Thyroxine Secretion Rates During Food Restriction in Rats.* (27600)

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Until recently studies of changes in thyroid gland function as a result of various degrees of food restriction were based upon morphological evidence(1), studies of altered I^{131} uptake(2), and upon thyrotropin content of blood(3). With availability of a direct method of estimating thyroxine secretion rate (TSR)(4) it is possible to obtain quantitative data on influence of various degrees of food restriction on TSR.

In studies of factors influencing TSR it is important to be able to differentiate the effect of experimental procedure *per se* and restriction of food consumption which may be associated with experimental conditions or endocrine gland removal. Reduction in TSR in mice associated with degrees of feed restriction has been reported(5). The present paper reports upon effect of feed restriction upon TSR of rats.

Materials and methods. Mature female Sprague-Dawley-Rolfsmeyer rats weighing approximately 250 g were maintained at temperature of 78 ± 1°F with artificial lighting provided during daylight hours. Control TSR values were determined while animals were on *ad libitum* diet. TSR determinations were repeated beginning 3 and 17 days after initiation of food restriction in each animal. The end-point of each determination required up to 12 days. Animals in lower TSR range reached end-point earlier than in higher range and were restricted for a shorter period. Each animal received 2 μc carrier free I^{131} and was allowed 48 hrs for uptake. Counts were made at 48-hr intervals and thyroxine (T_4) dosage was begun at 0.25 μg/100 g BW and without blockage of thyroidal I^{131} release (95% previous dose) increased by 0.25 μg at every counting interval. Purina Lab Chow in granular form was weighed out daily according to individual initial weights of animals. An 8, 17, 22 and 48% reduction from the normal mean value of 5.26 g/100 g BW was given to each animal(6). Body weights were taken on alternate days.

Results. Reduction of food intake depressed TSR depending on degree of food restriction in almost every case (Table I). During the first interval (3-14 days), an 8% reduction in food intake had no effect on TSR as shown by a non-significant increase in TSR ($t = 0.274$). Reductions in food intake of 17% resulted in an 8% reduction of TSR as compared to control value. A 22% food reduction depressed TSR by 13%. These reductions are non-significant. However, a reduction of 48% resulted in a 28% depression of TSR which was significant ($t = 2.3$).

During second interval of food restriction (17 to 27 days), more profound reductions in TSR were apparent. A 23% reduction in TSR occurred with an 8% reduction of food intake during this period. When food intake was reduced 17%, TSR was depressed by 15%. Statistically, these reductions in TSR are not significant. A 22% reduction of food results in a significant ($t = 2.445$) 42% depression in TSR while a food intake 48% below mean values resulted in a highly significant ($t = 4.74$) 54% depression in TSR.

Discussion. It should be pointed out that feed restriction in respective groups in this experiment is based on mean feed intake of a group of 24 animals of this strain. As shown in studies of this strain, a rather wide variation in food intake occurs(6). By analysis of frequency distribution reported, slightly more than 18% of a population will have normal feed intakes equal to or less than ($=<$) 4.86 g (21.4 Calories (C)) per 100 g BW or 8% below mean value. Similarly, about 16% will have a food intake $=<4.38$ g (19.3 C) per 100 g BW or 17% below mean and only 5.7% will consume an amount of food $=<2.75$ g (12.1 C) per 100 g BW which is 48% below mean consumption. Therefore, it is possible by chance to include animals in a

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TABLE I. Effect of Food Restriction on Thyroxine Secretion Rates in Rats.

Treatment	8% reduction†				17%				22%				48%			
Duration	No. animals	TSR	% TSR change	BW loss (g)	No. animals	TSR	% TSR change	BW loss (g)	No. animals	TSR	% TSR change	BW loss (g)	No. animals	TSR	% TSR change	BW loss (g)
Control	12	.916	—	—	12	.937	—	—	12	.937	—	—	12	1.08	—	—
1st interval 3-14 days	12	.979	+7	8	12	.863	−8	11	12	.812	−13	6	12	.77*	−28	11*
2nd interval 17-27 days	12	.708	−23	3	11	.795	−15	15	12	.541*	−42	22*	12	.50*	−54	24*

* Significant reductions ($P = < .05$).
† From mean of 5.26 g/100 g body wt.

group which require no more than restricted amounts of feed. Animals on various degrees of restriction of food consumption but whose feed intake is less than or equal to restricted diet would not lose body weight or suffer a reduction of TSR. Animals with food intake above normal would be expected to show greater losses in body weight and show greater reductions in TSR upon progressive reduction in food intake.

