Effects of Thiouracil on Turnover of Thyroidal I^{131} and Thyroxine Secretion Rate Measurement. (26233)

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Technics which involve simultaneous administration of thyroxine and thiouracil for determination of thyroxine secretion rate (1,2) have long been used, and require the sacrifice of the animals. Use of radioiodine has provided a much improved technic for determination of thyroxine secretion rate, and eliminates the need to sacrifice the animals. Reineke and Singh(3) devised a thyroxine-substitution method which permits determination of thyroxine secretion rate of individual rats based on inhibition of thyroidal I^{131}-output by progressively increasing doses of thyroxine. Recently, Pipes *et al.*(4,5,6) described a modification of the thyroxine substitution method in which thiouracil is used to prevent recycling of I^{131} from the metabolized thyroid hormone, and to enhance the sensitivity and accuracy of the assay. By this means they measured thyroxine secretion rate in cattle and chickens. The present work was designed to compare thyroxine secretion rate measured with thiouracil or without thiouracil, and to clarify the effects of thiouracil on thyroidal I^{131}-turnover in the chicken.

Methods. Four-week-old cockerels weighing 210 g, 12-month-old cocks weighing 1930 g, hens weighing 1730 g, and 24-month-old hens weighing 1680 g, all of White Leghorn stock, were used. Immature chickens were reared in an electric brooder kept at 80°F, and mature chickens were placed in battery cages at room temperature of about 70°F. The birds were maintained on the standard ration used at Nat. Inst. of Agric. Sciences. Thiouracil (Nutritional Biochemicals Corp., Cleveland) was given at the ratio of 0.1% in the ration. This amount of thiouracil blocks the synthesis of thyroid hormone by the thyroid in chickens(7). Immature and mature chickens were injected subcutaneously with 15 and 30 μc of carrier-free I^{131} as NaI, respectively. The γ-radiation from the thyroid region of immature and mature chickens was counted with a scintillation counter (Inst. of Physics and Chemistry, Ltd., Tokyo) with lead collimators with openings of 3 cm and 7 cm, respectively. The unanesthetized immature and mature chickens were placed in an immobilization apparatus during measurement of radioactivity at distances of 7 cm and 20 cm between the crystal in the counter and the surface skin of the thyroid region, respectively. Counts were corrected for background, and for physical decay at the measurement of release of I^{131}. Immature chickens were divided into 3 groups: one group maintained on normal diet, a second group on 0.1% thiouracil diet for 2 days before I^{131}-injection, and a third group on 0.1% thiouracil diet for 7 days before I^{131}-injection. Thiouracil feeding was continued after I^{131}-injection. Thyroidal uptake of injected I^{131} in percent was measured at 6 and 24 hr after injection. The apparent release rate of I^{131} from the thyroid was expressed as percentage loss of thyroidal I^{131} per day and calculated using following equation: Release rate (%) = 100–(48 hr uptake % /24 hr uptake %) x 100. Thyroidal I^{131}-uptake was measured *in vivo* using I^{131} standards in phantoms. These coincided with scintillation spectra of the thyroids of the chickens. In mature chickens thyroidal I^{131} uptake was measured at 6 and 24 hr after injection of I^{131}; the apparent thyroidal I^{131}-release rate during the period from 48 hr to 120 hr was calculated from the regression of points of the counts plotted semilogarithmically *vs.* time, and expressed as percentage loss of thyroidal I^{131} per day. Thiouracil feeding was started 120 hr after injection of I^{131} and thyroidal I^{131}-release rate for the first 24 hr after the start of thiouracil feeding was measured. For plasma studies, blood was taken from wing vein of five 12-month-old hens receiving 100 μc I^{131},

