Effect of Hydrocortisone Administration on Erythropoiesis in the Adrenalectomized Dog

In previous experiments it has been shown that adrenalectomy has the effect of reducing the hemoglobin formation in the dog[1]. This and other reported studies[2-5] definitely indicate that the adrenal gland influences erythropoiesis. However, there is little direct evidence that the adrenal cortical steroids are capable of exerting a significant influence on red cell production in this animal. Therefore, the present study was designed to investigate the influence of prolonged daily injections of hydrocortisone on erythropoiesis in adrenalectomized dogs.

Five adult mongrel dogs of both sexes were studied for 8 months divided into 4 periods. During an initial control period of 2 months a Cr^{51} total red cell volume and Fe^{59} plasma and red cell iron turnovers[6] were obtained. The animals were then bilaterally adrenalectomized and maintained in apparently normal health and vigour by injecting 5 mg of DCA i.m. weekly. The above determinations were repeated 4 months subsequent to the operation (period II). As can be seen in the Table, DCA-maintained adrenalectomized dogs showed a 40% decrease in the total circulating red cell volume and a concomitant 27% reduction in the plasma volume. The hematocrit value and hemoglobin concentration were slightly decreased. The mean radioiron disappearance half-life determined before adrenalectomy was 71 min compared to 133 min 4 months after adrenalectomy. The mean plasma iron turnover and the red cell iron turnover rates were 68 and 82% below control values, respectively. Therefore, anemia in the adrenalectomized dog appears to result from a diminished rate of erythropoiesis[1].

Following period II, the animals were given 2 mg of hydrocortisone acetate/kg (Merck, Sharp and Dohme, Argentina) body weight for 40 days (period III), but DCA was no longer injected. This dosage maintained normal activity and vigour of the animals. In comparison with period II values, there was a significant reduction in the plasma iron disappearance half-life and a marked increase in the rate of erythropoiesis, as judged by red cell iron turnover rate (see Table). This period of marked increase in red cell synthesis corresponds to the period of expansion of the total red cell volume, which increased 43% from the period II-value. The effect, although clear, is somewhat masked by the fact that the animals gained weight and presumably fatty tissue with low blood content during this period. These data are in agreement with a number of reports by other investigators[7-9] who have shown the stimulatory effect of oxycorticosteroids on erythropoiesis.

Treatment with hydrocortisone at a dosage of 4 mg/kg daily during the next 40 days (period IV) resulted in an increased erythroid marrow activity, if we accept that plasma iron turnover rate is a measure of the erythroid activity of the bone marrow[10]. At this moment this parameter was within normal values (see Table) and 2.6 times that of the 4 months adrenalectomized dog. This increased activity of the bone marrow was not reflected in the red cell utilization of radioiron, indicating a discrepancy between total and effective erythropoiesis. The average total erythropoiesis was 0.9 times normal, while the average effective erythropoiesis was about 0.4 times normal. Therefore, the average efficiency of the bone marrow was 44%, which means that, of all hemoglobin produced in the bone marrow, less than half was delivered effectively to the peripheral circulation. This can be considered as a disfunction of erythropoiesis, which indicates that the activity of the bone marrow is largely ineffective insofar as the delivery of viable erythrocytes to the circulation is concerned. This is supported by the fact that indirect bilirubin was slightly elevated in the dogs receiving hydrocortisone at this dosage compared with normal dogs.

The 'ineffective erythropoiesis' resulted in a decrease in the total red cell volume back to the level found 4 months after adrenalectomy. The hemoglobin concentration and the hematocrit value reached their lowest values. The plasma volume of the dogs receiving the increased dosages of hydrocortisone increased, reaching almost normal values, which would account for the great fall in hemoglobin concentration and hematocrit observed at the end of period IV.

