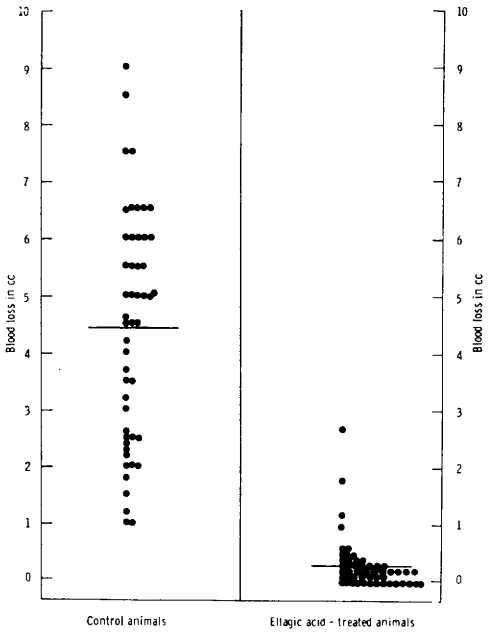

FIG. 1. Distribution of blood loss values (in ml) in control and ellagic-treated animals. Horizontal bars represent the mean in each group.

hemorrhage had stopped, during the 2-week period of observation. No untoward side effects of ellagic acid were noted.

Discussion. Ellagic acid induces increased coagulability presumably by activating Hageman factor(1,2,3). In the course of our experiments(3) it was noted that bleeding from vessel punctures was less in the ellagic acid treated animals than in controls.

The present controlled study has confirmed that ellagic acid in small amounts prevents bleeding, at least from the rat's tail, even though the duration of activity of ellagic acid is short in the rat (about 20 minutes) (3).

Presumably ellagic acid prevents bleeding by activating the coagulation mechanism at an early stage. Other mechanisms are possible but seem unlikely. Vasoconstriction could prevent bleeding but activation of Hageman factor should elicit vasodilation(4), and furthermore, with vasoconstriction only, there is usually delayed bleeding which was not seen in these animals. Ellagic acid might induce platelet plug formation at the site of vessel injury, a common method of control of hemorrhage(5,6) but there has been no evident effect of ellagic acid on platelet function(2,3).

The lack of toxicity and the failure to produce marked changes in the clotting mechanism, which might produce other complications, makes this a most interesting compound.

Summary. Intravenous injection of ellagic acid causes marked statistically significant decrease in bleeding after amputation of the tail in the rat.

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Growth Hormone-Releasing Activity of Crude Ovine Hypothalamic Extracts. (30480)

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Although it is now apparent that the hypothalamus exercises a controlling influence over the secretion of anterior pituitary hormones, evidence for hypothalamic control of growth hormone (GH) secretion has been slow to accrue. This is probably related to the complexity of the growth process. The finding of stunted growth after hypothalamic lesions by itself is not sufficient proof that secretion of GH is impaired(1,2). Reichlin(1) has re-

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ported that massive ventral hypothalamic lesions lowered the content of GH in the pituitary; however, the possibility of ischemia of the pituitary as a result of disruption of hypophysial portal vessels cannot be excluded in his experiments. Franz *et al*(3) were probably the first to report a growth-promoting effect of hypothalamic extracts from dogs. More recently, Deuben and Meites(4) showed that crude acidic extracts of rat stalk-median eminence (SME) tissue added to the pituitary in tissue culture increased the release of GH into the medium. Pecile *et al*(5) have now reported that the intracarotid (ic) administration of rat hypothalamic extracts can lower the content of GH in the pituitary. The results reported here show that ovine hypothalamic extracts can deplete hypophysial GH when administered parenterally by the intraperitoneal (ip), intravenous (iv), or ic route; and that several other substances are inactive.

Methods. Preparation of brain extracts. Ovine SME or cerebral cortical tissue was made into an acetone powder.† This powder was extracted with 4 M acetic acid at room temperature and centrifuged in a refrigerated centrifuge. The supernatant extract was lyophilized and the solid material extracted with glacial acetic acid. After dilution with 3-4 volumes of glass distilled water, the glacial acetic acid extract was lyophilized. The detailed procedure has been reported(6). The resultant powder was dissolved in saline, 0.1 N acetic acid, or in 0.1 M ammonium acetate buffer (pH 5.5) prior to injection into animals. Each animal received a dose of 4 mg/100 g body weight of the powder dissolved in 0.25 ml of diluent. One mg of the powder was roughly equivalent to one sheep SME with a wet weight of 60 mg.

