Effects of Parathyroid Extract, Calciferol, Hytakerol and Dihydrotachysterol Upon Thyroid Secretion Rate in Normal Female Rats.* (29463)

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It has been demonstrated conclusively that the intensity of milk production in large domestic animals is controlled in part by thyroidal preparations(1). This finding was later supported by investigations in other species(2-6), in which it was observed that thyroid hormone secretion might increase during lactation. It has been demonstrated that parathyroid extract, calciferol, Hytakerol and crystalline dihydrotachysterol increased milk secretion when administered to rats from day 7 to 20 of lactation(7). It seemed desirable therefore, to investigate the relationship between these compounds and thyroid gland function in the rat. However, it is difficult to conduct experiments using radiobiological techniques in lactating animals (for controls and experiments) since the period of lactation is short in the rat. The investigation reported herewith was made, therefore, in non-lactating rats.

Materials and methods. A total of 66 adult primiparous non-pregnant female rats of the Sprague-Dawley-Rolfsmeyer strain were kept under conditions of uniform temperature (78° ± 1F) and fed Purina Lab Chow and water *ad libitum*. Carrier-free ^{131}I was obtained from Nuclear Consultants Corp., St. Louis, Mo. and was diluted in distilled water to contain 15 μc/0.2 ml, and was administered to the rat subcutaneously. Standards were prepared by diluting 1.5 μc of the ^{131}I in distilled water and kept in small vials.

Measurements of ^{131}I were made with a scintillation detector, Nuclear Chicago (NC) model DS 5, connected to a rate meter, NC 1620B, with a maximum capacity of 10^6 cpm. Sodium L-thyroxine (T_4) was obtained from Travenol Lab., Ill., and a stock solution was prepared in 0.1 N NaOH. The solution was then precipitated with 0.1 N HCl and kept refrigerated. The required amount of this stock solution was diluted in alkaline 0.9% NaCl and contained 40 mg methimazole (Tapazole)/ml. The T_4 administered to each animal was contained in a volume of 0.1 ml.

The determination of thyroid secretion rate (TSR) used in this study was based on slight modification of the technique of Grosvenor and Turner(6). The rats were injected subcutaneously with 15 μc carrier-free ^{131}I and were allowed 48 hours for uptake by the thyroid glands. Thyroid counts were taken at 48 hour intervals. The animals were anesthetized with ether, and the thyroid region was gently pressed upon a scintillation detector. T_4 was administered daily (the first administration was 48 hours after that of ^{131}I) in increments of 0.25 μg/animal. The dose of administered T_4 which prevented further thyroidal ^{131}I output, that is, 95 to 100% of the previous count, was estimated as the TSR of the animals. Correction was made for the radioactive decay and background. Ten to 20 days after TSR was determined they were divided into 4 groups and treated as follows: 12 animals received 30 USP units of parathyroid extract§ (PTE)/100 g BW/day; 12 animals received 100 μg crystalline dihydrotachysterol‖ (DHT)/100 g BW/day; 12 animals received 125 μg Hytakerol (AT 10‖)/100 g BW/day and 12 animals received 200 μg

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2698. Approved by Director.

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‡ Aided by a grant from U.S. Atomic Energy Commission.

§ Kindly supplied by Eli Lilly and Co., Indianapolis, Ind.

‖ Crystalline dihydrotachysterol, calciferol and Hytakerol (AT 10) kindly supplied by Sterling-Winthrop Res. Inst. Hytakerol contains dihydrovitamin D_2II rather than dihydrotachysterol.

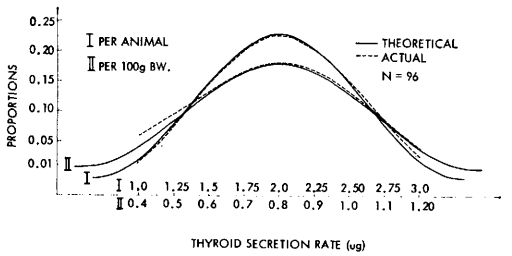

FIG. 1. Proportions graph of frequency distribution of thyroid secretion rate in 66 normal mature female rats.

crystalline calciferol‖/100 g BW/day. The materials were administered, subcutaneously, for 13 days. Three groups served as controls in this investigation. Six animals received 0.1 ml of a solution consisting of 1.6 g glycerin and 0.2 g phenol in 100 ml of distilled water/100 g BW; 6 animals received 0.05 ml of sesame oil/100 g BW and 6 animals received no treatment. The sesame oil served as a vehicle for AT 10, DHT and calciferol while the solution of 1.6 g glycerin and 0.2 phenol in 100 ml distilled water served as a vehicle for PTE.