It was noted that both degree and duration of food restriction affected TSR. Mild degrees of food restriction over an extended period of time can be almost as effective in depressing TSR as greater degrees of food restriction for short periods. Weight reductions up to 9 g resulted in a mean net reduction in TSR of .054 μg/100 g BW. A mean net TSR reduction of 0.187 μg/100 g BW occurred with weight losses of 10-19 g. Losses of 20-29 g resulted in a TSR reduction of 0.25 g/100 g BW and losses greater than 30 g resulted in a TSR reduction of 0.357 μg/100 g BW.

These data suggest that experimental treatments which depress food consumption and/or cause body weight losses may be expected to depress TSR and should be considered in interpretation of data. For example, optimal levels of thyroxine have been shown to influence mammary gland growth(7) and lactation(8). Reduction in TSR would be expected to unfavorably influence these processes. Following adrenalectomy and with salt replacement, it has been observed that food consumption was reduced. Reduction in TSR which ensued may have resulted from food reduction as well as operative procedure(9).

Summary. Mature female Sprague-Dawley-Rolfsmeyer rats with a mean food consumption of 5.26 g/100 g BW of Purina Lab Chow were subjected to 8, 17, 22 and 48% food restriction. Thyroxine secretion rates (TSR) were determined beginning 3 and 17 days later. Only 48% restriction resulted in a significant reduction in TSR during initial period (28%). In second period a 22% restriction resulted in a significant 42% and 48% restriction resulted in a highly significant 54% decrease in TSR.

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Chemical Studies of the Red Band Produced by *S. aureus* on Blood Agar. (27601)

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The development of an opaque red band surrounding the growth of certain strains of *Staphylococcus aureus* cultured on human blood agar has recently been reported(1). This band first appears after 4 to 5 days incubation at 37°C, takes final form after 18 additional hours either at 37°C or at room temperature, and when fully developed has a sharply demarcated edge surrounded by a narrower area of pale red. A similar red band was observed when sterile pieces of plain or blood agar near which *S. aureus* had been grown were placed on blood agar plates and incubated at 37°C. Production of the red bands appeared to depend upon a substance that diffused through the culture medium.

Direct observations of the red band indicated that it consisted of an accumulation of erythrocytes (red cells). The concept that red cells could aggregate in this way was novel and hence proof was sought that red cells actually moved in the agar. Preliminary efforts at sectioning the agar were unsuccessful. We therefore resorted to chemical means to provide proof of the phenomenon; the results are reported here. Since completion of these experiments we succeeded in sectioning the agar; these observations will be reported separately.

Methods and results. 1. *Hemin and nitrogen content in the red band and outside it. Staphylococcus aureus* strain Guy (hemolytic), was inoculated in the center of 10% human blood agar in plastic plates(1). After incubation, pieces of agar weighing between 0.05 and 0.50 g were cut out both from the red band which had already appeared and from areas outside of it. The pieces were immediately weighed on a Roller-Smith spring balance (accuracy of 0.1 mg), dissolved in 5 ml 0.5N HCl, capped with tinfoil, and placed in boiling water for one hour. Acid hematin was measured at λ 520 in the Coleman Junior Spectrophotometer, using standards established with CP hemin and gelatin solutions (2). The material was then washed into Microkjeldahl tubes and total nitrogen determined(3).

Table I summarizes 6 of the studies dealing with hemin and nitrogen content. In column 1 is shown the number of days that the plates had been incubated at 37°C, and then at room temperature before each assay was made. The red band had 18-61% more hemin and 8-25% more nitrogen than the area outside of it (see columns 5 and 9).

Chromium 51 studies. Although the above experiments provided some evidence that red blood cells are moved into the band, they were not conclusive. They did not exclude the possibility that the band consisted of red cells prevented from hemolyzing by the diffusible factor, plus hemoglobin from hemolyzed red blood cells outside the band which had diffused throughout the plate thus raising the total hemoglobin in the band. There-

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