Influence of Stress on the Growth Hormone (GH) Content of the Pituitary of the Rat.* (31205)

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Insulin-induced hypoglycemia or injection of α-2-deoxyglucose produced a marked depletion of GH from the hypophysis of the rat (1). On the other hand, repeated injections of glucose led to a slow accumulation of GH in the pituitary. A large dose of epinephrine had a biphasic effect producing first a short-lived depletion followed by a slight increase. Hypoglycemia, large doses of both deoxyglucose or epinephrine and even repeated injections of glucose could be considered as examples of non-specific damaging agents or as stressful situations. Therefore, it appeared worthwhile to study the influence on the GH content of the pituitary of other types of stress in which carbohydrate metabolism was unaffected in order to determine if stressful situations *per se* might alter GH secretion. The influence on pituitary GH content of a variety of stresses was evaluated in the present study.

Methods. Normal adult male rats of the Sherman strain weighing 200-220 g were subjected to the various treatments described below and were sacrificed at the appropriate time by stunning followed by exsanguination. The following types of stimuli were employed:

1) *Splenectomy.* The spleen was removed through an abdominal incision from rats which were anesthetized with ether. Care was taken to avoid injury to the pancreas.

2) *Formaldehyde.* Various dilutions and doses were given as described in the *Results*.

3) *Cold exposure.* In this case only, the rats were caged individually to prevent their congregating together and were placed in a room at 3°C for 1-3 hours.

4) *Sound.* A loud, high-pitched doorbell was placed in the cage and rung for 3 min out of every 5 min for 30 min.

5) *Fasting.* Groups of rats were deprived of food, but given free access to water for variable times prior to sacrifice.

At sacrifice pituitaries were removed from each group of experimental rats and from a group of untreated control animals. The glands were extracted in physiological saline solution, and the GH activity of the pooled pituitary extract from each group of rats was estimated by the tibial epiphyseal cartilage test of Greenspan *et al*(2). A dose of pituitary extract equivalent to 2 mg of pituitary was administered each day for 3 days. On the fourth day the width of the epiphyseal cartilage was determined. This bears a linear relationship to the log-dose of administered GH in the dose-range employed here. The width of the cartilage in hypophysectomized rats which were injected with pituitaries from the various experimental groups was compared to the width obtained with pituitaries from the controls in each experiment. Significant widening or thinning of the cartilage on comparison with the controls was taken to mean a significant increase or decrease, respectively, in pituitary GH. Significance of differences was determined by Student's t test. Full details of these procedures have been published(1,3).

Results. Splenectomy. There was a decrease in pituitary GH activity 15 minutes after splenectomy in 2 experiments (Table I, Exp. 6, 7). This change was significant ($P < .01$) in one experiment and highly significant ($P < .01$) if the results of the 2 experiments were pooled. At 30 minutes after the operation, variable results were obtained, a significant rise in one case and a significant fall in the other (Exp. 4 and 6). By one hour a significant elevation in GH activity was observed in 4 of 5 experiments (Exp. 1-5). This was still apparent at 3 hours in 2 of 3 trials (Exp. 1, 2, 4) and at 7 hours in a single experiment (Exp. 2).

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TABLE I. Effect of Splenectomy on Pituitary GH Activity.

Exp #	Treatment	Width of tibial epiphyseal cartilage of hypox recipients (μ)	Blood glucose (mg %)
1	Controls	233 ± 6.8 (6)*†	98
	Splenectomy—1 hr	273 ± 5.2 (6)[2]	162
	" 3 "	285 ± 5.0 (6)[2]	170
2	Controls	239 ± 2.4 (5)†	96
	Splenectomy—1 hr	282 ± 5.6 (5)[2]	202
	" 3 "	275 ± 4.0 (6)[2]	130
	" 7 "	293 ± 7.0 (6)[2]	111
3	Controls	277 ± 1.9 (5)‡	103
	Splenectomy—1 hr	301 ± 2.0 (6)[2]	147
4	Controls	259 ± 2.8(14)‡	97
	Splenectomy—½ hr	285 ± 5.3 (6)[2]	133
	" 1 "	274 ± 4.8 (6)[1]	129
	" 3 "	265 ± 3.6 (6)	114
5	Controls	301 ± 3.6 (5)‡	103
	Splenectomy—1 hr	298 ± 9.8 (6)	117
	" 3 "	305 ± 4.4(10)	110
6	Controls	303 ± 5.0 (5)‡	97
	Splenectomy—¼ hr	285 ± 7.7 (6)	132
	" ½ "	279 ± 7.1 (6)[1]	123
7	Controls	302 ± 3.1 (5)‡	101
	Splenectomy—¼ hr	278 ± 3.0 (6)[2]	133

Hypox = hypophysectomized.
* Mean ± SEM (No. of hypox rats).
† Diet not supplemented by meat.
‡ Diet supplemented by meat.
[1] $P < .05$ *vs* controls.
[2] $P < .01$ " " .

