SOME NEW ASPECTS OF THYROXINE DEGRADATION AS OBSERVED FROM STUDIES USING BOBWHITE QUAIL

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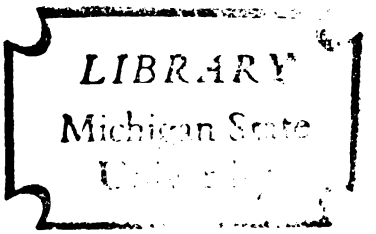

ABSTRACT

SOME NEW ASPECTS OF THYROXINE DEGRADATION
AS OBSERVED FROM STUDIES USING
BOBWHITE QUAIL

by Kevin M. Etta

In the unending search for more reliable indices of thyroid state and function, physiologists have employed many kinds of drugs. Perhaps the most commonly used are the anions like thiocyanate and perchlorate which have been known to block iodide trapping and goitrogens like tapazole and the thiouracils which block hormone synthesis. This study examined the more controversial subject of the possible extrathyroidal effects of thiocyanate and tapazol injection on the degradation of ^{131}I-L-thyroxine in adult male bobwhite quail. Blood samples were taken at regular intervals after drug and labelled thyroxine treatment. The longest period of continuing sampling was 45 hours. Some previous studies may have reported as long or even longer periods of sampling. However, none of the earlier studies known to this writer have ever reported a break in the degradation curve. The break in the control curves was exploited to yield a method for taking account of and mathematically correcting for extrathyroidal recycling of iodine. This is the first time that this correction has been made without the use of anions to block iodide trapping or goitrogens to block hormone synthesis.

Thiocyanate, by blocking recycling of metabolized iodine through the thyroid, offered a second method for estimating the best approximation to-date of fractional degradation per hour of L-thyroxine. These two methods served as checks, one for the other. Both thiocyanate and tapazole drastically and significantly reduced thyroidal retention of iodide. Thiocyanate depressed muscle retention to the same extent as it increased the retention of radioiodide by the gastrointestinal tract. Tapazole significantly increased liver retention as well as total body retention of radioiodine. Tapazole seemed to have the same effects thyroidally and extrathyroidally as have been reported for other goitrogens by many workers. More specifically, tapazole seemed to cause an impairment at some point in the metabolism of ^{131}I-L-thyroxine. This interference showed up as a greatly increased level of blood radioactivity, a reduced excretion of radioactive material via the gastrointestinal tract and a significantly increased retention by the liver of the bobwhite quail. Livers have been reported to be a major site of thyroid hormone deiodination. This would place the liver as a primary site of action of the interference of tapazole in peripheral degradation.

The observations concerning the extrathyroidal effects of tapazole and other goitrogens would put to serious question those methods for estimation of thyroxine

secretion rate which have used goitrogens either in the goiter-prevention technique or in the substitution methods where goitrogens served to give a steeper thyroidal output slope.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

LITERATURE REVIEW

Thyroid Hormone Synthesis

A tremendous amount of work has been done in thyroid physiology. Starting from just before the turn of the century when hardly anything was definitely known about the physiology of the thyroid gland and its products, thyroid physiology has come a long way to this date when an enormous number of thyroidal functions have been more or less accurately mapped out.

