 1.7 | |
| 10 | 8 | 337.6 ± 56 | 13.1 ± .90 | 46.6 ± 3.0 | 17.9 ± 2.3 | 35.6 ± 3.3 | 13.7 ± 1.1 | 38.5 ± 2.1 | |
| 20 | 7 | 334.4 ± 49 | 11.8 ± .77 | 45.3 ± 1.7 | 16.8 ± .64 | 38.5 ± 3.5 | 14.2 ± 1.1 | 37.1 ± 2.0 | |
| 60 | 6 | 361.8 ± 55 | 12.0 ± 1.2 | 50.5 ± 2.2 | 18.4 ± 1.1 | 42.3 ± 4.9 | 15.4 ± 1.7 | 36.4 ± 1.9 | § |
| | | | | Hydrocortisone—.5 mg/kg/day | | | | | |
| 0 | 8 | 302.3 ± 43 | 10.1 ± .60 | 44.1 ± 3.6 | 16.8 ± 1.3 | 42.3 ± 6.9 | 16.6 ± 1.4 | 38.2 ± 1.5 | |
| 20 | 8 | 315.1 ± 32 | 9.8 ± 2.20 | 45.4 ± 1.6 | 17.6 ± .71 | 48.0 ±11.6 | 18.8 ± 4.5 | 38.7 ± 1.6 | |
| 34 | 7 | 328.3 ± 44 | 10.7 ± .59 | 46.1 ± 2.7 | 16.4 ± .89 | 43.2 ± 1.7 | 15.4 ± 1.1 | 35.6 ± 1.8 | |
| 40 | 7 | 333.9 ± 45 | 11.2 ± .66 | 46.0 ± 2.8 | 17.3 ± .99 | 40.9 ± 2.8 | 15.3 ± 1.4 | 37.7 ± 3.1 | |
| 60 | 7 | 355.1 ± 52 | 12.6 ± .66 | 46.6 ± 1.3 | 18.0 ± 1.3 | 37.1 ± 2.2 | 14.3 ± 1.2 | 38.7 ± 1.8 | |
| 90 | 7 | 370.9 ± 56 | 10.8 ± 1.5 | 48.3 ± 3.3 | 17.4 ± .87 | 45.4 ± 7.1 | 16.3 ± 2.8 | 36.1 ± 2.4 | ‖ |
| | | | | Hydrocortisone—2 mg/kg/day | | | | | |
| 0 | 10 | 272.1 ± 24 | 9.5 ± .98 | 43.5 ± 2.2 | 16.2 ± 1.0 | 46.4 ± 5.6 | 17.1 ± 2.0 | 37.2 ± 1.0 | |
| 10 | 6 | 262.2 ± 27 | 10.5 ± 1.9 | 47.5 ± 4.4 | 17.3 ± 1.3 | 45.9 ± 6.8 | 16.9 ± 2.5 | 36.6 ± 1.2 | |
| 34 | 9 | 304.8 ± 29 | 11.5 ± 1.3 | 50.2 ± 1.7 | 18.3 ± 1.0 | 44.2 ± 5.6 | 16.1 ± 2.7 | 36.4 ± 1.8 | |
| 40 | 9 | 301.6 ± 28 | 12.3 ± .99 | 49.4 ± 1.6 | 17.6 ± .86 | 40.5 ± 3.0 | 14.4 ± 1.4 | 35.6 ± 2.0 | |
| 60 | 9 | 315.4 ± 28 | 13.9 ± 2.0 | 52.1 ± 1.5 | 18.0 ± 1.3 | 38.8 ± 8.3 | 13.3 ± 2.5 | 34.6 ± 1.8 | ¶ |
| 90 | 7 | 326.1 ± 11 | 12.1 ± 1.5 | 50.4 ± 1.1 | 18.6 ± .50 | 42.3 ± 5.4 | 15.6 ± 2.1 | 37.0 ± 1.6 | ** |
| | | | | Hydrocortisone—5 mg/kg/day | | | | | |
| 0 | 8 | 300.3 ± 26 | 9.4 ± .72 | 44.5 ± 2.7 | 15.8 ± .84 | 47.5 ± 3.4 | 16.9 ± 1.4 | 35.6 ± 1.1 | |
| 10 | 8 | 293.9 ± 36 | 11.8 ± 1.2 | 48.1 ± 4.0 | 18.7 ± 1.5 | 41.3 ± 6.8 | 16.0 ± 2.3 | 39.0 ± 2.2 | |
| 20 | 8 | 279.9 ± 35 | 12.6 ± 1.4 | 48.6 ± 3.9 | 18.1 ± .99 | 38.8 ± 3.4 | 14.4 ± 1.2 | 37.4 ± 2.0 | |
| 40 | 7 | 307.3 ± 37 | 12.1 ± .80 | | 18.7 ± .92 | | 15.4 ± 1.1 | | |
| 60 | 7 | 300.0 ± 41 | 12.0 ± 1.4 | 49.7 ± 5.7 | 18.3 ± 1.2 | 38.8 ± 4.9 | 15.4 ± 1.4 | 37.1 ± 2.5 | †† |