Blood sugar was consistently elevated within 15 minutes of splenectomy, but the change was minimal in the one experiment where no significant change in GH activity was observed 1 and 3 hours post-operatively (Exp. 5).

On the basis of all these experiments it would appear that splenectomy produces a transient fall in pituitary GH activity which is followed within an hour by a rise in this activity which may persist for at least 7 hours.

The width of the epiphyseal cartilage was reduced in all groups of hypophysectomized rats in which the diet was not supplemented by meat (Table I-V). This behavior of the cartilage has been noted earlier and was observed consistently in the present experiments.

Formaldehyde. These injections were uniformly followed by an increase of GH activity in the pituitary which was significant at 30 minutes in 2 of 3 experiments and at 60 minutes in both experiments in which it was estimated (Table II). The most clear-cut effect was observed in the second experiment where the large dose of formalin evoked systemic signs such as cyanosis and froth at the nose in addition to the signs of pain observed in all 3 experiments. No depletion was observed at 15 minutes in the one experiment in which this time interval was evaluated. Blood sugar was not elevated in any of the 3 experiments.

Sound. Similar results were observed as in the case of formaldehyde, but they were statistically significant in only one of 3 experiments (Table III). Since the controls in each experiment did not differ significantly, it was legitimate to pool the results of all 3 trials. Highly significant increases in GH activity were observed at 30 and 60 minutes with the pooled results. Blood glucose was

TABLE II. Effect of Formaldehyde Injections on Pituitary GH Activity.

Exp #	Dose of formalin	Treatment	Width of tibial epiphyseal cartilage of hypox recipients (μ)	Blood glucose (mg %)
1	.5 ml 2% s.c.	Controls	263 ± 2.8 (6)*†	96
		Formaldehyde—½ hr	269 ± 5.7 (6)	107
		" 1 "	279 ± 4.2 (5)[1]	101
2	.2 ml 20% i.m.	Controls	277 ± 7.7 (6)†	100
		Formaldehyde—½ hr	313 ± 8.2 (5)[1]	102
		" 1 "	324 ± 7.3 (4)[2]	96
3	.2 ml 10% i.m.	Controls	288 ± 5.9 (5)†	97
		Formaldehyde—¼ hr	299 ± 6.4 (6)	102
		" ½ "	306 ± 2.6 (5)[1]	92

Hypox = hypophysectomized. [1] $P < .05$ *vs* controls. [2] $P < .01$ *vs* controls.
* Mean ± SEM (No. of hypox rats).
† Diet supplemented by meat.

TABLE III. Effect of Sound on Pituitary GH Activity.

Exp #	Treatment	Width of tibial epiphyseal cartilage of hypox recipients (μ)	Blood glucose (mg %)
1	Controls	263 ± 2.8 (6)*†	
	Sound—1 hr	277 ± 3.6 (6)	
2	Controls	264 ± 4.7 (6)†	98
	Sound—½ hr	288 ± 5.3 (6)[1]	103
	" 1 "	281 ± 4.1 (5)[1]	96
3	Controls	277 ± 7.7 (6)†	100
	Sound—½ hr	295 ± 7.0 (5)	
Pooled	Controls	267 ± 2.8(18)	99
	Sound—½ hr	291 ± 4.4(11)[2]	102
	" 1 "	279 ± 2.8(11)[2]	98

Hypox = hypophysectomized.
* Mean ± SEM (No. of hypox rats).
† Diet supplemented by meat.
[1] P <.05 *vs* controls.
[2] P <.01 " " .

not altered in this situation.

Cold. In marked contrast to the preceding experiments the GH content of the pituitary of the rats exposed to cold showed a marked depletion at 1 hour with a return to normal or slightly elevated values at 3 hours (Table IV). Again, blood sugar was not significantly altered.

