Effect of Starvation on Pituitary and Plasma Growth Hormone in Rats[1]
(34991)

ALLEN TRENKLE
(Introduced by Wise Burroughs)
Department of Animal Science, Iowa State University of Science and Technology, Ames, Iowa 50010

Starvation of adult male rats has resulted in a decrease in the amount of growth hormone in the anterior pituitary as measured by biological assay (1, 2). Dickerman *et al.* (2) also reported lower levels of growth hormone activity in the plasma of starved male rats in comparison with rats fed *ad libitum*. Female rats have been found to have less growth hormone in the pituitary gland (3) and higher levels of the hormone in plasma (4) than males when radioimmunological methods were used to measure the hormone. The effect of starvation on plasma or pituitary growth hormone of female rats has not been reported. The purpose of the present experiment was to use radioimmunoassay techniques to study the effects of starvation of male and female rats for 2, 5, and 7 days on growth hormone concentrations in the anterior pituitary and blood plasma.

Materials and Methods. Adult male and female rats of the Wistar strain with average weights of 360 and 230 g, respectively, were used. The animals were kept in individual cages in a temperature-controlled room (24 $\pm$ 1°) with automatically controlled lights (14 hr of light daily). The rats had been maintained on a diet of Wayne Lab Blox pellets fed *ad libitum*. The rats were randomly divided into four groups consisting of four males and four females. The animals in group 1 were allowed to continue to consume the diet *ad libitum*. All feed was removed, the animals in groups 2, 3, and 4 were starved for 48, 114, and 163 hr, respectively. With minimum handling, the rats were removed from the cages and decapitated, and blood was taken from the neck in heparinized centrifuge tubes. After decapitation, the cranium was opened, and the posterior pituitary was dissected from the anterior lobe. Each anterior pituitary was weighed on a micro-Torsion balance and stored frozen in saline until extracted for assay. Growth hormone was extracted from the anterior pituitaries by grinding the glands in an all-glass tissue homogenizer with saline adjusted to pH 8.6 with 1 *N* NaOH. The homogenate was frozen and thawed before centrifuging to obtain the supernatant solution to be diluted for assay. Aliquots of the supernatant solution were diluted with sodium barbital buffer (pH 8.6, 0.1 ionic strength) containing 2.5 mg of bovine serum albumin per milliliter.

Rat growth hormone was measured by a modification of the coated-charcoal radioimmunoassay (5) using the cross-reactivity between rat growth hormone and guinea pig antiserum to porcine growth hormone (6). Purified porcine growth hormone (7) (a gift from Dr. C. H. Li) was labeled with ^{125}I (8) and just before use was further purified on a Sephadex G-100 column. Plasma samples or standards (porcine growth hormone) were incubated in quadruplicate with dilute guinea pig antiserum to procine growth hormone for 1 day at 4°. Sodium barbital buffer, pH 8.6, 0.1 ionic strength, containing 2.5 mg bovine serum albumin was used as diluent. The ^{125}I-labeled growth hormone was added to each tube, and the tubes were incubated another day at 4°. The separation of the free hormone from the hormone bound to antibodies was accomplished by adding 1.5 ml of albumin–dextran–charcoal suspension (7.5 g

[1] Journal Paper No. J-6546 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1790. This work was supported in part by NIH Grant AM-9227.

Norite A, 0.75 g dextran-80 and 3 g bovine serum albumin per 100 ml of the pH 7.4 buffer, defined by Herbert *et al.*, 5), followed by centrifuging 2 hr later. After removing the supernatant solution by aspiration, the radioactivity bound to charcoal was counted in an automatic well scintillation counter. Five point standard curves were plotted over the range from 0–1.5 ng of porcine growth hormone per assay tube. Regression coefficients of 33.7 for porcine growth hormone and 30.7 for an extract of a rat pituitary were obtained by plotting the percentage of labeled hormone bound to antibody against the logarithm of the amount of unlabeled hormone or extract added per tube. The similarity of the slopes of the two lines suggests that the antiserum did not distinguish between porcine and rat growth hormones over the range of concentrations used in this assay. Immunodiffusion in agar and gel filtration on Sephadex G-100 were also used to study the specificity of the assay. The single line of precipitation which developed between the antiserum and an alkaline extract of a pituitary fused with the line of precipitation between the antiserum and the purified procine growth hormone. The substance in the pituitary extract reacting with the antiserum was contained in a single symmetrical peak which was undistinguishable from porcine growth hormone with gel filtration.

