Inability of Growth Hormone to Prevent the Anemia which Follows Hypophysectomy.* (19944)

D. C. Van Dyke, M. E. Simpson, J. F. Garcia, and H. M. Evans
From the Institute of Experimental Biology and the Donner Laboratory of Medical Physics, University of California, Berkeley.

As part of a general reinvestigation of the entire problem of the endocrine control of red blood cell production, the effect of growth hormone on erythropoiesis has been investigated. When it was shown that after hypophysectomy there was a decrease in the concentration of red cells in the blood(1), hypoplasia of the erythrogenic elements of the bone marrow(2) and a decrease in the circulating red cell volume(3), it was necessary to determine which of the various hormones of the pituitary was responsible for maintaining red cell production at a normal level. To determine the importance of pituitary hormones in erythropoiesis one either removes a target organ, administers the target organ hormone, or administers the pituitary hormone. However, as growth hormone has no known target organ through which it acts, one can evaluate the importance of this hormone in maintaining the red cell volume only by the administration of growth hormone. Meyer, Stewart, Thewlis, and Rusch(4) reported that the administration of growth hormone to hypophysectomized rats for periods up to 9 weeks was invariably attended by reticulocytosis and bone marrow hyperplasia, but no increase in concentration of hemoglobin or erythrocytes. Boiling destroyed both the growth activity and the reticulocyte stimulating activity of the extract. Injection of growth hormone into normal rats produced no changes in the levels of reticulocytes, hemoglobin or erythrocytes. Overbeek(5,6) found no influence on the reticulocyte count of hypophysectomized rats after administration of growth hormone. Gaebler and Mathies(7) reported that the hemoglobin values of hypophysectomized rats injected with 1 mg growth hormone daily actually decreased during the treatment.

Because of this lack of agreement regarding the effect of growth hormone on erythropoiesis, an experiment was done to determine whether administration of growth hormone would prevent the decrease in circulating red cell volume which follows hypophysectomy.

Material and methods. Female rats of the Long-Evans strain (21 days of age) were placed in 3 groups of 17 animals each. The rats in one group were hypophysectomized, those in another were hypophysectomized and injected intraperitoneally with 1.25 mg of growth hormone daily for 30 days, and those in a third group were kept as normal controls. The growth hormone preparation used was potent in the standard 4-day tibia test when given at a daily dose of 0.025 mg. It was free from adrenocorticotropic hormone and follicle stimulating hormone, but contained some interstitial cell stimulating hormone and barely detectable traces of thyrotropic hormone at the 1.25 mg daily dose given for 30 days. Twenty-four hours after the last injection, the circulating red cell volumes were determined by the Fe^{59} labeled red cell dilution method (8).

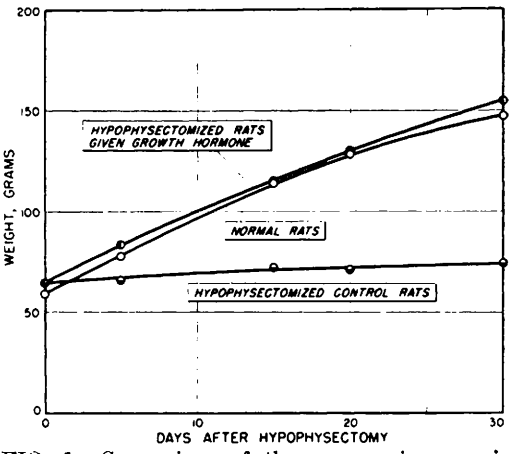

FIG. 1. Comparison of the average increase in body wt of normal rats, hypophysectomized rats, and hypophysectomized rats given growth hormone from the time of hypophysectomy.

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TABLE I. Hematological Data. 17 animals in each group.

Group	Body wt, g	Hematocrit, %	Hemoglobin, g/100 ml	Red blood cell vol/100 g body wt, ml	Red blood cells $\times 10^6$, mm^3
Hypophysectomized given 1.25 mg growth hormone daily for 30 days	156 ± 16*	37.7 ± 1.7	11 ± .8	1.78 ± .15	5.11 ± 1.17
Hypophysectomized controls	70 ± 7	39.5 ± 2.7	11.2 ± .7	1.87 ± .14	5.66 ± .61
Normal controls	149 ± 16	44.6 ± 2.1	13.7 ± 1.9	2.47 ± .16	6.02 ± .72

* Stand. dev. $= \sqrt{\frac{\Sigma (x)^2}{(N-1)}}$.

