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Received February 19, 1964. P.S.E.B.M., 1964, v116.

The Effect of Crystalline Dihydrotachysterol on Milk Secretion in Thyroparathyroidectomized Lactating Rats.* (29405)

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When lactating rats were thyroparathyroidectomized on day 4 of lactation and given thyroxine replacement therapy without parathyroid hormone, some mothers died of tetany, and the young either died or grew very poorly to day 14. Milk yield averaged 1.6 g on day 14 after a 10 hour separation period(1).

Replacement therapy was successfully achieved in thyroparathyroidectomized lactating rats using 60 to 100 μg Hytakerol (AT 10)/100 g BW/day given orally from day 7 to 13 post-partum(2). The effect of AT 10 on milk secretion in normal rats was also investigated and it was observed that subcutaneous injection of 125 μg of the material to normal rats from day 7 to 20 of the lactation period increased milk yield 57% and 15% on day 14 and 20 of lactation respectively(3). It was claimed that this material contained dihydrotachysterol. Biochemical study carried out to investigate the nature of commercial Hytakerol, however, indicated that this material did not contain dihydrotachysterol but was dihydrovitamin D_2II(4). Consequently the effect of dihydrotachysterol on milk secretion has not been explored. Preliminary study in this laboratory indicated that administration of crystalline dihydrotachysterol to normal rats increased milk secretion 8% on day 14 and 34% on day 20 of lactation. The hypercalcemic and hyperphosphatemic effects of crystalline dihydrotachysterol in rats has been reported(5).

Evidence is presented here which suggests that crystalline dihydrotachysterol is able to take the place of the parathyroid hormone in milk synthesis in lactating thyroparathyroidectomized rats.

Materials and methods. Pregnant rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages and on day 4 post-partum the litters were reduced to 6 young. The animal room was maintained at a temperature of 78° ± 1°F and the rats were fed Purina Lab Chow and water *ad lib.*

The dams were thyroparathyroidectomized (TPEX) on day 4 post-partum under sodium Nembutal anaesthesia with a dose of 30 mg/kg BW. One hour before the operation a blood sample was collected for serum calcium determination by the chloranilate method(6). In seeking evidence for the completeness of removal of the glands, a second blood sample was collected 8 hours after the operation for serum calcium determination. Animals which showed a fall of serum calcium of less than 3 mg% after thyroparathyroidectomy were excluded from the experiments and were considered as "failures." A third and fourth blood sample was collected on day 14 and 20 of lactation for determination of serum calcium. Controls were treated in the same way. The thyroid and parathyroid glands were exposed but not removed. Immediately after collection of the second blood sample, 23 animals were injected with 100 μg crystalline dihydrotachy-

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2697, approved by the Director. Aided in part by research grants from U.S.P.H.S.

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sterol‡ (DHT)/100 g BW/day with a daily supplement of 3.0 μg L-thyroxine (T_4)/100 g BW. The DHT was dissolved in sesame oil to contain 2 mg/ml and the T_4 was dissolved in alkaline 0.9% NaCl to contain 30 μg/ml. The sham-operated controls were injected daily with 0.05 ml sesame oil/100 g BW/day and 0.1 ml alkaline 0.9% NaCl/100 g BW. Weights of dams and litters were taken from day 7 to 20 of lactation and milk yields were determined on day 14, 16, 18 and 20 by the differences of the litter weight before and after nursing, after a 10-hour period of isolation from the dams. One USP unit of oxytocin was injected into the dams immediately before nursing to aid in milk removal. The deoxyribonucleic acid (DNA) of 6 posterior mammary glands per 100 g BW was determined at the end of each experiment expressed in mg and as an index of mammary gland growth or mammary gland involution by the method described previously(7).

