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Influence of Growth Hormone (GH) on Content of GH in the Pituitaries of Normal Rats. (30980)

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By contrast with the well known feedback mechanisms which operate between the hypothalamo-hypophyseal system and its respective target glands, the possibility of so-called auto-feedback mechanisms in which the level of a hormone would directly influence its own secretion from a gland is not well documented. That such a mechanism may exist in the case of insulin secretion was deduced from observations that insulin administration could decrease the beta cell granulation and insulin content of the pancreas(1,2).

In the case of anterior pituitary hormones, a similar mechanism may be operative for adrenocorticotrophin (ACTH) because chronic injections of ACTH were observed to decrease its content in the pituitary of adrenalectomized rats(3) and to elevate its content in the gland of normal animals(4). More recently, Szontágh and Uhlarik(5) showed that chronic administration of human chorionic gonadotrophin would reduce the hypophyseal content of LH. In the present paper, data are presented on the effects of administration of exogenous GH on its content in the pituitary which indicate that in this case also a similar auto-feedback mechanism may be postulated.

Methods. Two series of experiments were performed. In the first series, normal adult male rats of the Sherman strain (body weight 260-290 g with an average of 275 g) were injected with bovine GH (NIH, GH B7).‡ One group received a single dose of GH 24 hours before sacrifice, the second group was injected daily for 5 days and killed on the 6th day (24 hours after the last injection) and the third group was given GH for 10 consecutive days and sacrificed on the 11th day. Five animals were in each group. All the animals received 6 mg of GH per dose, that is about 2.2 mg/100 g/day. GH was given subcutaneously dissolved in physiological saline.

The second experiment was performed in the same manner, except that smaller animals were used (body weight 165-195 g with an average of 178 g) and the dose of GH was elevated to 10 mg per dose, so that the average dose per day was 5.6 mg of GH per 100 g body weight. Instead of a single control group, 3 groups of normal animals were used, which were injected with physiological saline following the same pattern as in the case of GH injections.

Both groups of experimental animals were killed by stunning followed by exsanguination, and immediately thereafter the whole pituitaries were removed. Pooled pituitary extracts from a given group of rats were

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TABLE I. GH Content, as Indicated by Tibial Epiphyseal Cartilage Width, Pituitary Weight and Blood Glucose in Animals Injected with GH.

Exp No.	Type of rat	Epiphyseal cartilage width (μ)	Donor pituitary wt (mg)	Donor blood glucose concentration (mg %)
1	Controls	222 ± 5.3 (9)*	9.5 ± .3†	102 ± 1.5‡
	GH, 1 inj§	262 ± 5.0 (5)¶	10.3 ± .4	101 ± 4.1
	GH, 5 "	229 ±10.1 (5)	8.4 ± .4	109 ± 2.7
	GH, 10 "	198 ± 4.6 (5)¶	8.5 ± .5	110 ± 6.1
2	Saline controls, 1 inj	277 ± 5.2 (6)	8.3 ± .4	97 ± 3.2
	Saline controls, 5 "	278 ± 4.0 (6)	8.8 ± .3	95 ± 4.3
	Saline controls, 10 "	288 ± 4.2 (6)	9.3 ± .4	102 ± 2.8
	GH, 1 inj	302 ± 5.4 (5)¶	8.7 ± .5	103 ± 5.1
	GH, 5 "	289 ± 3.8 (5)‖	8.3 ± .5	113 ± 3.7‖
	GH, 10 "	235 ± 2.5 (6)¶	7.9 ± .4	111 ± 4.1

* Values are mean ± standard error of mean (No. of rats).
† Five animals in each group of pituitaries.
‡ Five animals for each value of blood glucose.
§ inj = injections.
‖ $P < .05$ *vs* appropriate control.
¶ $P < .01$ *vs* appropriate control. In the first experiment, significance is determined against the single control group and in the second experiment against the proper control group.