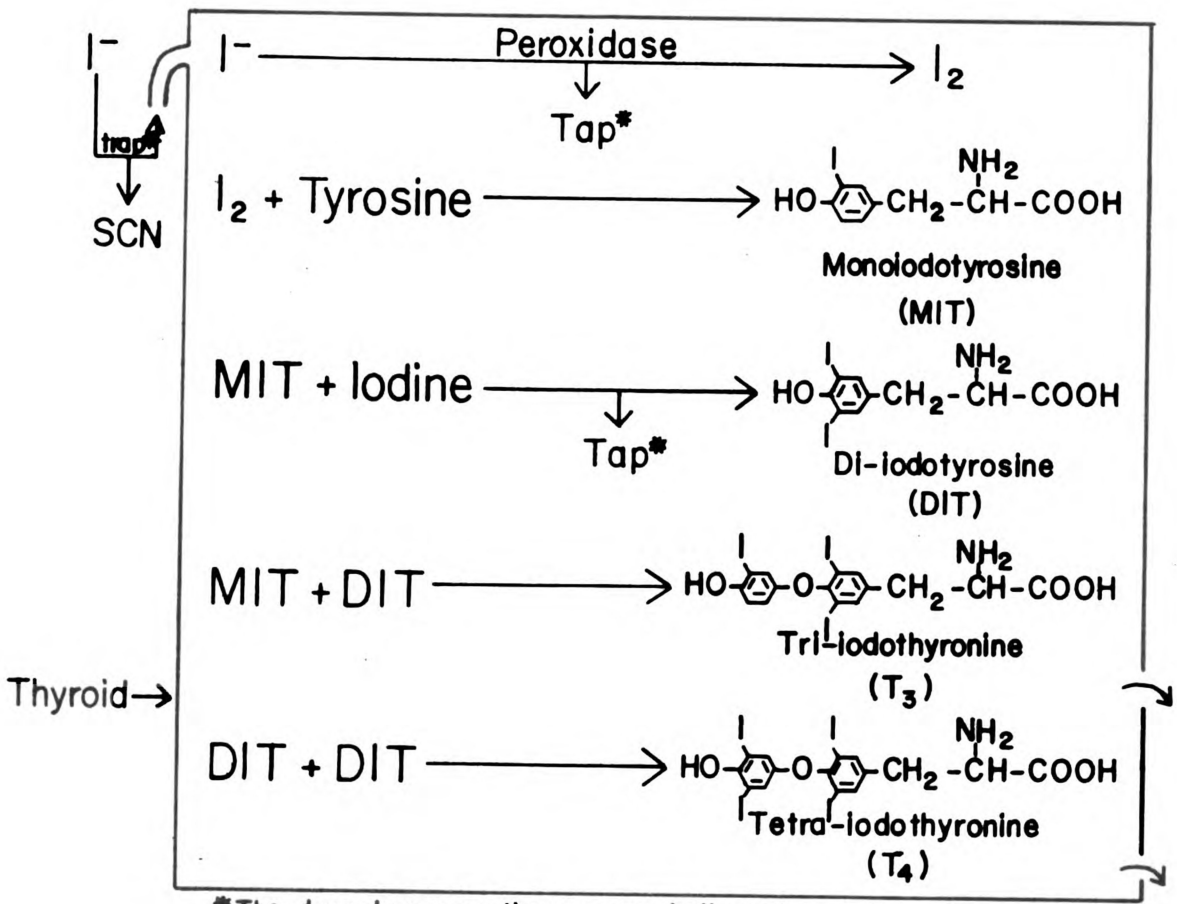
Avian thyroid glands are located ventrolaterally to the trachea just outside the thoracic cage. As in all vertebrate animals, the thyroids in birds are specialized for the uptake and retention of iodide. Twenty to forty percent of an injected tracer dose of ^{131}I is taken up by the thyroids. The bulk of the remainder is excreted in the urine such that 90 percent of the injected amount is accounted for by the combined thyroid uptake and urinary excretion in normal birds. Only small amounts of iodide appear in the gastric juice and saliva. Normal rats have been reported to be in iodide balance when they excreted 70 percent of their daily ^{131}I dose in their urine (Jones and Van Middlesworth, 1960).

For convenience of discussion, Richards and Ingbar (1959) have divided thyroid hormone synthesis into three major steps:

1. Thyroid gland concentration of inorganic iodide from extracellular fluid.
2. Oxidation of the iodide, by a peroxidase, presumably to elemental iodine within the thyroid. Iodine then iodinates tyrosyl residues to form first mono-iodotyrosine (MIT) and then di-iodotyrosine (DIT).
3. Coupling of the iodotyrosines to form iodothyronines like triiodothyronine (T3) and tetra-iodothyronine (T4). The probable sequence of reaction is summarized in Chart I.

The MIT, DIT, T3 and T4 all occur within the thyroid gland combined in a storage colloid glycoprotein named thyroglobulin. A protease cleaves the thyroglobulin to release T3 and T4 into the bloodstream while MIT and DIT are degraded by a scavenger deiodinase enzyme to release iodide within the thyroids. This iodide is again available for use by the thyroid glands via intrathyroidal recycling of iodine. The circulating hormone is deiodinated by a deiodinase and conjugated at peripheral tissue centers most important of which is the liver (Flock and Bollman, 1959). The resulting metabolites are secreted from the liver through the bile. Most of the iodide is excreted in the urine, only a small amount appearing in the feces in the normal animal. Some of the iodide released by deiodination is reabsorbed by peripheral

Thyroid Hormone Synthesis



*The drug depresses the process indicated.

Chart I

tissues and some is recycled back to the thyroid for further trapping and reuse--extrathyroidal recycling.

