Effect of Hormone Therapy on Body Weight During Protein Depletion and Repletion.* (20199)

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There is evidence that androgens(1) insulin (2,3), and growth hormone under certain conditions(4-7) promote protein anabolism whereas adrenocortical steroids of the cortisone type augment nitrogen excretion(8,9). It was felt that these hormones (if involved in the regulation of protein metabolism) might alter the rate of loss or gain in body weight during the course of protein depletion and repletion. In earlier experiments with rats, neither the weight loss during depletion nor the gain in body weight during repletion was influenced by testosterone treatment(10). In the present communication data are presented on the effects of insulin, growth hormone, and cortisone administration on the change in body weight of rats under conditions similar to the above.

Procedure. Two experimental rations were employed in the present investigation: diet A and diet B. Diet A was a protein-free ration of the following composition: sucrose, 80%; salt mixture,‡ 5%; cellulose,§ 5%; cottonseed oil (Wesson), 8%; and wheat germ oil (Vio-Bin), 2%. To each kg of the above diet were added the following synthetic vitamins: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 60 mg; ascorbic acid, 100 mg; biotin, 5 mg; 2-methyl-naphthoquinone, 5 mg; folic acid, 10 mg; para-amino-benzoic acid, 400 mg; inositol, 800 mg; vit. B_{12}, 150 μg; and choline chloride, 2 g. Diet B was similar to diet A except that casein‖ was incorporated in this ration at a level of 24% of the diet, replacing an equal amount of sucrose. Each rat also received once weekly 4.5 mg alpha tocopherol acetate and a vitamin A-D concentrate¶ containing 150 U.S.P. units of vit. A and 15 U.S.P. units of vit. D. Eighty-four male rats of the Wistar strain which had been raised from weanling on a natural food ration** were selected at an average body weight of 252.7 g (range 201-290 g) for the present experiment. Animals were placed in individual metal cages with raised screen bottoms and were fed for 15 days the protein-free ration diet A. For the next 15 days each rat received the complete ration diet B. Animals were fed *ad libitum* and food consumption was determined for each rat. Rats were divided into 7 groups of 12 animals:

Group I was administered 0.5 cc saline solution daily during both the depletion and repletion period. *Group II* received saline solution during the depletion period and 500 μg of growth hormone†† daily during the reple-

* This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and assigned No. 417 approved for publication. The views or conclusions contained are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army. Communication No. 338 from the Department of Biochemistry and Nutrition, University of S. California. Supported in part by the Williams-Waterman Fund of the Research Corporation.

† With the School of Medicine, University of S. California, Los Angeles, and Van Camp Sea Food Co., Terminal Island, Calif.

‡ Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Chagrin Falls, O.

§ Ruffex, Fisher Scientific Co., St. Louis, Mo.

‖ Vitamin-free Test Casein, General Biochemicals, Chagrin Falls, O.

¶ Nopco Fish Oil Concentrate, assaying 800000 U.S.P. Units of vitamin A and 80000 U.S.P. units of vit. D/g.

** Purina Chow.

†† Somatrofin (STH-Horner), Frank W. Horner, Montreal, Canada. When assayed in hypophysectomized female rats, 10 μg of this material daily, for 10 days resulted (according to Dr. L. Mitchell of Frank W. Horner) in average increment of 11 g body weight. This material was dissolved in alkaline aqueous solution and the volume adjusted to contain 1 mg Somatrofin/cc.

TABLE I. Comparative Effects of Saline, Cortisone, Insulin, and Growth Hormone Administration on the Loss in Body Weight of Adult Rats During Protein Depletion. (15 days on protein-free diet.)

Series	No. of animals	Initial body wt, g	Avg loss in body wt,* g	Avg food consumption/rat during depletion, g
Saline Groups I, II, III, IV	48	252.3	56.1 ± 1.2 (47)	184
Cortisone Group V	12	253.5	53.2 ± 2.8 (12)	186
Insulin Group VI	12	253.9	55.4 ± 1.7 (11)	181
Growth hormone Group VII	12	252.3	58.3 ± 2.0 (12)	179

* Including stand. dev. of the mean.
Values in parentheses indicate No. of animals which survived and on which averages are based.

