or brain after 17 days at 4°C in one of the snakes.

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Erythropoietic Stimulating Action of Acetylcholinesterase. (25956)

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This author reported production of experimental hyperchromic anemia in dogs by daily injection of acetylcholine(1). Liver extract or folic acid injections caused remission of this anemia with a concomitant rise in serum cholinesterase activity which was postulated to be the cause of remission. Other experiments(2) indicated that cobalt injection or production of marked anemia by phenylhydrazine caused significant increases in serum cholinesterase activity. Both of these procedures increase plasma erythropoietic stimulating factor (ESF or erythropoietin)(3,4,5). These facts suggested the possibility that cholinesterase, or part of its molecule, might be concerned with erythropoiesis, and prompted the present investigation.

Procedures and methods. Injections of Acetylcholinesterase* (200 units daily) were given subcutaneously to each of 7 normal rats weighing about 180 g. Another group of 7 untreated rats served as controls. Blood samples were obtained by cutting off the tip of the tail, before and at various intervals after commencement of daily injections. Erythrocyte counts and reticulocyte percentages were determined by standard methods, and hematocrit percentage of packed cells was determined using capillary tubes and a Clay-Adams micro-hematocrit centrifuge.

Results. Table I shows results of daily subcutaneous injections of 200 units (*i.e.,* 4 mg of commercial preparation in 1 cc of water) of Acetylcholinesterase into 7 normal rats. After 5 to 7 daily injections, reticulocyte percentages were elevated to 6.1% (average), which represented a 5-fold increase over average pre-experimental value. Erythrocyte counts were not significantly increased at this time, but after 2 weeks of daily injections, they had increased by 18% (average) or 1.4 million cells per cmm of blood. Hematocrit percentages of packed cells did not increase significantly at any time. The control group of 7 untreated rats showed no significant changes in the blood throughout period of experiments. Observations performed on only 2 experimental animals indicated that Acetylcholinesterase injections increased hemoglobin values about 10%, but caused no significant changes in total leukocyte counts.

* Acetylcholinesterase from bovine erythrocytes contained 20,000 Ammon units in 400 mg of material. (Nutritional Biochemicals Corp.)

TABLE I. Effect of Acetylcholinesterase on Blood of Rats. 200 units given daily to each experimental rat.

Group	Time of blood sampling	Reticulocytes, %	Erythrocytes in millions/mm³	Hematocrit, % cells
Exp. (7 rats)	Before start of inj.	1.2 (.8-1.7)*	7.90 (7.40-8.50)*	45.7
Control (")	Before start of exp.	1.1 (.6-2.2)	8.25 (7.50-8.75)	46.7
Exp.	After 5-7 daily inj.	6.1 (3.4-9.5)	8.15 (7.65-8.77)	45.9
Control	Untreated	1.2 (.8-1.8)	8.18 (7.51-8.55)	46.2
Exp.	After 12-16 daily inj.	2.3 (1.2-4.6)	9.30 (9.11-9.44)	46.3
Control	Untreated	1.3 (.8-2.0)	8.07 (7.76-8.78)	45.9

* Range given in parentheses.

Discussion. Acetylcholinesterase administration caused a marked increase in rate of formation or release of reticulocytes and erythrocytes. The fact that hematocrit values were not significantly changed, in spite of an increase of erythrocyte numbers, indicates that the Acetylcholinesterase causes an increased production of cells that are smaller than average in volume. Our present data do not indicate whether or not cholinesterase activity bears any relationship to plasma erythropoietic stimulating factor, although such a possibility is not precluded. Experiments are being continued in the effort to clarify this question.

Summary. Daily subcutaneous injections of 200 units Acetylcholinesterase into rats caused stimulation of erythropoiesis demonstrated by significant reticulocytosis within 7 days and increased erythrocyte counts after 14 days. Hematocrit values did not change significantly.

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Effects of Acute Hypoglycemia on Rat Testis. (25957)

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Hypoglycemia is known to exert a deleterious effect on certain endocrine glands and on cerebral structures(2,3,4) but no effect has been reported on male gonads. As these are known to consume glucose mainly(1), we attempted this histological study of the testis of rats submitted to acute hypoglycemia.

Material and methods. 318 male albino rats were used. They were separated as follows: A) Animals in which one hypoglycemic coma was induced: *Group 1*: 62 prepubertal rats weighing 35-50 g; 28 received 200 or 400 I.U. of regular insulin/kg, and 34 received Tolbutamide (2 or 3 g/kg). Insulin was injected subcutaneously and tolbutamide was given by gastric tube. Animals were killed or died in coma. *Group 2*: 84 adult rats weighing 180 to 440 g; 40 received insulin and 44 tolbutamide, at same doses and by same routes as in Group 1, and also killed or died in coma. *Group 3*: 26 adult rats weighing 200-400 g; 16 received Insulin and 10 received tolbutamide as in preceding groups, but these were injected intraperitoneally with 10 ml/kg of 10% glucose several times after administration of the hypoglycemic drug; these animals did not die in coma but were killed 5-8 hours after injection of hypoglycemic agent. B) Animals in which 2 to 5 hypoglycemic comas were induced. *Group 4*: 62