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Variations in Arginase Concentration in Livers of White Rats Caused by Thyroxine Administration.

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The literature concerning stored and ingested materials as energy sources in the increased rate of metabolism caused by thyroxine administration has been reviewed by Kendall[1] and Harington.[2] Nitrogen excretion after administration of thyroxine, or desiccated thyroid, has been found not to be in direct relation to changes in the basal metabolic rate. Protein breakdown is said to be initially accelerated by the hormone. Continued administration, however, appears to be accompanied by decreases in nitrogen output, to, or even below, the initial level. Nitrogen equilibrium can be maintained at times of high basal metabolic rate by medication on very low daily protein intakes, and by providing sufficient carbohydrate and fats to meet caloric requirements. The quantities of nitrogen excreted and protein catabolized are dependent upon the carbohydrates and fats available. Changes in nitrogen metabolism caused by thyroxine, therefore, depend upon caloric intake and type of food in relation to requirements.

Bodansky and Duff[3] have found pregnant rats have a marked tolerance to thyroxine. Daily administration of one milligram of the hormone during the last 10 to 12 days of gestation failed to interfere with gains in weight as compared with pregnant controls. Danforth and Loumos[4] have found that in the case of female rats at constant food intakes after feeding desiccated thyroid the increase in rate of oxygen consumption by pregnant animals is less than that by nonpregnant controls. Pregnant rats were able to maintain the rate of increase in weight on doses that caused marked decreases in nonpregnant rats.

Analysis of livers of white rats[5] after feeding at various levels

1 Kendall, E. C., *Thyroxine*, The Chem. Catalog Co., New York, 1929.

2 Harington, C. R., *The Thyroid Gland, Its Chem. and Physiol.*, Oxford University Press, London, 1933.

3 Bodansky, M., and Duff, V. B., *Endocrinology*, 1936, **20**, 537.

4 Danforth, D. N., and Loumos, S., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 870.

5 Lightbody, H. D., and Kleinman, A., *J. Biol. Chem.*, 1939, **129**, 71.

of protein intake have shown that the concentration of arginase in the livers, and the total arginase per unit of body weight, varies directly with the quantity of protein ingested. Similar analysis[6] of livers from fasted rats indicated a correlation between the quantities of arginase in the livers and the type of body materials, carbohydrates and fats, or protein that serve as primary energy sources at the time of sacrifice. Sex differences[7] in concentration of the enzyme characteristic of the reproductive period therefore seem likely to be related to sex differences in protein metabolism.

The object of the experiments reported here was to study the action of thyroxine upon the concentration of liver arginase when administered to male and to pregnant and nonpregnant female rats.

Procedure. One milligram of crystalline thyroxine was administered by subcutaneous injection daily, for 9 days, to rats of 3 groups. These groups consisted of from 8 to 10 animals each and were made up of males (I), nonpregnant females given thyroxine (II), and pregnant females given thyroxine (IV). A similar group (III) of pregnant animals was used as controls. The periods of injection were so selected that sacrifice was made during the 17th or 18th day of pregnancy and when the ages were 96 to 106 days. These are the approximate ages of greatest sex differences in concentration of arginase in the liver.[7] Daily records of food consumption of animals in Groups I and II were kept for a preperiod of 5 days and during the 9 days of thyroxine injection. Similar records were kept for pregnant animals of Groups III and IV only during thyroxine administration. The housing and feeding of the animals, and the methods of collection of tissue, and determination of arginase have been described elsewhere.[7] A summary of the results is given in Table I.

Discussion. The data show that the responses of the sexes to thyroxine injection were quite different. Of the 10 males injected (Group I) all but 1 lost weight. The average loss amounted to 8.8% of the average initial body weight. Three nonpregnant females in the group of 10 (Group II) given thyroxine lost some weight but the average change amounted to a gain of 2%. Food intake by the males was decreased during the treatment. The food intake of the nonpregnant females was not changed by thyroxine injection. The thyroxine-treated pregnant rats of Group IV did not show a marked change in food intake when compared with controls (Group

[6] Lightbody, H. D., and Kleinman, A., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 25.

[7] Lightbody, H. D., *J. Biol. Chem.*, 1938, **124**, 169.

TABLE I.
Summary of Analytical Data.
Except in first 3 columns values given are group means.