TABLE II. Summary of Data—Protein Repletion Experiment. (15 days on protein repletion diet.)

No. of animals	Material inj. during depletion period	Material inj. during repletion period	Avg food consumption/rat during repletion period, g	Avg gain in body wt over depletion wt,* g	Avg gain/100 g food ingested.* g
12	Saline	Saline	304.4	100.3 ± 2.9 (12)	33.0 ± 0.7
12	"	Growth hormone	325.5	117.4 ± 4.0 (12)	36.0 ± 0.8
11	"	Insulin	319.0	101.4 ± 6.6 (11)	31.7 ± 1.0
12	"	Cortisone	320.4	104.0 ± 4.9 (11)	32.5 ± 0.9
12	Cortisone	Saline	317.6	96.5 ± 7.1 (12)	30.3 ± 1.2
11	Insulin	"	316.7	100.8 ± 7.0 (8)	31.8 ± 1.3
12	Growth hormone	"	318.4	102.8 ± 5.1 (11)	32.3 ± 1.1

* Including stand. dev. of the mean.
Values in parentheses indicate No. of animals which survived and on which averages are based.

tion period. *Group III* received saline solution during the depletion period and 0.6 units insulin‡‡ daily during the repletion period. *Group IV* received saline solution during the depletion period and 2.5 mg cortisone acetate§§ daily during the repletion period. *Group V* received cortisone acetate in the above dosage during the depletion period and saline solution during repletion. *Group VI* received insulin in the above dosage during depletion and saline solution during repletion. *Group VII* received growth hormone in the above dosage during depletion and saline solution during the repletion period. The saline and hormone solutions were administered in divided doses twice daily. All injections were made subcutaneously.

Results are summarized in Tables I and II. Findings indicate that during the depletion period the rate of loss as well as the average total loss in body weight was similar for all

‡‡Protamin Zinc Insulin (Lilly) 0.6 unit diluted to 0.5 ml with water.

§§ Saline solution of cortone acetate, Merck and Co., Rahway, N. J., containing 25 mg cortisone acetate/cc. This material was diluted with saline solution to a concentration of 5 mg cortisone acetate/cc.

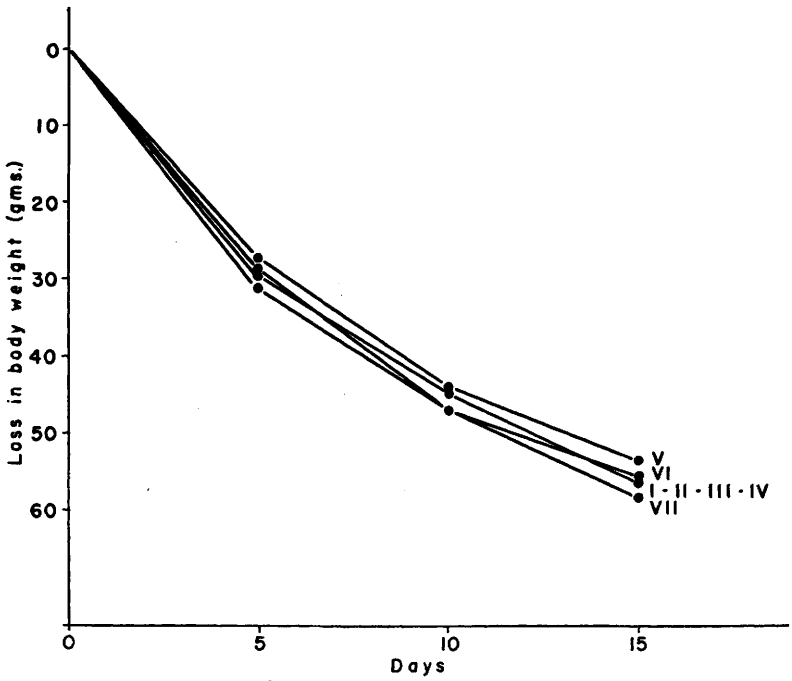

FIG. 1. Depletion experiment.
Group I, II, III and IV on saline; Group V, cortisone treated; Group VI, insulin treated; Group VII, growth hormone treated.