A second TSR determination of each animal was then undertaken. The difference between the TSR of the first and the second determination in the same animal was used as an indication of the effects of the various materials upon the functional activity of the thyroid gland.

Results. The 66 normal mature female rats showed a mean TSR of 2.22 μg T_4 per animal with a range of 1.0 to 3.0 μg. Estimated on a 100 g BW basis, the mean TSR was 0.88 μg with a range of 0.40 to 1.20 μg. The frequency distribution of the TSR per animal and per 100 g BW is shown in Fig. 1. No differences were observed in the first and second determination of TSR in animals receiving the control solution of 1.6 g glycerin and 0.2 g phenol in 100 ml distilled water, and in the non-treated group. The mean TSR of animals receiving sesame oil, however, was increased slightly (7%) but this change was not statistically significant.

Administration of 30 USP units of PTE/100 g BW/day for 13 days caused a significant 21% increase in TSR (Table I). The mean body weight of the animals was also increased slightly. Calciferol, AT 10 and DHT at the dosage given increased TSR 48%, 55% and 103% respectively. A severe body weight loss was observed in these groups. On the other hand, the control groups showed an increase in body weight.

Discussion. A relationship between vit. D and thyroid gland function was reported in the thirties. Histological studies showed that administration of vit. D caused hyperplasia of the thyroid glands of dogs(8). In their study of the effect of vit. D in thyroparathyroidectomized and intact dogs, Deutsch and coworkers(9) concluded that a calorigenic effect was exhibited by the vitamin via the thyroid gland. Administration of vit. D to rachitic rats was reported to increase the basal metabolic rate(10). On the other hand, no calorigenic effect was observed when a parathyroid extract was given to dogs(11). It was reported that parathyroid hormone reduced the uptake of ^{32}P in all hard tissue when it was given to kittens(12) and O^2 uptake in kidney and liver mitochondria was reported to increase by *in vitro* addition of parathyroid extract(13). Recently, it was reported that parathyroid extract given to rabbits increased the conversion of glucose and organic acids to CO_2 in bone slices(14). Although no functional relationship between parathyroid and thyroid glands has been reported, the investigations cited suggested the possible participation of parathyroid extract in the regulation of certain metabolic pathways.

The present technique used to measure the daily TSR in rats using ^{131}I as a tracer(6) enables one to measure thyroidal activity in rats more objectively in comparison with the methods using morphological and histological techniques. In the present study, the effect of crystalline calciferol on thyroid function was reinvestigated and its related compounds crystalline dihydrotachysterol and Hytakerol (containing dihydrovitamin D_2II). Data obtained from this preliminary study showed that administration of calciferol, dihydrotachysterol and dihydrovitamin D_2II for a period of 13 days to normal female rats induced a significant increase in TSR associated with a severe drop in body weight. On the other hand, parathyroid extract at a level of 30

TABLE I. Effects of Parathyroid Extract, Calciferol, Hytakerol and Dihydrotachysterol upon TSR in Intact Female Rat.

Before treatment			After treatment				
No. of animals	Body wt (g)	TSR (μg/100 g)	Treatment	Body wt (g)	TSR (μg/100 g)	Increase (%)	P*
6	222.00	.993 ±.050†	None	235.50	.963 ±.070†	−3.12	<.40 >.35
6	187.17	.786 ±.138	Sesame oil	221.00	.846 ±.133	7.00	<.40 >.35
6	207.33	.886 ±.114	Control solution consists of 1.6 g glycerin, 0.2 g phenol in 100 g water	220.67	.893 ±.097	.79	>.45
12	209.50	.835 ±.057	PTE, 30 USP units/100 g BW	222.50	1.010 ±.049	20.96	<.010 >.005
12	215.67	.940 ±.092	AT 10, 125 μg/100 g BW	155.67	1.457 ±.126	55.00	<.005
12	203.17	.582 ±.081	Calciferol, 0.2 mg/100 g BW	174.67	.862 ±.089	48.11	<.005
12	221.83	.723 ±.094	DHT, 100 μg/100 g BW	142.60	1.406 ±.089	102.59	<.005

* P students "t" test; level of significance before and after treatment of the same animals.
† Mean ± S.E.

