used for immunization, corresponding precipitation lines would be expected. But electrophoresis disclosed only a single line reactive with a-mouse TtH serum.

It is conceivable that antigens derived from the tumor induced and grafted in an isologous strain of mice circulate in the blood. They may react with antibodies prepared against native tumor proteins, but probably not with the a-mouse TtH prepared with highly purified hormone. It is noteworthy that the titer of the presumed antigen (TtH) was highest in athyroid hosts in which the TtT gave no gross or histologic evidence of autoimmunization. The titer dropped precipitously when the tumors acquired ability to grow in the normal host in which TtH production is restrained by the host's thyroid (TH). It is unlikely that such a change is paralleled with loss of specific tumor antigens.

For these reasons, we believe that the assays here reported are hormone specific. The alternate assumption that the antigen detected is tumor antigen other than hormone lacks supporting evidence. Tumor specific substances may be present in the blood. Their existence is of sufficient interest to suggest experiments aimed at their detection.

Summary. An antiserum prepared against purified murine thyrotropin precipitated specifically sera of thyrotropic tumor-bearing mice and extracts of thyrotropic tumors. The titer of precipitation reaction paralleled the bioassay titers for TtH. The test can detect TtH in 0.002 ml of serum of TtT mice but not in 0.2 ml of normal sera. In immunoelectrophoresis, a single TtH line was obtained in the globulin region, yet to be precisely localized. Slight or no group reactions were obtained among rat, beef, and mouse TtH preparations.

The receipt of purified murine TtH from Dr. Robert W. Bates and P. G. Condliffe are gratefully acknowledged.

1. Bates, R. W., Garrison, M. M., Howard, T. B., *Endocrinology,* 1959, v65, 7.

2. Bates, R. W., and Condliffe, P. G., *Rec. Prog. in Hormone Res.,* 1960, 16, 309.

3. Furth, J., Burnett, W. T., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 222.

4. McKenzie, J. M., *Endocrinology,* 1958, v68, 372.

5. Grabar, P., Williams, C. A., *Biochim. Biophys. Acta,* 1953, v10, 193.

6. *Immunoassay of Hormones,* Ciba Found. Coll. on Endocrinology, 1962, v14.

7. Werner, S. C., *In Thyrotropin,* Charles C Thomas, Springfield, Ill., 1963.

8. Furth, J., Clifton, K. H., *The Pituitary Gland,* Butterworth, London, in press, 1963.

9. Bates, R. W., Anderson, E., Furth, J., *Endocrinology,* 1957, v61, 549.

10. Kamat, V. B., Ph.D. Thesis, Univ. of Bombay, India, 1961.

11. Bates, R. W., *In Evaluation of Thyroid and Parathyroid Functions,* Sunderman, J. B. Lippincott Co., Philadelphia, 1964.

Received April 3, 1964. P.S.E.B.M., 1964, v116.

Effects of Parathyroid Extract, Calciferol, Hytakerol and Crystalline Dihydrotachysterol on Feed Consumption in Normal Lactating Rats.* (29330)

S. DJOJOSOEBAGIO† AND C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia

The normal mean and variation in feed consumption of female Sprague-Dawley-Rolfsmeyer rats has been reported as a preliminary to a study of the effect of food restriction on thyroxine secretion rates(1). In addition, it was observed that the feed intake of the rat increased with the advance of lactation to the 20th day, then declined rapidly

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2705. Approved by Director. Aided in part by grants from U.S.P.H.S.

† Kentucky Res. Foundation Fellow from College of Vet. Med. and Animal Husbandry, Univ. of Indonesia, Borgor-Indonesia.

TABLE I. Effects of Parathyroid Extract, Dihydrotachysterol, Hytakerol and Calciferol on Feed Consumption in Lactating Rats from Day 7 to 14 Post Partum.

No. of animals	Treatment	Dosage	Mean total daily feed consumption from day 7 to 14 of lactation: Mean ± S.E. (g)	Increase (%)	P*
34	None	—	29.69 ± .87†	—	—
14	Solution of 1.6 g glycerin, .2 g phenol in 100 ml distilled water	.3 ml/100 g BW	30.94 ± 1.07	—	—
15	Sesame oil	.05 ml/100 g BW	26.47 ± 1.03	—	—
22	Dihydrotachysterol	100 μg/100 g BW	26.77 ± 1.28	1.13	<.45 ‡ >.40
18	Hytakerol	125 μg/100 g BW	30.74 ± 1.00	16.13	<.005‡
24	Calciferol	.2 mg/100 g BW	31.59 ± .96	19.34	<.005‡
22	Parathyroid extract	30 USP units/100 g BW	33.87 ± 1.10	9.47	<.050§ >.025

* Student's "t" probability.
† Based on data of Anderson and Turner(3).
‡ Significant difference from animals treated with sesame oil.
§ Significant difference from animals treated with solution consisting of 1.6 g glycerin, and 0.2 g phenol in 100 ml distilled water.

following weaning(2,3). Studies on the effects of parathyroid extract, Hytakerol, crystalline calciferol(4) and crystalline dihydrotachysterol (unpubl.) on lactation showed that these materials increased milk secretion when they were administered to rats from day 7 to 20 of lactation. The present study was conducted to determine the effects of these materials on feed consumption of lactating rats.