Fasting. In this case the results were complicated. A marked depletion of GH was observed in both experiments at 16 hours after onset of the fast (Table V). The value had returned to base line (Exp. 1) or was elevated (Exp. 2) by 40 hours and declined again at 64 hours (Exp. 1) and at 88 hours (Exp. 2). In both instances blood sugar was already reduced by 16 hours, but there was a tendency for it to rise slightly at 40 hours.

TABLE IV. Effect of Cold on Pituitary GH Activity.

Exp #	Treatment	Width of tibial epiphyseal cartilage of hypox recipients (μ)	Blood glucose (mg %)
1	Controls	233 ± 6.8 (6)*†	98
	Cold—1 hr	199 ± 5.0 (5)[2]	118
	" 3 "	241 ± 3.4 (5)	116
2	Controls	247 ± 5.1 (6)†	87
	Cold—1 hr	214 ± 5.9 (6)[2]	80
	" 3 "	266 ± 3.9 (6)[1]	91
Pooled	Controls	239 ± 4.9(12)	93
	Cold—1 hr	207 ± 4.7(11)[2]	98
	" 3 "	254 ± 4.7(11)	103

Hypox = hypophysectomized.
* Mean ± SEM (No. of hypox rats).
† Diet not supplemented by meat.
[1] P <.05 *vs* controls.
[2] P <.01 " " .

Discussion. The effects of a number of acute stimuli on the GH content of the pituitary have now been evaluated. Insulin-induced hypoglycemia produced a fairly long-lasting decrease in GH with a return to normal after 3 hours(1). A similar depletion was observed in cold-exposed rats in the present study. Epinephrine(1), *a*-2-deoxyglucose(1), an inhibitor of carbohydrate metabolism, and splenectomy have also induced transient depletions. No decrease was observed in animals which were either injected with formalin or exposed to the sound of a doorbell.

TABLE V. Effect of Fasting on Pituitary GH Activity.

Exp #	Treatment	Width of tibial epiphyseal cartilage of hypox recipients (μ)	Blood glucose (mg %)
1	Controls	242 ± 3.4 (17)*†	86
	Fasting—16 hr	203 ±10.6 (9)[2]	61
	" 40 "	244 ± 4.9 (9)	80
	" 64 "	208 ± 5.0 (9)[1]	76
2	Controls	284 ± 2.1 (6)‡	98
	Fasting—16 hr	256 ± 5.3 (6)[2]	54
	" 40 "	313 ± 6.0 (6)[2]	65
	" 64 "	310 ± 6.7 (5)[2]	64
	" 88 "	269 ± 4.3 (5)[1]	58

Hypox = hypophysectomized rats.
* Mean ± SEM (No. of hypox rats).
† Diet not supplemented by meat.
‡ Diet supplemented by meat.
[1] P <.05 *vs* controls.
[2] P <.01 " " .

All of the acute stimuli with the exception of hypoglycemia, *a*-2-deoxyglucose, and cold produced a delayed increase in pituitary GH which was apparent within 30 to 60 minutes of application of the stress. Injection of glucose has similarly produced elevations in pituitary GH activity.

It is clear from these results that some of the stimuli used are associated with alterations in carbohydrate metabolism, such as insulin-induced hypoglycemia, but it is equally clear that a variety of stimuli which appear to have little influence on carbohydrate metabolism are also effective. We have no certain explanation for the differences in be-

havior of pituitary GH content in response to different stimuli, but it would appear that a variety of non-specific stimuli can influence GH secretion in the rat.

The acute depletions in pituitary GH are interpreted to mean that a release of the hormone has taken place. The subsequent elevations seen later may be related to increased synthesis of hormone, consequent to increased release, but we realize that such interpretations are only speculative in the absence of measurements of plasma GH. In man, it has been shown that insulin-induced hypoglycemia elevates plasma GH(4). The influence of non-specific stress is less clear, although it has been observed that exercise will also increase plasma GH(4).

Further evidence for an effect of stress on plasma GH in the monkey has just been reported by Knobil(5). He has observed elevations in plasma GH as determined by immunoassay following administration of epinephrine and large doses of vasopressin. Similar results have been obtained by Smith *et al*(6).

Fasting which might be considered a subacute stimulus produced a decline in GH activity at 16 hours with later rebound followed by still another decrease at 64 or 88 hours. Friedman and Reichlin(7) and Meites and Fiel(8) have also studied the effect of fasting on GH content. The latter authors reported a reduction in pituitary GH content 120-170 hours after initiation of the fast in agreement with our data, whereas Friedman and Reichlin(7) reported a progressive decrease in pituitary GH during fasting in a single experiment, instead of the biphasic response observed in the present experiments. The reason for the difference between their results and our own is not apparent. An elevation in GH in plasma of fasting humans has been reported by Roth *et al*(4), so the altered pituitary content of GH is probably a reflection of the balance between increased release and synthesis of the hormone.

