Pancreatic Hyperglycemic Factor and Growth.* (20029)

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Highly purified preparations of pituitary growth hormone have either a diabetogenic or a growth-stimulating effect depending on the type of experimental animal used(1). The role of the pancreas in the action of this hormone has received extensive study. There is some evidence that growth hormone suppresses insulin secretion(2), while other observations point to a stimulation(1,3). However, growth hormone affects the blood sugar level(4) and nitrogen excretion(5) under conditions in which changes in insulin secretion can not occur. There are a number of observations which suggest that the hyperglycemic factor from pancreas (HGF) is a true hormone originating in either the alpha or delta cells of the islets and that it is involved in the action of growth hormone. This substance has been isolated free from insulin activity from the pancreas of several species and has a specific action on liver glycogen which is demonstrable both *in vivo* and *in vitro*(6,7). It is present in normal amounts in the pancreas of the alloxan-diabetic rabbit and in increased amounts per unit weight in duct-ligated-sclerosed pancreatic tissue(6). Recently, it has been shown that a highly purified growth hormone preparation administered intravenously to normal men induces a rapidly-occurring, transient hyperglycemia(8) which resembles that caused by hyperglycemic factor given intravenously to several species including man(9). Bornstein *et al.* (10) reported that pancreatico-duodenal vein blood (but not femoral vein blood) from cats made diabetic with growth hormone contains a hyperglycemic - glycogenolytic substance when tested in the alloxan diabetic-hypophysectomized-adrenalectomized rat. These experiments are consistent with the hypothesis that pituitary growth hormone stimulates the release of hyperglycemic factor from the islets of Langerhans as suggested by Bornstein *et al.*(10). The fact that growth hormone may elicit its characteristic effects in depancreatized animals(11) is not conclusive evidence against this hypothesis, since hyperglycemic factor has been isolated from the gastric and duodenal mucosa of the dog and rabbit(6).

TABLE I. Assays of Hyperglycemic Factor (HGF) Preparations for Glycogenolytic Activity in Pigeon Liver Slices Incubated in 2.1 ml Saline-Phosphate Buffer (Ca^{++} Free).

Preparation	Min amt of preparation /ml incubation medium giving max glycogenolytic response, μg
HGF #3	2.4
#5	1.2
#208-65B-257	.7
Insulin used as source of HGF #3 and HGF #5	24

This paper reports briefly on some experiments which were done to determine whether the growth stimulating action of pituitary growth hormone could be mediated through hyperglycemic factor.

Experimental. The purified hyperglycemic factor preparations used in Exp. A and B were prepared from a sample of a low ash insulin† by the method of Sutherland *et al.*(12). The hypoglycemic action of insulin is completely destroyed during this procedure(13). Fraction No. 3 (HGF No. 3) of the Sutherland method was used in Exp. A and fraction No. 5 (HGF No. 5) was used in Exp. B. The hyperglycemic factor preparation (HGF No. 208-65B-257) used in Exp. C, prepared by Dr. Alfred Staub of the Lilly Research Laboratories, contained less than 0.1 unit of insulin per mg. All 3 hyperglycemic factor preparations were assayed by an *in vitro* method modified from that of Sutherland *et al.*(13). The modifications were the use of pigeon liver instead of rabbit liver and an in-

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† Kindly supplied by Dr. R. G. Romans of the Connaught Medical Research Laboratories, Toronto.

‡ Obtained from the Hormone Assay Laboratory, Chicago, Ill.

TABLE II. Exp. A: Effect of Hyperglycemic Factor #3, and Various Growth Hormone Preparations on the Proximal Tibial Epiphyseal Cartilage of Hypophysectomized Rat. (4 daily inj.)

