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Thyroid Activity of Spontaneous Hypertensive Rats[1] (38757)

MELVIN J. FREGLY

Department of Physiology, University of Florida, College of Medicine, Gainesville, Florida 32610

A sigmoid relationship exists between the weight of the thyroid gland and systolic blood pressure in rats made hypertensive by encapsulation of both kidneys with latex envelopes (1). The increase in thyroid weight is accompanied by an increase in acinar cell height measured microscopically (2). A negative correlation was found between maximal uptake of ^{131}I by the thyroid gland and systolic blood pressure of renal hypertensive rats, suggesting that uptake was reduced as blood pressure became elevated (2). Thus, a mechanism(s) is activated during the development of renal hypertension in the rat which is directed toward reduction in secretory activity of the thyroid gland. The increase in the weight of the thyroid gland appears to be a function of the elevated blood pressure rather than the method by which elevation of blood pressure is induced since an increase in thyroid weight has also been reported in rats made hypertensive by administration of *d*-aldosterone acetate (3).

Aoki *et al* (4) studied the histology of the thyroid and pituitary glands of spontaneous hypertensive (SH) rats. Prior to the development of an elevated blood pressure, they observed an increase in the height and area of follicular cells of the thyroid gland. The size and number of cells forming each follicle increased while the amount of colloid present decreased. In the case of the anterior pituitary, the basophils, which are considered to secrete TSH and gonadotrophic hormones were increased in number in the SH rats (5). When hypertension developed, the changes became more marked and the weight of the glands increased. Although Aoki *et al.* (4) pointed out that the changes in histological appearance of the thyroid and anterior pituitary glands observed in their animals could indicate hypersecretion of the thyroid gland, the same changes could also signify a reduction in secretion of thyroid hormone as observed by Astwood *et al.* (6) to accompany administration of antithyroid compounds. In both cases, the thyroid gland is responding to elevated levels of serum TSH from the anterior pituitary gland. Since Aoki *et al.* (4) used no antithyroid agents in their study and had not considered the possibility that such an agent might be produced endogenously, they postulated that the thyroid gland had increased its activity during the development of hypertension.

Yamabe (7) measured the ^{131}I uptake, rate of release, conversion ratio, thyroid hormone secretion rate and thyroid gland weight of SH rats at stages during the development of hypertension. Twenty-four hour uptake of ^{131}I was measured only once in male SH rats, i.e., during 4–6 mo of age, and was increased in the hypertensive group. However, uptake of ^{131}I was measured 3 hr after administration of the isotope and was reported to be increased in both male and female SH rats at 40–60 days, 4–6, 12–14, and 18–20 mo of age. There was, however, a marked variability among the SH groups and the mean 3-hr uptake ranged randomly from 2.3–18.3 % of the injected dose at the different stages tested. Yamabe (7) also reported an increase in the rate of release of ^{131}I from the thyroid glands of both male and female SH rats at 40–60 days, 4–6 and 12–14 mo of age. Thyroid hormone secretion rate was measured by the indirect technique of Reineke and Singh (8) at the ages mentioned above and appeared to be greater in SH rats than in controls although the significance of the differences between means of normotensive and hypertensive animals was not stated. The ratio of thyroid weight to body weight was increased only in the male SH rats at 4–6 mo of age. Thus, Yamabe (7) was unable to confirm

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consistently the findings of Aoki (4) of an increase in thyroid weight in SH rats but felt his data supported the hypothesis of Aoki (4) that thyroid activity was increased in SH rats. Yamabe (7) also reported no effect of either renal or DOCA-induced hypertensions on thyroidal ^{131}I uptake although the ratio of thyroid weight to body weight increased in the advanced stage of hypertension in both groups.

Since the report of Yamabe (7) contrasts with the reduced thyroid activity observed in renal hypertensive rats (2) and to some extent with the data of Aoki *et al.* (4), an objective of the present experiments was to attempt a resolution of these differences. This also seemed important because preliminary (abstract) reports of Manger *et al.* (9, 10) also suggested that spontaneously hypertensive rats may be hypothyroid relative to controls.