injected into hypophysectomized rats for determination of GH activity by the tibial epiphyseal cartilage assay of Greenspan *et al* (6) as described previously(7,8). Earlier experiments in this laboratory have shown that the width of the proximal tibial epiphyseal cartilage in this assay is directly proportional to the log-dose of administered GH in the range of dosage used here and that the response to NIH bovine GH standard and pituitary extract have not differed significantly from parallelism. Since GH standard was not employed in the present experiments, results are expressed directly in terms of the width of the epiphyseal cartilage. Values for cartilage width obtained with pituitaries from the control rats were compared with those observed in the same experiment with pituitaries from animals injected with GH. Significance of differences was determined by Student's t test. A significant alteration in width of the cartilage was taken to mean that a significant alteration in the content of pituitary GH had taken place.

The hypophysectomized test animals used for the GH assay in the first series were fed a regular laboratory diet; in the second experiment the diet was supplemented by daily doses of raw meat.

Blood sugar values of the experimental animals were measured at time of sacrifice by the method of Nelson(9). In the second experiment the body weight of all animals was measured several times during the injection period.

Results. The changes of GH in the pituitary followed roughly the same pattern in both experiments (Table I). Twenty-four hours after the single injection, there was a significant increase of the GH content in the pituitary. In the first experiment, where the lower dose of GH was used, this increase disappeared after 5 days of GH application and the GH content returned to the control level. In the second experiment (with a higher dose of GH), the increased GH level persisted even on the 6th day; however, in both experiments there was a marked decrease in pituitary GH after 11 days of administration of GH. The decrease was highly significant on comparison with the untreated control group of the first experiment and on comparison to the saline-injected control groups of the second series. Injection of saline alone failed to alter the GH content of the pituitary.

Epiphyseal cartilage width was consistently wider in all groups of Experiment 2 in which the diet of the hypophysectomized rats was supplemented with meat. The supplementation with meat is the cause of this difference rather than any change in the GH content of the pituitaries as was previously demonstrated(8).

The pituitary weights followed the same trend as the GH activity; however, these dif-

ferences were not statistically significant. In both cases there was a slight elevation of blood sugar level after 6 and 11 days of treatment with GH. Except after 6 days in the second experiment, these glucose elevations were not significant statistically.

Body weight gain was greater in rats which received GH than in the controls. For example, in the second experiment the controls gained 30 and 45 g on the average at 6 and 11 days, respectively, after beginning the experiment. The GH-injected rats gained 53 and 79 g, respectively, at the same time intervals. This indicates that an effective dose of GH was employed.

Discussion. From the data presented above it appears that injections of exogenous GH lead to marked changes in the content of GH in the pituitary gland. First there is an elevation in pituitary GH level, which is already apparent 24 hours after the initial dose of GH and which may persist for several days. This elevation is followed later by a diminution in GH content. There was a difference in the temporal course of the changes in the first phase, but since the dosage of GH differed also, it is quite possible that the different dosage was the responsible factor.

Our method of evaluation does not allow any conclusive statement about the actual changes in secretion of GH which accounted for these alterations, and the interpretation of the changes in GH content is therefore speculative. It may be assumed that the high level of circulating GH decreases its release by the hypophysis. This change would lead first to an accumulation of GH in the pituitary, as a result of its continuing elaboration, and later on to a decrease in content when its production is also suppressed, presumably by the accumulated GH. This explanation appears the more plausible, because it is difficult to conceive that a surplus supply of GH could cause first an increase in its production in the gland followed by increased release, which could of course produce the observed changes in glandular content.

The present studies provide no answer as to the mechanism of action of GH in altering its own secretion rate. The changes may be induced by a direct action of GH itself or may be induced by some metabolic effect of the hormone. Since only slight alterations in blood sugar were observed, it appears unlikely that the signal could be blood sugar, if a metabolic change is the causative factor.

Also, it is not clear whether GH acts centrally on either the hypothalamus, or on the pituitary directly to alter its own secretion. Recently, David *et al*(10) have given evidence that ACTH may act directly on the hypothalamus to inhibit its own release. They have shown that implants of ACTH into the median eminence reduce adrenal activity. Similarly implants of LH have been reported to suppress LH secretion(10,11), although the results of our colleagues to date (Antunes, Molitch and Nallar, unpublished) have been of only doubtful significance. Currently, experiments with implants of GH into the hypothalamus are in progress which may resolve this question of the locus of action of GH (Molitch and Katz, unpublished).