The results presented here regarding the erythropoietic effects of hydrocortisone are very difficult to explain in the light of present knowledge. Adrenal steroids are known to increase calorigenesis[7,11] and to inhibit protein

[1] C. E. BOZZINI, M. E. BARRIO RENDO and J. A. KOFOED, Acta physiol. latinoam., in press (1968).
[2] R. C. CRAFTS, Endocrinology *29*, 596 (1941).
[3] A. S. GORDON and H. A. CHARIPPER, Ann. N.Y. Acad. Sci. *48*, 615 (1947).
[4] S. J. PILIERO, D. LANDAU and A. S. GORDON, Science *112*, 559 (1950).
[5] W. W. SWINGLE, H. M. VARS and W. M. PARKINS, Am. J. Physiol. *109*, 488 (1934).
[6] R. L. HUFF, T. G. HENNESSY, R. E. AUSTIN, J. F. GARCIA, B. M. ROBERTS and J. H. LAWRENCE, J. clin. Invest. *29*, 1041 (1950).
[7] E. S. EVANS, D. C. VAN DYKE and J. L. ROSEMBERG, Endocrinology *75*, 758 (1964).
[8] J. W. FISHER, Proc. Soc. exp. Biol. Med. *97*, 502 (1958).
[9] W. W. SWINGLE, G. BARLOW, E. J. FEDOR, M. BEN, R. MAXWELL, E. COLLINS and C. BAKER, Am. J. Physiol. *173*, 4 (1953).
[10] C. A. FINCH and W. D. NOYES, J. Am. med. Ass. *175*, 1163 (1961).
[11] E. S. EVANS, A. N. CONTOPOULOS and M. E. SIMPSON, Endocrinology *60*, 403 (1957).

Hematologic data from dogs. Normal, adrenalectomized, adrenalectomized injected with hydrocortisone

	Period I	Period II	Period III	Period IV
Hemoglobin concentration, g/100 ml	14.6 ± 0.38	12.1 ± 0.34	13.1 ± 0.31	10.5 ± 0.30
Hematocrit, %	43.0 ± 1.35	41.0 ± 1.12	40.0 ± 1.19	34.0 ± 1.17
Red cell volume, ml/kg	31.5 ± 1.14	18.8 ± 1.01	27.0 ± 1.08	18.2 ± 1.36
Plasma volume, ml/kg	47.6 ± 2.94	34.0 ± 1.18	40.0 ± 2.01	45.6 ± 2.27
Plasma iron, µg/ml	1.35 ± 0.08	1.05 ± 0.06	0.97 ± 0.04	1.25 ± 0.09
Plasma Fe^{59} half life, min	71 ± 7.60	133 ± 8.24	70 ± 5.23	55 ± 4.99
Plasma iron turnover rate, mg/kg/ray	0.88 ± 0.13	0.28 ± 0.09	0.56 ± 0.11	0.74 ± 0.19
Red cell iron utilization, %	74 ± 3.40	37 ± 1.28	70 ± 2.97	35 ± 1.17
Red cell iron turnover rate, mg/kg/day	0.65 ± 0.15	0.11 ± 0.05	0.39 ± 0.09	0.26 ± 0.11

synthesis[12], and both effects are known to influence erythropoiesis in opposite ways. By combination of both at the highest dosage, the bone marrow could be stimulated in the presence of an impairment of its function with the resulting development of non-viable red cells. A suppressive effect of adrenal cortical steroids on erythropoiesis has already been reported[13,14]. Thus, GORDON et al.[14] have shown that high doses of methyl prednisolone depresses erythropoiesis in normal mice as the result of a decreased erythroid responsiveness to erythropoietin and a depression of erythropoietin formation. However, these mechanisms do not appear to be similar to those which are responsible for the results reported here[15].

Resumen. La administración de 2 mg/kg/día de hidrocortisona durante 40 días en perros adrenalectomizados 4 meses antes produjo elevación del volumen de la masa roja circulante y de la síntesis de hemoglobina. El tratamiento con la hormona en la dosis de 4 mg/kg/día durante los siguientes 40 días produjo más aumento de la actividad eritropoyética de la médula ósea, una gran proporción de la cuál fue inefectiva en términos de producción de eritrocitos, produciéndose un descenso de la masa roja circulante.

C. E. BOZZINI[16], M. E. BARRIO RENDO[16], J. A. KOFOED[16] and G. O. FREY

Cátedra de Fisiología, Facultad de Odontología, Universidad de Buenos Aires and Departamento de Aplicaciones, Comisión Nacional de Energía Atómica, Buenos Aires (Argentina), 19 March 1968.