Results. The effects of starvation on growth hormone concentrations in plasma and pituitaries in male and female rats are shown in Table I and Fig. 1. In comparison with males, females had more plasma growth hormone ($p<.01$) at each observation. In comparison with the fed animals, starved males had lower levels of growth hormone in the plasma at 48, 114, and 163 hr, but the

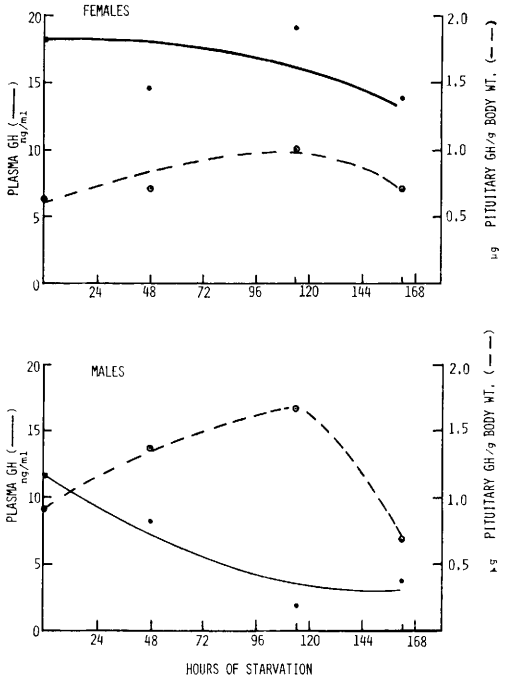

FIG. 1. Levels of growth hormone (GH) in the plasma and pituitaries of male and female rats starved from 0–163 hr.

TABLE I. Concentrations of Growth Hormone (GH) in Plasma and Pituitaries of Starved Male and Female Rats.

Sex	Starvation (hr)	Body weight (g)	Plasma GH (ng/ml)	Anterior pituitary (mg)	Pituitary GH	
					(µg)	(µg/mg)
Male	0 (4)[a]	342 ± 16.6[b]	11.7 ± 5.9	8.5 ± 0.6	312 ± 58.3	36.1 ± 4.8
	48 (4)	319 ± 19.0	8.3 ± 5.2	8.9 ± 1.0	442 ± 95.8	48.0 ± 6.2
	114 (4)	265 ± 2.7	1.8 ± 1.2	8.4 ± 0.3	440 ± 41.3	51.9 ± 3.4
	163 (4)	252 ± 7.4	3.7 ± 1.0	7.8 ± 0.5	169 ± 25.7	21.3 ± 2.0
Female	0 (4)	231 ± 6.4	18.4 ± 0.9	12.6 ± 0.9	148 ± 24.2	12.1 ± 2.6
	48 (4)	198 ± 5.3	14.6 ± 2.9	10.6 ± 1.1	140 ± 36.5	12.9 ± 2.5
	114 (4)	163 ± 4.4	19.0 ± 3.0	10.9 ± 0.9	164 ± 19.1	15.4 ± 2.2
	163 (4)	150 ± 4.7	13.8 ± 2.5	9.4 ± 0.2	106 ± 37.8	11.1 ± 3.7

[a] Number of animals per group.
[b] Standard error of the mean.

decrease was not statistically significant (NS). The females starved for 163 hr also had slightly lower (NS) levels of plasma hormone, but the decline was much less pronounced than that observed in the males.

Females had larger ($p<.01$) anterior pituitaries than males. Starvation resulted in a decrease in pituitary weight in both males and females ($p<.05$), but proportionately there was a greater loss in body weight than in weight of the anterior pituitary. The weight of the gland per unit of body weight (mg pituitary/100 g body weight) increased from 2.5 to 3.1 in the males and from 5.5 to 6.3 in the females, from 0–163 hr of starvation.

