Salivary Gland Function in Rats. II. Effect of Thyroid Function on Salivary Flow and Viscosity.* (24004)

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Our previous studies have established a relationship between thyroid gland function and experimental dental caries in the rat(1,2). It has been postulated that the mechanism by which this phenomenon occurs is mediated through the salivary glands(3,4) although exact details are not clearly defined. Histologic changes in salivary glands occur when thyroid function is disturbed, and these changes have suggested the possibility of alteration in the physical-chemical function of salivary glands (5). The present studies were designed to help clarify the role of the thyroid gland in regulating salivary gland function and to provide some clue to specific alterations in function which might explain the dental caries changes.

Method. This study was divided into 3 series. Series I contained 18 adult male Sprague-Dawley strain rats. Group 1 of this series received 10 μg of sodium thyroxine subcutaneously each day, a second group received 0.1% propylthiouracil added to stock diet,‡ while group 3 were controls. The drugs were continued for 10 weeks when salivary flow was measured as follows: animals were anesthetized with nembutal (37 mg nembutal/kg body weight; concentration of solution was 5 mg ml). Then, pilocarpine nitrate was given subcutaneously, 10 mg/kg of body weight. The volume of saliva, collected for 60 minutes, was determined by measuring in a calibrated syringe. Salivary viscosity was recorded as number of seconds required for saliva to pass between 2 marks 28 cm apart on finely drawn glass viscosimeter. Series II was composed of 2 groups. Group 1 consisted of 18 male weanling albino rats, 10 injected intraperitoneally with 500 μc of carrier-free radioactive iodine (I^{131}), while remaining 8 animals were controls. The base-line salivary flow and viscosity in radiothyroidectomized and control rats was determined after 90 days. Rats receiving I^{131} were given 10 μg of sodium thyroxine subcutaneously each day and salivary flow and viscosity again determined on third, fifth and ninth days following beginning of administration. Series III consisted of 63 male weanling rats divided into 5 groups: 1) controls; 2) animals thyroidectomized by administration of 600 μc of I^{131}; 3) radiothyroidectomized animals given 10 μg of sodium thyroxine daily; 4) radiothyroidectomized animals receiving 10 mg of testosterone§ intramuscularly every 3 days; and, 5) radiothyroidectomized animals receiving both thyroxine and testosterone at same levels as groups 3 and 4. Salivary flow and viscosity were measured one and 3 weeks following radiothyroidectomy and beginning of administration of thyroxine and testosterone. All animals were housed in raised screen cages and fed stock laboratory diet and distilled water *ad libitum*.

Results. The effect of thyroxine and thiouracil are seen in Table I, and indicate that thyroxine resulted in 33% increase in salivary flow as compared to 63% reduction by feeding propylthiouracil. The effect of thyroxine is not statistically significant ($p = 0.1$) although the effect of thiouracil is significant

TABLE I. Effect of Thyroxine and Thiouracil on Rat Salivary Flow (Series I).

Group	No. of animals	Avg body wt (g)	Salivary flow (ml/hr)
Control	4	360	2.9 ± .6*
Thyroxine	9	356	4.3 ± 1.2
Thiouracil	5	273	1.6 ± .4

* Stand. dev.

* Supported by Office of Surgeon General, Dept. of Army.

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‡ Composition of diet as follows (in %): yellow corn grits, 52.7; ground yellow whole corn, 11.3; powdered whole milk, 30; alfalfa, 4.8; iodized sodium chloride, 1; and irradiated yeast 0.2.

§ Supplied generously by Schering Corp., Bloomfield, N. J.

TABLE II. Effect of Thyroxine on Salivary Flow and Viscosity in Radiothyroidectomized Rats (Series II).

Group	No. of animals	Avg body wt (g)	Salivary flow (ml/hr)	Salivary viscosity (sec.)
		0 days		
Control	8	267	1.6 ± .5*	54 ± 12
I^{131}	10	126	.1 ± .1	100 ± 26
		3 days		
Control	8	275	1.1 ±1.1	46 ± 7
I^{131} + thy†	10	123	.1 ± .1	86 ± 14
		5 days		
Control	8	257	2.6 ±1.0	58 ± 14
I^{131} + thy	10	126	.4 ± .3	73 ± 20
		9 days		
Control	7	260	1.8 ± .7	49 ± 7
I^{131} + thy	8	130	1.2 ± .8	49 ± 4

* Stand. dev. † thy = thyroxine.

(p = 0.02).

Radiothyroidectomy resulted in significant reduction in salivary flow and significant increase in salivary viscosity (Table II). Thyroxine administered to thyroidectomized animals produced a gradual increase in salivary flow and reduction in salivary viscosity until, by end of ninth day, there were no significant differences between the 2 groups.