Day = Day of treatment; M.C.V. = Mean corpuscular vol; M.C.H. = Mean corpuscular hemoglobin.
Red cell vol (cc red cells/100 g body wt) are as follows:

| | | | |
|---|---|---|---|
| * 1.96 ± .07 | ‡ 2.85 ± .31 | ‖ 2.73 ± .30 | ** 2.80 ± .52 |
| † 2.28 ± .08 | § 2.90 ± .13 | ¶ 3.21 ± .46 | †† 2.90 ± .28 |

± = stand. dev.

lower than 60 day values but not to a significant degree (P values for red cell volume, hematocrit and erythrocyte counts were .40, .10 and .08 respectively).

Treatment with hydrocortisone at a dosage of 5 mg/kg daily resulted in a less pronounced rise in blood values than the 2 mg/kg dose, and may be the result of toxicity indicated by failure of rats in this group to gain weight. The 0.5 mg dose of hydrocortisone induced a slower and more gradual rise in blood values than either the 2 mg or 5 mg dose.

Rats treated with corticosterone at daily dosage of 5 and 10 mg/kg for 60 days showed a significant elevation in hematocrit, hemoglobin, red cell count and total red cell volume but to a lesser degree than those treated with hydrocortisone at 2 mg/kg. Sixty days of treatment with corticosterone at a daily dose of 10 mg/kg increased erythrocytes from 9.1

to 12.0 million cells/cu mm; the hematocrit, from 42.3 to 50.5%; hemoglobin, from 15.7 to 18.4 g/100 cc; and red cell volume to 2.9 cc/100 g body weight (1.48 times untreated controls). As with rats receiving hydrocortisone at a dosage of 2 mg/kg, these rats also showed a decreased mean corpuscular cell volume and mean corpuscular hemoglobin. The mean corpuscular hemoglobin concentration remained relatively normal.

A group of 10 rats not included in Table I was treated with 10 mg hydrocortisone/kg daily via subcutaneous injection. Progressive loss in weight and ulcerations at injection sites were noted. Only 2 animals survived in this group at 50 days and none at 60 days. Increases in erythrocytes, hematocrit and hemoglobin were noted at 10 days but these values gradually decreased to below-normal values as toxic signs appeared.

*Discussion.* Hydrocortisone and corticosterone produced elevation in erythrocytes, hematocrit, hemoglobin and total red cell volume (Table I) in many respects similar to that produced by ACTH(7), hypoxia(11) and cobalt(12). Two mg hydrocortisone/kg for 60 days (Table I) increased the hematocrit to approximately the same degree as rats treated for 40 days with 0.5 mg cobalt nitrate/day subcutaneously(12) or 1 mg ACTH intraperitoneally for 116 days(7). Hydrocortisone produced a more marked effect than corticosterone judged both by rate and magnitude of response shown in Table I.