Methods. Experiment 1. Seven spontaneously hypertensive male rats of the Manor Research strain, a derivative of the Wistar strain, weighing 250–300 g were used. Nine male normotensive rats of the Wistar strain born within 7 days of the hypertensive rats were used as controls. Approximately 2 wk after their arrival in the laboratory, weekly systolic blood pressure and body weight measurements were begun. Blood pressure was measured as described by Fregly (11) except that a transducer–monitor–coupler (NarcoBio Instruments) coupled to a Sanborn polygraph recorded the systolic blood pressure of each unanesthetized rat. Blood pressures and body weights were measured for 20 wk. All rats were given Purina Laboratory Chow[2] and tap water except as noted below.

During the 11th wk of the experiment, seven hypertensive and eight control rats were injected intraperitoneally with 10 μCi of carrier-free ^{131}I. Uptake of radioactivity by the thyroid gland was measured at 18, 21.5, 41.5, 63.5, and 68.5 hr after injection of the isotope. Twenty-four hours after injection of ^{131}I each rat was injected ip with 5 mg of methimazole. At this time each rat was also given drinking water containing 0.05% methimazole to prevent recycling of ^{131}I to the thyroid gland. Methimazole was discontinued when the last measurement of radioactivity was made. Radioactivity of the thyroid gland was measured by holding the rat's neck over a shielded scintillation detector and counting for 1 min intervals until successive counts agreed within 3%. Radioactivity of the thigh of the hind leg was counted and the radioactivity subtracted from the neck counts. This served to correct for residual tissue radioactivity. After 24 hr, approximately 30% of the injected dose remained within the body. Urine was collected during each 24-hr period for 3 days and the radioactivity of an aliquot measured in a well-type scintillation detector. All measurements of radioactivity were corrected for background radiation and for decay of the isotope.

During the 13th wk of the experiment, the rats were caged individually and food intakes were measured daily for 5 days. At the end of the 20th wk of the experiment the rats were killed with ether and the thyroid gland removed, cleaned of extraneous tissue, and weighed on a torsion balance.

Experiment 2. Five spontaneously hypertensive female rats of the Manor Research strain weighing 160–180 g were used. Five normotensive, age-matched female rats of the Wistar strain were used as controls. All rats had been bred and raised at the University of Florida. Purina Laboratory Chow[2] and tap water were provided *ad libitum.*

During the 12th wk, each rat was injected ip with 10 μCi of carrier-free ^{131}I and radioactivity of the thyroid gland measured at 17, 23, 44, 50, 74, and 80 hr after injection of the isotope. Radioactivities of the thyroid gland and urine were measured as described in Experiment 1.

During the 14th wk of the experiment, the rats were caged individually and food intakes were measured daily for 5 days. At the end of the 15 wk, all rats were killed with ether and thyroid gland removed, cleaned of extraneous tissue and weighed on a torsion balance. Twenty-four hours prior to death, each rat was injected ip with 5 μCi ^{131}I and placed in a metabolism cage. The urine excreted during the 24-hr period was collected, the volume measured, and the radioactivity of an

[2] Purina Laboratory Chow contains 2 mg of iodide/kg according to manufacturer's analysis.

aliquot measured in a scintillation detector. Radioactivity of the excised thyroid gland was also measured.

Experiment 3. Six spontaneously hypertensive male rats of the Manor Research strain and six male, age-matched, normotensive derivatives of the Wistar strain were used. Thyroxine secretion rate was measured by the technique of Reineke and Singh (8). Injections of thyroxine began 75.5 hr after the injection of ^{131}I.

