Thyroid Hormone and Lactation in the Rat.* (24559)

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Maximum lactation presumably occurs only when each hormone involved in secretion and evacuation of milk is present in adequate quantities. Similarly, any hormone, if present in inadequate amounts, may limit intensity of response though mammary gland growth and other hormone levels may be adequate. This has been shown clearly with regard to oxytocin in which administration of adequate levels to lactating rats resulted in significantly increased milk yields. When combined with growth hormone (GH), a further increase occurred with marked reduction in variability of response among members of the population(1). These data suggested GH also may be a limiting factor in milk secretion in this species. Extensive research, conducted mainly with large animals, has demonstrated that intensity of milk secretion is controlled to some extent by thyroid hormones and that a mild hyperthyroidism is generally beneficial to milk production(2). As it has been observed that milk yield(1,3) and thyroid hormone output(4) varied considerably among individual lactating rats, we have attempted in the present investigation to determine what effect administration of thyroxine alone, and in combination with GH and oxytocin, have upon milk yield. In this way, the extent to which thyroid hormones limit intensity of milk secretion in lactating rats was estimated.

Materials and methods. Ninety primiparous lactating rats of the Sprague-Dawley-Ralfsmeyer strain which had reached growth stasis were housed in individual cages under conditions of uniform temperature (78 ± 1°F) in a room artificially illuminated during daylight hours. They were fed Purina Lab Chow and water *ad libitum*. Shortly after birth each litter was adjusted to 6 young and, whenever possible, were equally distributed as to sex. Each lactating rat was injected subc. daily from days 7-13 postpartum with 3.0 μg/100 g l-thyroxine. Thirty received, in addition, GH‡ subc. at a dose of 1 mg/day over a similar period. On day 14, each litter was isolated from their mother for 10 hours, after which mother and young were reunited and permitted to nurse 30 minutes. A few minutes before nursing 30 lactating rats which were treated with thyroxine and 30 treated with thyroxine plus GH were injected subc. with 1 USP unit oxytocin§ to insure maximum milk withdrawal. Immediately after nursing each litter was removed, weighed, killed by decapitation and stomach contents removed and weighed to the nearest .1 g. Each mother also was killed shortly after nursing and her adrenal and pituitary glands rapidly removed, blotted to remove surface moisture and weighed to the nearest .1 mg. Daily body weights of each mother and her litter were recorded.

Results. Weight of milk, expressed as percent litter body weight, obtained on day 14 postpartum by litters of 30 lactating rats injected from days 7-13 with 3.0 μg/100 g/day l-thyroxine, averaged 5.2%, an increase of 37% over normal average yield of 3.8% (Table I). Injection of 1 USP unit oxytocin before nursing on day 14 resulted in a 19% greater average milk yield (6.2%). That obtained with aid of oxytocin from rats injected with l-thyroxine plus 1 mg GH daily from days 7-13 averaged 6.6% which represents a total increase in milk yield of 73.7% over untreated controls. Progressive reduction in variability among individual values also was obtained with addition of each hormone though in each case some overlap with control values occurred. Increased milk yield resulting from l-thyroxine, alone, and in combina-

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‡ The growth hormone (NIH-BGH-1) is a highly purified bovine preparation distributed by Nat. Inst. Health.

§ Kindly supplied by Armour Lab., Chicago.

TABLE I. Effect of l-Thyroxine Alone, and in Combination with Oxytocin and GH, upon Milk Removal by Litters of 6 Young on Day 14 Postpartum. 30 lactating rats in each group.

Treatment	Avg wt of: Milk (g)	Litter (g)	Milk / Litter (%)	Mother (g)	Pituitary (mg/100 g)	Adrenal (mg/100 g)
Control*	6.20	163.6	3.8 ± .29[1]	303.5	3.39[5]	23.41[9]
3 μg/100 g/day l-thyroxine subcut. days 7-13	9.55	182.4	5.2 ± .22[2]	285.9	3.71[6]	
Idem, then 1 USP oxytocin subcut. on day 14	11.00	176.6	6.2 ± .14[3]	301.8	3.81[7]	23.23
Idem + 1 mg GH/day subcut. days 7-13, then 1 USP oxytocin on day 14	11.98	181.4	6.6 ± .13[4]	300.5	3.49[8]	26.31[10]

* Milk yield data from Grosvenor and Turner(1).