Effects of Drugs on Hormone Synthesis and Metabolism

Thiocyanate: Drug Effects on Synthesis

Numerous drugs have been reported to modify or completely block some or most of the steps in thyroid hormone synthesis. Several such drugs have been employed as an aid in the study of thyroid physiology. One such group of anions has been known for a long time to affect the trapping or concentration of iodide by the thyroid gland. Perchlorate, thiocyanate and nitrates have been shown to block the ability of the thyroid to trap iodide to varying degrees. Barker (1936) first reported the occurrence of goiter in patients receiving thiocyanate therapy for hypertension and the correction of such goiter by the administration of desiccated thyroid. Jones and Van Middlesworth (1960) have shown perchlorate to inhibit the uptake of iodide by the thyroid without influencing thyroxine deiodination in rats. This was suggested by the observation that both control and potassium perchlorate-fed rats were in iodine balance when they excreted 70 per cent of their daily ^{131}I dose in urine. Franklin, Chaikoff and Lerner (1944) demonstrated that thiocyanate, but not thiouracil, interferred with thyroidal accumulation of iodine. Two years later, Vanderlaan and Bissel (1946a)

demonstrated even more conclusively that thiocyanate blocked thyroidal uptake of iodine. Vanderlaan and Vanderlaan (1947) also demonstrated that iodide accumulated during treatment of rats with thiouracil could be rapidly and quantitatively flushed out from the thyroid by the administration of thiocyanate. Wyngaarden, et al. (1952) demonstrated that perchlorate was ten times and nitrates one-thirtieth as effective as thiocyanate in flushing out concentrated iodide from propylthiouracil-treated thyroid glands. In an attempt to localize the effect of anions like thiocyanate, Vanderlaan and Greer (1950) suggested that the histologic effects of perchlorate would favor either the hypothesis of a thyrotropin regulation of the iodide trapping mechanism or a competition between blocking agents and the iodides for the ability to get trapped. The concept of competition has since been supported by experiments. The greater the iodide supply to rats, for instance, the less effective would the blocking agents be in their interference with the trapping mechanism. Vanderlaan and Caplan (1954) have supported the concept that iodide concentrating capacity was a more sensitive indicator of thyrotropin activity than thyroid weight in goiter studies. All the reports are thus unanimous on the concept that anions like perchlorate, thiocyanate and nitrates can block the iodide trapping mechanism to varying degrees. It is established that thiocyanate and similar types of

anions have little effect on the organification of iodine. Richards and Ingbar (1959) have reported that increasingly higher doses of such anions resulted in no appreciable alterations in the relative proportions of the individual iodinated amino acids. At extreme reductions of both total and organic uptake of iodine, however, a small decrease in the ratio of the di-iodotyrosine and mono-iodotyrosine occurred. This decrease indicated an impaired synthesis of thyroid hormone. Extreme doses of anions such as would give these extreme reductions of both total and organic uptake are presumably rarely used in studying normal thyroid physiology. It is quite possible that such impaired hormone synthesis may be secondary to the primary effect of the anions in blocking iodide trapping. This is speculative, however. It is also speculation whether or not perchlorate and thiocyanate have extrathyroidal effects.

Extrathyroidal Effects of Perchlorate and Thiocyanate

Jones and Van Middlesworth (1960) reported that both control and perchlorate-fed rats were in iodine balance when they excreted 70 percent of their daily ^{131}I dose in urine. If the route and quantity of excreted iodine was any index to the state of peripheral metabolism of iodinated thyronines, the report would tend to suggest that perchlorate had no effect on peripheral metabolism, specifically, the deiodination of thyroid hormones. Raben

(1949) and Greer, et al. (1966) have shown anions like thiocyanate and perchlorate to depress organic binding by the thyroid. Yamada (1967) surmised that such anions probably altered the peripheral metabolism of thyroid hormone by releasing some of it from its binding plasma proteins (Robbins and Rall, 1957), (Ingbar and Freinkel, 1960). In accordance with this hypothesis Yamada reported markedly decreased protein-bound iodine values ($p < 0.001$) in rats as a result of perchlorate and thiocyanate treatment. He also observed an increase in urinary iodine as well as an increased uptake of iodine by skeletal muscles. It is interesting to note that Yamada did not observe any difference in the muscle retention of iodine between control and perchlorate-treated rats until at least a ten-fold dilution of the plasma from the muscle samples was attained. The reduced PBI could be due to an increased rate of excretion along with a block in recycling. It is more difficult to consider muscle retention to be consequentially altered when the samples needed a ten-fold dilution to show a difference. The odds are, therefore, still strongly in favor of the concept that thiocyanate and perchlorate do not have extrathyroidal effects.

Goitrogens: Effects of Thiouracils and Methimazole on Hormone Synthesis

Thiouracil and Methimazole have been reported to block hormone synthesis at one or other of three possible stages:

1. The oxidation of iodide to iodine.
2. The iodination of mono-iodotyrosine to di-iodotyrosine.
3. The coupling of tyrosines to form thyronines.