just before the start of thiouracil feeding at 96 hr after injection of I^{131}, at 24 and 48 hr after the start of thiouracil feeding. Total plasma I^{131} and TCA-precipitable I^{131} (PBI^{131}) were measured with a well-crystal scintillation counter (Nuclear - Chicago Corp.) From both values inorganic I^{131} ($iodide^{131}$) was obtained by difference(8). To measure the extent of thyroidal recycling of I^{131} from metabolized thyroid hormone, normal diet-fed immature and mature chickens and thiouracil-fed immature chickens were injected intravenously with 0.2 μg L-thyroxine labelled with 8 μc I^{131} (Abbott Labs., Oak Ridge). Rate of recycling I^{131} was measured at 6 and 24 hr after injection of radiothyroxine in immature chickens, and at 24 hr in mature chickens, because the peak recycle value of immature and mature chickens was observed around 6 and 24 hr after injection, respectively. In another group of immature chickens thiouracil was given 48 hr after injection of I^{131}, and thyroidal I^{131}-release rate was expressed as percentage loss of thyroidal I^{131} for the first 24 hr after start of thiouracil feeding. For measurements of thyroxine secretion rate, immature chickens were divided into normal diet-fed and thiouracil-fed groups, the latter being pre-fed thiouracil before the thyroxine substitution as follows: for 24, 48 or 120 hr, and thiouracil was continued after the start of the substitution, which was started at 4 weeks of age. I^{131} was injected subcutaneously 72 hr before the substitution in each case. Thyroid count and body weight were measured daily. Daily graded increasing levels of L-thyroxine (Nutritional Biochemicals Corp., Cleveland), dissolved in an alkaline solution, of 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 and 18 μg per 100 g body weight were injected. In most cases of thiouracil-fed chicks, some of the described doses were skipped. Thyroxine secretion rate was estimated as a given dose of thyroxine which resulted in coming to an equilibrium or to a little rise in comparison with the preceding count as indicated by the external thyroid count. When a thyroxine dose producing

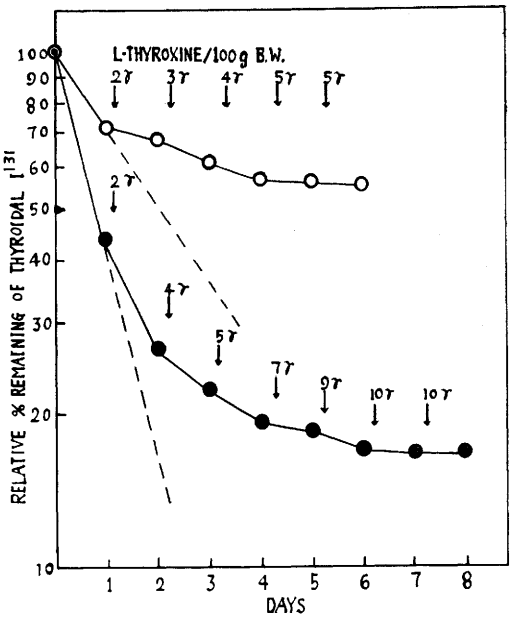

FIG. 1. Typical procedures for determination of thyroxine secretion rates in 4- to 5-wk-old cockerels. ○—○ Normal diet-fed bird. ●—● 0.1% thiouracil-fed bird.

equilibrium or a rise in external thyroid radioactivity of I^{131} was found, the same dose of L-thyroxine was injected repeatedly (Fig. 1). Estimated true release rate of I^{131} from the thyroid(T) was calculated from the apparent release rate of I^{131} from the thyroid(A) and rate of peak recycling of I^{131} from metabolized thyroid hormone(R), using a equation of $T(\%) = A(\%) \times 100/100\text{-}R(\%)$.

Results. The effect of thiouracil on turnover of I^{131} by the thyroid in 4-week-old cockerels is summarized in Table I. Maximum percent uptake of I^{131} by the thyroid was observed 6 hr after I^{131} injection. Administration of thiouracil had a statistically significant body growth depressant effect, and it significantly reduced uptake and recycling of I^{131} by the thyroid in immature chickens. Rate of recycling of I^{131} derived from the metabolized thyroid hormone, measured by accumulation of I^{131} from I^{131}-labelled L-thyroxine, was always a little less than rate of uptake of I^{131} by the thyroid. Both the uptake and the recycling of thyroidal I^{131} significantly decreased with

TABLE I. Effect of Thiouracil Feeding on Turnover of I^{131} by Thyroid of 4-Week-Old Cockerels.

		0.1% thiouracil in diet	
	Normal diet	Fed for 2 days before inj.	Fed for 7 days before inj.
Body wt at I^{131} inj., g	208 ± 3 † (90)	190 ± 4 * (20)	189 ± 3 * (20)
6 hr uptake, %	43.58 ± .73 (80)	31.10 ± 4.40* (10)	8.79 ± .68* (10)
24 " " , %	34.65 ± .80 (80)	20.08 ± 2.97* (10)	2.92 ± .66* (10)
6 " recycle,‡ %	37.71 ± 1.30 (10)	23.48 ± 2.57* (10)	—
24 " " , %	29.07 ± 1.21 (10)	9.54 ± 3.05* (10)	2.24 ± .96* (10)
Apparent release rate,§ %	27.54 ± 1.29 (40)	67.03 ± 4.24* (10)	77.96 ± 1.81* (10)

* Difference from value of birds maintained on normal diet statistically significant at 1% level.