Student's "t"	Probability
1-2,3,4	.001
2-3,4	.001
3-4	.06
5-6,7	.001
5-8	.1
9-10	.01

tion with GH were not reflected in significant increases in litter growth as analyzed by percent weight gain from days 6-14 postpartum. Body weights of mothers were similarly unaltered (Table II). Thyroxine caused a significant increase in wet weight of maternal pituitary gland. This did not occur, however, in rats treated with thyroxine plus GH (Table I). Adrenal gland weight was significantly increased in lactating rats injected with thyroxine plus GH but was unaffected by thyroxine alone (Table I).

Discussion. It has been suggested that if less than an optimal rate of thyroxine is secreted by dairy cattle, milk production may be depressed below the potential capacity of the animal. By increasing the level of circulating thyroid hormone in such animals, intensity of milk secretion may be increased(2). However, as thyroid hormones are general metabolic stimulants, it is important that levels are administered which stimulate milk secretion maximally without causing undue stress to the animal. In the present investigation daily administration of thyroxine at a level equal to the highest thyroxine secretion rate (TSR) (3.0 μg/100 g) previously observed in our rats during mid lactation(4) did not adversely affect mother body weight indicating that the level employed did not produce severe hyperthyroidism, yet evoked a 37% increase in milk yield. In a previous

TABLE II. Effect of l-Thyroxine Alone, and in Combination with GH, upon Body Weight Gain of Lactating Rats and Their Litters from Days 6-14 Postpartum. Litter weights at day 14 corrected for weight of milk obtained during 30 min. nursing.

Treatment	No. of rats	Avg wt (g) of Litter: Day 6	Day 14	Gain	% gain	Mother: Day 6	Day 14	Gain	% gain
Control	30	72.0	157.4	84.6	117.5 ± 1.2[1]	297.1	303.5	6.4	2.2 ± .42[4]
3 μg/100 g/day l-thyroxine subcut. days 7-13	30	74.4	165.6	91.2	123.6 ± 1.2[2]	280.1	285.9	5.8	2.1 ± .41[5]
Idem + 1 mg/GH /day subcut. days 7-13	30	77.3	171.1	93.8	122.1 ± 1.1[3]	295.4	300.5	5.1	1.7 ± .52[6]

Student's "t"	Probability
1-2,3	.2
4-5,6	.5

study 1 mg GH/day for same period of time evoked a similar increase in milk yield and when combined with oxytocin greater and more uniform milk yields were obtained than with GH or oxytocin alone. This suggested GH and oxytocin were limiting factors in milk secretion intensity in lactating rats(1). The combination of GH, oxytocin and thyroxine employed in the present investigation elicited still greater average milk yield with pronounced uniformity among individual values. These data suggest suboptimal thyroxine secretion may be limiting intensity of milk secretion in many lactating rats. However, increase in milk secretion as a result of thyroxine administration was not reflected in significant increase in litter weight as reported by Desclin(5). It has been questioned whether, in intact healthy rats, more intense milk secretion can produce litter growth in excess of the inherent capacity of the species (1). Maternal adrenal gland weight per 100 g was not affected by thyroxine, or, in a previous study by GH(1). A combination of 2 hormones, however, produced a significant increase. The observation that thyroxine nullified the GH-induced increase in body weight previously reported for lactating rats(1) suggests heightened glucocorticoid secretion may occur in the enlarged adrenals. Similar effects of thyroxine and GH upon adrenal function have been reported in non-lactating rats(6). Wet weight of the maternal pituitary was increased significantly as a result of thyroxine treatment presumably due to blockage of thyrotropin discharge and subsequent accumulation of the hormone. Combined treatment of thyroxine with GH resulted in no increase over untreated controls. Experiments conducted after those described in the present study demonstrated GH caused significant elevations in TSR and rate of thyroidal I^{131} release in lactating rats(7). Thus the thyrotropin-blocking dose of 3.0 μg/100 g l-thyroxine for normal lactating rats employed in the present investigation might not be sufficient, at least in some instances, to block release of the hormone in animals treated with GH. The possibility also exists that elevation of milk yield of the GH-thyroxine-oxytocin treated group in the present study, over those treated with thyroxine and oxytocin may partially be due to elevated thyroid hormone output in excess of that exogenously administered.

Summary. We have investigated effects of l-thyroxine alone, and in combination with oxytocin and growth hormone (GH) upon lactation in the rat. Amount of milk obtained by a litter of 6 during 30 minutes nursing on day 14 postpartum, expressed as percent litter body weight, was used as the index of response.