All rats were maintained in individual metabolism cages and were given a Purina Laboratory Chow diet[2] and tap water *ad libitum.* Each rat was administered 10 μCi of carrier-free ^{131}I ip and radioactivity of the thyroid gland was measured at 24, 48, 68.5, and 75.5 hr after injection of the isotope. At the end of the 48-hr measurement the rats were administered methimazole as described in Experiment 1. At 75.5 hr, each rat was injected ip with 0.50 μg thyroxine/100 g body wt. At approximately 24-hr intervals thereafter, the rats received 0.75 and 1.00 μg thyroxine/100 g body wt. Radioactivity of the thyroid gland was measured in a 4π scintillation detector. Each rat was placed into a cylindrical, close-fitting mesh wire restraining cage. The cage, in turn, was placed into a lead-shielded cylinder, 0.25 in. thick, in such a way that only the head protruded from the shielded cylinder. The advantage of the use of the 4π scintillation detector was its efficiency (approximately 40%). Earlier studies showed that after 48 hr the radioactivity of the head region measured in this fashion was largely due to the thyroid gland (approximately 80%). Measurements of radioactivity were calculated as percent of the injected dose and were corrected for radioactive decay of the isotope as well as for background radiation and extrathyroidal radioactivity. Thyroid secretion rate was determined by plotting radioactivity as a percentage of the previous day's radioactivity and graphing this value against the injected dose of thyroxine. The dose which prevented further thyroidal ^{131}I output, i.e., 100% of previous day's radioactivity, was estimated as the thyroxine secretion rate. Urine was collected under light mineral oil daily and the radioactivity of aliquots measured in a scintillation detector.

One week after completion of this study all rats were killed with an overdose of sodium pentobarbital and thyroid gland removed, cleaned of extraneous tissue and weighed on a torsion balance.

Statistical analysis of the data in all experiments was made by means of Student's *t*-test for the 95% confidence limit (12).

Results. Experiment 1. Mean systolic blood pressures of the seven hypertensive rats exceeded that of controls throughout the experiment. Maximal levels of 180–190 mmHg were reached by the sixth experimental week. Systolic blood pressures of the control group remained at approximately 140 mmHg throughout the 20 wk of the experiment. In contrast, body weight of the hypertensive group was significantly less ($P < 0.01$) than that of controls throughout the experiment.

Measurement of radioactivity of the thyroid gland at intervals after injection of ^{131}I revealed a reduced maximal uptake at 21.5 hr after injection and a reduced rate of release of radioactivity (Fig. 1A). The mean half-time for release of thyroidal radioactivity in the control group was 37.8 $\pm$ 3.1 (SE) hr compared to 54.8 $\pm$ 7.2 hr for the hypertensive group. The difference between groups was significant ($P < 0.05$). The results suggest that hypertensive rats have not only a reduced uptake of ^{131}I, but also a reduced rate of release compared to controls. Male hypertensive rats excreted a greater urine volume than controls ($P < 0.05$) but cumulative output of ^{131}I into urine did not differ significantly from that of controls (Table I).

Mean food intake of the SH rats was 4.50 $\pm$ 0.24 (SE) g/100 body wt/day while that of controls was 4.90 $\pm$ 0.08. The difference between means was not statistically significant.

At autopsy the ratio of thyroid weight to body weight of the SH group (6.6 $\pm$ 0.5 SE) was significantly greater ($P < 0.01$) than that of controls (5.1 $\pm$ 0.2).

Experiment 2. The uptake and rate of release of radioactivity from the thyroid gland of hypertensive female rats was significantly ($P < 0.01$) less than that of the control group (Fig. 1B). Maximal uptake occurred in approximately 23 hr after injection of the isotope in both groups. Half-life of thyroidal radioactivity was 32.2 $\pm$ 1.2 (SE) hr for control rats and 84.1 $\pm$ 4.1 hr for hypertensive

