

Biliary Excretion of Thyroid Hormones in Heat-Exposed Rats* (34090)

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The thyroid hormones are secreted by the thyroid gland, circulated throughout the animal, and eventually metabolized to less active or inactive forms by the muscles, kidneys, and liver. The metabolites of the thyroidal hormones are excreted into the urine and bile; however, whether the major route for the excretion of these iodinated metabolites is urine or bile depends upon the species of animal and the dose of thyroid hormones administered (1). For example: in man the administration of a physiological dose of ^{131}I -labeled-1- T_4 is primarily eliminated via the urine as inorganic iodine, while in the rat most of the hormonal iodine is excreted into the feces after hepatic conjugation and biliary excretion of the hormone (2).

In the rat several factors have been shown to affect hepatic metabolism and biliary and fecal excretion of organic iodine; namely, an increase in fecal volume, the ingestion of salicylates, and lactation (3-5). An additional factor affecting biliary and fecal organic iodine excretion is a decrease in environmental temperature. Cottle (6) found an increase in biliary and fecal clearance of endogenous thyroid hormone in cold-acclimated rats. This worker concluded that biliary excretion of thyroid hormones by the liver is the major mechanism controlling the rate of excretion in the feces. The effects of a high environmental temperature on thyroxine secretion and disappearance rates were investigated by Yousef and Johnson (7) who noted that these parameters were decreased in growing heat-exposed male rats.

This study had two objectives; namely, to measure the biliary excretion rate of (1) exogenous ^{131}I -labeled-1-thyroxine ($^{131}\text{I}-\text{T}_4$) and

(2) endogenous ^{131}I -labeled thyroid hormones in albino rats exposed to an elevated environmental temperature.

Materials and Methods. The methods in this experiment consisted of determining the percentage of ^{131}I appearing in bile after the injection of $^{131}\text{I}-\text{T}_4$ in heat-exposed rats and of calculating the biliary clearance of endogenous ^{131}I -labeled thyroid hormones in similarly treated rats.

Sprague-Dawley rats weighing 150-200 g were randomly divided into a normal control and a heat-exposed group. Rats selected as normal controls were kept five per cage in air-conditioned animal quarters where the temperature ranged between 22 and 25°. Animals to be heat-exposed were placed in a wire cage inside a wooden box that was partially open at each end. An electric heater was placed at one opening and the warmed air circulated toward the opposite end. A thermoregulator and a Tempscribe recording thermometer were placed opposite the heated opening. Rats selected for heat exposure underwent a "thermal adjustment" period of 3 days, during which time the temperature was gradually raised from room temperature to an environmental temperature of $34^\circ \pm 2$. Both the normal control and heat-exposed groups were given food and water *ad libitum*. The amount of food consumed per day and the gain in body weight were calculated weekly during a 3-week period in 15 control and 15 heat-exposed rats.

In the first experiment the percentage of ^{131}I appearing in the bile after injecting $^{131}\text{I}-\text{T}_4$ was determined by the following procedure: at the end of the 3-week period, heat-exposed and normal control animals were anesthetized with ether and the bile duct cannulated with polyethylene tubing.

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Before closing the abdomen, the animals were injected with 2.0 ml of warmed tap water into the lumen of the duodenum. The abdominal muscles and skin were closed and the cannula extended about 10 cm from the abdomen. The animals were placed in a restraining cage and allowed to recover from the anesthetic. $^{131}\text{I-T}_4$ (Abbott Laboratories) was injected intravenously via the tail vein until each rat received 80–100 μCi or 1 μg $\text{T}_4/100$ g body weight. Bile was collected continuously in chilled graduated centrifuge tubes for 6 hours and at various times 0.1-ml aliquots were taken. The radioactive iodine in the aliquots was measured in a Tracerlab well-type scintillation counter and the amount present was calculated as a percentage of the injected dose.

