Degradation of Thyroxine Confounded by Thyroidal Recycling of Radioactive Iodine[1] (36480)

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Birds such as chickens and quail have a rapid thyroxine (T_4) turnover rate and short biological half-life ($t_{1/2}$) and a very low level of protein-bound iodine (1). However, estimates of $t_{1/2}$ vary widely. In chickens $t_{1/2}$ values of 3.25 (1), 7.6 and 11.4 (2) and 22.5 hr (3) have been reported. Japanese quail have been reported to have $t_{1/2}$ values ranging from 4.6 hr (1) to 30.4 hr (4). It has been reported (5) that there are as many as four rate constants involved in the disappearance of both T_4 and triiodothyronine from blood plasma of the chicken up to 4 days postinjection, probably representing distribution in various body pools. Little attention has been given to the possible influence of recycling of metabolized radioiodine back through the thyroid on the T_4 disappearance rate. The experiments reported herein were designed to determine whether any such recycling would have a significant effect on apparent T_4 turnover rate or $t_{1/2}$ in the bobwhite quail.

Materials and Methods. Adult male bobwhite quail (*Colinus virginianus*) from the Michigan State University poultry flock were used in two experiments. In each experiment the birds of one group were subcutaneously injected with 20 mg of NaSCN in aqueous solution 30 min before radiothyroxine injection to block the thyroid I^- trap. In Expt. 1, where blood sampling extended over a period of 44 hr, the NaSCN injection was repeated at 12 hr intervals. In each experiment a group of birds not receiving SCN^- was designated as the normal control.

Each quail in Expt. 1 was injected with 30 μCi of ^{131}I-L-T_4[2] in the left brachial wing vein at the beginning of the experiment. In Expt. 2 (5.5 hr duration) 5 μCi were given.

Heparinized blood samples were drawn from the right brachial wing vein of each quail at several intervals. Sampling extended 44 hr after ^{131}I-T_4 injection in Expt. 1 and 5.5 hr in Expt. 2. Radioactivity counts were taken on an aliquot of each plasma sample and standards, employing a well counter and radiation analyzer. For each experiment ^{131}I-L-T_4 standards were set up in triplicate to contain 0.01 times the injected dose. Percentage of the injected dose of radioactivity in each plasma sample (% dose) was calculated by the formula:

$$\% \text{ dose} = \frac{\text{plasma cpm} - \text{background cpm}}{\text{injected cpm} - \text{background cpm}} \times 100.$$

The log of % injected dose/ml plasma was regressed against time in hr by the method of least squares (6). In Expt. 1, log % dose/ml in plasma was also regressed against log time. At the conclusion of each experiment the whole thyroids were excised from each bird at necropsy. Their radioactivity was also expressed as % injected dose. Biological half-life ($t_{1/2}$) was calculated by the equation: $t_{1/2} = 0.301/b$ where b = the regression coefficient.

Results. The ^{131}I disappearance curves for both experiments are presented graphically in Fig. 1. The SCN^- treated group in Expt. 1 showed a significant linear regression ($r = -0.99$) between log % dose/ml plasma and

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[2] ^{131}I-Labeled L-thyroxine obtained from Abbott Radiopharmaceutical Laboratories, North Chicago, IL.

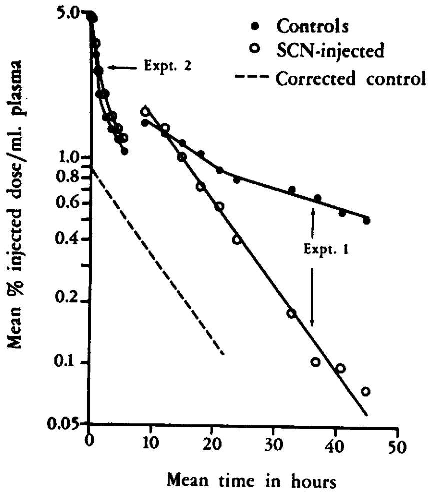

FIG. 1. Mean ^{131}I-L-T_4 degradation curves for uncorrected control, thiocyanate-treated and corrected control groups of bobwhite quail.

time through the whole sampling period that extended from 9 to 44 hr after radiothyroxine injection. Data for the controls showed a significant break in the disappearance curve (Table I) at about 20 hr, indicating the operation of more than one exponential. It was suspected that both slopes of this curve were influenced by (1) disappearance of the radiothyroxine initially injected and (2) dilution of the initial dose of labeled T_4 by newly labeled hormone produced by recycling of ^{131}I through the thyroid. In order to correct for this, the calculated line for the second slope was extrapolated back to zero time. Values for this slope were then subtracted from those of the first slope at several time intervals to obtain the corrected slope (Fig. 1). This slope had a mean $t_{1/2}$ of 8.6 ± 1.36 hr that was not significantly different ($p > .10$) from the $t_{1/2}$ of 6.7 ± 0.71 hr for the SCN^- treated birds. In Expt. 2 it was judged from the curves that about 2 hr had been required for mixing and equilibration of the radiothyroxine in the various body pools. Therefore, $t_{1/2}$ was calculated from the 2.5 to 5.5 hr segment of the ^{131}I disappearance curve for both control and SCN^- treated birds. There was no significant difference between the two groups (Table I). The % dose/ml plasma (Y) of control birds (Expt. 1) for 9 to 44 hr after radiothyroxine injection was also regressed against time (X) by the log–log equation,