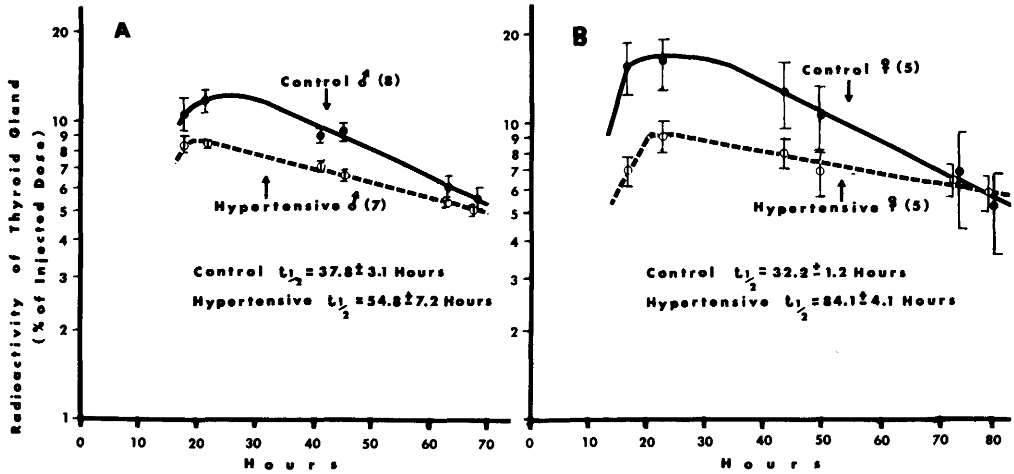

FIG. 1 Radioactivity of the thyroid gland (percentage of the injected dose) of male (A) and female (B) spontaneous hypertensive and normotensive rats at intervals after injection of ^{131}I. One standard error is set off at each mean. Biological half-life of ^{131}I for each group is shown in the figures.

rats ($P < 0.01$). Again no differences in cumulative loss of urine radioactivity were observed between groups (Table I). In the case of the female rats, urine volumes of hypertensive rats were consistently greater than those of controls but the difference between groups was not significant.

Mean food intake of the SH rats was 5.91 ± 0.43 (SE) g/100 g body wt/day while that of controls was 6.47 ± 0.18. The difference between means was not statistically significant. At death, the ratio of thyroid weight to body weight in hypertensive rats (7.4 ± 0.9 SE) was significantly ($P < 0.05$) greater than that of controls (5.4 ± 0.2). Uptake of ^{131}I at 24 hr, as measured in the excised thyroid gland of the hypertensive rats (4.9 ± 0.9 SE percent of injected dose), was significantly less ($P < 0.01$) than that of controls (13.2 ± 2.5). Again, neither urine volume nor the amount of ^{131}I excreted into urine differed significantly from controls.

Experiment 3. The SH male rats again showed a significant depression in their uptake (% injected dose) of ^{131}I 24 hr after injection of the isotope compared to the normotensive control group (8.9 ± 0.6 SE and 11.9 ± 1.2 respectively). The mean half-life of thyroidal radioactivity calculated prior to injection of the lowest dose of thyroxine was 31.6 ± 4.1 hr for the control group and 44.4 ± 2.5 hr for the hypertensive group ($P < 0.02$). Urinary excretion of ^{131}I did not differ significantly between groups at any time during the experiment.

The thyroidal secretion rate estimated by this technique was 0.97 μg thyroxine/100 g body wt/day for the control group and 1.35 μg/100 g body wt/day for spontaneously hypertensive rats (Fig. 2).

The possibility existed that expression of thyroid weight of SH rats on a body weight basis may have artifactually increased the value above that of control because of their reduced body weight. Therefore, thyroid weight of male SH and control rats in Experiments 1 and 3, as well as three additional male SH and three additional male control rats from the animal colony, were graphed against their body weight at death. Figure 3 shows that at any body weight, the thyroid weights of all SH rats fell above the line representing the relationship for controls.

Discussion. The role of the thyroid gland in the development and maintenance of hypertension in rats, dogs and humans has been studied by a number of investigators (1, 2, 13–19). In the case of the rat made hypertensive either by means of administration of aldosterone acetate or latex encapsulation of the kidneys, the ratio of thyroid weight to body weight bears a sigmoid relation to blood

TABLE 1. URINE VOLUME AND RADIOACTIVITY DURING 3 DAYS FOLLOWING ADMINISTRATION OF RADIOACTIVE IODIDE TO NORMOTENSIVE AND HYPERTENSIVE RATS