The second experiment consisted of measuring the biliary clearance of endogenous thyroid hormones by the following method (8). Essentially, this method consists of injecting subcutaneously the normal and heat-treated rats with 100–120 μCi of carrier-free ^{131}I as inorganic iodide. Three days later the bile duct was cannulated under ether anesthesia and 0.5 ml of blood was collected by cardiac puncture at the beginning and then at the end of the bile-collection period. Before closing the abdominal muscles and skin, 2.0 ml of warmed tap water or of a choleric, sodium dehydrocholate (Decholin, Ames Co.), was injected into the duodenum. Bile was collected for 6 hours in graduated centrifuge tubes while the rats were in the restraining cages.

The determination of radioactive protein-bound iodine PB^{131}I in plasma was calculated by centrifuging both samples of blood. After centrifugation, 0.2 ml of plasma from each sample was placed in duplicate 12 \times 75-mm test tubes. The plasma proteins were precipitated with 2.0 ml of 10% trichloroacetic acid and then dissolved in 1 ml of 2 *N* NaOH. The average counting rate was obtained for the PB^{131}I samples taken from each animal. Duplicate samples of bile (0.2 ml) were transferred to the counting test tubes and diluted to 1.0 ml with distilled water and counted in the scintillation counter. The method used for estimating biliary clearance

rate of plasma thyroid hormone was calculated by dividing the average PB^{131}I in 0.2 ml of plasma into the total ^{131}I in 0.2 ml of bile to obtain the bile-to-plasma ratio. The bile-to-plasma ratio was then multiplied by the total volume of bile collected from the respective rat for the 6-hr collection period.

Results and Discussion. The adverse effects of a high environmental temperature on body growth in male albino rats are shown in Table I. The rats exposed to ambient environmental temperature gained 68.3 g compared to 40.1 g for the heat-exposed rats which was significantly different at the 0.1% level. Significantly, the heat-exposed rats also consumed less food per day than the controls. The above results simply confirm the effects of heat exposure on growth made earlier by other investigators (9). The effects of a high environmental temperature on biliary excretion of exogenous $^{131}\text{I-T}_4$ were inconclusive. The mean percentage of dose of $^{131}\text{I-T}_4$ excreted during 6 hr into the bile of nine normal control rats was 15.2% with a standard error of 0.7. Five heat-exposed rats injected with $^{131}\text{I-T}_4$ excreted 14.3% with a standard error of 0.2. The difference between the mean percentage of $^{131}\text{I-T}_4$ in the bile of these two groups was not significantly different. The normal control rats in this first experiment produced 5.3 ± 0.3 ml of bile during this 6-hour collection compared to 4.4 ± 0.3 ml of bile produced by five heat-exposed rats. The difference between the means was significant only at the 10% level of probability. The results of the above work are not in agreement with Yousef and John-

TABLE I. Effects of High Environmental Temperature on Growth and Food Consumption during a 3-Week Treatment Period.

Treatment	No. of rats	Gain in weight (g)	Food consumption (g/day)
Normal control	15	68.3 ± 2.4^a	13.6 ± 0.2
Heat-exposed	15	40.1 ± 3.2 ($p < .01$) ^b	9.4 ± 0.2 ($p < .01$)

^a Mean \pm SE.

^b p = significance of difference between means of control and heat-exposed rats.

TABLE II. Biliary Clearance of Endogenous ^{131}I -Labeled Thyroid Hormones in Heat-Exposed and Non-Heat-Exposed Rats.

Treatment	No. of rats	Bile/plasma ratio	Bile volume (ml/6 hr)	Biliary clearance rate (ml plasma/6 hr)
Normal control	16	1.8 ± 0.2^a	5.3 ± 0.2	9.5 ± 0.8
Heat-exposed	19	1.8 ± 0.1 (NS)	3.8 ± 0.2 ($p < .001$)	6.8 ± 0.4 ($p < .01$) ^b

^a Mean \pm SE.

^b p = significance of difference between means of control and heat-exposed rats.

son (7) who noted a decrease in thyroxine degradation rate in heat-exposed rats injected with a tracer dose of ^{131}I - T_4 (0.1 μg). A possible explanation for the lack of significance noted in biliary excretion of ^{131}I between controls and heat-exposed rats may be the larger quantity (1–2 μg) of ^{131}I - T_4 injected into each rat in this first experiment. Moreover, Flock and Bollman (2) reported that the biliary excretion of exogenous ^{131}I - T_4 in control rats during a 6-hr period was 10.2% while we have reported 15.2% during the same time period.