Results. Crystalline DHT at a level of 100 μg/100 g BW in conjunction with 3.0 μg T_4/100 g BW given daily to TPEX lactating rats from day 4 to 20 appears capable of replacing the parathyroid gland function of the animals. The effectiveness of replacement therapy was improved with the advance of the administration of the material as shown by the milk yields determined on days 14, 16, 18 and 20 (Table I). Similarly, serum calcium values increased gradually (Table II). The litter weights, however, were lighter than the sham-operated controls (Table I). Significant differences were not observed in the DNA content of the mammary glands of the two groups (sham-operated: 9.10 ± 0.42 mg; TPEX: 8.33 ± 0.20 mg; $0.15 > p > 0.10$). It was shown also that serum calcium of the sham-operated group decreased with the intensity of milk secretion (Table II).

Discussion. The importance of calcium and of the participation of the parathyroid glands in milk secretion has been indicated by several authors, primarily based on the

‡ Kindly supplied by Sterling-Winthrop Res. Inst., Rensselaer, N. Y.

TABLE I. The Effect of Crystalline Dihydrotachysterol on Milk Yield and Litter Weight in Thyroparathyroidectomized Rats.

No. of animals	Treatments	Litter wt (g)†		Litter wt gain (g)†	Milk yields*(g)†			
		Day 7	Day 20	Day 20	Day 14	Day 16	Day 18	Day 20
13	Sham-operated; .05 ml/100 g BW sesame oil + .1 ml/100 g BW alkaline .9% NaCl	79.6 ± 8.3	197.1 ± 7.5	117.5 ± 6.3	8.7 ± 1.3	11.2 ± 1.4	12.7 ± 1.0	12.2 ± .7
23	TPEX; 100 μg/100 g BW DHT + 3.0 μg T4/100 g BW	72.0 ± 7.1	160.3 ± 6.9	88.3 ± 6.8	7.9 ± .5[1]	9.3 ± .5[2]	12.9 ± 1.0[3]	13.0 ± .4[4]

* Litter was reduced to 6.
† Mean ± S.E.
TPEX = thyroparathyroidectomy. DHT = crystalline dihydrotachysterol. T4 = L-thyroxine.
Students "t" probability of significance of difference from controls: [1] $.20 > p > .15$, [2] $.050 > p > .025$, [3] $.45 > p > .40$, [4] $.10 > p > .05$.

TABLE II. Serum Calcium of Rats Before and After Thyroparathyroidectomized on Day 4 of Lactation and After Administration of Crystalline Dihydrotachysterol.

No. of animals	Treatments	Serum calcium (mg %), mean ± S.E. Day 4 Pre-operative	Day 4 8 hr post-op	Day 14	Day 20
13	Sham-operated; .05 ml/100 g BW sesame oil + .1 ml/100 g BW alkaline .9% NaCl	10.81 ±.39*	10.19 ±.79	8.72 ±.82	9.72 ±.30
23	TPEX; 100 μg/100 g BW DHT + 3.0 μg/100 g BW T4		6.43 ±.28†	10.54 ±.50[1]	10.50 ±.31[2]

* Data from 64 animals. † Data from 44 animals.

Students "t" probability, significant difference from sham-operated controls: [1] .10 > p > .05, [2] .15 > p > .10.

effect of removal of the glands on milk yield of experimental animals(8-13).

Dihydrotachysterol is a steroid related to vit. D and can be obtained by reduction of tachysterol(14). Chemically, this compound is different from parathyroid hormone. The data presented show that DHT is able to take the place of parathyroid hormone in milk synthesis when it is given daily to thyroparathyroidectomized lactating rats from day 4 to 20 post-partum at a dose level of 100 μg/100 g BW in conjunction with 3.0 μg T_4/100 g BW. DHT maintained serum calcium levels on days 14 and 20 of lactation slightly above that of the controls. While the milk yield of the experimental animals was below that of the controls on days 14 and 16, it increased gradually and exceeded that of the normal rats on days 18 and 20. The lighter litter weight and lower milk yield of the experimental group might be due to the fact that the most effective plasma pool of DHT was not immediately achieved after early administration of the material. This has been shown to be true of the administration of thyroxine(15). Replacement therapy with a larger dose at the beginning and then a gradually decreasing dose might have had a more favorable action throughout the period of lactation.