† Mean and stand. error.

‡ Expressed as % uptake of liberated I^{131} by thyroid 6 hr after radiothyroxine inj.

§ " " " " loss of thyroidal I^{131}/day between 24 and 48 hr after inj.

Figures in parentheses = No. of birds used.

length of the period of thiouracil feeding. Thiouracil feeding caused a significant increase in release rate of I^{131} from the thyroid. This effect of thiouracil became progressively more pronounced as length of time of thiouracil feeding before I^{131} dosage was increased. Thyroxine secretion rate measured by substitution with L-thyroxine in 4 to 5-week-old cockerels is shown in Table II. Thyroxine secretion rate expressed either in μg/100 g body weight or per bird significantly increased in thiouracil-fed chickens compared with that in normal diet-fed chickens, and further increased as length of period of thiouracil feeding was increased. Thiouracil feeding also significantly increased release rate of thyroid I^{131} in mature cocks and hens (Table III). The maximum uptake of I^{131} by the thyroid of mature chickens occurred around 24 hr after injection of I^{131}. The percent recycling of I^{131} from metabolized thyroid hormone was somewhat lower than percent uptake of I^{131} by the thyroid. Both uptake and release rate of thyroidal I^{131} were lower in cocks and hens than in immature chickens. The observed release rate of thyroidal I^{131} during the first 24 hr following thiouracil feeding was much higher than the estimated true release rate of I^{131} calculated from the apparent release rate and rate of peak recycling. Such an increase in release rate of thyroidal I^{131} is not only due to blocking the recycling of I^{131} by the thyroid but also to increase of the release rate of I^{131} from the thyroid by thiouracil. Plasma iodide^{131} concentration increased 2-3

TABLE II. Effect of Thiouracil Feeding on Thyroxine Secretion Rate Measured by Substitution with L-Thyroxine in 4- to 5-Week-Old Cockerels.

		0.1% thiouracil in diet		
	Normal diet	Fed for 24 hr before substitution	Fed for 48 hr before substitution	Fed for 120 hr before substitution
No. of birds	40	20	15	10
Body wt at estimation, g	311 ± 6*	309 ± 5	285 ± 10†	292 ± 4
Thyroxine secretion rate, μg/100 g body wt/day	4.61 ± .19	7.00 ± .53‡	7.97 ± .71‡	11.67 ± 1.18‡
Thyroxine secretion rate, μg/bird/day	14.40 ± .70	21.62 ± 1.55‡	23.00 ± 1.82‡	33.89 ± 3.19‡

* Mean and stand. error.

† Difference from value of birds maintained on normal diet statistically significant at 5% level.

‡ Difference from value of birds maintained on normal diet statistically significant at 1% level.

TABLE III. Effect of Age on Uptake, Recycling, and Release of I^{131} by the Thyroid of the Chicken.

	Body wt, g	6 hr I^{131} uptake	24 hr I^{131} uptake	24 hr I^{131} recycling	I^{131} release rate/day	Estimated true release rate/day	I^{131} release rate 1st 24 hr after 0.1% thiouracil feeding
		%					
4-wk-old cockerel	208	43.58 ± .73† (80)	34.65 ± .81 (80)	29.07 ± 1.21 (10)	27.54 ±1.29 (40)	44.21	57.71 ± 2.78 (40)
12-mo-old cock	1928	17.06 ± 1.60 (10)	20.80 ± 2.92 (10)	17.84 ± 3.36 (10)	5.63 ± .93* (10)	6.85	12.57 ± 3.27 (10)
12-mo-old hen	1726	13.28 ± 1.11 (10)	16.40 ± 1.63 (10)	13.99 ± 1.91 (10)	3.80 ± .50* (10)	4.42	7.87 ± .97 (10)
24-mo-old hen	1676	10.94 ± 1.75 (10)	11.47 ± 1.90 (10)	10.88 ± 1.27 (10)	2.60 ± .60* (10)	2.92	—

* Calculated from regression of plots for 4 days. † Mean and stand. error.
Figures in parentheses = No. of birds used.

times 24 hr after the start of thiouracil feeding as compared with the level before the treatment, while plasma PBI^{131} level remained at pre-treatment level until 48 hr after the start of treatment.