In the second experiment, the biliary clearance of endogenous ^{131}I - T_4 hormones was examined in heat-exposed rats. The results from this experiment may be seen in Table II. The volume of plasma cleared of organic iodine was 9.5 ml in 6 hr in control rats which was significantly greater than 6.8 ml of plasma cleared in heat-exposed rats.

The above observations are consistent with the work published by Cottle (6) who showed that rats acclimated to cold significantly excreted more endogenous thyroid hormones via the bile than rats kept at a much warmer environment. However, Harland and Goldberg (10) found that the biliary clearance of endogenous thyroid hormones was not significantly different in cold-exposed rats compared to controls and that mice kept at 29° concentrated organically linked iodine in significantly larger quantities in the liver than did cold-exposed mice.

In this second experiment, the decrease in hepatic secretion of endogenous ^{131}I -labeled thyroid hormones in heat-exposed rats may have been simply a function of bile flow. The above statement is partially supported by the results in Table II that biliary clearance

is a function of the mean bile to plasma concentration ratio of organic ^{131}I times the mean bile volume during the 6-hr collection period. The mean bile volume in a 6-hr collection period was 5.3 ml in 16 control rats which is significantly greater than 3.8 ml, the mean bile volume for 19 heat-exposed rats; however, there was no significant difference between the mean bile-to-plasma ratio in heat-exposed and normal rats.

The proposed hypothesis that the reduction in biliary clearance of endogenous ^{131}I -labeled thyroid hormones in heat-exposed rats was due to the observed reduction in bile flow was tested by the following procedure. At the beginning of the bile collection period, Decholin instead of warmed tap water, was injected into the duodenum in a group of control and heat-exposed rats. The results of this experiment may be seen in Table III. As expected, the bile volume in both groups of rats increased during the 6-hr collection period; but, unlike the rats injected with tap water, there was no significant difference between the mean bile volume ($9.9 \text{ ml} \pm 1.2$ to 8.1 ± 0.8). Heat-exposed rats injected with the choleric have a significantly lower mean bile-to-plasma concentration ratio of ^{131}I than controls and the biliary clearance was 6.8 ml of plasma cleared in heat-exposed rats compared with 12.1 ml of plasma cleared of organic ^{131}I in controls.

In conclusion, the biliary clearance of circulating endogenous organic ^{131}I was reduced in heat-exposed rats. The reduction in bile flow in heat-exposed rats may be due to the smaller food intake observed in these rats or to an elevation of body and hepatic temperature. Brauer *et al.* (11) reported that, as the temperature of the perfusate increased, there

TABLE III. Biliary Clearance of Endogenous ¹³¹I-Labeled Thyroid Hormones in Heat-Exposed and Non-Heat-Exposed Decholin-Treated Rats.

Treatment	No. of rats	Bile/plasma ratio	Bile volume (ml/6 hr)	Biliary clearance rate (ml plasma/6 hr)
Normal control	5	1.3 ± 0.1 ^a	9.9 ± 1.2	12.2 ± 0.8
Heat-exposed	6	0.8 ± 0.1 (<i>p</i> < .05)	8.1 ± 0.8 (NS)	6.8 ± 0.7 (<i>p</i> < .01) ^b

^a Mean ± SE.

^b *p* = significance of difference between means of control and heat-exposed rats.

was a reduction in bile flow in isolated rat liver.

Summary. Heat-exposed rats gained less weight, consumed less food, and, when bile was collected, secreted less bile than the controls. When heat-exposed rats were injected with ¹³¹I-T₄, this hormone was excreted into the animals' bile at the same rate as exogenous ¹³¹I-T₄ is eliminated in controls. However, heat-exposed rats cleared less plasma per unit time of endogenous ¹³¹I-thyroid hormones than did normal controls. It was concluded that this decrease in hepatic secretion of endogenous labeled ¹³¹I-thyroid hormones is not due to the observed reduction in bile flow in heat-exposed rats, since a choleric did not increase biliary clearance of ¹³¹I in heat-exposed rats.

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