Changes in Submaxillary Gland Ribonucleic Acid Following Hypophysectomy, Thyroidectomy and Various Hormone Treatments.* (22998)

DAVID BIXLER,† JOSEPH C. MUHLER, RICHARD C. WEBSTER, AND WILLIAM G. SHAFER
Departments of Chemistry and Anatomy and School of Dentistry, University of Indiana, Bloomington.

Recent studies have indicated that granule content of the tubular portion of rat submaxillary glands is inversely related to incidence of experimental dental caries(1-3). Proteins, or closely related organic substances, seem to be directly involved in this relationship, since proteolytic activity of the rat submaxillary gland appears directly related to dental caries incidence(3). Screebny(4) has proposed that the granular tubule is the site of this proteolytic activity. Since ribonucleic acid is intimately associated with protein metabolism, it seemed desirable to know what changes, if any, in ribonucleic acid content of the granular cells were taking place under those experimental conditions known to alter proteolytic activity and tubule granule content. In addition, this study compares the effects in the submaxillary glands with those observed in the parotid and sublingual rat salivary glands since little information is available on enzymatic aspects of these glands.

Materials and methods. This study consisted of 2 experiments. *Series I* was composed of 40 male Sprague-Dawley strain rats hypophysectomized at 45 days of age, and 6 male unoperated Sprague-Dawley strain rats of approximately 55 days of age, which served as controls. Five days following surgery the animals were divided into 4 groups and fed *ad libitum* a special diet of milk, bread, dog food, carrots and oranges. Group 1 received daily injections of 10 μg of aqueous sodium thyroxine subcutaneously and 200 μg of testosterone propionate in olive oil intramuscularly. Group 2 received daily intramuscular injections of 2.5 mg of cortisone acetate in aqueous suspension. Group 3 received all 3 hormones daily at the same concentrations described above. Group 4 received no supplements and served as the hypophysectomized control. Group 5 was composed of unoperated animals which served as controls. The animals were sacrificed after 21 days and the salivary glands were removed and fixed in 10% neutral formalin. *Series II* was composed of 30 weanling male rats of the same strain equally divided into 5 groups. Groups 1-4 were intraperitoneally injected, respectively, with 100, 250, 500 and 750 μc of carrier-free radioactive iodide. All animals were fed a stock laboratory corn diet previously described(5). One hundred days after injections, all animals were sacrificed and major salivary glands and thyroid glands were removed and fixed in 10% neutral formalin. Sections of submaxillary, sublingual and parotid glands were cut at 8 μ from both series of animals and stained by the methyl green pyronine method described by Kurnick (6). This method is specific enough to yield semi-quantitative data on ribonucleic acid (RNA) distribution and concentration. Additional sections from each gland were incubated in a sterile solution of crystalline ribonuclease‡ (0.25 mg RNase/ml) for 2 hours at 37°C, then stained by methyl green pyronine. Any staining material persisting after the RNase digestion was considered to be material other than RNA.

Results. Ribonucleic acid in all 3 major salivary glands was confined to the acinar portion of the gland; no RNA was found in the duct system. For this reason the data obtained from Series I and II, which appear in summary form in Tables I and II respectively, pertain only to the acinar portion of the 3 glands.

Series I. *Submaxillary Gland.* Hypophysectomy markedly depleted the RNA content

* This work was supported in part by Department of Army, under Contract.

† Postdoctoral fellow, N.I.H.

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

TABLE I. Effect of Hypophysectomy and Hormone Replacement Therapy upon RNA Content of the Major Salivary Glands.

	Submaxillary gland		Sublingual gland		Parotid gland	
	*	†	*	†	*	†
Hypo + testosterone & thyroxine	BND	4	BN	4	BND	3
" + cortisone	BN	1	"	1	BN	1
Idem + testosterone & thyroxine	"	3	"	2	"	2
Hypophysectomized	"	0	B	0	"	1
Control unoperated	"	2	"	2	"	2

* Denotes location of RNA: B = Basal portion of cell; N = Nucleolar; D = Diffusely spread in cytoplasm.

† Denotes estimated amount of RNA as judged by intensity of staining reaction. 0 = essentially none present; 4 = very heavy conc. of RNA.

TABLE II. Effect of Loss of Thyroid Function on RNA Content of Major Salivary Glands.

Thyroid histology		Submaxillary gland		Sublingual gland		Parotid gland	
		*	†	*	†	*	†
100 μc I^{131}	Normal	BN	4	BN	4	BD	4
250	Some degeneration but still functional	"	3	"	4	"	4
500	Atrophic	BND	2	BND	2	"	3
750	"	"	2	"	2	"	3
Control		BN	4	BN	4	"	4

* Denotes location of RNA: B = Basal location; N = Nucleolar; D = Diffuse.

