thelium. The latter compound is PAS positive, its sulfate staining is unaffected by testicular hyaluronidase, and it loses its alcianophilia after the periodic acid, N,N-dimethyl-m-phenylenediamine treatment. These histochemical properties mitigate against the presence of a CHS-like material in the fundic crypt epithelium, suggesting the existence of a sulfated glycoprotein instead. Such a material may be akin to sulfated glycoproteins chemically separated from dog gastric mucosa (7).

Summary. The correlation of chemical, electrophoretic and histochemical findings herein reported is consistent with the concept that the chondroitin sulfate A- or C-like material found in canine fundic gastric juice is derived from chief peptic cells.

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Effects of Thyroid Status and Adrenergic Blocking Drugs on Isoproterenol-Induced Enlargement of the Salivary Glands.* (31927)

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There is a substantial body of evidence suggesting that the response of certain adrenergic receptor mechanisms is altered by thyroid status whereas other responses are unaffected(1-3). For example catecholamine-induced lipolysis *in vitro* is diminished in adipose tissues from hypothyroid rats(4) and enhanced in adipose tissue from hyperthyroid rats(5). Similarly depletion of cardiac glycogen by isoproterenol is diminished in hypothyroid rats(3). Studies from several laboratories have shown that isoproterenol increases the wet and dry weight of submaxillary and parotid glands of the rat(6-8) and that this response is inhibited by dichloroisoproterenol(9). The following experiments were conducted to investigate the effect of thyroid status and adrenergic blocking drugs on the response of rat salivary glands to isoproterenol.

Methods and materials. The male Holtzman rats used in these experiments were housed in a constant temperature room and fed Purine Labena Chow and tap water *ad lib.* Thyroidectomy was performed as previously described(10) at least 4 weeks before the beginning of an experiment and the adequacy of operation was evaluated by cessation of growth(11). A diet containing 2.5% pancreatin (Viokase) was fed for 6 days. Isoproterenol was diluted each day from com-

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TABLE I. Effect of Growth, Thyroidectomy, Isoproterenol and Pancreatin on Salivary Gland Weight of Rats.

Group	Age, wk	Treatment	No. of animals	Body wt	Salivary gland wt, mg		Wet wt / Body wt, mg/g
					Wet	Dry	
Normal	8	None	6	151 ± 32*	350 ± 9.7	76 ± 1.5	2.27 ± .04
	12	"	6	245 ± 12	470 ±27	101 ± 7	1.91 ± .06
Hypothyroid	12	None	5	179 ± 6	287 ±16	63 ± 3.5	1.58 ± .06
	12	Isoproterenol†	5	168 ± 5	356 ± 4	73 ± 1.4	2.12 ± .08
	12	Pancreatin‡	6	174 ± 9	431 ± 4	103 ± .8	2.38 ± .08

* Mean ± S.E.
† Isoproterenol, 15 mg/kg, i.p., on 1st, 3rd and 5th day.
‡ Viokase, 2.5% in diet—6 days.

mercial preparations (Isuprel 1:200) or dissolved in .15 M NaCl. Triiodothyronine (Glaxo Labs. Ltd.) was dissolved in alkaline saline and injected subcutaneously each day for 8-10 days in doses of 3, 5 or 15 μg/day. Propranolol (kindly provided by Dr. Sahigian-Edwards, Ayerst Labs.) was prepared in .15 M saline and phenoxybenzamine was diluted from vials of Dibenzyline® (kindly provided by Smith, Kline and French Labs.). Each drug was injected subcutaneously for 8 days in doses of 1 and 5 mg/kg respectively. Dicholoroisoproterenol (DCI) was dissolved in .15 M saline and injected subcutaneously each day for 8 days in a dose of 20 mg/kg.

At the end of the treatment periods submandibular and submaxillary salivary glands were removed and weighed on a torsion balance. In some instances dry weight was determined by heating at 100°C to constant weight.

Results. After thyroidectomy, growth stopped and the weight of the salivary glands decreased (Table I). Four weeks after sham operation the normal salivary glands had increased by 120 mg and body weight by 94 g. Thyroidectomy reduced the weight gain to between 17 and 28 g and produced a weight loss of 63 mg in the salivary glands. Isoproterenol increased the weight of the salivary glands in hypothyroid rats from 287 mg to 356 mg. Pancreatin added to the diet further increased the weight of the salivary glands to 431 mg (Table I).