The labeled cells were obtained from a Long-Evans donor previously injected with Fe^{59}. The experimental animals were injected through the saphenous vein with 0.1 ml of donor blood containing 0.03 μc Fe^{59} in the erythrocytes. After allowing 6 minutes for the blood to mix, the abdomen was opened and a sample of blood was drawn from the vena cava into a heparinized syringe. A known volume of this blood was pipetted into a vial and its activity counted directly in a scintillation counter(9). The total blood volume was calculated from the fraction of the injected activity recovered in this sample. The hematocrits were determined in Wintrobe tubes. The total blood volume thus calculated multiplied by the hematocrit gave the total circulating red cell volume. The values for the total circulating red cell volumes were then divided by the values for the body weights of the animals and the results are presented in terms of ml of red blood cells per 100 g body weight. The hemoglobin concentration was determined by the method of Turner(10). The adrenals, thyroids and testes were weighed and sectioned in order to show the completeness of hypophysectomy and to serve as a biological assay of the injected growth hormone.

Results. The weight gain of the 3 experimental groups is plotted in Fig. 1. It will be seen that the dose of growth hormone given to the hypophysectomized animals was sufficient to maintain a normal rate of growth. The hematological data are presented in Table I. The hypophysectomized controls (uninjected) developed the typical post-hypophysectomy anemia during the course of the experiment. The circulating red cell volume was 1.86 cc per 100 g body weight as compared to that of normal controls where there were 2.48 cc per 100 g. The hypophysectomized rats injected with growth hormone sufficient to maintain a normal rate of growth throughout the experiment (30 days) developed the same degree of anemia as the uninjected hypophysectomized controls.

Summary. In the search for the factor in the anterior pituitary responsible for maintenance of the circulating red cell volume, the effect of growth hormone in maintaining the red cell volume of hypophysectomized rats was investigated. Administration of doses of growth hormone sufficient to maintain a normal rate of growth for 30 days failed to prevent the development of the anemia which characteristically follows hypophysectomy.

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Influence of Tetraethylthiuram Disulfide (Antabuse)* on Duration of Action of Thiopental. (19945)

WALLACE D. WINTERS,† F. E. SHIDEMAN,† R. K. RICHARDS, AND J. D. TAYLOR.

From the Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Mich. and the Department of Pharmacology, Abbott Research Laboratories, North Chicago, Ill.

It has been established that severe liver dysfunction in the mouse, rat, and man(1,2) results in a significant prolongation of action of thiopental. Giarman *et al.*(3) have reported a 60-fold increase in the duration of action of this drug in mice as a result of pretreatment with tetraethylthiuram disulfide (Antabuse). In view of the known inhibitory effects of this latter compound on xanthine oxidase(4), Giarman *et al.* have suggested the possibility that this enzyme may function in the metabolism of thiopental to its carboxylic acid derivative(5). If such is the case, it should be possible to demonstrate such an action of xanthine oxidase *in vitro* and an important step in the metabolism of thiopental would have been established. Prior to the institution of such *in vitro* studies it was felt that the experiments of Giarman *et al.*(3) should be repeated and extended to animal species other than the mouse. The following report is the result of such studies in the mouse, rat, and dog.

Methods and results. In Table I are summarized the data obtained from experiments on 5 groups of mice. Animals in group A received 1 g/kg/day of Antabuse orally as a 10% suspension in peanut oil. On the day following the last of 3 such administrations, these mice and 11 controls received 30 mg/kg of sodium thiopental intravenously. Duration of action was determined as the time elapsing between loss and return of the righting reflex. In the remaining 4 groups each animal served as his own control and received the same dose of thiopental as did those animals in group A. Thiopental was first administered one to 6 days before Antabuse and again 24 hours after the last dose of Antabuse. Groups B and C received Antabuse (1 g/kg) orally as a 10% suspension in peanut oil on 3 successive days. Group D received 25 mg of Antabuse (aqueous suspension) per day for 3 days. Animals in Group E received the same dose of Antabuse as animals in groups A, B, and C except that the drug was suspended in a solution of tragacanth and administered for 5 days. In none of these groups was there a significant prolongation of the action of thiopental (Table I).

Five mongrel dogs received 100 mg/kg of Antabuse (orally in capsules) daily for 3 successive days. Six days prior to the administration of Antabuse and 24 hours after the last dose of this drug each animal received 20 mg/kg of sodium thiopental intravenously and its duration of action determined as the time elapsing between the loss and return of the righting reflex. It is apparent from the data in Table II that there is no statistically significant difference between the values before and after Antabuse treatment ($p > 0.4$).

Three groups of rats (Table III) received a single dose of 1 g/kg of Antabuse‡ (10% suspension in peanut oil) by the oral route. Three to 5 days prior to the administration

* Antabuse, tetraethylthiuram disulfide, was supplied by Ayerst, McKenna and Harrison, Ltd.

† Present address: University of Wisconsin, Madison, Wisc.

‡ Daily administrations of Antabuse at a dose level of 1 g/kg for 3 days were lethal to all 10 rats in one group, therefore, a single dose of 1 g/kg was employed.