Table III shows data obtained in Series III. Radiothyroidectomy again resulted in reduction in salivary flow, thyroidectomized animals producing an average of 0.4 ml of saliva/hour as compared to 1.1 ml in controls after 3 weeks. Radiothyroidectomized animals receiving testosterone, thyroxine or combined, after 1 week showed no distinctive changes from I^{131} animals receiving no therapy. However, by end of 3 weeks, significant differences in salivary flow were found between controls and I^{131} animals. Animals receiving thyroxine, or thyroxine plus testosterone had a significant increase in salivary flow. Testosterone alone was partially effective in returning flow to the level of the control group. There were changes in salivary viscosity which generally paralleled flow but while directional, were not significant.

Discussion. Changes in salivary gland function, as evaluated by salivary flow and viscosity, parallel what might be expected to explain the effects of thyroid dysfunction on dental caries incidence. It is known, for example, that a marked reduction in salivary flow, brought about by extirpation of salivary glands, results in a significant increase in incidence of dental caries in the rat(6). Similarly, administration of I^{131} or propylthiouracil significantly increases dental caries(7). If the effects of these 2 agents are directly mediated through the salivary glands, causing a physiologic reduction in salivary flow, then one could readily explain the effect of thyroid gland function on dental caries on this basis. Our studies lend credence to this hypothesis by proving that thyroxine not only increases salivary flow, but that thiouracil and I^{131} reduce salivary flow. These observations correspond also with similar histologic changes suggestive of this effect(8,9).

Decreased salivary flow in Series I and II may conceivably be related to smaller body size of thiouracil-treated and radiothyroidectomized animals. This objection is not valid however, since animals in Series III which received thyroxine and testosterone weighed less than control animals yet produced far more saliva. The relationship of salivary flow to weight of salivary glands rather than body weight must await further study.

Summary. 1) We determined the effects of thyroid gland dysfunction on salivary flow and viscosity in the rat. Results indicate that

TABLE III. Effect of I^{131} Thyroidectomy and Replacement Therapy on Rat Salivary Flow and Viscosity (Series III).

Group	No. of animals	Avg body wt (g)	Salivary flow (ml/hr)	Salivary viscosity (sec.)
		1 wk		
Control	5		1.2 ± .8*	18.0 ± 4.9
I^{131}	8		.5 ± .6	17.1 ± 1.3
I^{131} + test.†	5		.5 ± .3	24.0 ± 6.9
I^{131} + thy.†	5		.9 ± .8	11.3 ± 1.0
I^{131} + thy. + test.	3		.5 ± .0	13.8 ± .3
		3 wk		
Control	4	166	1.1 ± .2	32.0 ± 8.8
I^{131}	5	120	.4 ± .3	41.4 ± 2.9
I^{131} + thy.	6	114	1.9 ± .9	28.5 ± 7.3
I^{131} + test.	4	123	.9 ± .5	40.9 ±10.0
I^{131} + thy. + test.	5	116	2.0 ± .6	27.9 ± 4.6

* Stand. dev.

† test. = testosterone; thy = thyroxine.

propylthiouracil and radiothyroidectomy reduced salivary flow and increased salivary viscosity. This can be reversed and function restored by administration of thyroxine. Testosterone partially restores salivary flow. 2) These data provide one explanation for the mechanism of alteration in dental caries incidence in the rat following disturbance in thyroid function, although the relationship is probably far more complex than this.

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Isolation of Tumor Cell Colonies in Tissue Culture.* (24005)

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Marcus, Puck, and Cieciura(1,2) described methods for isolation of "clones" of HeLa cells in petri dish tissue culture. They emphasized the necessity for the presence of more than a single cell to obtain growth. Goldstein(3) pointed out that it is not possible with this procedure to be certain that colonies originated from a single cell. He described a method for isolation of clones by which all but a single cell in a Carrel flask are destroyed by local heat with electric soldering iron. However, this cannot be done if single isolated cells fail to grow. The present study was made in an attempt to obtain colonies of cells which failed to grow singly and to avoid the difficulty in microscopic observation of cells grown in a petri dish. The ascitic form of a mouse ependymoma grown in A mice(4) previously could not be propagated from single cells isolated from droplets in a micropipet and injected subcutaneously in suckling mice (unpublished data). This procedure was successfully used elsewhere for growth of single tumor cells in mice(5,6). Likewise our attempts to grow single cells in a variety of media in capillary pipets(7) or on cover slips failed. These ependymoma cells are readily cultivated on glass in tissue culture. Isolated colonies originating from single cells were obtained as follows.

Materials and methods. After good growth of cells was obtained at 37°C in Kolle flasks containing a casein hydrolysate medium(8) with 10% horse serum, the cells were removed from the glass by contact at 37°C with 0.25% trypsin, pH 8, for 2-3 minutes. Serial dilutions of cells were made in chicken plasma, one drop placed near one end of each of 7-8 rectangular cover slips (11 x 32 mm) contained on moist filter paper in a small petri dish. An attempt was made to have 1-20 cells/drop after dilution. A second drop of plasma containing several hundred cells was placed near the other end of each cover slip. A drop of filtered chick embryo extract (CEE) was added first to the plasma drop containing diluted cells. The drop was mixed (with a fine glass rod bent to form an L) and spread

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