The effect of triiodothyronine, isoproterenol and adrenergic blocking drugs on the weight of salivary glands has been examined in 3 experiments. The first experiment compares the effect of isoproterenol, 2.5 mg/kg, and dichloroisoproterenol (DCI), 20 mg/kg, on the salivary gland weight of normal and hypothyroid rats (Table II). The salivary glands of the normal rats were heavier than those of the hypothyroid rats and both groups showed an increase in weight after 8 days of treatment with isoproterenol. Simultaneous administration of DCI and isoproterenol completely blocked the effect of isoproterenol in increasing the weight of normal salivary glands but only partially inhibited this effect in thyroidectomized rats. In the second experiment phenoxybenzamine (5 mg/kg) and propranolol (1 mg/kg) both significantly reduced the weight of salivary glands in hypothyroid rats receiving isoproterenol but only propranolol reduced it significantly ($P<.05$) in thyroidectomized rats treated with T-3 (Table III). When the dose-response relationship was investigated isoproterenol was found to produce a dose-related increase in the weight of the salivary glands in hypothyroid rats. However, neither 3 nor 15 μg triiodothyronine daily potentiated this effect of isoproterenol (Table IV). In fact, the higher dose of triiodothyronine seemed to reduce the sensitivity of the salivary glands to the lower doses of isoproterenol. Triiodothyronine increased heart rate in this experiment from 337 ± 19 beats/minute in hypothyroid rats to 390 ± 15 beats/minute with 3 μg/day and to 405 ± 21 beats/minute with 15 μg/day, but isoproterenol did not affect the heart rate. Triiodothyronine also increased the weight of the heart, from 472 ± 17 mg in hypothyroid rats to 633 ± 19 in rats treated with 3 μg/d and to 738 ± 45 mg in

TABLE II. Effect of Isoproterenol and Dichloroisoproterenol on Weight of Salivary Glands of Normal and Hypothyroid Rats.

	Treatment	No. of animals	Body wt, g	Salivary gland wet wt, mg	Wet wt / Body wt, mg/g	P§
Normal	NaCl	5	189 ± 10*	478 ± 36	2.53 ± .19	
	Isoproterenol†	5	198 ± 10	671 ± 47	3.48 ± .10	<.001
	Isoproterenol + DCI‡	5	198 ± 7	434 ± 10	2.19 ± .06	
Thyroidectomy	NaCl	4	175 ± 21	313 ± 12	1.67 ± .16	
	Isoproterenol	4	189 ± 14	524 ± 58	2.77 ± .07	<.001
	Isoproterenol + DCI‡	5	159 ± 10	358 ± 18	2.32 ± .11	<.01

* Mean ± SEM.
† Isoproterenol (Iso), 2.5 mg/kg, s.c., each day for 8 days.
‡ Dichloroisoproterenol, 20 mg/kg, s.c., each day for 8 days.
§ Comparison with saline-treated controls.

rats receiving 15 μg/day.

Discussion. Ohlin has shown that thyroid hormone and testosterone increase the weight and the secretory response of rat salivary glands(12). The present studies have extended this observation by showing that treatment with triiodothyronine increased the weight of the salivary glands and that thyroidectomy reduced their weight. However, thyroidectomy did not abolish or reduce the hypertrophy of the submaxillary salivary glands in rats fed pancreatin or treated with isoproterenol. Indeed thyroidectomy may have potentiated the response to isoproterenol and treatment with triiodothyronine decreased it. Thus, hypertrophy of the salivary glands induced by isoproterenol, like certain other responses to catecholamines, is not potentiated by thyroid hormone(3).

