Growth Hormone and Carbohydrate Tolerance in Adrenalectomized Rat.*† (25656)

NORMAN B. MARSHALL† AND FRANK L. ENGEL

Depts. of Physiology and Medicine, Division of Endocrinology, Duke University Medical Center, Durham, N. C.

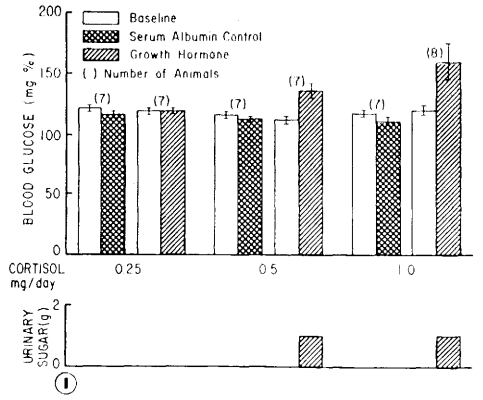
Little information is available on the adrenal requirement for producing growth hormone diabetes in the rat. It was stated earlier that anterior lobe pituitary extract was diabetogenic in the partially pancreatectomized-adrenalectomized rat only when adrenal cortical extract was administered simultaneously with the diabetogenic one(1). However, the significance of this finding is clouded by the fact that the amounts of adrenal cortical extract used in these experiments themselves induced glycosuria. The paucity of information as to diabetogenic effect of purified growth hormone in the adrenalectomized rat prompted the present investigation of adrenal requirement for production of hyperglycemia and glycosuria in the rat. Using a recently published procedure(2), which obviated use of partially pancreatectomized animals, the hyperglycemic influence of purified growth hormone was investigated in the tube-fed adrenalectomized rat maintained with various forms of adrenal cortical supportive therapy.

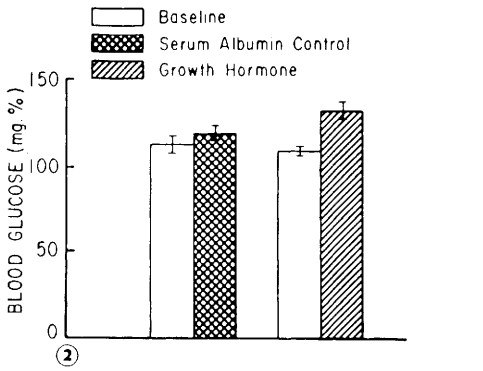
Materials and methods. Male rats of the Vanderbilt strain, weighing approximately 250 g at start of experiment, were housed in individual metabolism cages in a temperature controlled room maintained between 78-80°F. They were adapted to tube-feeding of a high carbohydrate diet(3) during an initial 5-day period. Thereafter, they received a total of 28.0 ml of diet in 2 equal feedings per day (full tube-feeding) at 8:00 a.m. and 4:00 p.m. On the 8th day of full tube-feeding, bilateral adrenalectomy was performed and the animals were placed into 6 groups which received the following types of supportive therapy: (1) 1% saline *ad lib.*, (2) 0.5 mg DOCA/day, (3) 0.1 mg DOCA/day, (4) 0.25 mg cortisol/day, (5) 0.50 mg cortisol/day, (6) 1.0 mg cortisol/day. All injections were by the subcutaneous route and were continued throughout experiment. On 8th and 9th days following adrenalectomy, baseline values for blood glucose and urinary sugar were obtained. From the 10th through the 13th days following adrenalectomy, each animal received either 2.0 mg of growth hormone/100 g of body weight or a control injection of bovine serum albumin at time of morning feeding. On the 12th and 13th days, blood glucose and urinary sugar values were obtained. This dose of growth hormone regularly induces hyperglycemia and glycosuria in intact tube-fed rats(4). Blood glucose was determined by the glucose oxidase method(5) on tail vein blood obtained 3 hours following injection of growth hormone or serum albumin and tube feeding. Urinary glucose was determined on 24-hour collections by the method of Benedict (6). Completeness of adrenalectomy was determined at end of the experiment by ability of the rats to survive a potassium load and by direct observation at autopsy.