Group	No. of rats	Day after administration of ^{131}I: 1		2			3		
		Urine vol. (ml)	Urine radio-activity (% Inj. dose)	Urine vol. (ml)	Urine radio-activity (% Inj. dose)	Cumulative output of ^{131}I (% Inj. dose)	Urine vol. (ml)	Urine radio-activity (% Inj. dose)	Cumulative output of ^{131}I (% Inj. dose)
Experiment 1 (male rats)									
Control	9	10.2	73.5	8.2	5.1	78.5	5.2	1.6	80.2
		±1.0*	±3.9	±0.8	±0.4	±3.9	±0.8	±0.2	±3.8
Hypertensive	7	13.9	71.6	15.5	11.5	83.0	12.4	3.0	85.8
		±1.1‡	±2.9	±0.3†	±3.1	±3.4	±2.5†	±0.3†	±3.3
Experiment 2 (female rats)									
Control	5	13.9	76.9	14.7	2.7	79.6	18.4	0.08	79.7
		±3.0	±3.6	±2.5	±0.6	±3.1	±2.9	±0.01	±3.1
Hypertensive	5	17.4	83.3	18.1	1.5	84.8	19.3	0.05	84.9
		±2.7	±0.7	±1.4	±0.2	±0.6	±2.9	±0.006	±0.6

* One standard error of mean
† Significantly different from control ($P < 0.01$)
‡ Significantly different from control ($P < 0.05$)

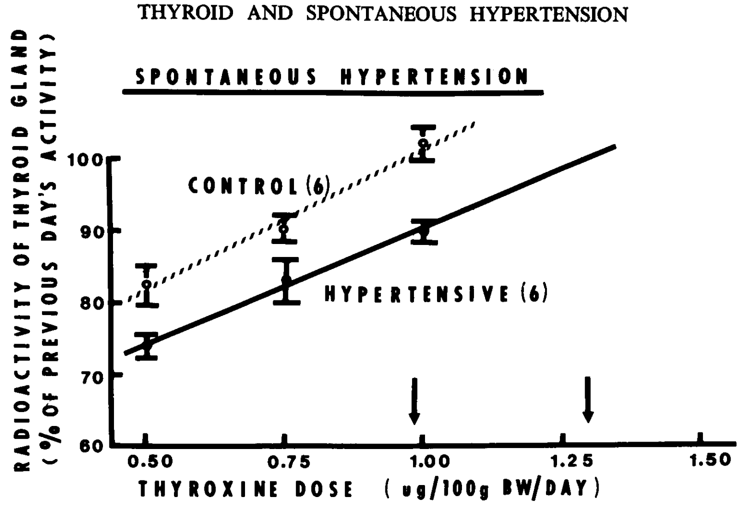

FIG. 2 Mean radioactivity of the thyroid gland (percentage of previous day's radioactivity) at each dose of thyroxine administered is shown for six spontaneous hypertensive and six control rats. One standard error is set off at the means. Arrows indicate estimated thyroxine secretion rate for control and hypertensive rats.

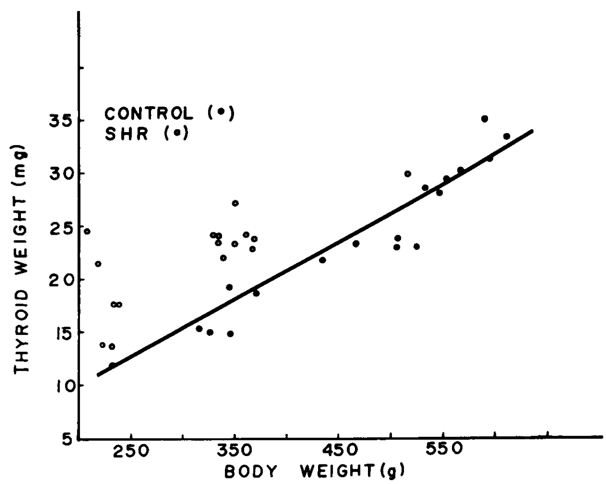

FIG. 3 Thyroid weight (mg) is graphed against body weight (g) at death. The equation of the line representing data of control rats is $Y = 0.52 \times -0.165$, $r = 0.91$, $n = 18$, $P < 0.01$.