Bioassay of GH-releasing activity. Normal adult males of the Sherman strain (200-220 g) were used throughout with the exception of one experiment in which rats of different sizes were employed. The extract or drug to be evaluated was injected ip, iv, or ic, and at various intervals after injection the animals were killed by stunning followed by exsanguination from the heart. The rats were lightly anesthetized with ether prior to iv or ic injections.

Whole pituitaries were removed immediately, weighed on a torsion balance and stored on dry ice. All glands belonging to one experimental group (usually 5-6) were pooled, homogenized in physiologic saline (5 mg/ml), and the homogenate centrifuged. The supernatant was stored in the refrigerator during the time required for assay which was begun immediately. Just prior to use, an aliquot of the extract was diluted with saline so as to contain the equivalent of 2 mg of fresh pituitary per ml. This dose was then injected subcutaneously into young hypophysectomized female rats‡ once daily for 3 successive days. The hypophysectomized test animals were killed on the fourth day, and the GH content of the pituitary extract evaluated from the width of the tibial epiphyseal cartilage by the method of Greenspan *et al* (7).

Several dose-response curves with both pituitary extracts and the NIH bovine GH standard§ were performed during the course of the whole experiment. The slopes of the log dose-response lines for pituitary extract and standard did not differ significantly from parallelism. The mean lambda of 6 successive experiments was 0.140. Details of the dose response relationship and of the care of the hypophysectomized animals will be described elsewhere.

Because GH standard was not used in each experiment, it is not possible to express the changes in pitutary GH content in terms of the standard. Instead, the results are expressed directly in terms of the width of the epiphyseal cartilage. The values obtained with glands from animals injected with hypothalamic extract were compared with those obtained in the same experiment with pituitaries from control animals, which either received an injection of diluent or no treatment. Significance of differences was deter-

† We are indebted to Mr. James Justra and Christopher Wohrle of Miller Abattoir, North Bergen, N. J. for generous supplies of hypothalami.

‡ Received from Hormone Assay Laboratories, Chicago, Ill.

§ We are indebted to the Endocrinology Study Section for supplies of purified bovine growth hormone.

TABLE I. Effect of ip or ic Injection of Hypothalamic Extracts Dissolved in Saline on Pituitary GH Activity (30 Min After Injection).

Treatment	No. of test rats	Width of tibial epiphyseal cartilage (μ)*
ip saline	6	247 ± 5.1
ip SME extract	6	202 ± 6.8†
ic saline	6	265 ± 4.0
ic SME extract	6	255 ± 4.8

* Mean ± SEM.
† $P < .01$ *vs* appropriate control.

TABLE II. Effect of ip Injections of Crude Hypothalamic Extracts Dissolved in Acetic Acid on GH Activity in the Pituitary.

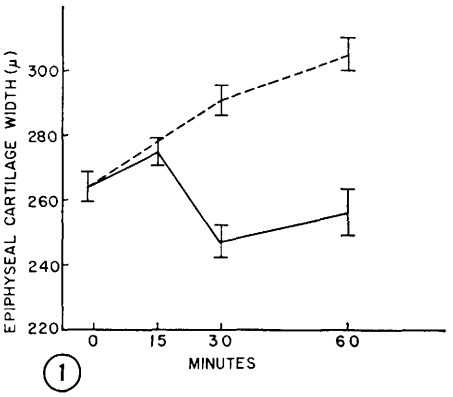
Treatment	No. of test rats	Width of tibial epiphyseal cartilage (μ)*
Non-injected controls	11	264 ± 4.4
Acetic acid-injected controls (30 min after injection)	4	291 ± 4.2
Acetic acid-injected controls (60 min after injection)	6	305 ± 5.2
SME extract (15 min after injection)	6	275 ± 3.8
SME extract (30 min after injection)	5	247 ± 5.0†
SME extract (60 min after injection)	6	256 ± 7.2†

* Mean ± SEM.
† $P < .01$ *vs* appropriate control.

mined by Student's "t" test. A significant decrease in width of epiphyseal cartilage in rats injected with pituitaries from animals receiving extract was taken to mean that a significant decrease in pituitary GH had occurred.

