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Thyroid Compensation Under the Influence of Dietary Nitrate.* (27769)

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The ability of thiocyanate, perchlorate, nitrate and other anions to interfere with normal thyroid function has been the basis of numerous investigations(1,2). Of these ions, nitrate is unique in that it is found in many plant species(3) and in well water(4,5) commonly consumed by domestic animals and man. Recent studies(6) have shown that dietary nitrate interferes with the normal metabolism of injected I^{131}. These studies were conducted on rats and sheep fed nitrate for less than one week. Under normal conditions rats fed KNO_3 show a slight depression in rate of gain; however, when cold stress was applied, the rate of gain was significantly decreased.† This would indicate that rats can compensate for a nitrate load under the usual environmental conditions but not under cold stress. This investigation was undertaken to determine whether or not the thyroid compensates for the nitrate effect on iodine metabolism.

Materials and methods. Sixteen 150 g male rats, originally of the Wistar strain, were divided into 2 groups of 8 each. The animals were maintained on a basal diet of the following % composition: ground yellow corn 58.5, soybean oil meal 35.0, calcium carbonate 1.5, dicalcium phosphate 1.5, sodium chloride 0.2, and Vit. B mix 0.8 (diet calculated to contain 0.83% K and 0.17% Cl). The animals were fasted 24 hours then fed the basal diet supplemented with 2.5% KNO_3 or 1.8% KCl plus 0.7% cerelose. Six hours after the diets were offered, the animals were injected intraperitoneally with 400 μg tapazole per 100 g body weight. One hour later

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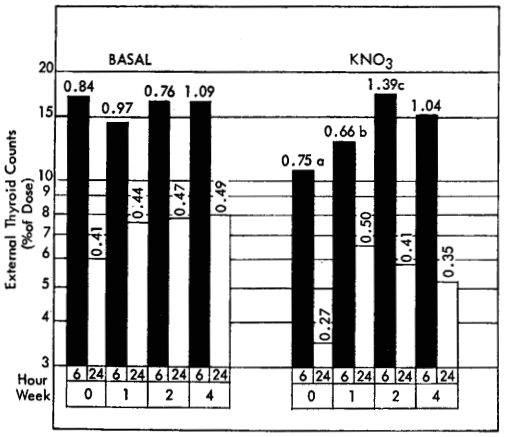

FIG. 1. Compensation of the rat thyroid in overcoming 2.5% dietary KNO_3. b is significantly higher than a, $p < .01$. c is significantly higher than a or b, $P < .01$. Values at top of bars represent standard error of mean.

the rats were injected intraperitoneally with 3 μc I^{131}. Subsequently external thyroid counts(7) were made at 6 and 24 hours. The supplemented rations were fed to the rats for 5.5 weeks and average intake was 14.3 g per day in each group. Injection of tapazole and I^{131}, and the subsequent 6- and 24-hour external counts were repeated at 1, 2, and 4 weeks without 24-hour fast. Tapazole was used so that iodide "trapping" mechanism could be measured without interference from protein binding. Since tapazole caused I^{131} to remain as inorganic iodide, measurements of the iodide flushing action of KNO_3 on the thyroid could be made.

The last injection of I^{131} at 5.5 weeks was without tapazole so that serum PBI^{131} could be measured. The animals were sacrificed by guillotine 36 hours after I^{131} injections. Weights of the thyroids, adrenals, and livers as well as values for serum PBI^{131}(8) and the blood KNO_3(9) were determined.

Results. The results are shown in Fig. 1. Six-hour external counts represent uptake while the difference in 6- and 24-hour counts show the degree of flushing. Rats fed KNO_3 showed a lower uptake of I^{131} at 0 week and lost the majority of this I^{131} within 18 hours. By the end of the second week KNO_3-fed rats had a greater uptake than the KCl group. The greater uptake was not reflected in the ability to overcome the flushing action of KNO_3 as the 24-hour count was still lower than in the control animals. The fourth week showed a slight decrease in uptake with an even greater decrease in the ability to hold the I^{131}.