The physiological utility of these autofeedback mechanisms remains to be clarified. They may operate only at high blood levels of the respective hormones which are above those required to produce their physiological effects as, for example, ovulation in the case of LH and nitrogen retention in the case of GH.

Summary. Alterations in the content of pituitary GH as estimated by the tibial epiphyseal cartilage assay were produced in rats by the daily, subcutaneous injection of large doses (2 and 6 mg/100 g/day) of bovine GH. An elevation of pituitary GH was observed one day after the first injection of GH. This level returned towards normal after 6 days and the GH content was depressed at 11 days following the initial injection. The injection of saline was without effect on pituitary GH. Blood glucose concentration was slightly elevated at 6 days after the first injection of the higher dose of GH. The rate of weight gain of rats treated with GH was increased. It is suggested that large doses of exogenous GH may alter its secretion by the adenohypophysis.

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Distribution of $Co^{60}B_{12}$ in Blood of Chickens and Rabbits.* (30981)

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Doran and Gregory(1) recently reported that 43 to 91% of chicken blood vit B_{12} is present in the plasma. In contrast to these results, Rosenthal and Brown(2) found that only 12% of chicken blood vit B_{12} is present in the plasma, while in mammals the vitamin is almost equally distributed between the cells and the plasma. Baker *et al*(3,4) found that red cells from normal human subjects contained about 20% of the blood vit B_{12}. Furthermore, Yamamoto *et al*(5) indicated that dog erythrocytes contain no microbiological B_{12} activity while Whipple *et al*(6) found dog red cell stroma protein to be rich in radioactivity following injection of Co^{60} cyanocobalamin during active blood regeneration.

The present study reports on the distribution of fed and injected Co^{60} cyanocobalamin in blood of rabbits and chickens in an effort to resolve the discrepancies between various laboratories by a method which is essentially unequivocal.

Materials and methods. Exp. 1. Two White Rock pullets (1.5 kg) and one Rhode Island Red hen (2.4 kg) were injected I.M. daily with 0.1 ml $Co^{60}B_{12}$† for 18 days while being fed a vit B_{12}-deficient pelleted diet.‡ Three days after the last injection, blood was obtained by heart puncture.

Exp. 2. Sixteen newly-hatched chicks of mixed sex and mixed Red and White Rock strains were obtained from a commercial hatchery. The chicks were placed on a ground purified vit B_{12}-deficient diet‡ supplemented with 8 μg $Co^{60}B_{12}$/kilo diet for 11 weeks. Two chicks died during the first 2 weeks. Seven chicks were exsanguinated by heart puncture for an initial estimation of radioactivity. The remaining 7 chicks were fed the deficient diet without added B_{12} for an additional 16 days in order partially to deplete the vit B_{12} stores and a final blood sample was obtained.

Exp. 3. Seven 30-day-old New Zealand white rabbits of mixed sexes weighing 500 g were fed a ground commercial rabbit diet (Purina Rabbit Checkers) supplemented with 2 μg $Co^{60}B_{12}$/kilo diet for 8 weeks. Two additional rabbits were fed a commercial vit B_{12}-deficient diet‡ supplemented with 2 μg $Co^{60}B_{12}$/kilo. Twenty ml blood was obtained by cardiac puncture for an initial determination. The animals were then placed on commercial pelleted rabbit diet for an additional 14 days with no added $Co^{60}B_{12}$ and a final blood sample was obtained.

All blood samples were heparinized. Whole blood and plasma radioactivity was determined in a well-type scintillation detector,

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† The activity of $Co^{60}B_{12}$ (cyanocobalamin) used throughout this study was about 1μc/μg/ml, and was generously supplied by Dr. Elmer Alpert of Merck, Sharp & Dohme Research Laboratories, West Point, Pa.

‡ General Biochemicals.