

A Dialyzable Heat Stable Factor in Acromegalic Plasma Associated with Increased *in Vitro* Growth Hormone Release¹ (36816)

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A number of reports in the recent literature have suggested the possibility of persistent hypothalamic control in acromegaly (1-3), and further that a primary pathogenetic defect may be characterized by excessive secretion of hypothalamic growth hormone releasing factor. In a previous communication from this laboratory we have shown greater *in vitro* release of monkey pituitary growth hormone in the presence of acromegalic plasma than in the presence of normal plasma (4). It is the purpose of this report to further characterize this observation.

Methods. Intact rhesus monkey pituitary glands were obtained and handled in a manner previously described (4). The incubation mixture of 10 ml included lactated ringers 7.5 ml, containing 50 μ g/ml neomycin, and a glucose concentration of 100 mg/100 ml, to which 2.5 ml aliquots of plasma or dialysates of plasma with added albumin were added for study purposes. All incubations were carried out in a Dubnoff shaker at 37°, aliquots for immunoreactive growth hormone were obtained at 30 sec after the start of an incubation, during and at the conclusion of two hour incubation periods. Growth hormone was assayed using minor modifications of a solid phase radioimmunoassay (5), with highly specific rabbit anti-growth hormone antibodies, produced in our laboratory. The standard is purified human growth hormone, NIH HS-1147.

Three types of experiments were carried out. In one, whole pituitaries were incubated for 2 hr periods, first either in the presence of

normal or acromegalic plasma, washed and transferred to a different plasma and then finally back to the original medium. Three intact glands were started first in acromegalic plasma, transferred to normal plasma and back for the final 2 hr of incubation to acromegalic plasma. Two pituitary glands were incubated in reverse order.

In a second experiment, 5 ml of the normal and 5 ml of the acromegalic plasma were dialyzed against an equal volume of lactated ringers for 24 hr at 4°. Human serum albumin was added to the final dialysates to achieve a protein concentration approximating that of normal plasma. Whole pituitaries were sectioned into halves, one half incubated in medium containing whole plasma, normal or acromegalic, and the other incubated in medium containing either dialyzed plasma or dialysate from the respective acromegalic or normal materials. Four whole pituitaries were used, and all incubations were continued for 2 hr.

In a third experiment, whole pituitaries were again sectioned into halves, one half incubated for 2 hr in control plasma, normal or acromegalic, the other in the same respective plasma after gentle heating to 60° for 5 min. The acromegalic and normal plasma used in this experiment were from the same pools used previously; growth hormone concentrations were, respectively, 126 and 3.6 ng/ml.

Results. Experiment I. Effect on monkey pituitary growth hormone release of incubating in medium containing normal or acromegalic plasma. Although the patterns of growth hormone release were similar in each of the experiments as noted in Figs. 1 and 2, the actual quantities released vary between

¹This research was supported by U.S. Public Health Service Grants T01-AM-05170, RR-55, Fisher Endocrine Research Fund and the William R. C. Murphy Research Funds.

glands. In order to assess statistical significance, it was elected to arbitrarily assign a value of 1 μg to one of the normal incubations in each experiment, and express the corresponding acromegalic incubations at the respective multiple. This latter value is $2.68 \pm 0.36 \mu\text{g}$ (SEM) which is significantly greater ($p < .001$). Importantly, too, in the series begun in acromegalic plasma, the rate of release in the third incubation period, although slightly depressed, almost nearly approximated that of the first incubation.

Experiment II. Effect of dialysis on the growth hormone releasing property of normal and acromegalic plasma. In this experiment growth hormone released into the media has been expressed as total micrograms of growth hormone released per milligram of pituitary tissue. Although the glands were sectioned by hand it is noteworthy that total growth hormone content of each incubated pituitary half was within 10% of the total content of the contralateral half. In order to more closely equate results, however, total growth hormone released into whole plasma containing media has been expressed as one, and the total growth hormone released from the contralateral half as a fraction thereof. From Fig. 3 it can be seen that dialysis reduced the growth hormone releasing property of these plasmas by just about 50% and that 50% of this activity was recoverable in the dialysate

of the respective plasma. The anticipated growth hormone in plain lactated ringers is approximately 30–40 $\mu\text{g}/\text{mg}$ pituitary at the end of 2 hr (4). This amount represents less than one tenth of the total growth hormone release in either of the dialysate-containing media.

Experiment III. Effect of heating on the growth hormone releasing property of acromegalic and normal plasma. Total growth hormone released from pituitary halves over 2 hr, expressed as micrograms of GH released per milligram pituitary tissue, was quite similar in unaltered and previously heated plasma, indicating heat stability of the factor or factors involved (Table I).