groups (Table I; Fig. 1). In the repletion period the initial body weight was regained in all groups after 7 days of feeding. During this period of repletion the growth increment was practically identical for all groups (Fig. 2). Subsequent to this period, however, the growth increment of animals administered growth hormone during the repletion period was greater than that of other groups. Average food consumption per rat was similar in all groups in both the depletion and repletion period. The efficiency of food utilization as judged by gain in weight per 100 g of food ingested during repletion was slightly greater for animals in group II than for other groups tested.

Discussion. Previous findings indicate that the change in body weight of rats during protein depletion and repletion was not affected by testosterone administration(10). Present data indicate that the administration of insulin and cortisone acetate was similarly ineffective. Growth hormone was without effect on the loss in body weight during protein depletion and on the gain in body weight during repletion until such time as initial body weight was regained. Once pre-depletion weight was attained, however, the growth increment was greatest in the group administered growth hormone. These findings suggest that under conditions of the present experiment exogenous sources of testosterone, insulin, cortisone, or growth hormone were without significant effect on protein depletion and repletion in the rat (as judged by change in body weight), at least in the dosage at which these hormones were administered. It is possible that larger or smaller doses of the various hormones may have yielded different results. The dosages employed, however, appeared to be of physiological "magnitude" and represented quantities which when injected into rats in other experiments showed typical hormonal effects. It is also possible that the above hormones (although without effect on body weight during protein depletion and repletion) may have exerted differential effects on the composition of the tissues. Kochakian's observation(11) that testosterone propionate hastened the replenishment of protein