Discussion. This study as well as that of Pipes *et al.*(5,6) demonstrates that thiouracil feeding significantly augments release rate of I^{131} from the thyroid. Pipes *et al.*(5,6) attributed this increase in the rate to blocking of reutilization of I^{131} derived from metabolized thyroid hormone, and stated that the rate observed in thiouracil-treated birds was a true rate of release. However, our work shows that this increase is a composite of the blocking of reutilization of hormonal I^{131} as well as increase of release rate of I^{131} itself. Furthermore, the rate of loss is accelerated as length of the period of thiouracil feeding increases. Recently, Kobayashi and Gorbman(9) reported that thiouracil blocked hormone synthesis at the monoiodotyrosine stage in the young chicken. Administration of thiouracil may result in prompt release of $iodide^{131}$ from the thyroid, suggested by the present work. Rosenberg *et al.*(8) reported that injection of TSH resulted in a prompt release not only of PBI^{131} but also of $iodide^{131}$ from the thyroid. Rate of recycling of I^{131} always proved a little less than rate of uptake of I^{131} by the thyroid (Table I, III). The theoretical I^{131}-release rate calculated using the equation by Brownell(10) in this experiment is too high, but it is in good agreement with the estimated true release rate obtained from the correction for recycling of I^{131} from I^{131}-labelled thyroxine. Thyroidal uptake of I^{131} greatly decreases in thiouracil-fed birds(9,11) and decreases further with the advance in length of the period of thiouracil administration, as shown by the present work.

The present work demonstrates that the thyroxine secretion rate expressed either in μg/100 g body weight or per bird, increases (more than 50%) in thiouracil-fed chickens as compared with that in normal diet-fed chickens, and increases further with length of the period of thiouracil feeding. This result shows that the thyroxine substitution using thiouracil and external I^{131} counting (5,6) is not adequate for precise determination of thyroxine secretion rate, at least in the chicken; the value obtained using this procedure is high. This increase in thyroxine secretion rate measured by the thyroxine substitution by thiouracil may be due to augmenting the output of TSH from the pituitary(12). Reineke and Singh(3) reported estimated thyroxine secretion rate was about 11% higher in thiouracil-treated than in normal rats. They began the thyroxine substitution at the same time thiouracil administration started. This method of substitution may reduce TSH production by the pituitary encountered after longer periods of goitrogen treatment. Recently, Mellen and Wentworth (13) reported that the thyroxine secretion rate measured by the thyroxine substitution method with thiouracil and radioiodine was

50% higher than the value obtained from the classical goiter-prevention assay without radioiodine in the young chicken. The value derived from the classical goiter-prevention assay may be closer to normal secretion rate, because in this way thyroxine substitution usually starts on the same day as the thiouracil feeding, and the increase in TSH production by the pituitary by thiouracil is inhibited in chickens receiving the successive excess dose of thyroxine.

Summary. The effects of thiouracil on turnover of I^{131} by the thyroid in immature and mature chickens were studied. Rate of uptake of I^{131} by the thyroid was significantly reduced and release rate of I^{131} from the thyroid was significantly augmented in thiouracil-fed birds. Rate of recycling of I^{131} from the metabolized thyroid hormone measured by accumulation of I^{131} by the thyroid from I^{131}-labelled thyroxine injected was always a little less than rate of uptake of I^{131} by the thyroid. Both uptake and recycling of thyroidal I^{131} decreased with length of the period of thiouracil feeding. Rate of release of I^{131} from the thyroid increased with the lengthened time of thiouracil feeding. The thyroxine secretion rate expressed either per 100 g body weight or per bird measured by the thyroxine substitution significantly increased in thiouracil-fed birds compared with normal diet-fed birds. It also increased with length of thiouracil feeding. Use of thiouracil is not adequate for precise determination of thyroxine secretion rate in the chicken.

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Incorporation of Tritiated Thymidine into DNA after Oral Administration.* (26234)

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Recent studies of cellular proliferation utilizing parenteral tritiated thymidine, H^3TDR, have demonstrated widespread incorporation of this DNA precursor into proliferating cells(1,2,3). Metabolic studies with H^3TDR in man have shown prompt utilization of this compound for DNA synthesis with degradation of approximately one-third of the injected dose to tritiated water and excretion in the urine of only small amounts as non-volatile tritiated products (4). No information is available on the fate of ingested H^3TDR. Such studies are needed for evaluation of the potential hazard of this material in respect to its laboratory use. In this paper autoradiographic (ARG) studies will demonstrate absorption following oral administration and subsequent incorporation

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