It was demonstrated conclusively in this investigation that DHT could function fairly well in the absence of the parathyroid glands in the maintenance of milk secretion. The effect is probably due to the maintenance of a normal serum calcium level available to the mammary glands for milk synthesis.

Summary. Daily administration of 100 μg crystalline dihydrotachysterol/100 g BW from day 4 to 20 of lactation in conjunction with 3.0 μg L-thyroxine/100 g BW was able to maintain lactational performance in thyroparathyroidectomized rats. Milk yields of the thyroparathyroidectomized animals were elevated to the level of the sham-operated group by day 14 of lactation but litter weights of the thyroparathyroidectomized animals were smaller than the sham-operated controls. It is suggested that the role of crystalline dihydrotachysterol in maintaining milk secretion is mediated by the mobilization of a level of serum calcium adequate for the synthesis of milk.

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Received February 28, 1964. P.S.E.B.M., 1964, v116.

Comparison of Pharmacologic Activity in a Series of Benzimidazole Compounds. (29406)

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Hunger and associates(1) reported on the synthesis of a series of 1-(2-dialkylaminoethyl)-2-benzyl-5-nitro-benzimidazoles. These compounds were subsequently described as a new class of potent analgetics(2). Following these reports, several others appeared describing the synthesis and analgetic activity of a number of analogs in which various substitutions on the 2-benzyl benzimidazole nucleus were made. The structure-activity relationships of these compounds were reviewed by Beckett and Casy(3). The purpose of this study was to explore the structure-activity relationships of some 1-(2-dialkylaminoethyl)-2-phenoxymethyl benzimidazoles produced by varying substitutions on the benzimidazole and phenyl rings. In addition, the effects of alteration of the alkyl groups on the nitrogen of the ethyl amino side chain of certain derivatives were compared.

Methods. Analgetic activity as well as acute toxicity was determined in male, Swiss-Webster mice using a modification of the hot plate method of Woolfe and MacDonald(4). The heat source was a 250 watt infrared lamp (General Electric) to which 18 volts were applied from a variable transformer. One mouse at a time was placed on the surface of the bulb and the time recorded until the animal jumped off. The sum of 4 consecutive trials was recorded as the reaction time. Reaction times were recorded 3 times for each mouse approximately 20 minutes apart before a drug was administered. Any animal showing a reaction time greater than 6 seconds on the third trial was discarded. The reaction times of the animals were then determined at 30, 60 and 90 minutes following subcutaneous administration of drugs. A 60 second cut off time was utilized for mice which did not jump off the bulb. Since no significant difference could be demonstrated between reaction times determined 30 and 60 minutes following administration of any of the drugs tested, it was decided to use a function of the 30 minute post drug reaction time as the response metameter. Since animals which displayed reaction times greater than 6 seconds on the third trial before drug administration were discarded, the number of animals in dose groups were frequently unequal, ranging from 6 to 10 animals per group. For this reason the unsymmetrical bioassay described by Finney (5) was used to compare statistically the analgetic activity of the analogs to a dose-response regression derived from responses to 2, 4 and 8 mg/kg of morphine sulfate. This statistical method provides for the assessment of potency relative to a standard. A value of 100 has been assigned to the potency of morphine sulfate in this bioassay, hence the relative potency (R) calculated for each analog is in terms of % of morphine sulfate activity.

Acute toxicity, as reflected by lethality within 24 hours following injection of drugs was conducted in Swiss-Webster mice. Intraperitoneal LD-50 values were obtained by determining the effect of 3 dose levels yielding between 10 and 90% effect. At least 10 mice were utilized in determining each point. The computations for fitting the probit-log dose regression line determining the LD-50 and 95% confidence intervals were done ac-