

Growth Hormone and Thyroxine: Effects on Submaxillary Gland of Hypophysectomized Rat.* (24641)

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Considerable work indicates that thyroid function and submaxillary gland histology and physiology in the rat are intimately related, and that testosterone is synergistic with thyroid hormone in maintaining normal histologic appearance and enzymatic activity of submaxillary glands(1-5). Shafer *et al.*(4) indicated that either cortisone or growth hormone may also increase proteolytic activity of salivary glands of hypophysectomized rats but to a lesser degree than thyroxine. Subsequently, cortisone revealed that it actually inhibited the effects of thyroxine, in maintaining normal salivary function and enzymatic activity. The present study is concerned with a more detailed investigation of the effects of growth hormone, both alone and in combination with thyroxine, upon rat submaxillary gland.

Materials and methods. Forty male albino rats, hypophysectomized[‡] at 4 months of age, were equally divided into 4 groups. Group I received daily subcutaneous injections of growth hormone[§] (1 mg/kg reconstituted in distilled water). Group II was given 20 mg of sodium thyroxine subcutaneously each day, and Group III growth hormone and thyroxine in same concentrations as used above. Group IV received no hormones or special supplements. Six unoperated rats of same age were controls. All 46 animals received *ad lib.* laboratory chow supplemented by special diet of bread, oranges, carrots and milk. After 24 days animals were sacrificed by chloroform and one submaxillary gland from each animal was assayed for proteolytic activity(4). The remaining submaxillary gland was fixed in Bouin's solution for histologic study. Parotid

gland, stomach, pancreas, adrenal and thyroid glands were also fixed for histologic study. Paraffin sections of glands were cut at 8 μ and stained by (1) hematoxylin and eosin; (2) methyl green-pyronine for ribonucleic acid(7); and (3) periodic acid-Schiff method. Additional sections from each gland were incubated in sterile solution of crystalline ribonuclease|| (0.25 mg/ml for 2 hours at 37°C), then stained with methyl green-pyronine.

Results. Table I shows the effect of various hormone supplements upon body weight. These data confirm our original observation that growth hormone is effective in maintaining normal body weight in the hypophysectomized rat. Thyroxine administration, on the other hand, resulted in an even greater weight loss than that observed in non-treated hypophysectomized rat. The effect on body weight of thyroxine and growth hormone administered together indicates that weight-gain inhibitory effects of thyroxine predominate since these animals gain weight at only a minimal rate in spite of growth hormone supplements.

Measurements of the diameter of submaxillary gland tubules indicate that hypophysectomy markedly reduces size of tubules. Administration of either thyroxine or growth hormone only partially prevents atrophic changes (Table I). The 2 hormones administered together maintained normal tubule size. Similarly, atrophic histologic changes seen throughout the submaxillary gland of the hypophysectomized rat were only partially prevented by either thyroxine or growth hormone alone, while the 2 in combination produced histologic appearance indistinguishable from the normal. Such results are unexpectedly similar to those previously published concerning the efficacy of thyroxine and testosterone in preventing hypophysectomy-induced submaxillary gland changes(4,6).

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[§] Growth hormone was generously supplied by Dr. Irby Bunding, Armour Labs., Chicago, Lot #M208.

|| Previously heat-treated at 90°C for 10 min. to destroy any contaminating desoxyribonuclease.

TABLE I. Effect of Growth Hormone and Thyroxine upon Body Weight, Proteolytic Activity, and Submaxillary Gland Tubules in the Hypophysectomized rat.

	No. ani- mals sur- viving	Final body wt (g)	Wt change (g)	Diam. of submax. tubules	Avg wt submax. gland (mg)	Submax. wt (mg/100 g body wt)	Relative activity†	Activity/g fresh tissue‡
Control	5	345	+40	44.3 ± 1.7*	270.3	77.8 ± 4.7*	12.051	9.086 ± .678*
Hypox	9	240	-27	30.7 ± .7	143.7	59.8 ± 4.4	2.308	3.282 ± .296
Hypox + GH	8	305	+41	38.1 ± 2.8	194.2	63.7 ± 3.8	4.734	4.951 ± 1.254
Hypox + thy- roxine	8	220	-32	40.1 ± 2.2	165.2	76.5 ± 4.5	4.181	5.163 ± .958
Hypox + GH + thyroxine	7	284	+17	45.3 ± 1.2	251.4	88.6 ± 4.0	11.085	8.988 ± 1.472

* Stand. dev.

† Expressed as ratio between color produced by total gland and color of tyrosine standard.

‡ Expressed as ratio between color produced/gg of gland tissue and activity of tyrosine standard.