USP units/100 g BW given daily for the same period caused a less marked increase in TSR accompanied by a slight increase in body weight of the experimental animals. The inorganic phosphate level of the blood was reported to increase when L-thyroxine or L-triiodothyronine was given to man(15). The effect of thyroid hormone on oxygen consumption and on carbohydrate metabolism is well known. A possibility that the metabolic effect exerted by the parathyroid extract reported by other investigators(12-14) was mediated by the thyroid gland is, therefore, not excluded.

Nothing is currently known concerning the mode of action of the parathyroid hormone and the irradiated ergosterols in stimulating an increased thyroxine secretion rate. It is expected that the most likely mode of action would involve the increased secretion and discharge of the thyrotropic hormone. However, it is not impossible that these substances may act directly upon the thyroid gland to increase the responsiveness of the glands to a given level of TSH.

Summary. The influence of a parathyroid hormone extract, calciferol and its related compounds on thyroxine secretion rate (TSR) of normal female mature rats was studied with the aid of ^{131}I. Administration of 30 USP U of parathyroid extract/100 g BW/day for a period of 13 days induced a significant 21% increase in TSR associated with a slight increase in body weight. The TSR was increased 48%, 55%, and 103% respectively and accompanied by a dramatic loss of body weight when 200 μg calciferol/100 g BW, 125 μg Hytakerol/100 g BW or 100 μg crystalline dihydrotachysterol/100 g BW were given daily for the same period.

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Received March 31, 1964. P.S.E.B.M., 1964, v116.

The Chromosomes of Tumors Induced in Mice by Human Adenovirus, Type 12.* (29464)

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Human adenovirus, type 12, when injected into newborn hamsters(1), rats(2), mice(3), or mastomys(4) induces undifferentiated tumors at site of injection. This communication concerns a chromosome study of tumors so induced in mice.

Materials and methods. Cells were studied from primary tumors in four C_3HfGs mice and from 71 tumors representing the first (37 animals) and second (34 animals) passages of these tumors. The 4 primary tumors were obtained from animals (2 females, 2 males) injected intraperitoneally when newborn with 0.05 ml of human adenovirus type 12 ($A_{12}S_9$). Most of the animals used for tumor passage were males and were injected with tumor mince or cell suspensions when adult. The resulting tumors were harvested after 2-6 weeks of growth. Two of the animals with primary tumors were treated with colchicine and 2 were untreated. All of the animals carrying tumor passages were colchicine treated (1 μg per gram body weight). The animals were injected with colchicine 1-3 hours before they were sacrificed by rupture of the cervical spinal cord. Tissue culture studies were done on all animals, and sections for pathology on most. Routine air dry techniques and temporary squash methods were used in making the chromosome preparations from finely minced tumor tissue. Most of the studies for structural aberrations were done from temporary orcein squash preparations, since these had a higher proportion of cells with chromosomes lying separately in one plane. The minced tissue was suspended in saline, which was subsequently diluted 1:4 with distilled water, allowed to stand 7-20 minutes, and then centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted, and 1 ml of fixative (60% acetic acid) was layered over the button of cells. After one hour or longer the acid was decanted, and 1 ml of acid orcein dye (2% orcein in 60% acetic acid) was added. The cells, resuspended in the solution, were used for making squash preparations. Terminology for chromosome variation is employed in accordance with definitions used in classical genetics adapted to present day cytogenetics(5).

Results and discussion. Results of the investigation are summarized in Tables I and II. Material from the original tumors, passage tumors, and tissue cultures showed essentially the same findings: aneuploidy, polyploidy, mixoploidy, low rates of endoploidy (5/2001 cells), and relatively few structural aberrations (17/804). Very few chromosomes other than normal telocentrics were present. Most of the cells (2001) analyzed for chromosome number (Table I) were in the diploid range of 30 to 50, 56% having the normal number of 40 telocentric chromosomes per cell. Fig. 1, showing the distribution of the chromosome number in the original tumors and the first and second passages of the tumors, demonstrates the likely presence of mixoploidy. All show a peak at 42 ($2N = S = 2x + 2 = 42$) and 55 ($2N = S = 2x + 15 = 55$) as well as peaks at 3x (excepting original tumors), 4x, 5x and higher. There were no marker chromosomes present, or any

* Supported by grants from the Anna Fuller Fund and Nat. Cancer Inst., U.S.P.H.S.

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