THE RELATIONSHIP BETWEEN THE HISTOLOGY OF THE THYROID AND THE SALIVARY GLANDS AND THE INCIDENCE OF DENTAL CARIES IN THE RAT

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AN INVERSE relationship appears to exist between the activity of the thyroid gland and the incidence of dental caries in the rat.[1-4] Thus, the feeding of dessicated thyroid,[1, 2, 4] or the injection of thyroxine,[5] significantly reduces dental caries, and the administration of antithyroid compounds, such as thiouracil,[1, 2, 4] or radioactive iodine,[3, 4] significantly increases dental caries. Five hundred microcuries of I^{131} in one dose significantly increased dental caries and was sufficient to inactivate completely the secretory activity of the thyroid gland.[3] However, it was also found that a total of 500 μc of I^{131} administered, either as 2 equally spaced injections of 250 μc, or 5 equally spaced injections of 100 μc each, was without significant effect on the dental caries experience although a marked atrophy of the thyroid gland was apparent as a result of both series of injections. The purpose of this experiment was to investigate the effect of a single injection of various graded doses of I^{131} upon the thyroid gland and dental caries in order to establish, if possible, what level of thyroid inactivity is correlated with histologically apparent alterations in the salivary glands and the incidence of dental caries.

EXPERIMENTAL

A total of 125 weanling male Sprague-Dawley rats was equally divided as to initial body weight into 5 experimental groups. Group 1 was injected intraperitoneally with 100 μc of radioactive, carrier-free I^{131} as the sodium salt. Groups 2, 3, and 4 received single intraperitoneal injections of 250, 500, and 750 μc, respectively, of I^{131}. The remaining group received no I^{131} and served as a control. All animals were fed ad libitum a coarse corn cariogenic diet[6] and distilled water for the experimental period of 140 days. The animals were housed in pairs in raised screen cages in a temperature-controlled room. At the termination of the experimental period the animals were sacrificed with ether and the heads removed for dental caries evaluation.[7] The thyroid and salivary glands were removed, fixed in Bouin's solution, and examined histologically.

The diameter of the granular tubule of the submaxillary glands of 5 rats in each group was determined with the aid of a micrometer eyepiece. All measurements for each group were averaged and these results subjected to statistical analysis.

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RESULTS

Graded doses of I^{131} produced a graded response in dental caries (Table I). Although 100 μc of I^{131} was without a significant effect, each higher level of I^{131} produced a higher caries incidence, with the group receiving 750 μc showing the highest experience. Similarly, as the dosage of I^{131} was increased, an increase was also noted in both the number of molars affected and the severity of the carious lesions.

TABLE I
EFFECT OF GRADED DOSES OF I^{131} ON DENTAL CARIES IN THE RAT

I^{131} ADMINISTERED (μC)	NO. ANIMALS	FINAL BODY WT. (GM.)	MEAN NO. OF LESIONS	MOLARS AFFECTED	SEVERITY*
100	23	377	7.4	3.9	2.6
250	21	366	9.2	4.4	2.7
500	19	239	9.6	4.8	2.8
750	17	194	10.4	4.9	2.9
Control	26	361	7.3	3.6	2.3

*Based on the following system: Tooth 0 to ¼ destroyed—1; tooth ¼ to ½ destroyed—2; and tooth ½ to completely destroyed—3.

Thyroid Gland.—The thyroid glands from both the control and 100 μc groups were essentially the same, although in the latter group the glands appeared to have a slightly increased amount of connective tissue (Table II). The nuclei were normal and the epithelium of the follicles varied from columnar to low cuboidal. In the larger follicles the colloid was frequently observed to be vacuolated. The parathyroid glands were normal in appearance in both groups.

TABLE II
EFFECT OF GRADED DOSES OF I^{131} UPON THE HISTOLOGY OF THE THYROID GLAND

I^{131} ADMINISTERED (μC)	NO. THYROID GLANDS	EPITHELIUM OF FOLLICLE	NUCLEAR APPEARANCE	AMOUNT OF CONNECTIVE TISSUE INVASION*	FUNCTIONAL ACTIVITY OF GLAND†
Control	6	Cuboidal to columnar	Normal	0	+ + + +
100	6	Cuboidal to columnar	Normal	0 (+)	+ + +
250	6	Cuboidal	Normal and pycnotic	+	+
500	6	Low cuboidal	Pycnotic	+ +	0
750	6	Low cuboidal	Pycnotic	+ + +	0

*Plus signs indicate increasing amounts of connective tissue.
†Estimation based upon predominant type of epithelium, presence and appearance of colloid in follicle, amphoteric nature of colloid, and nuclear morphology.