The effect of hormone supplements on submaxillary gland weight is also shown in Table I. Hypophysectomy markedly reduced gland weight, and growth hormone was ineffective in preventing weight loss. Thyroxine, in contrast, was completely effective in preventing submaxillary gland weight loss. Apparently growth hormone is effective in maintaining body weight but has little effect upon submaxillary gland weight. Thyroxine, on the other hand, maintains submaxillary gland weight while it is ineffectual in maintaining body weight.

Table II also indicates alterations observed in proteolytic activity of the submaxillary gland following hypophysectomy and hormone treatments. Hypophysectomy reduced proteolytic activity by about 65% as compared to control values. Administration of either growth hormone or thyroxine alone, was partially effective in maintaining normal proteolytic activity. Only combined administration of growth hormone and thyroxine re-

sulted in submaxillary gland proteolytic activity within the normal range. Shafer *et al.* (4) reported that of many hormones tested only testosterone and thyroxine together produced normal submaxillary proteolytic activity values in the hypophysectomized rat. However, combinations of thyroxine with other hormones were not attempted. In this study, combination of growth hormone and thyroxine seems quite comparable to that of testosterone and thyroxine. The reason for this result remains obscure. Perhaps the preparation of growth hormone used contained a trace of gonadotrophic hormone which had a stimulatory action on gonadal tissue. However, it seems equally probable that the effect is merely a metabolic one in that growth hormone and testosterone may help to restore a general metabolic balance to the hypophysectomized animal.

It is important to know what effects growth hormone and thyroxine have upon other organs. Ribonucleic acid content of the sub-

TABLE II. Location and Distribution of Ribonucleic Acid in Organs of Hypophysectomized Rat Treated with Growth Hormone and Thyroxine.

	Submaxillary gland*		Pancreas†		Stomach‡	
	Cone.	Location	Cone.	Location	Cone.	Location
Control	+++	BP	+++	PB	++	P
Hypox	+	B	+	P	+	P
GH	+	B	+(+)	P	+	P
Thyroxine	++	BD	++	PD	+	P
GH + thyroxine	+++(+)	BPD	+++	PD	++	PD

* Found only in acinar cells, no RNA evident in duct cells except nucleolus.

† " " " " " , none in islet cells except nucleolus.

† " " " zymogenic cells. Parietal cells exhibited little if any RNA except in nucleolus.

R—Basal, D—Diffuse, P—Perinuclear.

maxillary gland has already been shown to be affected by hypophysectomy, thyroidectomy and thyroid hormone supplements(5), and so its concentration and distribution, as judged histochemically, were used as criteria for the action of these hormones upon stomach, pancreas, and submaxillary gland. These data are summarized in Table II.

RNA in submaxillary gland and pancreas was confined to cell cytoplasm of acinar portions of the glands. No RNA was found in cells of the duct systems or islets of Langerhans except in the nucleolus. In the stomach, RNA was found in zymogenic cells but not parietal cells and the concentration also appeared to be less than that observed in acinar cells of submaxillary gland and pancreas. Hypophysectomy depleted RNA content of cells of all 3 glands, and that which was present remained in close proximity to the cell nucleus. Injection of growth hormone did little to restore RNA concentration and distribution to normal in any of the 3 glands. Thyroxine, on the other hand, did partially prevent loss of acinar cell RNA in both submaxillary gland and pancreas. RNA present was diffusely spread throughout the cytoplasm rather than concentrated in basal or perinuclear position. No changes were observed in cellular RNA content of the stomach following thyroxine administration. RNA concentration in the stomach seemed little modified by any procedures in this study. Combined administration of growth hormone and thyroxine maintained normal levels of RNA in cells of all 3 glands. Cellular distribution was very similar to that observed following administration of thyroxine.

Our results emphasize the action of thyroxine upon rat submaxillary gland. The action of such hormones as cortisone(4,5) testosterone(1,2,4,5,6), and growth hormone are

likely to be metabolic in nature, as most certainly is the case with thyroxine, but without pronounced stimulatory effect upon the submaxillary gland which thyroxine has demonstrated. This study also indicates the possibility that other organs, such as pancreas, may be similarly affected by thyroid hormone.

Summary. Submaxillary glands of hypophysectomized male albino rats injected with growth hormone and thyroxine, both alone and in combination, were studied histologically and enzymatically. Growth hormone and thyroxine separately were only partially effective in preventing hypophysectomy-induced enzymatic and histologic changes in the submaxillary salivary gland. The 2 hormones when administered together, however, maintained normal histologic and enzymatic activity. These results are comparable to those previously reported utilizing testosterone and thyroxine in combination. Apparently the stimulatory action of thyroxine upon the submaxillary gland function may be enhanced by simultaneous administration of either testosterone or growth hormone, both of which are deficient in the hypophysectomized animal.

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