$$\log Y = \log A + b \log x.$$

This yielded the prediction equation,

$$\log Y = 0.841 + (-0.673 \log X)$$

and a correlation coefficient of −0.99.

An average of 2.58% of the injected dose of radioiodine was retained in control thyroids 5.5 hr after labeled T_4 injection compared to 0.08% in thyroids of SCN^- treated birds. Forty-four hours after labeled T_4 injection, the respective retention figures for control and SCN^- treated thyroids were 23.65 and 0.48% (Table II).

Discussion. These experiments clearly establish that measurements of T_4 degradation rate or $t_{1/2}$ in normal bobwhite quail are seriously confounded by the recycling through the thyroid of I^- metabolized from the labeled hormone. The influence of recycling can be minimized by injecting a compound such as NaSCN that will block the thyroid I^- trap, by correcting for I^- recycling (Fig. 1), or by collecting the series of blood samples at short intervals as soon as the labeled hormone has become equilibrated in the body systems. In the bobwhite quail the equilibration time is about 2.5 hr. The concept of recycling is supported by the massive accumu-

TABLE I. Biological Half-life ($t_{1/2}$) of ^{131}I-L-T_4 in Bobwhite Quail (Mean ± SE).

	n	$t_{1/2}$ (hr)	p^a
Experiment 1 (Sampling period: 44 hr)			
Control:			
1st slope (9–20 hr)	5	16.0 ± 1.63	
			< .01
2nd slope (24–44 hr)	5	34.0 ± 4.14	
Corrected slope	5	8.6 ± 1.36	
SCN^- injected	5	6.6 ± 0.71	
Corrected control vs. SCN^- injected			> .10
Experiment 2 (Sampling period: 5.5 hr)	n	$t_{1/2}$ (hr)	p
Control	6	6.7 ± 1.27	
			> .50
SCN^- injected	6	7.1 ± 1.85	

^{a} Student t test.

TABLE II. Radioiodine Retention by Whole Thyroids as Percent of Injected Dose (Mean ± SE).

Post injection of ^{131}I-L-T_4 (hr)	Control group	SCN^- treated group	Signif. of difference from controls
5.5	2.58 ± 0.41 (6)[b]	0.08 ± 0.03 (6)	($p < .01$)[a]
44	23.65 ± 3.10 (5)	0.48 ± 0.18 (5)	($p < .01$)

[a] Student t test.
[b] Number of samples in group mean.

lation of iodine label in the thyroids of control quail compared to a small accumulation in SCN^- treated birds.

Recycling probably starts soon after labeled hormone injection at which time some 10% of the labeled iodine has been shown to be unbound (1). This radioiodine is immediately available for thyroid trapping. As more of the injected hormone is metabolized and radioiodine is released, more of this iodine gets trapped, reorganified, secreted into the blood and remetabolized. Thyroxine degradation rates would thus be continuously confounded by recycling and, therefore, should be multiexponential curves. This is borne out by the log–log regression ($r = -0.99$) of control data from experiment I. The concept of curvilinearity in T_4 degradation curves would explain the widely varying biological half-lives that have so far been reported. Values of 22.5 hr reported in chickens (3) would result from a curve that extended for days after radiothyroxine administration. On the other hand, a $t_{1/2}$ value of 4.6 hr reported for bobwhite quail (1) would result from a sampling period of about 12 hr following labeled hormone injection. The double log plot thus indicates that varying $t_{1/2}$ values would be obtained even in the same species of animal depending on what part of the degradation curve was involved in the sampling period. Consequently, without some drug such as thiocyanate to block the bulk of recycling, $t_{1/2}$ values calculated about 3 to 12 hr after ^{131}I-L-T_4 injection would more nearly represent the true degradation rate of the original labeled T_4 dose.

Summary. Data are presented to show that measurements of T_4 degradation rate in bobwhite quail, when using radioactive T_4 as a tracer, are confounded by recycling through the thyroid of I^- metabolized from the labeled hormone. The effects of this can be minimized by (1) blocking the thyroid I^- trap with SCN^-, (2) taking the blood samples over a short period of time after equilibration of the injected radioactive T_4 or (3) mathematically correcting for recycling in data collected over an extended period. The biological half-life for the disappearance of injected radioactive thyroxine in two experiments, including four groups of birds, ranged from 6.6 to 8.6 hr.

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