Group No.	No. in Group	Sex	Body wt, gain or loss, %	Uterus and fetuses, total wt, g	Liver			
					Solids, %	Dry, per 100 g rat, g	Arginase units*	
							Per mg dry liver	Per 100 g rat $\times 10^{-3}$
I	10	M	— 8.8	—	30.8±0.2	1.06±0.02	243.9±8.5	259.9±10.1
II	10	F	+ 2.0	—	30.4±0.3	1.23±0.02	223.9±6.6	273.9± 6.8
IV	10	F(P)	+22.3	22.0	29.7±0.2	1.29±0.02	232.3±5.2	303.5± 8.6
			Controls (uninjected).					
V†	18	M	—	—	33.7±0.2	1.15±0.02	219.3±7.2	252.9± 9.5
VI†	12	F	—	—	33.8±0.4	1.18±0.03	176.9±4.2	207.8± 4.7
III	8	F(P)	+29.9	22.9	32.8±0.3	1.36±0.05	174.9±6.4	237.9±12.0

*The arginase unit is that quantity of the enzyme which will liberate urea equivalent to one micromole (M $\times$ 10^{-6}) of carbon dioxide under the experimental condition used.

†The values for these controls are those obtained after feeding a diet containing 25% of milk proteins. These results have been previously reported[5] and are repeated here for convenience.

The average daily food consumptions in grams per 100 g body weight per day were as follows:

Group I, preperiod 6.5, thyroxine injection period 4.9.
Group II, preperiod 6.0, thyroxine injection period 6.0.
Group III, preperiod —, control period 7.3.
Group IV, preperiod —, thyroxine injection period 6.9.

III) during a similar period of gestation. The livers from all groups of rats (I, II, IV) given thyroxine were found to have lower percentage of solids than did those from non-treated groups (III, V, VI). The quantity of dry liver tissue per 100 g of body weights was found to be greater in pregnant rats than in others of the same sex. The administration of thyroxine was found to have not significantly changed this value. Neither did the hormone cause loss, or changes in weight of the fetuses and associated tissues.

The quantity of total arginase in livers of male rats given the hormone (Group I) was not different from that in livers of similar animals fed a diet near the optimum in protein content (Group V). Unlike male rats fasted for 2 days,[6] which had lost a comparable percent in body weight, the thyroxine-treated males maintained the quantity of total liver arginase at levels characteristic of well fed animals. Thyroxine administration to male rats did not cause marked increases in liver arginase as did the feeding of diets high in protein.

Livers of pregnant rats (Group III) were found to contain more arginase than those from the nonpregnant controls (Group VI). The increase was due to the greater size of the organ rather than to a difference in concentration. The arginase concentration and total arginase in the liver of the nonpregnant female rats given

thyroxine (Group II) were found to be considerably greater than in the livers of the Group VI controls and to have become comparable to similar values found in livers of male rats (Group V) after feeding a diet of near optimum protein content. Similar changes in arginase values in the livers of female rats have been found after feeding high protein diets[5] and after fasting.[6] High protein feeding, as previously reported,[5] caused increases both in concentration of the enzyme and in size of the organ. Fasting caused increases in concentration but liver sizes were decreased. Pregnant rats given thyroxine (Group IV) did not increase in body weight as much as did the pregnant controls (Group III). They did, however, increase both the quantity of the liver tissue and the concentration of the enzyme. The values found for liver size, concentration, and total quantity of enzyme, upon analysis of the livers of these animals are closely comparable to those previously found for livers of female rats fed diets containing 75% protein for 21-25 days.

If the quantity of liver arginase per unit of body weight is used as an index of the demands placed upon the enzyme system concerned with protein catabolism, the metabolic responses of male and female rats to thyroxine administration are indicated by these data to be different. Livers from treated males contain quantities of arginase quite comparable to those found in untreated males. It seems likely, therefore, that any readjustment in enzyme systems caused by the hormone must have been made primarily in systems other than those concerned with protein catabolism. The quantities of the enzyme found in livers of female rats, both pregnant and nonpregnant, are similar to those found in livers of female rats fed diets high in proteins, or after fasting for long periods. It seems likely, therefore, that a readjustment in the enzyme system concerned with protein catabolism is caused by thyroxine when injected into female rats.

Summary. Analysis of the livers of white rats for arginase activity after the administration of thyroxine have been made. The data show that, although the hormone in the dosage used caused male rats to lose body weight, the arginase content of the livers remained unchanged. Livers from pregnant and nonpregnant female rats given thyroxine contained quantities of the enzyme comparable to those found after feeding diets high in protein or after long fasting. Possible relations of these changes in enzyme concentration to protein catabolism are discussed.