† Denotes estimated amount of RNA—judged by intensity of staining reaction. 0 = essentially none; 4 = heavy RNA conc.

of acinar cells in the submaxillary gland. The small amount observed was located in basal portions of the cytoplasm and in the nucleolus of each cell. Injection of cortisone (2.5 mg day) had only a slight effect on RNA content while administration of both testosterone (200 μg/day) and thyroxine (10 μg/day) dramatically increased the acinar RNA content. In this instance a small amount of RNA was observed evenly distributed throughout the cytoplasm. Location of acinar RNA following simultaneous administration of all 3 hormones was essentially the same as that observed following only testosterone and thyroxine administration. However, intensity of the RNA stain was not so great as that observed in the group receiving only testosterone and thyroxine. Apparently, cortisone may have an inhibitory effect on cytoplasmic RNA under these conditions.

Sublingual and Parotid Glands. Results for these 2 glands were essentially the same as described for the submaxillary gland. That is, hypophysectomy markedly depleted RNA content in both mucous acini and serous acini of the 2 glands. Cortisone had some slight ability to relieve these hypophysectomy-induced changes. However, simultaneous administration of testosterone and thyroxine resulted in an even greater RNA content than in the unoperated animals. When cortisone was used it appeared to exert a damping action on the apparent RNA stimulating ability of testosterone and thyroxine (Table I).

Series II. *Submaxillary Gland.* Ribonucleic acid in control animals was predominately in the basal portion of the cell and in close relationship to the nuclei. Following treatment with 100 μc of I^{131} concentration and location of RNA remained unchanged, while rats which received 250 μc of I^{131} seemed to give slightly less intense RNA reaction. Administration of 500 μc of I^{131} noticeably decreased RNA reaction and, in addition, RNA no longer appeared to be concentrated in basal portion of the cell but distributed diffusely throughout the cell cytoplasm. This same histologic picture was ob-

served in the 750 μc group.

Sublingual Gland. The RNA of normal sublingual gland is localized in the basal portion of the mucous acinar cell and lies in close proximity to the basement membrane and nucleus. The groups which had received 100 and 250 μc of I^{131} had essentially the same concentration and distribution of RNA. However, administration of 500 and 750 μc of I^{131} reduced RNA content of cells by approximately one-half and this RNA was not concentrated in any one portion of the cell but diffused throughout the cytoplasm.

Parotid Gland. Control animals had parotid glands which showed RNA concentrated in the basal portion of the acinar cell. Administration of radioactive iodine to any group did not change cellular location or concentration of RNA in any of the 4 experimental groups.

Histologic study of thyroid glands from Series II animals revealed that increasing the dose of radioactive iodide resulted in progressive destruction of thyroid tissue. Both control and 100 μc animals receiving I^{131} had identical, normal appearing thyroid glands. Thyroid glands from the 250 μc group showed definite but minimal effects of radiation. Many cells exhibited a frothy appearing colloid while a few had no colloid at all. Only an occasional pycnotic nucleus was observed and the epithelium in the majority of follicles was low cuboidal. Some connective tissue invasion was apparent.

The glands from the 500 and 750 μc groups were essentially identical and will be described together. Only a few follicles remained in these glands and these were atypical and non-functional in appearance. The bulk of the gland in both groups was composed of fibrous connective tissue. These results appear in Table II. The effects of altered thyroid function on salivary gland RNA also appear in Table II.

Discussion. The results of these experiments indicate that ribonucleic acid content of major salivary glands is related to normal thyroid and testicular function. This appears to be true since administration of thyroxine and testosterone completely inhibited depletion of RNA following hypophysectomy. Administration of the 2 hormones to hypophysectomized rats resulted in what appeared to be supranormal amounts of RNA in the acinar cells. It is felt that food intake plays a negligible role in explaining the results of this study because of the short duration of the experiment, and since the final body weights were essentially identical, the hypophysectomized group having a mean weight of 103 g and the hypophysectomized group receiving testosterone and thyroxine 110 g.

The thyroid gland appears to have the most prominent effect upon acinar RNA content and localization, since a progressive loss of thyroid tissue was paralleled by progressive loss of RNA and a change in its cellular location. Shafer *et al.* have shown that only thyroxine and testosterone can prevent marked decrease in proteolytic activity of the rat submaxillary gland following hypophysectomy (7). They also found that other hormones such as cortisone, or growth hormone may partially alleviate these changes.