The thyroid glands from the 250 μc group showed definite effects of the radiation. The follicles were reduced in size; approximately 50 per cent of them contained colloid and this appeared frothy and vacuolated. The nuclei were often normal appearing, although occasionally pycnotic nuclei associated with an atrophied follicle were observed. The epithelium of the majority of the follicles was cuboidal and a pronounced connective tissue invasion was apparent. The glands gave the appearance of having been almost entirely

destroyed by the initial dose of radioactive iodine, but subsequently had regenerated to a limited degree. Thus, a small amount of what appeared to be functional thyroid tissue was present in each gland examined, although the major portion of the gland was atrophic. The parathyroid glands appeared normal.

Both 500 μc and 750 μc of I^{131} appeared to have the same degree of destructive effect upon the thyroid gland. The glands themselves were mostly connective tissue and the few follicles which remained were atypical in appearance. The nuclei were pycnotic and the epithelium was low cuboidal. The thyroid glands of both these groups gave a striking picture of inactivity and atrophy. It was particularly difficult in the 750 μc group to find any thyroid tissue at all. Connective tissue composed almost the entire gland with only a few scattered, nonfunctional follicles present.

The parathyroid glands in these groups seemed to show some slight effect of the radiation, although the cell nuclei themselves were normal in appearance, and the over-all histologic picture was one of a normally functioning gland.

Submaxillary Gland.—Since previous reports have clearly indicated that the structure of the submaxillary gland is regulated at least in part by the thyroid gland,[8, 9] the effects cited here (Table III) are most likely the direct result of loss of thyroid function.

TABLE III
EFFECT OF GRADED DOSES OF I^{131} ON SIZE OF GRANULAR TUBULES OF SUBMAXILLARY GLAND

I^{131} ADMINISTERED (μC)	NO. SUBMAXILLARY GLANDS	NO. MEASUREMENTS	MEAN DIAMETER OF GRANULAR TUBULES	P VALUE†	TUBULES SHOWING GRANULES (%)
Control	5	100	54.9 ± 3.7*	–	98
100	5	100	51.5 ± 3.9	.007	57
250	5	100	43.3 ± 2.8	.001	22
500	5	100	35.8 ± 2.2	.001	0
750	5	100	30.1 ± 1.1	.001	0

*Standard deviation.
†Each mean diameter compared to that of the succeeding group rather than each one to the control.

The administration of 100 μc of radioiodine significantly reduced the diameter of the granular tubules but was without significant effect upon the other structural components of the gland. However, one striking alteration was noted in the cells composing the granular tubule. These cells ordinarily are filled with large, highly refractile granules. In the 100 μc group there was approximately a 40 per cent reduction in the number of tubules which contained these granules. This same group exhibited no significant alterations in the thyroid glands or in the incidence of dental caries.

The injection of 250 μc of I^{131} produced a significant reduction in tubule size in comparison to that observed following administration of 100 μc (Table III), and a further reduction in the number of tubules showing granules was observed. No other histologic changes were noted.

The administration of 500 μc and 750 μc of I^{131} produced nearly the same effect on the submaxillary gland, i.e., complete loss of granules from both the acini and the intralobular duct system, a significant reduction in tubule diameter, the appearance of vesicular atypical nuclei and, apparently, a marked retardation in the development of the duct system. Thus, the control and lower I^{131} concentration groups had many convoluted appearing tubules, whereas the higher I^{131} groups had uniformly small and oval-shaped tubules.

DISCUSSION

The results of this experiment show that the activity of the thyroid gland, as judged by histologic study, is closely related to the incidence of dental caries in the rat. The animals receiving a concentration of radioiodine (100 μc) which did not significantly alter the structure of the thyroid gland had a normal, expected incidence of dental caries but higher levels of I^{131}, which resulted in pronounced atrophic changes in the thyroid gland, were associated with significant increase in dental caries.