Results. The influence of growth hormone administration on glycemia and glycosuria of adrenalectomized rats receiving various amounts of cortisol supportive therapy is illustrated in Fig. 1. There was no significant difference between baseline values and those obtained following administration of bovine serum albumin in all 3 groups. Growth hormone administration to the animals maintained on 0.25 mg of cortisol/day had no effect on blood glucose level and did not result in glycosuria. However, growth hormone administered to rats receiving 0.5 mg cortisol per day resulted in a significantly greater blood glucose level ($p<.01$) than in the injected control rats. Six of the 7 animals in this group developed a mean glycosuria of 1.04 ± 0.22 g/rat/day during the 4-day growth hormone treatment period.

* Supported by grants from Cancer Soc., Nat. Inst. Arthritis and Metab. Dis., and Office of Surgeon General, Dept. of Army.

† Present address: Dept. of Nutrition, Upjohn, Kalamazoo, Mich.





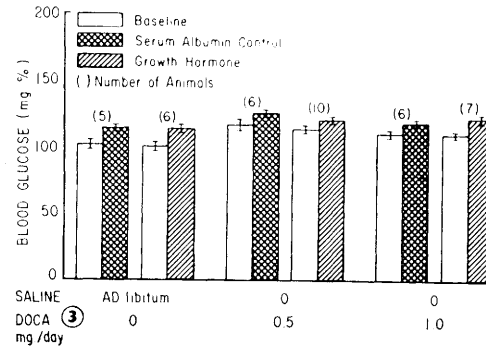


FIG. 1. Influence of growth hormone on glycemia and glycosuria of adrenalectomized, tube-fed rats maintained on various levels of cortisol.

FIG. 2. Influence of growth hormone on glycemia of adrenalectomized rats after prolonged maintenance on 0.25 mg cortisol/day.

FIG. 3. Influence of growth hormone on glycemia of adrenalectomized, tube-fed rats maintained on saline *ad libitum* or DOCA.

In the group maintained with 1.0 mg cortisol/day and receiving growth hormone, there also was a significant elevation ($p<.01$) of blood glucose over the baseline and the injected control value. Seven of the 8 growth hormone-treated rats in this group exhibited glycosuria during the 4-day treatment period which amounted to 1.0 ± 0.23 g per rat per day.

In both groups with glycosuria, the amount of sugar was slight on the first day of growth hormone administration, reached a maximum on the 2nd and 3rd days and became minimal again on the 4th day.

Administration of growth hormone to the rats receiving 0.25 mg of cortisol was repeated at a time when these animals had received cortisol for a period of 5 weeks (Fig. 2). A small but statistically significant ($p<.01$) elevation of blood glucose concentration was observed, but no glycosuria. This may have been due to the cumulative effect of the microcrystalline suspension of cortisol.

The results of growth hormone administration to adrenalectomized rats receiving either saline or DOCA in doses of 0.5 and 1.0 mg/day as supportive therapy are illustrated in Fig. 3. Growth hormone had no significant influence on blood glucose concentration or urinary glucose excretion under these conditions.

Discussion. The results of this study are consistent with the report that anterior lobe pituitary extract induces glucosuria in pancreatectomized-adrenalectomized rats maintained with adrenal cortical extract, but not saline(1). In the earlier work with anterior pituitary extract the significance of the response was uncertain since the dose of adrenal extract used itself permitted glycosuria. The present study was designed to eliminate this defect by using subdiabetogenic supportive doses of cortisol and, in addition, employed purified growth hormone.

The results obtained support the concept of a species difference regarding the influence of the adrenal cortex and its secretory products in production of growth hormone diabetes. Thus the rat, which requires a glucocorticoid for development of hyperglycemia in response to growth hormone, is unlike the dog, which does not require any adrenal cortical hormonal substitution therapy(7-9), and the cat, which seems to need a functional adrenal gland(10,11). The results with the small dose of cortisol (0.25 mg/day) indicate that magnitude and duration of adrenal substitution therapy are important factors in the rat. Pre-

sumably, this may reflect a critical level of adrenal cortical activity which must be attained in the rat to permit the hyperglycemic response to become apparent. In our experiment this may be attributed to an accumulation of depot cortisol over the 5-week period.