It was noted that RNA of the parotid gland was least affected by either destruction of the thyroid gland with I^{131} or by throxine administration. The submaxillary and sublingual gland RNA, however, demonstrated a pronounced responsiveness to altered thyroid function. More interesting, the tubular portion of the submaxillary gland, which several workers have associated with dental caries experience, contained no RNA. Since it was the acinar portion of the gland which showed alterations in RNA following various hormone treatments, these results would seem to indicate that the study of a dental caries-salivary gland relationship must include both a study of the entire gland as well as a study of each one of the salivary glands.

Baker and Abrams(8) have studied the effect of hypophysectomy and hormone replacement on the weight and histologic appearance of the major salivary glands. They found that of the major salivary glands the parotid gland is most severely affected by hypophysectomy. In addition, thyroxine did not restore the gland weight loss following hypophysectomy. No gland weights were

taken in this study, but, histologically, the submaxillary gland appeared the most drastically affected by hypophysectomy.

Summary. 1) A histochemical study of location and concentration of RNA in the major salivary glands was made in animals which had been subjected to various experimental procedures. It was noted that only the acinar portion of the salivary gland exhibited RNA. None was found in the duct portion of any of the 3 major salivary glands. In the hypophysectomized rat RNA is strikingly reduced in the acini of all major salivary glands. Injection of cortisone does not appreciably affect this condition whereas thyroxine and testosterone in combination restore the RNA to normal concentration and location. 2) A progressive loss of thyroid function produced by injecting graded doses of I^{131} resulted in progressive loss of cytoplasmic RNA. This effect was obtained in submaxillary and sublingual glands. The parotid gland RNA was essentially unaffected by thyroidectomy.

1. Muhler, J. C., and Shafer, W. G., *J. D. Res.*, 1952, v31, 798.

2. Shafer, W. G., and Muhler, J. C., *ibid.*, 1953, v32, 262.

3. ———, *ibid.*, 1955, v34, 531.

4. Screebny, L. M., *ibid.*, 1953, v32, 686.

5. Muhler, J. C., Nebergall, W. H., and Day, H. G., *ibid.*, 1954, v33, 33.

6. Kurnick, N. B., *Stain Technology*, 1955, v30, 213.

7. Shafer, W. G., Clark, P. G., and Muhler, J. C., *Endocrinology*, 1956, v59, 516.

8. Baker, B. L., and Abrams, G. D., Chapter II, *Hypophyseal Growth Hormone, Nature and Actions*, Smith, R. W., Gaebler, O. H., and Long, C. N. H., Ed., McGraw-Hill, New York, 1955.

Received January 8, 1957. P.S.E.B.M., 1957, v94.

Effect of Estrogens on Myocardial Sensitivity to Toxic Effects of Digoxin.* (22999)

EDWARD H. GRINNELL† AND PAUL W. SMITH

Department of Pharmacology, University of Oklahoma School of Medicine, Oklahoma City.

In experiments previously reported at the Federation meetings, 1955, it was found that a single dose of 0.15 mg of digoxin/kg body weight, given intravenously to dogs under pentobarbital anesthesia, produced an arrhythmia characterized by complete A-V dissociation and persistent idioventricular tachycardia. The interval between administration of the glycoside and onset of idioventricular rhythm varied from 5 to more than 30 minutes. It was of considerable interest to us that female animals, without exception, exhibited the greatest delay in onset of arrhythmia. This observation suggested that susceptibility to this particular manifestation of digitalis intoxication might be influenced by estrogenic hormones. To investigate this possibility, a new series of experiments was instituted, in which the time interval was measured with the greatest possible accuracy.

That such an influence exists is shown by data presented below, which includes experiments on normal males, normal females in anestrus, normal females in natural estrus, castrate females and castrate females treated with estrogenic drugs.

Materials and methods. Forty-four healthy, adult, mongrel dogs of both sexes, weighing 7 to 20 kg, were used. Of the 44, seven were normal males, 4 were normal females in anestrus, 3 were normal females in natural estrus and 30 were castrate females. The castrate females were spayed by technic which included both bilateral oophorectomy

* This research was supported by grants from Council on Pharmacy and Chemistry, Amer. Med. Assn. and Research Com., University of Oklahoma Medical Center.

The digoxin used was given to us by Burroughs Wellcome & Co., (U.S.A.). The estradiol was synthesized in the laboratory of Dr. Max N. Huffman.

† Research Fellow of American Heart Assn.