pressure (1, 3). This relationship suggests that thyroid weight ratio remains constant over a range of systolic blood pressures varying from 120 to 150 mmHg. When blood pressure exceeds the range of 150–159 mmHg, thyroid weight increases rapidly with further increases in blood pressure (1, 3). The significance of this relationship is not completely understood but has led to the suggestion that the thyroid gland plays a secondary role in the development of these types of experimentally-induced hypertension. It is therefore of interest that SH rats also have increased thyroid weight ratios (4). Unfortunately, the present data are insufficient in number to determine whether a sigmoid relationship exists between blood pressure and thyroid weight in SH rats.

To assess thyroid function, both the uptake and rate of release of ^{131}I from the thyroid glands of the male and female SH rats were studied. Both were reduced below the level of the control group (Fig. 1). The reduced uptake was not the result of increased urinary excretion of ^{131}I (Table I).

A similar reduction in uptake and rate of release of ^{131}I by thyroid glands of chronic renal hypertensive rats was reported recently (2). The results of the present experiments also agree with the studies of Aoki et al (4) who reported an enlargement of the thyroid gland in SH rats but disagree with the interpretation of his results with respect to activity of the thyroid.

The measurement of thyroid secretion rate in SH rats suggested an increase compared to controls. This finding at first glance seems incompatible with the results of Experiments 1 and 2 as well as with the reduced ^{131}I uptake and rate of release in Experiment 3. The results of Experiment 3 become compatible with all other experimental data when it is recognized that the method of Reineke and Singh (8) estimates thyroxine secretion rate indirectly, i.e., by reducing rate of secretion of thyroid stimulating hormone (TSH) from the anterior pituitary gland. The results suggest that SH rats secrete more TSH than normotensive rats and therefore a greater amount of thyroxine must be administered to inhibit rate of secretion completely. This interpretation of the results reconciles the findings of Yamabe (7) with those reported here. This interpretation also emphasizes the fact that the indirect method for estimation of TSR will yield artifactually high values when TSH secretion is elevated and thyroxine production is partially or completely blocked at the level of the thyroid gland.

TSH secretion by SH rats was measured by Manger *et al.* (9, 10) and was elevated significantly above the level of normotensive controls. In addition, serum T_4 concentrations were reduced significantly in SH rats but serum T_3 concentrations were not. Further, Aoki *et al.* (4) showed an increase in the number of basophils in the pituitary of SH rats. These studies, considered together, are consistent with the suggestion that the SH rat is hypothyroid relative to control. The reduced growth rate of SH rats may also be related to reduced thyroid activity.

The question arises as to why SH rats have a reduced ^{131}I uptake and rate of release in the presence of an increased rate of secretion of TSH. While a complete answer cannot be given, it is possible that a thyroid depressing factor (TDF) of renal origin may interfere with the response of the thyroid gland to TSH. Earlier studies from this laboratory showed that the kidneys of normotensive and hypertensive rats contain a factor, extractable in saline, which can reduce both uptake and binding of ^{131}I by the thyroid gland *in vivo* and *in vitro* and can increase thyroid weight in weanling rats (20, 21). There appears to be a greater amount of the factor recoverable from the kidneys of rats made hypertensive (either by renal encapsulation with latex envelopes or by administration of deoxycorticosterone trimethylacetate) than from the kidneys of normotensive controls. In view of this fact, it seems likely, although not certain, that the rate of synthesis and/or release of the factor from the kidney is a function of blood pressure level. This statement is supported by the fact that the potency (by bioassay) of TDF isolated from the kidneys of a group of hypertensive rats was linearly related to blood pressure measured just prior to death (21). It is visualized at present that an increased rate of secretion of TDF reduced thyroid function initially, and consequently the rate of secretion of TSH increases. The time course has not been precisely established but uptake of ^{131}I by the thyroid gland of renal hypertensive rats was significantly reduced below control level by 7 wk after renal encapsulation (2). The effects of hypertension on the thyroid gland, as observed here and elsewhere (1, 3), are reminiscent of the effects of certain antithyroid drugs on the thyroid gland (15, 22). The fact that the thyroid gland of the hypertensive rat does not enlarge to the extent observed with antithyroid agents suggests either that inhibition is incomplete and TSH is not secreted at maximal levels or that the sensitivity of the thyroid gland to TSH is reduced. Thyroid depressing factor of renal origin (rat) is capable of blocking TSH-stimulated iodide uptake by bovine thyroid slices (23). Earlier studies from this and other laboratories showed that administration of antithyroid drugs protected against the rise of blood pressure to the level of untreated hypertensive controls (14, 15, 19, 22). Apparently this experimental approach may mimic the physiological response of the rat to induction of hypertension.