Vanderlaan and Vanderlaan (1947) reported that thiouracil prevented the oxidation of iodide to iodine. This step is presumably catalyzed by a peroxidase enzyme within the gland. Slingerland, et al. (1959) reported that propylthiouracil treatment increased the mono-iodotyrosine to di-iodotyrosine as well as the tri-iodothyronine to tetra-iodothyronine ratios. The same study also showed that the conversion of mono-iodotyrosine to di-iodotyrosine was more sensitive to propylthiouracil treatment than the iodination of tyrosine to mono-iodotyrosine. These results suggested that propylthiouracil probably blocked thyroid hormone synthesis at the MIT to DIT or the T3 and T4 steps. Richards and Ingbar (1959) reported that propylthiouracil affected not only the initial oxidation of iodine and the resulting mono-iodination of tyrosine but also the di-iodination of tyrosyl residues as well as their coupling to form the hormonally active iodothyronines. Whatever the precise stage at which the blocking effect of goitrogens is exerted, there can be no doubt as to the fact that goitrogens do in fact block thyroid hormone synthesis.

Effect of Goitrogens on Thyroidal Output of Iodine

Albert and Tenney (1951) reported that thiouracil accelerated "thyroidal secretion" of iodine six-fold in rats. Flamboe and Reineke (1959) reported a more rapid ^{131}I output rate in goats treated with thiouracil compared to untreated goats. Tanabe, et al. (1965) reported that thiouracil, propylthiouracil and methimazole all significantly increased the release rate of ^{131}I from the thyroid gland. Grosvener (1963) demonstrated that propylthiouracil rapidly increased the thyroidal release of iodine. Goitrogens, therefore, have marked effects on the rate at which the thyroid gland releases iodine in addition to their effects on thyroid hormone synthesis. Such effects are apparently shared by all goitrogens to differing degrees. Thiouracil is apparently the most potent in accelerating iodine output followed by propylthiouracil and then methimazole (Tanabe, et al., 1965). Regarding the interference of goitrogens with thyroid hormone synthesis by blocking recycling of iodine, methimazole is said to be the most effective (Grosvener, 1963; Premachandra, et al., 1958; Pipes, et al., 1963). Methimazole has also been employed to obtain a steeper output slope in the substitution method of estimating thyroxine secretion rates by Brooks, et al. (1962) and Romack, et al. (1964). But do tapazole and other goitrogens have any extrathyroidal effects?

Extrathyroidal Effects of Goitrogens

Goitrogen Depression of Metabolic Rate

Barker, et al. (1949); Andik, et al. (1949); Barrett and Gassner (1951) and Stasilli, et al. (1960) have all reported that the thiouracils inhibited the rise in oxygen consumption or metabolic rate that would normally follow the administration of exogenous thyroxine. There is some more or less direct relationship between the metabolic effectiveness of the thyroid hormone and its catabolism in the tissues via the dehalogenating pathways. Variations in the rates of peripheral deiodination of the hormone and its metabolic effectiveness may, therefore, be different indices of the disturbance of metabolic parameters (Escobar and Escobar, 1961).

Goitrogen Effects on PBI, Routes of Iodine Excretion and Tissue Retention of Iodides

Several other observations have led to the suggestion that goitrogens may affect the peripheral metabolism of thyroid hormones. Jagiello and McKenzie (1960) reported that rats maintained on 2 µg of thyroxine per day and treated with propylthiouracil produced normal concentrations of thyroid-stimulating hormone but showed protein-bound iodine values that were twice those of normal rats. This suggested that propylthiouracil might have extrathyroidal effects. Stasilli (1960) reported that thiouracil probably inhibited peripheral utilization and modified

gastrointestinal excretion of iodinated thyronines and iodides. What doubts that could be entertained about Stasilli's findings were completely dispelled by Escobar and Escobar (1961) who reported the following:

1. The over-all peripheral deiodination of L-thyroxine sharply and rapidly decreased following the administration of thiouracil, methylthiouracil or propylthiouracil. This decrease was shown to be the first effect of the thiouracils clearly detectable, and the delay in the increase of fecal excretion was not due only to the time necessary for the formation of feces. It took four days for a sharp decrease in urinary iodide and an increase in biliary radioactivity to become apparent. Whereas some decrease in urinary excretion was observed 5 hours after the onset of PTU administration, a corresponding increase in the intestinal and fecal radioactivity was not observed until 20 hours after the onset of PTU administration.
2. The decreased deiodination could not be due to a decreased availability of thyroid hormone to peripheral tissues, for even where there was an increased fecal and a decreased urinary excretion of iodide, the concentration of iodide in the tissues of such rats was not significantly lower in thiouracil-treated rats relative to normal rats. Besides, a decrease in urinary iodide was

clearly demonstrable long before biliary secretion and/or intestinal plus fecal contents of iodinated compounds were altered.

In the light of such eloquent evidences of the extrathyroidal effects of goitrogens, one is a little surprised to find the rather profuse use of goitrogens in the estimation of thyroxine secretion rates.