Several groups(9-11), including our own (3), have reported the existence of a GH-releasing factor (GH-RF) in the hypothalamus as determined by both *in vivo* and *in vitro* tests. The active factor has recently been purified and separated from other releasing factors(12). The *in vivo* test for the GH-RF has been based on depletion of pituitary GH 30-60 minutes after the intraperitoneal, intravenous, or intracarotid administration of hypothalamic extract. Since a variety of non-specific stimuli are also capable of depleting pituitary GH, this raises a question about the specificity of the results obtained with GH-RF. It is noteworthy that pituitary GH remains low for at least 60 minutes after injection of GH-RF(3) whereas it had returned to normal or was elevated within 30-60 minutes of application of most non-specific stimuli. Furthermore, cerebral cortical extracts, vasopressin, α melanocyte stimulating hormone, and a variety of inactive hypothalamic fractions failed to mimic the effect of GH-RF(3,12). Consequently, it would appear that a specific GH-RF does exist, but it is also becoming clear that a variety of apparently non-specific stimuli can evoke alterations in GH secretion. Presumably these stimuli reach the hypothalamus by way of various afferent pathways and there trigger release of GH-RF which causes release of GH. Further work is required to determine if changes in availability of carbohydrates as exemplified by insulin-induced hypoglycemia and fasting operate as non-specific stresses or in a more specific fashion, *i.e.*, by influencing hypothetical gluco-receptors in the hypothalamus. In any event, it is clear that GH secretion is not static but is often modified by a variety of stimuli to a degree reminiscent of adrenocorticotrophin secretion.

Summary. Pituitary GH activity was estimated by the tibial epiphyseal cartilage test in hypophysectomized rats. Experimental animals were exposed to 5 different stimuli. Splenectomy evoked a transient decrease in pituitary GH which was followed by a rise in GH activity apparent within 60 minutes of operation. Formalin injections similarly produced an elevation of pituitary GH activity at 30 to 60 minutes after injection. Ringing a doorbell in the animal's cage intermittently for 30 minutes also elevated pituitary GH. Exposure to a temperature of 3°C for 60 minutes produced a depletion of pituitary GH. A biphasic response was observed in

fasting, that is an initial depletion, return to base line, and finally depletion observable 64-88 hours after onset of the fast. It was concluded that a variety of apparently unrelated stimuli could alter the GH content of the pituitary. Taken together with other evidence it appears that GH secretion in the rat is very labile and influenced by a variety of stresses as well as by alterations in the supply of available carbohydrate.

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Gestational and Dietary Influences on the Lipid Content of the Infant Buccal Fat Pad.* (31206)

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The buccal fat pad contributes significantly to the prominence of the cheek of the newborn and infant. It is well developed in the premature fetus, appears to be one of the earliest sites of well developed fetal adipose tissue deposition, and probably is important in the sucking mechanism.

The preservation of the buccal fat pad during emaciated states of infancy and childhood remains unexplained. Early investigators(1) believed that compositional differences might explain this phenomenon; however, Shattock(2) in 1909 reported that the chemical composition of the buccal pad was identical to subcutaneous fat utilizing iodine values and melting points. Inadequate methodology prevented detailed chemical analysis. Recent technologic advances now make it possible to obtain an accurate fatty acid analysis with relative ease. To determine whether biochemical differences explain the resistance of this tissue to mobilization during emaciated states, this specialized adipose tissue was analyzed and its composition compared to that of abdominal subcutaneous adipose tissue during various stages of intrauterine and post-partum development.

Methods. Obtaining the sample. The buccal pad is easily exposed through a small cruciate incision in the buccal mucosa inferior to the zygomatic bone in a fossa formed by the masseter, buccinator, and platysma muscles. Small pieces of the right buccal fat pad and abdominal subcutaneous fat were obtained at necropsy from unfed infants who expired within 24 hours after birth. Infants of mothers with known metabolic disease were excluded from this study. Comparable specimens were obtained from older children who died from non-metabolic causes.

Preparation and analysis of the sample. Fragments of tissue were pulverized with a glass rod during lipid extraction in a 1:1 mixture of petroleum ether (BP 30°-60°C) and isopropyl alcohol. Fatty acid methyl esters

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