Metabolic Effects of Growth Hormone in Fasting, Phlorhizinized and Adrenalectomized Rats. (20292)

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In the rat, anterior pituitary growth hormone has been clearly shown to promote nitrogen storage(1) and to diminish the proportion of carcass fat(2). In accord with this is the report(3) that in the fasted normal rat there is a lowering of the blood sugar level, decreased urinary nitrogen excretion and increase in ketone body production after administration of anterior pituitary preparations rich in growth factor. The relationship of these phenomena to the adrenal cortex is, however, not clear. In this same study(3) there is also reported a decrease in endogenous protein catabolism and increased ketonuria after saline A.P.E. in the adrenalectomized rat. Several other authors(4-7) have reported that the adrenal gland is not necessary for the ketogenic response to growth hormone. On the other hand, it has been reported(8-10) that the ketosis caused by anterior pituitary growth preparations is diminished or completely inhibited in the absence of the adrenals.

In 1940 Gaebler and Zimmerman(11) reported on the effect of a crude anterior pituitary extract on the metabolism of phlorhizinized fed dogs. They reported a diminution of glucose and nitrogen output which was not at the expense of increased fat oxidation. Since the metabolic action of growth hormone in the adult dog differs from that in the rat, a study was undertaken of the effect of purified growth hormone on the metabolism of fasted phlorhizinized rats. In addition, the relationship of the adrenal cortex to the effects of growth hormone both in the unphlorhizinized and phlorhizinized animal was studied.

Material and methods. Young mature female rats of the inbred Wistar strain, weighing from 150 to 225 g, were used. Bilateral adrenalectomy was performed in a single stage through a dorsal cutaneous incision under ether anesthesia. Following the operation the animals were fed Purina Laboratory Chow for 2 days. On the 3rd post-operative day food was removed and on the 4th day the animals were placed in metabolism cages and 50 mg/100 g body weight of phloridzin glucoside as 10% solution in propylene glycol was injected subcutaneously(12). 0.5 mg/100 g body weight of growth hormone* was administered intraperitoneally on each of 2 days prior to and on the day of urine collection. Throughout the course of the experiments all animals received 1% saline rather than tap water. Twenty-four hour urine specimens were collected in clean vessels and preserved with a crystal of thymol and one g of citric acid. Washings from the funnels were added to the day's collection. Urine glucose was determined by the Nelson Modification of the Folin Wu Micro-Method(13). Urinary NPN was determined after deproteinization by the Micro Kjeldahl procedure(14). Acetone was determined by the method of Levine and

TABLE I. Effect of Growth Hormone on Nitrogen and Acetone Body Excretion of Normal and Adrenalectomized Fasted Rats.

	mg/100 g/24 hr	
	Range	Mean ± S.E.†
Nitrogen		
Normal (12)*	48.5–79.1	60.3 ± 2.4
Normal + G. H. (14)	34.0–70.0	53.7 ± 3.2
Adrenalectomized (12)	51.0–65.3	55.5 ± 2.4
Adrenalectomized + G. H. (22)	35.5–62.7	45.0 ± 4.1
Acetone		
Normal (12)	0 –2.2	.95 ± .21
Normal + G. H. (14)	.76–4.14	1.87 ± .30
Adrenalectomized (12)	.64–1.14	.87 ± .09
Adrenalectomized + G. H. (22)	.67–3.69	1.33 ± .34

* Figures in parentheses indicate No. of animals.

† S.E. = Standard error of mean = $\sqrt{\Sigma(x-\bar{x})^2/n(n-1)}$.

* The growth hormone used was Lot No. GH-3, which has 25% activity as compared with the standard preparation $22KR_{21}$ kindly supplied by Armour and Co. through the courtesy of Mr. Irby Bunding.

Taterka(15). The animals were divided into 8 groups, which are represented in Table I. For each experiment a series of 6 animals was used and identical experiments were repeated 2 to 4 times.

Results. I. *Effect of growth hormone pretreatment in fasted normal and adrenalectomized rats.* A) *Normal*: As seen from Table I, the fasted normal rat had a mean 24 hours urinary nitrogen excretion of 60.3 ± 2.4 mg/100 g and 0.95 ± 0.21 mg/100 g of acetone. Pretreatment with growth hormone caused a significant increase in acetone body excretion ($P < .05$) to 1.87 ± 0.30 mg/100 g and a moderate decline in nitrogen excretion to 53.7 ± 3.2 mg/100 g, which is not statistically significant. This failure of growth hormone to reduce materially the nitrogen excretion of the fasted normal rat is in accord with a statement of Bennett and coworkers(4) that "the effect of these hormones upon the nitrogen excretion of the fasted rat is not as striking as their effect upon the nitrogen excretion in the fed rat." Similarly, Harrison and Long(3) state "that when saline A.P.E., rich in growth factor was administered on the first and second day of fasting, the nitrogen excretion for the second day is not significantly less than that observed in saline controls." The increase in acetone body excretion elicited by this growth hormone preparation is not of the same magnitude as that previously reported by others(3,4). This difference is probably due to differences in the extract, dosage levels and experimental design.