Total anterior pituitary content of growth hormone tended to increase in both sexes with 48 and 114 hr of starvation, and then decreased with 163 hr of starvation ($p<.05$ for males only). Males had more ($p<.05$) total growth hormone in the anterior pituitary at 0, 48, and 114 hr than the females; but after 163 hr of starvation, the pituitary content of the hormone was similar in both sexes. Starvation of the males for 48 and 114 hr resulted in an increase ($p<.05$) in concentration of growth hormone in the anterior pituitary, while starvation for 163 hr caused a decrease ($p<.05$). The same trends in concentration of the hormone in the glands were observed with the females, but the differences were not statistically significant. Starvation of the females for 163 hr did not significantly decrease concentration of growth hormone in the pituitary in comparison with the animals that had been fed. Males in comparison with females had higher pituitary concentrations of growth hormone at each time of observation. Data in Fig. 1 indicate there were no significant relationships between pituitary content of growth hormone and plasma levels of the hormone.

Discussion. The data obtained in this study indicate that starvation of rats for up to 7 days results in lower levels of growth hormone in plasma and the anterior pituitary in both males and females. The response, however, was more pronounced in males. The reason for this difference between males and females is not obvious. The results obtained in this study with radioimmunoassay agree with data obtained by bioassay of plasma and pituitaries from starved male rats (1, 2).

The increase in growth hormone content in the pituitary with 48 and 114 hr of starvation, when plasma levels of the hormone were decreasing, suggests there might have been less stimulation by the growth hormone-releasing factor from the hypothalamus in the starving animals. A reduction in hypothalamic concentration of the growth hormone-releasing factor in male rats after starvation for 5 days (9) and 7 days (2) has been reported. The decrease in concentration of growth hormone in the pituitary observed at 7 days may have been an indication of decreased synthesis of the hormone because of less available energy and amino acids during starvation. Srebnick *et al.* (10) reported that feeding rats protein-free diets for 5 weeks reduced the amount of growth hormone in the anterior pituitary and plasma. The differences between males and females in pituitary content and concentration of growth hormone agree with the results reported by Birge *et al.* (3) who proposed that the gonadal hormones accounted for these differences in quantity of pituitary growth hormone. The higher levels of plasma growth hormone along with lower concentrations of the hormone in the pituitaries of the females observed in this study as well as by others (3, 4) suggest greater secretion of growth hormone in females than in males.

There was no positive relationship between the amount of growth hormone in the pituitary and the plasma. Concentrations of growth hormone in the pituitary were higher in the males than in the females, whereas plasma levels of the hormone were higher in the females than in the males. Fasting of the male rats for 48 or 114 hr tended to increase the amount of growth hormone in the pituitary, while the amount in the plasma decreased.

Summary. Growth hormone was measured by radioimmunoassay in anterior pituitaries and plasma obtained from adult male and female rats that had been fed or starved for 48, 114, and 163 hr. Starvation for 163 hr resulted in lower levels of growth hormone in

plasma and pituitary of both sexes. Pituitary content and concentration of growth hormone were both increased with 48 and 114 hr of starvation and then were decreased at 163 hr in comparison with the animals fed *ad libitum*. The males consistently had smaller anterior pituitaries, higher concentrations of growth hormone in the piutitary, and lower levels of the hormone in plasma in comparison with females.

The author acknowledges the technical help of Kathy Hopkins.

1. Friedman, R. C., and Reichlin, S., Endocrinology **78**, 787 (1965).

2. Dickerman, E., Negro-Vilar, A., and Meites, J., Endocrinology **84**, 814 (1969).

3. Birge, C. A., Peake, G. T., Mariz, I. K., and Daughaday, W. H., Endocrinology **81**, 195 (1967).

4. Schalch, D. S., and Reichlin, S., Endocrinology **79**, 275 (1966).

5. Herbert, V., Lau, K-S, Gottlieb, C. W., and Bleicher, S. J., J. Clin. Endocrinol. **25**, 1375 (1965).

6. Trenkle, A., and Li, C. H., Gen. Comp. Endocrinol. **4**, 113 (1964).

7. Papkoff, H., Li, C. H., and Lui, W-K., Arch. Biochem. Biophys. **96**, 216 (1962).

8. Greenwood, F. C., Hunter, W. M., and Glover, J. S., Biochem. J. **89**, 114 (1963).

9. Meites, J., and Fiel, N. J., Endocrinology **77**, 455 (1965).

10. Srebnik, H. H., Nelson, M. M., and Simpson, M. E., Proc. Soc. Exp. Biol. Med. **101**, 97 (1959).

Received May 22, 1970. P.S.E.B.M., 1970, Vol. 135.