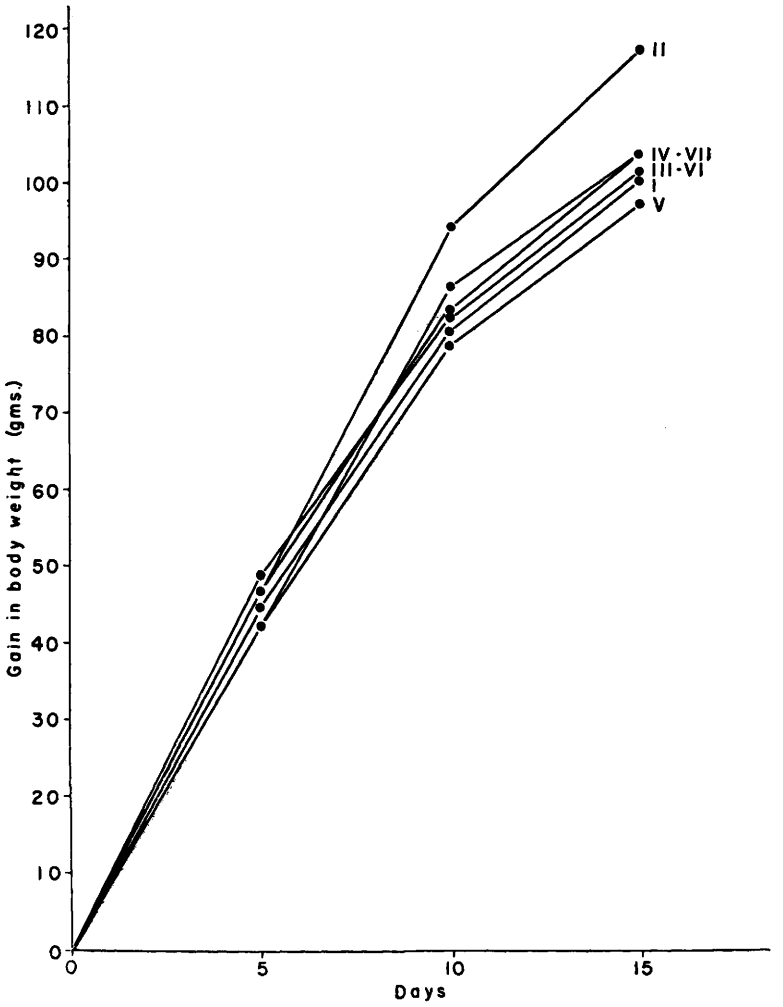

FIG. 2. Repletion experiment.
Group I, saline treated (during depletion, saline); Group II, growth hormone treated (during depletion, saline); Group III, insulin treated (during depletion, saline); Group IV, cortisone treated (during depletion, saline); Group V, saline treated (during depletion, cortisone); Group VI, saline (during depletion, insulin); Group VII, saline (during depletion, growth hormone).

in starved rats may be pertinent in this regard.

It seems likely that during protein depletion and repletion all available regulative mechanisms including hormonal factors are mobilized by the body in an attempt to maintain or restore homeostasis. This increased anabolic tendency finds its expression in the fact that the body weight lost during 15 days of depletion is restored within 5 to 7 days during repletion. It would seem plausible that in the normal intact animal the anabolic mechanisms of the tissues have an upper limit which cannot further be increased by exogenous hormone supplies.

Summary. The effects of growth hormone, insulin, and cortisone acetate administration was determined on the change in body weight of rats during protein depletion and repletion. Neither the weight loss during depletion nor the gain in body weight during repletion was affected by the administration of these hormones. After pre-depletion weight was regained, however, the growth increment for animals administered growth hormone was

larger than for the other groups.

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Received February 11, 1953. P.S.E.B.M., 1953, v82.

Complex Formation and Chemical Specificity of Boric Acid in Production of Chicken Embryo Malformations.* (20200)

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Boric acid, when injected into the yolk sac of developing chicken eggs, acts as a potent teratogenic substance(1). Following treatment at 4 days of incubation a typical syndrome arises, with features of shortening (often extreme) of the mandible, facial coloboma and cleft palate, shortening and bending of the tarsometatarsus, and poor development of the toes or their complete lack, the fourth toe being affected preferentially. Length of femur and tibiotarsus may also be reduced, depending on the amount of injected boric acid(2). Among hatched chicks the most prominent symptom is curled-toe paralysis.

These effects of boric acid on development assume particular interest on account of varied evidence, also previously discussed, pointing to the conclusion that the malformations are caused by an interference with the embryos' supply of riboflavin. In the experiments to be reported here we sought information on two points: 1. Will substances which are known to form complexes with boric acid, reduce or abolish the teratogenic qualities of the latter when they are dissolved in the boric acid solution prior to treatment or when they are used as supplements? 2. Is the teratogenic action specific for boric acid or is it, more generally, a quality of boron?

Methods. Sterile solutions of the compounds to be tested were made up in saline with 0.25% phenol added. The injections were given into the yolk sac in volume of 0.05 cc using a tuberculin syringe and No. 27 needle. The experiments were carried through to hatching. The results are based on all survivors of the 13th day of incubation.

Results. The results of experiments with compounds which are known to form complexes with boric acid are shown in Tables I and II. In a first series of tests (Table I) the teratogenic effects of boric acid were compared with the consequences of simultaneous but separate injection of boric acid and either D-ribose or pyridoxine hydrochloride. It can be seen that the addition of D-ribose or pyridoxine hydrochloride increased the chances of embryos to develop normally and reduced the incidence of every one of the different types of malformations. Considered individually, many of the differences between the group treated with boric acid and the three other groups are on the borderline of significance; but, if all features are taken into consideration, the differences become highly significant. D-

* Supported by a grant from the American Cancer Society, on recommendation by the Committee on Growth, National Research Council.