Enlargement of the salivary glands can be produced by amputating the incisor teeth as well as by feeding pancreatin or injecting isoproterenol(13,14). An analysis of these phenomena has indicated that the effects of amputation of the incisors or feeding pancreatin are mediated through both the sympathetic and parasympathetic nervous system and that presumably isoproterenol mimics the effects of stimulation of the sympathetic nervous system. The nature of the adrenergic receptors mediating this phenomenon is as yet unclear. Adrenergic receptors have been classified by Ahlquist(15) into two types. Alpha receptors are defined as those where the response to norepinephrine is greater than to isoproterenol while beta receptors are those with a greater response to isoproterenol. On this basis, the hypertrophy of salivary glands should be mediated by beta receptors since isoproterenol causes significant hypertrophy

TABLE III. Effect of Triiodothyronine, Isoproterenol and Adrenergic Blockade on Enlargement of Salivary Glands of Thyroidectomized Rats.

	Hypothyroid				Treated with triiodothyronine‖			
Treatment	No. animals	Body wt, g Initial	Body wt, g Change	Salivary gland wet wt, mg/g B.W.	No. animals	Body wt, g Initial	Body wt, g Change	Salivary gland wet wt, mg/g B.W.
Vehicle (.15M NaCl)	7	152	+8	1.68 ± .08*	5	208	+13	1.73 ± .05
Isoproterenol†	7	151	+3	2.86 ± .10¶	6	192	+ 7	2.45 ± .06¶
Isoproterenol and phenoxybenzamine‡	7	160	+2	2.43 ± .10**¶	6	149	— 2	2.46 ± .07¶
Isoproterenol and propranolol§	6	160	+2	2.17 ± .09††¶	6	142	+20	2.24 ± .07**¶
Phenoxybenzamine‡	6	167	—6	1.67 ± .04	6	228	— 6	1.82 ± .09
Propranolol§	6	159	0	1.76 ± .08	6	222	+14	1.79 ± .04

* Mean ± SEM—All animals treated 10 days. † Isoproterenol, 2.5 mg/kg/d. ‡ Phenoxybenzamine, 5 mg/kg/d. § Propranolol, 1 mg/kg/d. ‖ Triiodothyronine, 5 μg/d (T-3). ¶ P <.01 (compared to groups treated with vehicle or triiodothyronine alone). ** P <.05 (compared to isoproterenol-treated group). †† P <.001 (compared to isoproterenol-treated group).

TABLE IV. Effect of Triiodothyronine and Isoproterenol on Salivary Gland and Body Weight of Thyroidectomized Rats.

Isoproterenol,* mg/kg	Body wt, g						Salivary gland $\frac{\text{Wet wt}}{\text{Body wt}}$, mg/g		
	Triiodothyronine,* μg/d								
	0		3		15		0	3	15
	I†	Δ‡	I	Δ	I	Δ			
0	170	+3	168	+39	179	+11	1.59 ±.08§	1.74 ±.05	2.23 ±.19‖
0.1	168	0	178	+33	174	—12	1.91 ±.14	1.87 ±.09	2.03 ±.32
1.0	171	+3	169	+13	170	— 8	2.52 ±.09¶	2.28 ±.15‖	2.39 ±.14
10.0	176	+5	173	+19	164	— 5	3.11 ±.14¶	2.82 ±.11¶	2.99 ±.32

* Isoproterenol + triiodothyronine daily for 10 days. † I = Initial weight. ‡ Δ = Weight change during treatment. § Mean ± SE (5 animals per group). ‖ $P < .05$ (compared to untreated group). ¶ $P < .01$.

(6-8) and norepinephrine is without effect (16). Dichloroisoproterenol, a drug which blocks beta-adrenergic receptors, reportedly blocks the response to isoproterenol(9) and this has been confirmed in both normal and hypothyroid rats. Propranolol, a potent inhibitor of beta-adrenergic receptors, has been found more effective than phenoxybenzamine, an inhibitor of alpha receptors, in blocking isoproterenol-induced hypertrophy of the salivary glands of hypothyroid and triiodothyronine-treated rats. Thus, it appears that salivary gland receptors mediating isoproterenol-induced hypertrophy have more similarity to beta receptors than to alpha receptors.

Summary. Thyroidectomy reduced the weight of rat salivary glands and triiodothyronine increased it. The hypertrophy in salivary glands induced by isoproterenol was unaffected by thyroidectomy or pretreatment with triiodothyronine. Propranolol inhibited the isoproterenol-induced hypertrophy to a greater extent than phenoxybenzamine suggesting that the adrenergic receptors are primarily beta in type.

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