Results. Effect of ip or ic injection of SME extract dissolved in physiological saline. A significant depletion in pituitary GH as evidenced by a decrease in width of the tibial epiphyseal cartilage was seen 30 minutes after the ip injection of extract on comparison with the saline-injected controls (Table I). No significant change in GH activity was observed 30 minutes after the ic administration of extract; however, it is noteworthy that the level of GH activity in the controls given ic saline was significantly higher than that seen in controls which were injected with saline ip.

Effect of ip injection of SME extract dissolved in 0.1 N acetic acid. Because the hypothalamic powder was not readily soluble in saline, it was placed in 0.1 N acetic acid in which it dissolved easily. The injection of the acetic acid diluent produced a significant elevation in pituitary GH activity ($P < .01$) as evidenced by a widening of the epiphyseal cartilage on comparison with that observed with pituitaries from uninjected control rats (Table II, Fig. 1). The ip injection of hypo-



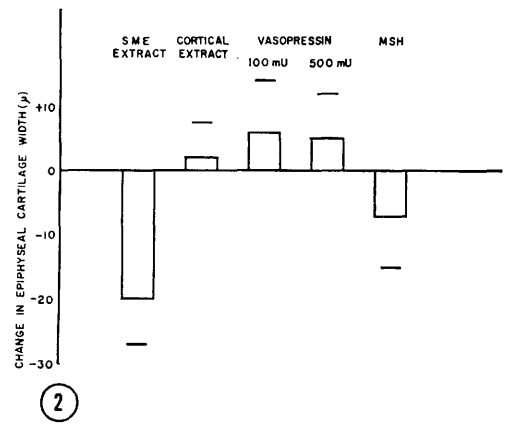


FIG. 1. Effect of ip injection of an acetic acid extract of ovine hypothalamus on pituitary GH activity (epiphyseal cartilage width). Time in min after injection is given on abscissa. Solid line represents values for pituitaries from animals treated with hypothalamic extract. Dashed line gives values for pituitaries from animals injected with acetic acid diluent. Vertical lines give standard error.

FIG. 2. Effect of various substances on pituitary GH activity. Results are expressed in terms of the change in epiphyseal cartilage width of rats receiving pituitaries from animals injected with the substance in question from the width of the cartilage in rats injected with pituitaries from control rats injected only with diluent. Dashes give standard error.

TABLE III. Effect of Different Routes of Injection of Hypothalamic Extracts Dissolved in Ammonium Acetate.

Route of application	Treatment	Time after inj (min)	No. of test rats	Width of tibial epiphyseal cartilage (μ)*
ip	Controls—ammonium acetate	30	5	269 ± 6.8
	SME extract	30	6	243 ± 5.8†
	" "	60	4	245 ± 2.7†
iv	Controls—ammonium acetate	30	6	272 ± 2.4
	SME extract	30	7	252 ± 7.3†
ic	Controls—ammonium acetate	30	5	279 ± 3.6
	SME extract	30	6	257 ± 6.7†

* Mean ± SEM.
† $P < .05$ *vs* controls.

thalamic extract had a marked effect in depleting pituitary GH significantly at both the 30- and 60-minute time intervals after its administration. Both values are significant against the acetic acid-injected controls. The value at 30 minutes is also significantly lower than that for uninjected controls or for the animals sacrificed 15 minutes after injection of hypothalamic extract.

Effect of different routes of administration of extract. The hypothalamic extract dissolved in 0.1 M ammonium acetate buffer at pH 5 caused a significant depletion of pituitary GH when injected ip, iv, or ic (Table III). In this case ic application was also effective in contrast to the first experiment. There was no significant difference in the magnitude of depletion of GH between the different modes of injection.

TABLE IV. Effect of Age of Experimental Animals on Response to Hypothalamic Extract (30 Min After iv Injection of Extract or Diluent).

Wt of the animals (g)	Treatment	No. of test rats	Width of tibial epiphyseal cartilage (μ)*
500	Controls	6	289 ± 4.9
	Extract	5	287 ± 4.8
200	Controls	5	277 ± 1.4
	Extract	5	261 ± 4.7†
100	Controls	6	269 ± 4.3
	Extract	6	253 ± 5.4‡

* Mean ± SEM.
† $P < .01$ *vs* control.
‡ $P < .05$ *vs* control.