B) *Adrenalectomized.* The saline treated fasted adrenalectomized rat excreted 55.5 ± 2.4 mg/100 g of nitrogen. This is not significantly less than the normal which is in accord with the findings of Ingle(16). Also, the acetone body excretion 0.87 ± 0.09 mg/100 g was almost identical with that of the normal controls. A similar type of result has been reported from *in vitro* work(17) in which it was shown that ketone body production by liver slices from normal and adrenalectomized rats does not differ. After growth hormone administration nitrogen excretion was 45.0 ± 4.1 mg/100 g and acetone body excretion was 1.33 ± 0.34 mg/100 g, neither of which are significantly different from the untreated adrenalectomized rat. The failure of growth hormone to increase acetone excretion in the adrenalectomized rat is in accord with the findings of some authors(8-10) but not with that of others(4-7). As stated above this may be due to differences in the type of extract or the experimental design.

TABLE II. Effect of Growth Hormone on Glucose, Nitrogen, and Acetone Body Excretion of Normal and Adrenalectomized Fasted Phlorhizinized Rats.

	mg/100 g/24 hr	
	Range	Mean ± S.E.†
Glucose		
Normal (24)*	178–616	381 ± 21.9
Normal + G. H. (12)	98–391	228 ± 30.1
Adrenalectomized (22)	102–232	144.5 ± 12.1
Adrenalectomized + G. H. (21)	43–284	137 ± 12.0
Nitrogen		
Normal (24)	75–143	115.7 ± 4.3
Normal + G. H. (12)	42–104	69.8 ± 5.0
Adrenalectomized (22)	13– 84	50.1 ± 4.1
Adrenalectomized + G. H. (21)	34– 80	52.0 ± 2.4
Acetone		
Normal (24)	1 –32	14.9 ± 6.3
Normal + G. H. (12)	.2–31	8.7 ± 8.9
Adrenalectomized (22)	0 –11.7	2.7 ± .76
Adrenalectomized + G. H. (21)	.5– 9.0	2.5 ± .55

* Figures in parentheses indicate No. of animals.

† S.E. = Standard error of mean = $\sqrt{\Sigma(x-\bar{x})^2/n(n-1)}$.

II. *Effect of growth hormone pretreatment in fasted phlorhizinized normal and adrenalectomized rats.* A) *Phlorhizinized normal.* The normal phlorhizinized animal excreted 381 ± 21.9 mg/100 g of glucose, 115.7 ± 4.3 mg/100 g of nitrogen, and 14.9 ± 6.3 mg/100 g of acetone per 24 hours (Table II). The values for glucose and nitrogen excretion are not significantly different than those reported by Winters *et al.*(12) using a similar technic. There are, however, quantitative differences between these results and those reported by Wells and Kendall(18) and Segaloff and Many(19). These differences are probably the result of differences in procedure, batch of phlorhizin and strain of rat. Pretreatment with growth hormone depressed markedly the excretion of glucose to 228 ± 30.1 mg/100 g and nitrogen to 69.8 ± 5.0 mg/100 g. There was, however, no significant alteration of the

rate of acetone body excretion (8.7 ± 8.9 mg/100 g) (Table II). This marked reduction in nitrogen excretion of the phlorhizinized animal is in accord with recent thought that the greatest apparent effectiveness of growth hormone is in situations where large amounts of amino acids are available for protein synthesis(20).

B) *Phlorhizinized adrenalectomized.* The phlorhizinized adrenalectomized rat excreted 50.1 ± 4.1 mg/100 g of nitrogen, 144.5 ± 12.1 mg/100 g of glucose and 2.7 ± 0.76 mg/100 g of acetone in 24 hours. The values for glucose and nitrogen excretion are almost identical with those reported by Wells and Kendall(18) for the phlorhizinized saline treated adrenalectomized rat. The marked lowering of the glucose, nitrogen, and acetone body excretion in this preparation as compared to the normal is also in accord with results of other investigators(18,19,21). After administration of growth hormone, glucose excretion was 137 ± 12 mg/100 g, nitrogen excretion was 52.0 ± 2.4 mg/100 g, and acetone excretion was 2.5 ± 0.55 mg/100 g. These values are identical with those obtained in adrenalectomized animals not receiving growth hormone (Table II).