[12] C. N. H. LONG, Endocrinology *30*, 870 (1942).
[13] J. B. TIERRSCH, L. CONROY, A. R. STEVENS and C. A. FINCH, J. Lab. clin. Med. *50*, 174 (1952).
[14] A. S. GORDON, E. A. MIRAND and E. D. ZANJANI, Endocrinology *81*, 363 (1967).
[15] This work was supported by Grant No. 1126d from the Consejo Nacional de Investigaciones Cientificas y Técnicas de la República Argentina.
[16] Member of the Carrera del Investigador Científico del Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

The Effect of Conflicting Unconditioned Reflexes on Serum Cholesterol Level in the Rat

Plasma lipids, particularly cholesterol, are thought to be involved in numerous pathological conditions, hence, their regulation in the body may bear some clinical importance. That the functional state of the central nervous system (CNS) affects serum cholesterol level has been demonstrated by many investigators. Some have found an increase in serum cholesterol following augmented strain on the central nervous system[1,2], whereas others have revealed a diminution[3] or phasic changes[4] in the cholesterol concentration of blood plasma. There is reason to believe that the discrepancy between these results was due to the differences in the method employed, species, and in previous condition of the nervous system. The present work was undertaken to clarify whether and to what extent the activity level of the CNS interferes with the response in serum cholesterol level to a nervous strain if other variables, i.e. species and experimental conditions, are kept constant. Also, several endocrine aspects of the problem have been investigated.

Methods. Experiments were made on a total of 538 Wistar inbred male rats weighing 180–220 g. The rats were fed a standardized semisynthetic diet, 15 g/day. Conflicting of the thirst and escape reflexes was undertaken after water deprivation for 40 h, in a special cage shown in Figure 1, over periods of 45 min. When the animals attempted to drink, they received an electric shock, 40–50 V[5]. After 45 min of conflicting, the rats were transferred into their boxes, where they were given water ad libitum. Spontaneous intake of water was observed for periods of 15–30 min. 90 min after conflicting of the reflexes had been started, the animals were decapitated, and total cholesterol in blood serum was determined according to the method of BLOOR[6], using a Beckman DU-G 2400 spectrophotometer. About half of the animals served as controls in each group; these were treated similarly, except that they were not subjected to the conflict situation.

In some of the animals, the above treatment was preceded by similar conflicting for 1 week, 2 or 3 weeks, 3 times every week. Another group of rats was subjected only to 1 conflict situation, but methimazol (1-methyl-5-mercaptoimidazol) was given for 5 days before decapitation, in daily doses of 2.5 mg/100 g body weight, dissolved in 0.5 ml of physiological saline; the controls were given the same amount of 0.9% saline, s.c.

The effect of 40–50 V electric shocks alone was checked also in a group of rats not subjected to water deprivation and conflict situation. The shocks were applied as many times as the number of voluntary drinking attempts had been observed in the experimental group.

In some of the rats, attempts were made by a paper chromatographic method[7] to establish whether the factors causing statistically significant changes in blood cholesterol levels also affected corticosterone in the serum.

Statistical analysis of the results was undertaken by using Student's *t*-test.

Results. (1) Single conflicting resulted in a statistically significant reduction of serum cholesterol by 15 mg% (column C in the Table). Previous administration of the thyroid inhibiting agent prevented this diminution to occur (E), while physiological saline injection did not (D). Electric shocks, in the absence of conflicting, failed to affect serum cholesterol level (B).

(2) If conflicting was preceded by similar conflict situations for 3 weeks, 3 times a week, serum cholesterol

[1] N. FRIEDMANN, R. ROSEMANN and V. CARROL, Circulation *17*, 852 (1958).
[2] G. A. GOLDBERG and V. P. MILLITIN, Terap. Arkh. *37*, 71 (1965).
[3] C. HOROVITZ and B. BRONTE-STEWART, Am. J. med. Sci. *244*, 272 (1962).
[4] P. S. KHOMULO, Bull. exp. Biol. Med. U.S.S.R. *10*, 44 (1964).
[5] T. SZAMOSI, J. I. SZÉKELY and J. ZOMBORY, Kiserl. Orvostud. *17*, 144 (1965).
[6] W. R. BLOOR, J. biol. Chem. *77*, 53 (1916).
[7] P. WEISZ, E. GLÁZ, M. DEMECZKY, E. FARAGÓ and V. LAX, Kiserl. Orvostud. *13*, 54 (1961).