Extrathyroidal Effects of Goitrogens Versus Their Use in Methods for the Estimation of Thyroxine Secretion Rates

Van Middlesworth, et al. (1959) reported that propylthiouracil-fed rats developed goiter in the presence of normal concentrations of plasma PBI. This finding seemed to bear out the earlier contention of Goldberg, et al. (1957) that thyroid-pituitary relationships were not fully explained by a simple feedback model. These findings could mean that a goiter can develop despite low plasma levels of thyroid-stimulating hormone, although this is very unlikely. Alternatively, it might mean that supernormal levels of thyroxine would be required to reduce thyroid weight from a goitrous to a normal state, which is a more acceptable possibility. If this were so, then all the goiter prevention methods that have been used by Tanabe, et al. (1961); Mellen (1961) and Turner, et al. (1959) were in error. In fact, Reineke and Singh (1955) reported that

estimates of thyroid secretion rate in rats given thiouracil were about 10 percent higher than those in rats not receiving the drug. It would then appear that goitrogens could not be expected to give accurate estimates of thyroxine secretion rates if such goitrogens did, in fact, have extrathyroidal effects. But, do all goitrogens share the same extrathyroidal effects as have been reported for the thiouracils? More specifically, does tapazole have the extrathyroidal effects that have been reported for the thiouracils?

Effects of Tapazole Versus Those of Other Goitrogens, e.g., The Thiouracils

Hershman and Van Middlesworth (1962) reported among several other findings that the effectiveness of inhibiting deiodination of thyroxine did not correlate with the antithyroid activities of goitrogens. Further, tapazole was reported to be ineffective in inhibiting deiodination. The two findings were also reported by Tanabe, et al. (1965). Tanabe and his group further found tapazole to have the same blocking effect on iodide recycling, thyroidal output of iodide and the same thyroxine secretion rate in cockerels receiving tapazole compared to that for the birds receiving other goitrogens. And yet, tapazole was still interpreted as being different in its lack of inhibition of deiodination compared to other goitrogens. Ingbar and Freinkel (1955), in presenting the thyroxine turnover method for the estimation of thyroxine secretion

rate, reported that the amount of thyroxine degraded by the body per day was equal to the amount of thyroxine synthesized by the thyroid for the same period. Emphasizing much the same concept, Flamboe and Reineke (1959) and Turner, et al. (1959) reported that since thyroidal iodine release was a measure of rate rather than quantity, neither this parameter nor thyroid uptake of iodine could be considered reliable indices of thyroidal functioning as would be reflected by thyroxine secretion rate. Premachandra, et al. (1958) had observed only a slight relationship between estimated thyroxine secretion rates and thyroidal iodide release rate in fowls. In a survey of thyroid physiology, Turner, et al. (1959), therefore, concluded that the rate of thyroidal release of iodine would only provide an accurate biological half-life of thyroidal iodine if recycling were blocked. "Only the estimation of thyroxine secretion rate would provide quantitative data on thyroid gland function," since the thyroidal uptake or release of iodine were useful only in qualitative studies of thyroid function. If thyroxine degradation accurately reflected thyroxine synthesis (Ingbar and Freinkel, 1955), and if synthesis matched thyroxine secretion into the blood since the colloid pools of the hormone remained constant, then the only other reliable index for thyroid function is thyroxine degradation. In the light of this, it would be extremely hard

to see how tapazole would have similar effects with the thiouracils with regard to thyroxine secretion rate without having analogous effects with regard to peripheral degradation or deiodination. Tapazole may, in fact, have similar effects to the other goitrogens and the difference could be in the extent of their effect or the exact point in the degradation process of such effects. Tanabe, et al. reported the following values of thyroxine secretion rates obtained by their modification of the radioiodine technique and by the goiter prevention assay technique:

Goitrogen	Dose	Radioiodine Assay	Goiter Prevention Assay
Thiouracil	0.1% of diet	1.47	1.69
Tapazole	0.1% of diet	1.42	1.71
Tapazole	0.05% of diet	1.52	1.36
Tapazole	0.025% of diet	1.40	1.63

The values of thyroxine secretion rate are given in μg of L-T4 per 100 gm body weight per day. First, it is disputable that TSR can be accurately estimated by either of these methods. Secondly, Tanabe and his group have themselves pointed out a difference in potency of these goitrogens regarding iodine release from the thyroid. Could such a difference in potency not explain the emergence, or lack of emergence of significant differences, in both the thyroxine secretion rates and effects on deiodination following the treatment of the cockerels with goitrogens?