Group	No. of animals	Daily dose in μg	Cartilage plate response, $\mu \pm$ S.E.*	Significance of response: P†
Control	5	0	109 ± 11.6	
Horner growth hormone #20-1-2-J51	5	10	150 ± 11.8	<.05
Li growth hormone	5	10	175 ± 17.5	<.02
Hyperglycemic factor #3	4	25	144 ± 10.6	<.1
" " "	2	100	176, 178‡	

* The low values in this experiment were due to a different method of making measurements from that used in Exp. B and C.
† Obtained from Fisher's table of t.
‡ Individual values.

TABLE III. Exp. B: Effect of Hyperglycemic Factor #5, and Various Growth Hormone Preparations on the Proximal Tibial Epiphyseal Cartilage of Hypophysectomized Rat. (4 daily inj.)

Group	No. of animals	Daily dose in μg	Cartilage plate response, $\mu \pm$ S.E.	Significance of response: P*
Control	10	0	166.9 ± 6	
Li growth hormone	11	10	253.6 ± 7	<.001
Horner growth hormone #20-1-2-J51	11	10	246.8 ± 4.7	<.001
Hyperglycemic factor #5	9	10	202.5 ± 7.1	<.01
" " "	9	40	201.1 ± 4.8	<.001

* Obtained from Fisher's table of t.

cubation medium of 2.1 ml of a saline-phosphate buffer(14) (Ca^{++}-free) instead of 1.2 ml of chloride-phosphate buffer. Glucose determinations in the assays were done by the Hagedorn-Jensen method(15). Female rats‡ of the Sprague-Dawley strain hypophysectomized at 26-28 days of age were used. They were fed on a diet of canned dog food, whole wheat bread, milk, and 5% glucose. Injections of hyperglycemic factor, growth hormone or saline (controls) were begun 12-14 days after hypophysectomy. They were given intraperitoneally in a volume of 0.5 ml once daily for 4 days (Exp. A and B) or for 9 days (Exp. C). Twenty-four hours after the last injection the animals were killed with chloroform and the tibias examined for changes in the width at the proximal epiphyseal cartilage according to the method of Evans *et al.* for the bioassay of hypophyseal growth hormone(17).

Four highly purified growth hormone preparations§ were tested for activity along with the hyperglycemic factor preparations: 1) Horner Growth Hormone No. 20-1-2-J51: prepared from beef pituitaries by the Wilhelmi method(18). 2) Li Growth Hormone: the electrophoretically homogeneous preparation of Dr. C. H. Li. 3) Horner Growth Hormone No. PR-1: prepared from hog pituitaries by the Rabens method(19). 4) Armour Growth Hormone No. 264-159A.

Results. Table I shows the results of the assays for glycogenolytic activity of the purified hyperglycemic factor preparations and of the insulin used as the source of HGF No. 3 and HGF No. 5. HGF No. 3 was 10 times and HGF No. 5 20 times as active as the source material. HGF No. 208-65B-257 was the most active of the three.

Tables II, III and IV summarize the results of the tibia test growth assay. Two of the 3 hyperglycemic factor preparations in daily doses of 10 μg, and 40 μg caused statistically significant increases (31-36 μ) in the width at the proximal epiphyseal cartilage of the tibia as compared with controls. HGF

§ Kindly donated by Dr. L. Mitchell of Frank W. Horner, Ltd., Montreal.

TABLE IV. Exp. C: Effect of Hyperglycemic Factor #208-65B-257, and Various Growth Hormone Preparations on Proximal Tibial Epiphyseal Cartilage of Hypophysectomized Rat. (9 daily inj.)

Group	No. of animals	Daily dose in μg	Cartilage plate response, $\mu \pm$ S.E.	Significance of response: P*
Control	8	0	137.8 $\pm$ 2.9	
Horner growth hormone #20-1-2-J51	8	10	185 $\pm$ 3.5	$<.001$
Horner growth hormone #PR-1	8	10	173 $\pm$ 1.5	$<.001$
Armour growth hormone #264-159A	8	10	162 $\pm$ 2.7	$<.001$
Hyperglycemic factor #208-65B-257	7	10	169 $\pm$ 7.9	$<.01$
Horner growth hormone #20-1-2-J51	8	25	256 $\pm$ 7.3	$<.001$
Horner growth hormone #PR-1	8	25	190 $\pm$ 2.3	$<.001$
Armour growth hormone #264-159A	8	25	184 $\pm$ 2.7	$<.001$
Hyperglycemic factor #208-65B-257	5	40	150 $\pm$ 4.9	$<.05$

* Obtained from Fisher's table of t.