Several factors in addition to the present results suggest that adrenocortical secretions play a significant role in regulation of hematopoiesis. Removal of the adrenal gland in the rat results in a temporary anemia(13,14), corrected upon development of accessory adrenals(13). Administration of adrenocortical extract in rats(15) or cortisone in rats and dogs(15,16) repaired the anemia of adrenalectomy. Cushing's disease is accompanied by a polycythemia(17). An increase in "stem" and erythroid cells was noted in bone marrow from rats treated for a short time with corticosterone(2). Adrenalectomized rats did not show the characteristic polycythemic response to cobalt injections (13).

Consideration should be given to the possibility of dissociation of total body growth and growth of red cell mass. However, we have found that animals treated with 2 mg hydrocortisone/kg for 60 days have a significant elevation in red cell volume for animals of their age, and weight. Therefore, elevation in total red cell volume cannot be due to a repression of normal weight gain.

Recent work has shown that adrenalectomized rats respond to bleeding with an increase in plasma erythropoietin, but the response is less than that shown by normal rats (18). The demonstration by Gley(5) of the existence of more than one erythropoietin in plasma could explain the response of adrenalectomized animals to bleeding.

The increased red cell volume after treatment with the adrenocortical hormones (Table I) is interesting when considered along with the greatly increased adrenal weights and erythropoietic stimulation in rats maintained at depressed oxygen(19) or treated with cobalt(13). The mechanism by which bone marrow is stimulated by adrenocortical hormones is not clear. The work of Gross(20) with bone marrow *in vitro* would indicate that the adrenocortical hormones stimulate bone marrow directly.

*Conclusions.* 1. Administration of hydrocortisone or corticosterone for 60 days to normal rats elevated the total circulating red cell volume from 1.45 to 1.64 times that of normal untreated controls. 2. Rats treated with 0.5, 2 or 5 mg hydrocortisone or 5 or 10 mg corticosterone/kg body weight for 60 days, showed a significant elevation in erythrocyte, hematocrit and hemoglobin values.

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## Hemolytic Anemia in Rabbits Following Injection of Bacterial Endotoxin. (23788)

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Alterations in the numbers of circulating white cells and platelets after injections of Gram negative bacterial endotoxins are well known(1,2). Corresponding changes in the red cells have not been as clearly defined. Braude *et al.* found variable effects of *E. coli* endotoxin on the blood volume and hematocrit of rabbits, with little change in the circulating red cell mass, although leukopenia and neutropenia occurred(3). Similarly, Kopp noted little change in the hemoglobin levels of patients receiving typhoid-paratyphoid (TAB) vaccine for production of therapeutic fever(4). Cartwright *et al.*(5) found that injecting 5 doses of typhoid vaccine intramuscularly into dogs did not induce the hypoferremia and anemia that occurs during infection. Willison observed that various bacterial exotoxins depressed the reticulocyte response to a constant blood loss anemia in rabbits but the effect of bacterial endotoxins was not investigated(6). It will be shown here that rabbits may develop a mild hemolytic anemia after a single injection of endotoxin and that they invariably develop such anemia after multiple injections of endotoxin derived from typhoid bacilli.

*Materials and methods.* Male albino buck rabbits, weighing 5-6 lb at the beginning of the experiments, were used. *Endotoxin.* Trypsin treated *S. typhi* (Ty2) endotoxin was prepared as previously described(7). Briefly, an overnight culture† was killed with chloroform, digested with trypsin, precipitated with acetone and extracted by repeated alcohol-ether precipitations from aqueous solution. The final solution, in physiological saline, was heated at 56° for 30 minutes in a sterile vial and stored at −25°C. The $LD_{50}$ of this endotoxin injected intravenously into 5-6 lb rabbits varied between 0.01-0.1 ml of an undiluted solution containing 10 mg per ml of endotoxin by dry weight. *Hematological studies.* Whole blood and plasma hemoglobin concentrations, reticulocyte counts, and mechanical and osmotic fragility of red cells were determined by the usual methods(8). The hemoglobin levels of apparently normal rabbits were found to vary from 10.5 to 14 g %, and the reticulocyte counts from 0.6 to 3.0%. *Labelled red cells.* Five ml of blood was drawn by cardiac puncture and the red cells labelled with 40-70 microcuries of radioactive sodium chromate ($Na_2Cr^{51}O_4$) according

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