Yamada (1967) has, for instance, reported that the administration of 1 or 3 µg T4 daily for 3 weeks to tapazole-treated rats resulted in a progressive increase in PBI with increasing doses of thyroxine. The point is that there is an increase in the protein-bound iodine level which most probably reflects some extrathyroidal effect. Absolute proof of the precise extrathyroidal effects of tapazole may yet come, but evidence certainly makes it extremely hard, if not impossible, to accept the contention that tapazole has no extrathyroidal effects. Even if we were to doubt the possible extrathyroidal effects of tapazole, we cannot but accept the impressive volume of evidence of the extrathyroidal effects of other goitrogens. Nor can we doubt the correspondence of the thyroidal effects of tapazole and those of other goitrogens.

Biological Half-Life (t1/2)

Biological half-lives in different birds do not appear to be widely different. Different groups of workers have, however, reported widely varying values, sometimes for the same species of birds. Biological half-lives are, of course, much shorter in birds than in mammals. Heninger and Newcomer (1964) reported mean half-lives of 4.9 hours in the cardiac tissue of chickens. This value is close to the t1/2 of T4 in the chicken plasma observed in the study by Singh, Reineke and Ringer (1968). McFarland, Yousaf and Wilson (1964) reported much longer fractional turnover

rates of T4 in Japanese quail which when expressed as t1/2 ranged approximately from 17 to 27 hours at 70-90°F. Hendrich and Turner (1967), sampling at 8-hour intervals, reported t1/2 values of 11.4 hours at normal environmental temperatures for fowl. The degradation curves reported in the literature (where such are shown or discussed) usually indicated that these were obtained without correcting for recycling in non-goitrogen-treated control birds. If the animals were treated with goitrogens, then the observations that such drugs had extrathyroidal, as well as thyroidal, effects were not considered. Some studies did not mention the methods of estimation of biological half-lives. It seems that recycling and the effects of goitrogens would have to be accounted for before any reasonably accurate t1/2 values can be obtained.

CHAPTER II

MATERIALS AND METHODS

Chemicals and Drugs

^{131}I-carrier-free L-thyroxine was obtained from Abbott Laboratories as 50 percent propylene glycol solutions. The specific activities of the consignments used in the first and second experiments were 23.5 and 41.4 mc per milligram, respectively. In the first experiment, the labelled thyroxine was diluted with 0.5 ml quail plasma, 4.1 ml of 0.9 percent normal physiological saline solution and 2.9 ml of ^{131}I-L-thyroxine solution. In the second experiment, 0.2 ml of quail plasma and 2.4 ml of ^{131}I-L-thyroxine solution were diluted with 1.4 ml of 0.9 percent NaCl solution. The quail plasma used in both experiments was to reduce adsorption of the radioactive material to glassware. Labelled thyroxine solutions were made up to 66.7 µc/ml and 117 µc/ml, respectively. The standards for the first experiment had dilution factors of 200 and 160; for the second experiment, the standard had a dilution factor of 50.

A freshly made up 40 mg/ml solution of sodium thiocyanate was used in both experiments. Only 0.5 ml of this solution was used in each subcutaneous injection per bird. This way, 20 mg of sodium thiocyanate was delivered to the

bird at each injection. A freshly made up 40 mg/ml solution of tapazole (1-methyl-2-mercaptoimidazole) or methimazole was used in the second experiment. By using 0.5 ml of this solution per injection 20 mg of tapazole was delivered to each bird per injection.

Experimental Animals

A total of 36 adult male bobwhite quail (Colinus virginianus) were used in this study. The 21 birds used in the first experiment had weights ranging from 140-170 grams. The weights of the 15 birds used in the second experiment ranged from 175 to 209 grams. The quail were all obtained from the poultry pens of the Michigan State University Poultry Science Department.

Experiment I

Fifteen of the 21 bobwhite quail used in this experiment were cannulated by their left external jugular veins according to the Weeks chronic infusion technique. A piece of silastic rubber tubing* 1.70 cm long and internal diameter 0.012 cm was just sufficient to run from the middle of the extended adult male bobwhite quail neck to the interior of the left auricle. Provided the tubing was kept flushed with at least a 0.9 percent NaCl solution, blood clotting within the tubing did not occur. The outer end of the tubing was then heat-sealed. Figure I shows a

*Dow Corning, Midland, Michigan.

diagrammatic representation of the silastic tubing and its connections. The part of the tubing outside the vein was folded back behind the dorsal part of the neck and firmly sutured between the two raised collars (Fig. 1) down over the back of the quail. This technique allowed the bird to feed without hindrance.