Discussion. The basal rate of protein catabolism as reflected in the urinary nitrogen excretion is almost identical in the fasted normal or adrenalectomized and the phlorhizinized adrenalectomized animal and is not influenced by growth hormone (Tables I and II). On the other hand in the normal rat where the rate of protein breakdown was augmented by phlorhizination, growth hormone was markedly effective in causing nitrogen retention and a proportional decrease in glucose output. These results are in complete agreement with those previously reported for the fed phlorhizinized dog(11).

The results of this study suggest further that the effect of growth hormone on ketogenesis is independent of its nitrogen retaining effect. On the one hand, in normal rats a non-significant change in nitrogen excretion was accompanied by a significantly increased acetone body excretion. On the other hand, in phlorhizinized fasted rats there was a marked reduction in urinary nitrogen with no change in acetone body excretion. This concept is supported by reports that pituitary preparations of equivalent growth hormone potency have different effects on ketogenesis by liver slices and on fat mobilization(22,23).

In addition, the ketogenic activity of growth hormone apparently requires the presence of the adrenal cortex so that even under the powerful stimulus of phlorhizination growth hormone pretreatment did not increase the acetone body excretion of fasted adrenalectomized rats. This is supported by studies on hypophysectomized rats in which it was shown that the injection of growth hormone within 15 days after hypophysectomy repairs the ketogenic defect of liver slices, whereas growth hormone is without apparent effect after 50 days unless cortisone is administered simultaneously with it(22). These interpretations are also in accord with the findings that the liver fat is not increased by growth hormone preparations in either normal or adrenalectomized mice unless the animals are pretreated with ACTH or cortisone(23).

The relationship of these actions of growth hormone to its diabetogenic action is not well understood. Other workers have shown that in fed alloxanized(24) or ACTH treated(25) rats, glycosuria is augmented by growth hormone. Conversely, the present study shows that in the phlorhizinized fasted rat, glycosuria is diminished by pretreatment with growth hormone, due apparently to decreased gluconeogenesis from protein. The explanation for this difference may be that the nitrogen retaining effect of growth hormone with its associated diminution in gluconeogenesis requires a high level of insulin production.

Another explanation has been suggested, namely, that a relative preponderance of catabolic activity presumably by increased adrenal steroid levels is necessary for the diabetogenic action of growth hormone(25). However, recently we showed that the blood sugar of the adrenalectomized dog is raised by growth hormone(26), presumably due to inhibition of peripheral glucose uptake. It seems that all experimental conditions under which a diabetogenic action of growth hormone has been demonstrated in the rat, have in common an increase in available glucose

either by gluconeogenesis in the liver or from exogenous sources. In the phlorhizinized fasted rat, the action of growth hormone in inhibiting peripheral glucose uptake cannot be demonstrated since peripheral glucose utilization is already at a minimum. Therefore, under these conditions its effect in reducing hepatic gluconeogenesis from protein predominates. On the other hand in the partially pancreatectomized or ACTH treated rat the growth hormone induced inhibition of gluconeogenesis from protein is interfered with and the peripheral action of growth hormone predominates. This latter action then appears as an increase in urinary glucose output. It can be concluded then that since growth hormone has a dual function the apparent action will depend to a large extent on the metabolic status of the animal at the time of administration.

Conclusions. 1. Growth hormone causes a marked reduction in urinary nitrogen output in the fasted phlorhizinized normal rat but does not have this effect in either the phlorhizinized adrenalectomized or the unphlorhizinized normal or adrenalectomized animal. 2. In view of the ketogenic effect of growth hormone in the normal fasted rat and the absence of this effect in either the adrenalectomized, the phlorhizinized normal or phlorhizinized adrenalectomized rat the hypothesis is advanced that the ketogenic action of growth hormone is independent of its nitrogen retaining action and apparently requires the presence of the adrenal cortex for its appearance. 3. Growth hormone caused a marked reduction of glycosuria in the phlorhizinized fasted rat. This together with the reported increased glycosuria of alloxanized or ACTH treated rats after growth hormone lead to the conclusion that the diabetogenic action of growth hormone is indicative of a relative preponderance of the inhibitory action of growth hormone on peripheral glucose uptake over its action in reducing gluconeogenesis from protein in the liver.

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