Discussion. The results of these studies confirm our previous finding that a factor or factors exists in peripheral plasma with the property of stimulating *in vitro* monkey pituitary growth hormone release. Since acromegalic plasma is clearly more potent in this phenomenon, it seemed important to ascertain whether acromegalic plasma contains different growth hormone releasing factors or

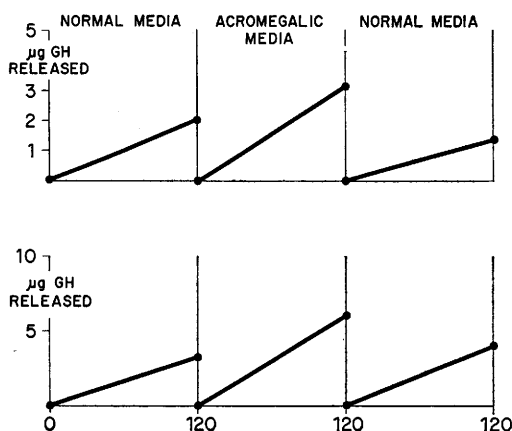


FIG. 1

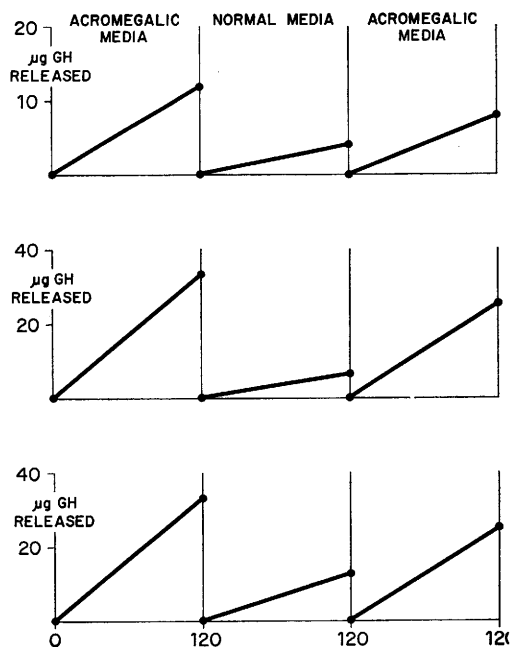


FIG. 2

FIGS. 1 and 2. Pattern of growth hormone release from intact monkey pituitary glands transferred, in the manner shown, between media containing normal or acromegalic plasma.

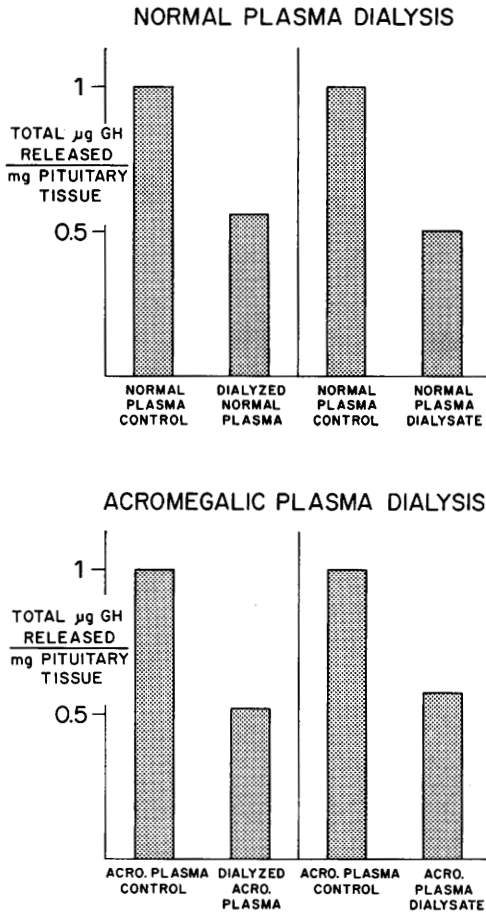


FIG. 3. Total growth hormone release from hemipituitaries incubated in plasma (normal or acromegalic) compared with growth hormone release from hemipituitaries incubated in dialyzed plasma or the corresponding dialysate.

simply more of the same material present in normal plasma capable of releasing growth hormone from incubated glands. The virtually identical behavior of both the normal and acromegalic plasma after dialysis and heating clearly lends support to the belief that a similar factor, present in greater quantity in acromegalic plasma, is responsible for this effect.

If the above speculation proves correct, then the property of stimulating *in vitro* pituitary growth hormone release is dependent upon factors which normally circulate. Current understanding of pituitary hormone release suggests, however, that hypothalamic

factors ultimately mediate growth hormone release in man (6). Many of the physiologic and pharmacologic factors known to result in augmented pituitary growth hormone release appear to work through stimulation of hypothalamic release centers (7). *In vitro* studies have additionally noted that growth hormone release from isolated pituitaries can be inhibited by altering physicochemical characteristics of the bathing medium (8).

TABLE I. The Effect of Heating Whole Plasma to 60° for 5 min on *in Vitro* Pituitary Growth Hormone Release.^a

	GH released ($\mu\text{g}/\text{mg}$) pituitary tissue/2 hr	
	Control	Heated
Normal plasma	0.25	0.30
Acromegalic plasma	0.58	0.47

^a One hemipituitary was incubated in each control plasma and its contralateral half was incubated with the respective heated plasma.

We believe, therefore, that these observations suggest the presence of hypothalamically originated growth hormone releasing factors in the peripheral circulation, the concentration being higher in patients with acromegaly. A recent report supports the existence of measurable amounts of luteinizing hormone release factor in the peripheral circulation (9). Similarly, results from some animal studies do support the belief that quantities sufficiently high for measurement are present in the extracranial circulation (10). Although the dialyzable nature and heat stability of this factor or factors indicate it may be of molecular size comparable to that proposed for other pituitary hormone releasing factors (11), confirmation of this thesis will largely depend upon studies subsequent to the elucidation of the structure of growth hormone release factor. Until that time it is as possible that some peripherally generated material is responsible for directly stimulating pituitary growth hormone release and perhaps synthetic activity as well.

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Received May 15, 1972. P.S.E.B.M., 1972, Vol. 141.