An influence of the kidneys on the thyroid gland was also observed by Nakamura et al

(24). These investigators reported an increased uptake of tritiated thymidine by the thyroid and pituitary glands of uninephrectomized rats within 1 day following operation. A greater uptake of thymidine than observed in control animals continued through the 21-day experiment. Histological section of the thyroid gland of a uninephrectomized rat 5 days after operation was shown and is characteristic of a thyroid gland under the influence of increased TSH secretion. If TDF normally acts in opposition to TSH, removal of one kidney might be expected to reduce, at least temporarily, blood level of TDF and hence, to increase thyroid activity. These results are important to the notion that the effect of hypertension on the thyroid gland may be mediated by way of the kidney. The weight of the kidneys is known to increase as blood pressure rises (1, 25, 26).

The chemical characteristics of the renal-thyroid depressing factor are being pursued currently. Studies from this laboratory suggest that it is neither renin nor associated with renin (20). It does not have vasopressor activity. In addition, graded doses of angiotension failed to mimic the effect of the renal factor on ^{131}I uptake in rats (20, 21). It is also unlikely that renal factor is prostaglandin E_1, E_2, B_1 or $F_{1\alpha}$ since these are reported either to increase or to have no effect on thyroid activity (27). Characterization of the renal factor will require further studies.

Ramirez *et al.* (28) studied patients in renal failure and observed a prevalence of goiter (58% of patients) and low serum thyroxine. Fang *et al.* (29) recently reported high serum TSH concentrations in patients with renal failure. These observations parallel those reported for hypertensive rats and suggest that the changes in thyroid activity observed may be related to renal damage accompanying the elevated systemic blood pressure. Ramirez *et al.* (28) were unable to determine the cause of goiter in their patients and postulated that "goitrogenic substances" of unknown origin were involved. It is possible that the unknown goitrogenic substance may be the factor described above.

Summary. Thyroid activity of both male and female spontaneous hypertensive (SH) rats was studied by measurements of uptake and rate of release of ^{131}I, urinary excretion of ^{131}I, and thyroxine secretion rate (TSR). In addition, thyroid glands were removed at death and weighed. Radioactivity of the thyroid gland of male rats measured at intervals after administration of ^{131}I revealed a significantly reduced maximal uptake at 21.5 hr after injection and a reduced rate of release. The mean biological half-life of ^{131}I for the control group was 37.8 $\pm$ 3.1 (SE) hr compared to 54.8 $\pm$ 7.2 hr for hypertensives ($P < 0.05$). Similar results were observed for females in that biological half-life of ^{131}I was 32.2 $\pm$ 1.2 hr compared with 84.1 $\pm$ 4.1 hr for hypertensives ($P < 0.01$). Urinary excretion of ^{131}I by hypertensive rats at 24, 48, and 72 hr after injection of ^{131}I did not differ from control in either experiment. Thyroid weight at autopsy was increased significantly above that of normotensive controls. TSR was measured indirectly in a third group of male spontaneously hypertensive and normotensive rats. TSR of control rats was estimated to be 0.97 μg T_4/100 g body wt/day and 1.35 μg T_4/100 g body wt/day for SH rats. The results are consistent with the suggestion that the method for measurement of TSR in hypertensive rats gives an artifactually high value because TSH secretion is elevated.

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