The histologic appearance of the submaxillary gland, particularly the tubular portion of the intralobular duct system and its granule content, was closely related to the activity of the thyroid gland. Large tubules and many granules in the tubule cells were found in the groups with normal caries indices (control and 100 μc I^{131}), whereas a marked reduction in size of the tubules and the absence of granules were found in groups with the highest caries indices (500 and 750 μc I^{131}). Shafer, Clark, and Muhler[10] and Sreebny[11] proposed that these granules represent sites of proteolytic activity, since their absence is associated with low activity and an abundance of these granules is associated with normal proteolytic activity. In addition, Shafer and Muhler[12] have proposed that an inverse relationship exists between the presence of the tubule granules and dental caries incidence. This study shows that dental caries incidence can be correlated with the size of the granular tubules and the presence of their granules. Since these same histologic structures in the submaxillary gland are shown to be closely related to the activity of the thyroid gland, as judged by histologic criteria, it seems a possibility that the effects of altered thyroid activity on dental caries[1-4] may actually be expressed through the activity of the salivary glands.

SUMMARY

The administration of single doses of 100, 250, 500, and 750 μc of radioactive iodine (I^{131}) to 4 groups of weanling rats produced a graded increase in their dental caries experience. Histologic study of the thyroid glands from the various groups revealed that 100 μc of I^{131} was without significant effect upon the thyroid gland, whereas each higher dosage of I^{131} produced a greater and more pronounced atrophy. Thus, a direct relationship between thyroid function and dental caries experience in the rat seems apparent. Furthermore, histologic study of the submaxillary glands of the animals in the various groups revealed a progressive nonfunctioning, atrophic appearance with increasing concentrations of radioactive iodine. From these results it appears that loss

of thyroid function may be intimately associated with loss of salivary gland function. The salivary glands then are considered as possible intermediates in a thyroid gland–dental caries relationship in the rat.

REFERENCES

1. Muhler, J. C. and Shafer, W. G.: Experimental Dental Caries IV. The Effect of Feeding Desiccated Thyroid and Thiouracil on Dental Caries in Rats, *Science* **119**: 687, 1954.
2. *Idem:* Experimental Dental Caries VIII. Effect of Thyroid-Testosterone Administration on Dental Caries in Rat, *Proc. Soc. Exper. Biol. & Med.* **88**: 191, 1955.
3. Bixler, D., Muhler, J. C., and Shafer, W. G.: The Effect of Radioactive Iodine on Dental Caries in the Rat, *J. A. D. A.* **53**: 667, 1956.
4. Muhler, J. C., and Bixler, D.: The Relation of Systemic Fluoride and Thyroid Gland Activity to the Incidence of Dental Caries in the Rat, *J. D. Res.* **36**: 304, 1957.
5. Bixler, D., Muhler, J. C., and Shafer, W. G.: Unpublished data.
6. Muhler, J. C. and Day, H. G.: Effects of Stannous Fluoride, Stannous Chloride and Sodium Fluoride on the Incidence of Dental Lesions in Rats Fed a Caries-Producing Diet, *J. A. D. A.* **41**: 528, 1950.
7. Muhler, J. C., Nebergall, W. H., and Day, H. G.: Comparison of Different Preparations of Stannous Fluoride With Sodium Fluoride in the Prevention of Dental Caries in the Rat, *J. A. D. A.* **46**: 290, 1953.
8. Grad, B. and LeBlond, C. P.: The Necessity of Testes and Thyroid Hormones for the Maintenance of the Serous Tubules of the Submaxillary Gland in the Male Rat, *Endocrinology* **45**: 250, 1949.
9. Raynaud, J.: Action of Thyroxine on Submaxillary Gland of the Castrated Mouse, *Compt. rend. Soc. biol.* **134**: 223, 1950.
10. Shafer, W. G., Clark, W. G., and Muhler, J. C.: The Inhibition of Hypophysectomy-Induced Changes in the Rat Submaxillary Gland, *Endocrinology* **59**: 516, 1956.
11. Sreebny, L. M.: Histologic and Enzymatic Differences in the Submaxillary Glands of Normal and Hypophysectomized Male and Female White Rats, *J. D. Res.* **32**: 686, 1953.
12. Shafer, W. G., and Muhler, J. C.: Experimental Dental Caries VI. The Effect of Hypophysectomy on Dental Caries and the Salivary Glands of the Rat, *J. D. Res.* **34**: 531, 1955.