Table I shows organ weights and serum PBI^{131} in the absence of tapazole. Thyroid weights were significantly higher in the KNO_3-fed animals but there was no difference in adrenal weights. The PBI^{131} was higher in the KNO_3 group. There was no significant difference in weight gains during the 5.5-week period.

Discussion. Mitchell and O'Rourke(10) have recently shown that an increase in thyrotropin (TSH) will overcome the thyroid inhibiting effect of blood thiocyanate. An increased TSH in the KNO_3-fed animals would possibly explain the increased size of the thyroid gland and the progressive increase in uptake of I^{131} over the 4-week period.

A possible explanation for the higher serum PBI^{131} in the KNO_3-fed group after 5.5 weeks is that dietary KNO_3 decreased the inorganic iodine pool in the animal. This concept is supported by the flushing of the thyroidal I^{131} under the influence of KNO_3. Fitting(11) has reported that the circulating inorganic I^{127} is apparently the major determining factor in quantitative fixation of injected I^{131}.

The data presented here show the progressive thyroidal compensation of the rat subjected to excess dietary nitrate under normal environment. The validity of using PBI^{131} as an index of thyroid function in rats that consume perchlorate, thiocyanate or nitrate

TABLE I. Organ Weights and PBI^{131} after 5.5 Weeks on KNO_3 Diet.

Dietary supplement	Thyroid wt, mg/100 g B.W.	Adrenal wt, mg/100 g B.W.	36-hr PBI^{131}, cts/min/ml	Blood KNO_3, mg/100 ml
KCl	8.0 ± .69	11.9 ± .70	3684 ± 534	.3
KNO_3	11.5 ± .48*	12.6 ± .46	7332 ± 665*	24.3

* Significantly different, $P < .01$, from control.

for several weeks is certainly open to question.

Summary. Rats maintained in a normal environment showed a decreased thyroidal I^{131} uptake after 7 hours on a nitrate supplemented diet but were able to overcome this effect after two weeks. Initially the nitrate flushed I^{131} from the thyroid gland; however, this effect was overcome in one week. After 5 weeks, in the absence of tapazole, the nitrate-fed rats fixed more I^{131} as PBI^{131} than did the controls.

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Effect of Thyroxine on Maturation of the Testes and Prostate Gland of the Rat. (27770)

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Injection of thyroxine beginning at birth has been shown to accelerate such maturational processes as opening of the eyelids, hair growth, epiphyseal plate closure and tooth eruption in rats(1,2,3). Attempts to advance differentiation of the gametogenic cells of the testis or to induce earlier and more rapid growth of the male accessory reproductive glands by thyroid hormone administration have uniformly failed(4,5,6). In these latter studies, however, hormone administration was not begun until the animals were 2 to 3 weeks of age. Since spermatogenesis is well under way at this time(7,8) it seemed possible that the thyroid hormone had been administered at too late an age to significantly accelerate this process. For this reason in the present study thyroxine was administered from birth at which time the earliest Type A spermatogonia are not yet recognizable(8).

Methods and materials. Forty-seven rats from Wistar stock were raised in a room maintained at 76°F and 50% relative humidity. All animals used in this study were obtained from litters which had been reduced to 7 at birth and contained at least 3 males. Day-old males from each litter were divided into 3 groups. The animals in 2 of these groups were injected subcutaneously daily from birth to 36 days of age with DL thyroxine* in alkaline saline. The control group received alkaline saline only.

The thyroxine solutions were prepared at 2 concentrations: 6 μg/0.05 ml ("A" dose) and 2 μg/0.05 ml ("B" dose). Fresh solutions were prepared at 7-day intervals. The daily dose given to the animals in each experimental group was maintained at 6 μg and 2 μg until the rats were 10 days of age. At 11, 16, 21 and 28 days of age the 2 doses were increased so that the "A" dose was maintained at about 9 times the normal physiological level and the "B" dose at 3 times this level. Six micrograms per 100 g of body weight was considered normal.

Necropsy was performed on the day after the last injection. The testes and ventral prostate gland were weighed and the testes

* Thyroxine Crystals Squibb, E. R. Squibb and Sons, New York.