1. 3.0 μg/100 g/day l-thyroxine, a level equal to the highest daily thyroid secretion rate previously observed, injected daily from days 7-13, increased average milk yield 37%. That obtained with aid of oxytocin, to insure maximum milk removal, in rats injected for 7 days with l-thyroxine alone, or with 1 mg GH/day, was increased 63.2 and 73.7%, respectively. With addition of each hormone there resulted also a progressive reduction in variation among individual milk yields. It is suggested suboptimal thyroid hormone secretion may limit intensity of milk secretion in many lactating rats. 2. Increased milk secretion, obtained with l-thyroxine with or without GH was not reflected in significant increases in litter growth as analyzed by percent weight gain during period of treatment. Thyroxine had no adverse effect on maternal body weight. Increase in maternal weight due to GH was offset by simultaneous treatment with thyroxine. 3. Thyroxine induced a significant increase in maternal pituitary gland weight which was offset when administered with GH. Adrenal gland weight was significantly increased in lactating rats injected with l-thyroxine plus GH but was unaffected by l-thyroxine alone.

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Thiocyanate Formation by Extracts of *Escherichia coli* and of Liver.* (24560)

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Previous work(1,2,3) has demonstrated the presence of a desulfurase enzyme system in *E. coli* which cleaves β-mercaptopyruvate to free sulfur and pyruvate at pH 7.4. This system appears to be similar to that found in liver although comparison of the properties of the bacterial and mammalian enzymes has not been reported. The present work shows that *E. coli* extracts incubated with β-mercaptopyruvate at pH 8.0 or above do not release free sulfur but transfer it to an acceptor in the environment in a manner analogous to the liver system(4). When cyanide is present, the transferred sulfur readily reacts to produce thiocyanate.

Materials and methods. Escherichia coli (wild type) was grown in the liquid medium of Monod and Williams(5) with addition of 1 g of yeast extract per liter. Ten liters of this medium were inoculated with 100 ml of a 2-day culture and the mixture was incubated at room temperature with vigorous aeration. After 36 hours the cells were collected in a Sharples centrifuge, washed once with distilled water and lyophilized.

Bacterial enzyme preparation. Two g of dried cells and 20 g of alumina were ground in a ball mill for 4 hours at room temperature. One hundred ml 0.01 M K_2HPO_4 were then added and grinding continued an additional 30 minutes. The creamy mixture was centrifuged at 3000 x g to remove the alumina and larger particles and then at 20,000 x g for 1 hour. The supernatant liquor was diluted to 100 ml with distilled water and 5 ml of M $MnCl_2$ were added to remove nucleic acids. The resulting precipitate was removed by centrifugation; excess Mn++ was removed by passing the supernatant through a column of Dowex 50 cation exchange resin, NH_4+ form. This preparation was without thiosulfate transsulfurase activity.

Rat liver preparation. A lyophilized enzyme preparation, made from rat liver acetone powder by the method of Meister *et al.* (1), was purified as described by Kun(6). This material exhibited both thiosulfate and β-mercaptopyruvate transsulfurase activities. *Ammonium β-mercaptopyruvate.* This substrate was prepared by the method of Parrod as modified by Kun(7). *Pyruvate assay.* Pyruvic acid, enzymatically produced from β-mercaptopyruvate, was determined by the method of Friedmann and Haugen(8). *Standard enzyme assay.* The enzyme (0.3 ml) was mixed with 0.5 ml of substrate (84 μmoles) and one ml of 0.2 M "Bis"‡ buffer was added to give a final pH of 9.1. This mixture was incubated for 15 minutes at 37°. Two-tenths ml of KCN solution (28 μmoles) were added and incubation continued for another 15 minutes. Enzyme action was stopped with 0.5 ml of 40% formaldehyde and the volume was made up to 5 ml with water. One ml of Goldstein's reagent(9) was added and the color produced was determined photometrically. The substrate for determination of thiosulfate transsulfurase was $Na_2S_2O_3$ and for β-mercaptopyruvate transsulfurase was NH_4 β-mercaptopyruvate.

Results. The *E. coli* enzyme system was found to be relatively stable at 100° for 5

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‡ Bis = bis(hydroxymethyl)aminomethane or 2-amino-2-methyl-1,3 propanediol.