No. 3 caused substantial increases but owing to the small numbers of animals used statistical significance could not be established. The tibial response was roughly parallel to the glycogenolytic potency of the 3 hyperglycemic factor preparations, *i.e.*, the order of potency of the preparations was the same by the 2 assay methods. In 2 of the 3 experiments (B and C) increasing the dose of hyperglycemic factor did not increase the tibial response. In one experiment (A) there was a substantial increase in the tibial response when the dose of hyperglycemic factor was raised but in this group only 2 of the 5 animals survived the injection period. In all experiments doses of hyperglycemic factor greater than 25 μg proved toxic (mortality 20-60%).¶ The responses obtained with the Li Growth Hormone (Tables II and III) were somewhat less (by about 25%) than that reported in the literature(17).

¶ The animals often went into coma following the injections. Some could be revived by glucose administered intravenously. In human experiments, done since this paper was submitted, highly purified preparations of hyperglycemic factor (containing less than 0.005 unit of insulin/mg) caused hypoglycemia, following the usual hyperglycemia. Recent work by Foa(16) suggests that hyperglycemic factor induces a release of insulin from the pancreas.

Discussion. It appears that small amounts of purified pancreatic hyperglycemic factor are capable of increasing the width of the epiphyseal cartilage of the hypophysectomized rat, an effect which is characteristically obtained with pituitary growth hormone(17). However, in only one of the 3 experiments was an increase in response observed when the dose of hyperglycemic factor was raised. This may have been due to the toxicity of the preparations in doses greater than 25 μg. It may also be that hyperglycemic factor belongs to a group of hormones (thyroxin, prolactin, and testosterone) which at certain dose levels increase the width of the cartilage plate 20-30 μ above control levels but not more when the dose is increased(17). The possibility remains that both the diabetogenic and growth-stimulating actions of growth hormone are mediated, in part or wholly, through the release of hyperglycemic factor from the pancreas and gastrointestinal tract.

The observation that hyperglycemic factor increases the width of the epiphyseal cartilage is in itself of some theoretical interest. Sutherland and Cori(20) have demonstrated that hyperglycemic factor causes a rapid increase in the concentration of phosphorylase in liver tissue. Significant amounts of phos-

phorylase as well as other enzymes of phosphorylative glycogenolysis have been found in preosseous, epiphyseal cartilage(21,22), and phosphorylative glycogenolysis appears to be essential for the *in vitro* calcification of cartilage(21). Cartilage cells preparatory to calcification accumulate large cytoplasmic stores of glycogen which disappear just prior to or coincident with the appearance of bone mineral in the adjacent cartilage matrix (23,24). If hyperglycemic factor stimulates glycolysis by causing an increase in the phosphorylase activity of tissues it might be that this is the mechanism whereby it stimulates growth. It is of interest, in this connection, that there is a disappearance of glycogen during cell growth and mitosis(25) and that increased rates of glycolysis have long been associated with the accelerated growth rates characteristic of tumor and embryonic tissue as compared with normal adult tissue.

Summary. Purified preparations of pancreatic hyperglycemic factor have been shown to increase the width of the epiphyseal cartilage of the hypophysectomized rat when assayed by the tibia test used for the bioassay of pituitary growth hormone. The activity of the preparations by this assay parallels their glycogenolytic activity *in vitro*. These observations are discussed from the standpoint of the mode of action of pituitary growth hormone and the process of growth at the enzyme level.

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