Influence of age of the test animals. Table IV shows that only the older, 500 g rats failed to respond within 30 minutes with a significant depletion of GH to the iv injection of extract. A positive response of similar magnitude was seen with both the 100 and 200 g animals. The dose of extract was adjusted on a weight basis to minimize any change in blood level of injected extract (4 mg/100 g body weight).

Lack of effect of other substances on pituitary GH content. By contrast with the effectiveness of SME extract in depleting GH was the ineffectiveness of other agents (Fig. 2). Cerebral cortical extracts prepared similarly and given at the same dose were inactive in 2 separate experiments. Synthetic arginine vasopressin‖ was inactive at either a 100 or 500 mU dose, and 1 mg per dose of a purified preparation of α melanocyte stimulating hormone was also inactive.

Discussion. In our opinion the above experiments show quite conclusively that injections of crude extracts from sheep SME are effective in depleting GH from the pituitary. The depletion reaches its maximum approximately 30 minutes after injection and results in all probability from a rapid discharge of GH from the gland. The GH depleting activity seems to be confined to hypothalamic extracts, as shown by the negative results with a similarly prepared extract from the cerebral cortex, and by the ineffectiveness of vasopressin and melanocyte stimulating hormone.

It is unlikely that the decrease in GH content was the result of a sudden cessation of GH synthesis, in the light of our own results to be published elsewhere, which demonstrated a similar depletion of pituitary GH in insulin-induced hypoglycemia. Hypoglycemia is known to stimulate GH release in man(8). Furthermore, appropriately placed hypothalamic lesions can prevent the insulin-induced decline in pituitary GH(9). Thus, GH release from the pituitary appears to be under hypothalamic control by means of a

‖ Kindly provided by Dr. S. Gimpel of Sandoz, Inc., Hanover, N. J.

GH-releasing factor (GRF) manufactured in the hypothalamus and carried to the pituitary gland *via* the hypophysial portal system. We have now succeeded in purifying the GRF and separating it from other hypothalamic releasing factors(10).

In the single experiment in which rats of different sizes were used, the older, 500 g test rats failed to respond to the hypothalamic extract. This observation suggests that older animals may become refractory to GRF.

It is noteworthy that a rise in pituitary GH activity followed injection of the diluent in several instances. This is probably a reflection of non-specific stress which has been found capable of elevating pituitary GH activity (Krulich, unpublished data).

The relative inefficacy of the ic route of injection in our hands is surprising; it was effective in one instance and ineffective in the other. This lack of effect could perhaps be related to the stress which accompanied the injection procedure. From our data there is no evidence that iv injection was any more effective than ip injection; however, it is possible that the use of different doses of extract might bring out a difference in sensitivity between these two methods of injection. Quite clearly either ip or iv injection of GRF is a convenient means of administration.

Summary. Crude extracts of ovine SME tissue were effective in depleting pituitary GH of rats at 30 and 60 minutes after injection. Depletion of pituitary GH was estimated by the tibial epiphyseal cartilage test performed in hypophysectomized rats. Injection of extracts into the carotid artery effectively depleted pituitary GH in one of 2 trials, whereas ip or iv injection uniformly evoked GH depletion. There was no apparent difference in the degree of response produced by the 2 latter routes of injection. Extracts were dissolved in several solvents such as physiological saline, 0.1 N acetic acid, or 0.1 M (pH 5.5) ammonium acetate buffer. Extracts dissolved in any of these 3 solvents possessed GH-releasing activity. The ip administration of the acetic acid diluent produced a significant elevation in pituitary GH activity, which is attributed to the irritant effects of the acid. Hypothalamic extract was administered to rats of different sizes in one experiment. GH depletion occurred in rats which weighed either 100 or 200 g, but failed to occur in animals which weighed 500 g. Since the same dose on a weight basis (4 mg/100 g) was given to each group, these results suggest that responsiveness to the GH-releasing factor may decline in older animals. In contrast to the effectiveness of hypothalamic extracts in depleting pituitary GH was the lack of activity of cerebral cortical extracts, synthetic arginine vasopressin, and α melanocyte stimulating hormone. It is concluded that ovine hypothalamus contains a GH-releasing factor.

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