At least three days after cannulation, five of the quail were subcutaneously injected with 20 mg sodium thiocyanate solution, all of the cannulated birds received a 10 µc dose of carrier-free ^{131}I-L-thyroxine through the cannulae. A three-way valve with two attached syringes was used for injecting the labelled hormone. One syringe contained the precise dose of ^{131}I-L-T4. The other syringe contained 0.9 percent NaCl solution. First, some of the saline was flushed through the cannula to make sure that it was open and that fluid could be easily flushed through it. The labelled hormone was then injected slowly and carefully. Blood was drawn back, just up to the tip of the 27-gauge needle and flushed back in. This drawing back of blood and flushing in procedure was repeated three times to ensure that the blood would pick up as much of the labelled hormone clinging to the walls of the cannula as possible. Just to make sure that all of the labelled material was flushed into the heart, the 0.9 percent NaCl solution was again flushed through the

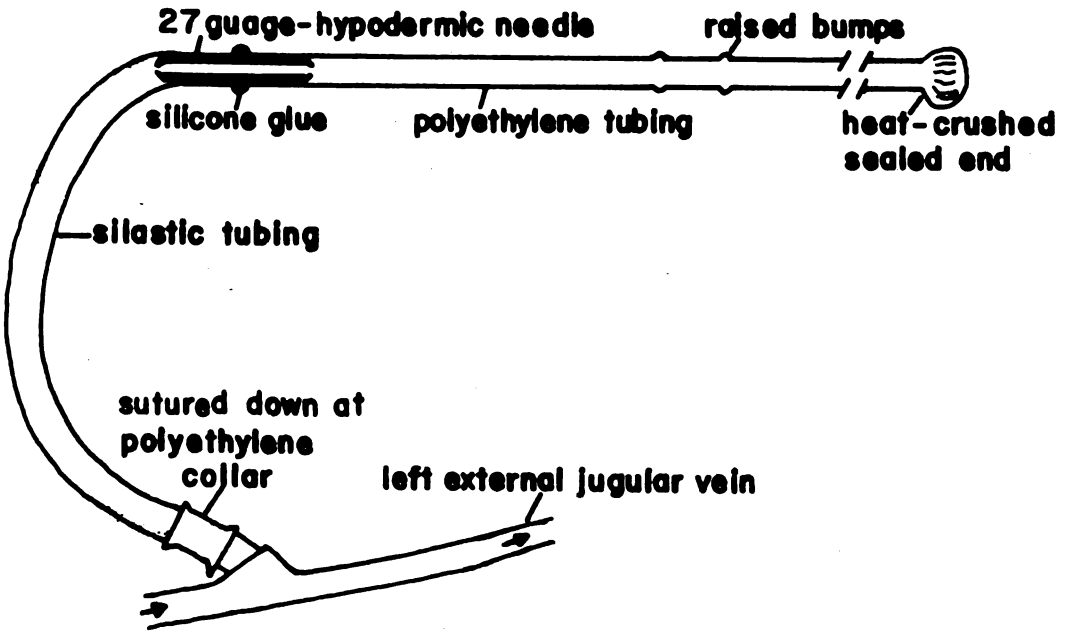


Fig. 1. Diagram of in-dwelling silastic cannula.

cannula. It was believed that this technique would ensure almost a 100 percent injection of the radioactive material. The injected material would go directly into active circulation from the heart and in drawing blood samples one would be spared the tortuous problem of hitting the tiny bobwhite veins. In order to check what effect, if any, the Weeks chronic infusion technique (1964) had on our results, six other birds were given 10 µc of ^{131}I-L-thyroxine by their right brachial (wing) veins. Blood sampling was from the wing vein opposite to that into which the labelled hormone was injected for the uncannulated birds. In the cannulated birds, sampling was from the cannula. The cannulae would then be flushed with more saline solution and heat-sealed after each sampling. Sampling commenced three hours after labelled hormone injection. Subsequently, each bird was sampled at three-hour intervals for nine more hours. This gave four blood samples per bird.

Only 0.3 ml of blood was drawn per sample. This meant a total of 1.2 ml of blood was taken from each bird in 12 hours. Since bobwhite quail probably have a blood volume of 8-10 ml per 100 gm body weight, the sampled volume would not produce an unphysiological condition in the bird by dangerously depleting its blood volume.

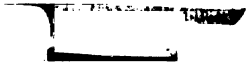
Each blood sample was spun down by centrifugation at 1000 rpm for two minutes. From each spun sample, 0.1 ml

plasma was carefully pipetted using 100 microliter (µl) micropipettes. Background counts were taken on a scintillation counter (Nuclear Measurements Corporation) just prior to plasma counts. Plasma counts were then taken for at least a minute per sample on the same scintillation counter. If the plasma counts were less than ten times the background counts, then counts were taken for at least two minutes. The counts per minute, per 0.1 ml of plasma, were then multiplied by ten to express them in terms of counts per minute, per ml of plasma.