The glucocorticoid requirement for growth hormone hyperglycemia in the rat, demonstrated herewith, agrees with data indicating an adrenal steroid requirement for the inhibitory effect of anterior pituitary extract and growth hormone on glucose uptake by the isolated rat diaphragm. Growth hormone injected into adrenalectomized rats has been found incapable of inhibiting glucose uptake by the diaphragm *in vitro* unless the animals were pretreated with cortisone(12-14).

Summary. Growth hormone in a dose which regularly leads to hyperglycemia and glycosuria in normal tube-fed rats has no such effect in saline or DOCA maintained tube-fed adrenalectomized rats. Treatment with subdiabetogenic doses of cortisol restores the hyperglycemic response to growth hormone in adrenalectomized rats. These results are compared to previous studies of growth hormone effects in adrenalectomized cats and dogs.

We are indebted to Upjohn, Kalamazoo, Mich., for hydrocortisone (Cortef) aqueous suspension; to Ciba Pharmaceutical, Summit, N. J., for desoxycorticosterone acetate in sesame oil (Percorten) and to Endocrinology Section, Nat. Inst. Health, for growth hormone.

1. Long, C. N. H., Katzin, B., Fry, E. G., *Endocrinology,* 1940, v26, 309.

2. Owen, J. A., Engel, F. L., *ibid.,* 1957, v60, 698.

3. Bogdonoff, M. L., Scherr, E. H., Lister, L., Owen, J. A., Jr., Engel, F. L., *ibid.,* 1955, v57, 272.

4. Marshall, N. B., Engel, F. L., *Fed. Proc.,* 1959, v18, 98.

5. Saifer, A., Gerstenfeld, B., *J. Lab. Clin. Med.,* 1958, v51, 448.

6. Benedict, S. R., *J. Am. Med. Assn.,* 1911, v57, 1193

7. Houssay, B. A., Foglia, V. G., Pasqualini, C. D., *Rev. Soc. Arg. Biol.,* 1946, v22, 147.

8. Houssay, B. A., Biasotti, A., *ibid.,* 1938, v14, 308.

9. Houssay, B. A., Penhos, J. C., *Endocrinology,* 1956, v59, 637.

10. Long, C. N. H., *Harvey Lect.,* 1936-37, v32, 194.

11. Lockett, M. F., Reid, E., Young, F. G., *J. Physiol.,* 1953, v121, 28.

12. Park, C. R., Brown, D. H., Cornblath, M., Dunaghady, W. H., Krahl, M. E., *J. Biol. Chem.,* 1952, v197, 151.

13. Bornstein, J., Park, C. R., *ibid.,* 1953, v205, 503.

14. Randle, P. J., Young, F. G., in *Hormonal Regulation of Energy Metabolism,* L. W. Kinsell, ed., C. C. Thomas, 1957.

Received December 21, 1959. P.S.E.B.M., 1960, v103.

Acute Splenic Tumor Produced in Rats by a Mucoprotein Obtained from Rabbit Urine.* (25657)

G. HODGSON,† I. ESKUCHE, S. FISCHER AND M. RUBIO (Introduced by C. C. Congdon)
Inst. de Fisica y Matematicas, Lab. Fisiopatologia, Lab. Parasitologia, Universidad de Chile

When mucoprotein fractions from phenylhydrazinized rabbits' urine were assayed for erythropoietic activity, it was noted that spleens of receptor rats showed marked increase in weight, within 48 hrs. after the first of 2 injections of extracts given one every 24 hours. No correlation was seen between splenic and erythropoietic response, as measured by plasma iron turnover, and uptake of Fe^{59} in erythrocytes. Greatest splenic responses were noted in rats injected with extracts of urine of normal rabbits devoid of erythropoietic activity. Histology of hypertrophic spleens was strikingly similar to that described by Rich(1,2) for acute splenic tumor, and now recognized as a characteristic response of spleen to antigens(3). In view of the magnitude of the response observed in re-

* This work was aided in part by grant from Rockefeller Fn.

† Present address: Division of Biol., California Inst. of Technology, Pasadena.