Materials and methods. A total of 115 pregnant rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages, fed Purina Lab Chow and water *ad lib.* The animal room was maintained at a uniform temperature of 78° ± 1°F. On the fourth day post partum the litter was reduced to 6 and mother and young were transferred into 8″ × 10½″ × 6″ metabolism cages. From day 7 to 14 of lactation, the animals were treated as follows: 22 animals were injected (subc.) with 100 μg crystalline dihydrotachysterol‡ (DHT)/100 g BW/day, 18 animals with 125 μg Hytakerol‡/100 g BW/day, 24 animals with 200 μg crystalline calciferol‡/100 g BW/day and 22 animals with 30 USP units of parathyroid extract§ (PTE)/100 g BW/day. One group of control animals was injected with 0.3 ml/100 g BW solution consisting of 1.6 g glycerin and 0.2 g phenol in 100 ml distilled water and another group with 0.05 ml sesame oil/100 g BW/day. The sesame oil served as a vehicle for Hytakerol, crystalline dihydrotachysterol and calciferol and the solution of 1.6 g glycerin and 0.2 g phenol in 100 ml of water served as a vehicle for the parathyroid extract. Beginning on day 7 of lactation daily feed consumption of the mother was estimated by the method described previously(3).

Results. Little difference was observed in the daily feed consumption of the untreated animals(3) and the animals treated with the solution of 1.6 g glycerin and 0.2 g phenol in 100 ml water. The group treated with sesame oil, however, showed a decrease in feed consumption compared with the other control groups (Table I). Administration of 100 μg DHT/100 g BW from day 7 to 14 of lactation in intact rats did not show a significant increase in daily feed intake. Animals receiving 125 μg Hytakerol/100 g BW or 200 μg calciferol/100 g BW on the other hand, increased their feed consumption by 16% and 19% respectively above the animals treated with sesame oil. In comparison with the animals injected with the solution consisting of

‡ Kindly supplied by Sterling-Winthrop Res. Inst., Rensselaer, N. Y. Trade name for AT 10 claimed to contain dihydrotachysterol. Shown to contain dihydrovitamin D_2II (5).

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

1.6 g glycerin and 0.2 g phenol/100 ml of distilled water, the animals treated with 30 USP units of PTE/100 g BW showed an increase of 9.5% in their feed intake (Table I).

Discussion. It is recognized that the maternal nutrient state is very important during pregnancy and lactation, since considerable amounts of extra energy are needed for growth of the fetus and for production of milk. The relationship between feed intake and intensity of lactation in rats has been reported(3). The thyroid hormone is known to have a calorigenic effect and the amount of feed consumed by animals might therefore, influence thyroid hormone output. It was reported that thyroxine secretion rates (TSR) of the mouse was decreased when subjected to feed restriction(6). Similar results were obtained in rats(7). On the other hand experimental hyperthyroidism by administration of L-thyroxine to rats of both sexes caused an increase in feed consumption depending on the dose of thyroxine given(8). In a study of the effect of parathyroid extract, calciferol, A.T. 10 and crystalline dihydrotachysterol on TSR in normal non-lactating rats in this laboratory, it was observed that these materials induced a condition of hyperthyroidism as measured by their TSR when the materials were given for a period of 13 days (in preparation).

The present study showed that administration of 30 USP units of PTE/100 g BW from day 7 to day 14 of the lactating period increased feed consumption compared with the control animals. Similarly the feed intake of animals receiving calciferol and Hytakerol was higher than that of animals given sesame oil. However, little diferences was observed in feed consumption of the animals treated with crystalline dihydrotachysterol and of those given sesame oil. The lack of effect of this material on feed consumption was manifested also on its lack of effect on milk secretion on day 14 of lactation (unpubl.). It is expected that a more prolonged administration of DHT might cause an increase of feed consumption of the experimental animals. It is suggested that the effect of PTE, calciferol and Hytakerol in elevation of feed intake is mediated by an increase in thyroid hormone output and/or as a compensation for the increased energy requirements for increased production of milk. A possibility that the materials acted directly on the feed intake regulatory center in the hypothalamus is not excluded. The low feed intake of the animals treated with sesame oil in comparison with the untreated group suggests that, to a certain extent, sesame oil may have been used as an energy source by the animals. It was observed, also, that administration of sesame oil for a period of 13 days caused an increase of 7% in TSR compared with the untreated group (in preparation).

Summary. Daily administration of 30 USP units of parathyroid extract/100 g BW, 0.2 mg calciferol/100 g BW or 125 μg Hytakerol/100 g BW from day 7 to 14 of lactation in rats increased the feed intake by 9.5%, 19% and 16% respectively. On the other hand, crystalline dihydrotachysterol at a dosage level of 100 μg/100 g BW given for the same period did not show a significant increase in feed intake. It is suggested that the increases of feed intake caused by administration of the materials was mediated by increases in the estimated thyroxine secretion rate and/or by the increases in the energy requirements stimulated by increases in the milk yields observed.

1. Grossie, J., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 748.

2. Nelson, M. M., Evans, H. M., *Milk,* 1961, v2, 137.

3. Anderson, R. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 334.

4. Djojosoebagio, S., Turner, C. W., *Endocrinol.,* 1964, v74, in press.

5. Terepka, A. R., Chen, P. S., Jr., Jorgensen, B., *ibid.,* 1961, v68, 996.

6. Pipes, G. W., Grossie, J., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 491.

7. Grossie, J., Turner, C. W., *ibid.,* 1962, v110, 631.

8. ———, *ibid.,* 1961, v107, 520.

Received March 9, 1964. P.S.E.B.M., 1964, v116.