Experiment II

Fifteen bobwhite quail, split into three groups of five each, were used. Each of the fifteen birds was intravenously injected with 29.25 µc of ^{131}I-carrier-free L-thyroxine by the left brachial (wing) vein some nine hours before the first blood sampling. Thirty minutes after injecting labelled hormone, five of the birds were given 20 mg of sodium thiocyanate solution subcutaneously. Five more quail got 20 mg of tapazole solution subcutaneously; the remaining five were used as controls and were untreated with drugs.

The birds were first sampled nine hours after the injection of labelled hormone. Sampling continued every three hours for about 15 more hours from the first sampling. After a nine-hour break, sampling was resumed at four-hour intervals for twelve more hours. Hendrich and Turner



(1967) had taken eight-hour samples for 40 hours following ^{131}I-L-T4 injection into chickens. It was felt that such extended periodicity of sampling would gloss over any parallel processes taking place along with the degradation of thyroxine. The three-or four-hour periodicity of sampling was thus preferred to much longer sampling intervals. This, it was hoped, would help document better than ever before the true shape of the degradation curves for labelled thyroxine.

Only 0.1 ml of blood was drawn for each of ten samples taken over a period of 45 hours. By prior experimentation, it was estimated that 0.1 ml of blood, when spun down, yielded at least 20 µl plasma volume. The small volume of blood sampling spread over 45 hours would thus obviate the danger of depleting quail blood volume to an unphysiological state. At the same time, the high dose of radioactivity in the injected labelled hormone would ensure high enough counts to be obtained even with such small plasma volumes as 20 µl. Such counts would then be computed per milliliter plasma.

Tissue Retentions

In experiment I, counts of samples of liver, kidney and spleen as well as whole thyroids were taken. All tissue samples counted were weighed. In experiment II, whole thyroids, livers, kidneys, and washed gastrointestinal tracts were counted and weighed. Two chunks of

breast muscle, one from either side of the clavicles, were also counted and weighed. Counts for the entire body muscle weight were then computed from the counted pieces of muscle. In this experiment, absolute percentages of the injected doses in the thyroids were expressed as percent of total body retention. The absolute percentages of the injected dose in all other tissues were expressed as percent of total extrathyroidal retention. Total body retention was taken to be the summation of the retention by liver, kidney, intestine, muscle and thyroid glands. This presumed that the retention by the exo- and endo-skeleton of the birds would be negligible.

In experiment II, TDS for the control birds was calculated from the first and uncorrected curve. TDS is defined as the space which would be occupied by the labelled hormone if the concentration of the hormone in the tissues were exactly the same as that in the plasma. In controls, this figure does not seem to be affected by the processes involved in degradation and recycling. The corrected curve would, therefore, not give a true reflection of distribution space. TDS for the other groups of birds is calculated from the first curve in tapazole-treated birds or from the only curve in thiocyanate-treated birds.

Computations

I. Percent Injected Dose per Milliliter of Plasma

The percentage of the injected dose represented by 1 milliliter plasma count was computed by the following standard procedure:

$$\frac{\text{Plasma counts/min. - Background counts/min.}}{\text{Standard counts/min. - Background counts/min.}} \times 100$$

Background counts/min.:	30
0.1 ml plasma count per min.:	4881
0.1 ml plasma count/min. - Background count/min.:	4851
Plasma count/min./ml:	48510
Mean Standard Count/min.:	19182
Mean Standard Count/min. - Background count/min.:	19152
Dilution factor:	200

No. of injected counts in standard:

$$19152 \times 200 = 3830400$$

$$\text{Percentage injected dose} = \frac{48510}{3830400} \times 100 = 1.2664$$

Since tests by thin layer chromatography (by F. L. Lorscheider) have shown that only 90% of the total injected radioactive material was in the form of ^{131}I-L-thyroxine, the calculated percent injected dose in each milliliter of plasma sample was then raised by 1.1111 to correct for the 10% free radioiodine.

$$1.2664 \times 1.1111 = 1.4071$$

Percent injected dose retained in the tissues counted was similarly computed.

II. Statistical Constants of Curves and TDS Computation

In experiment I the mathematical constants describing the T_4 degradation curve were determined by graphical analysis. Percent radioiodine dose per ml of plasma was plotted on the log scale (y) of semi-log paper against

time plotted on the arithmetic scale (x), and a line was fitted by inspection. Data of this type fit the general equation,

$$\log y = a + bx \qquad (1)$$

where,

a = the log y; intercept at injection time and
b = the slope of the regression line.

Equation (1) when transformed to natural logarithms becomes,

$$y = e^{-xt} \qquad (2)$$

where,

y = % injected dose/ml plasma at time t
e = the base of natural logarithm and
x = b x 2.302

where,

2.302 = the factor used to transform $\log_{10}$ to natural logarithms. The quantity e^{-x} was obtained from a table of descending exponentials*.

$$\text{Fractional degradation/hr.} = 1 - e^{-x} \qquad (3)$$

$$t_{\frac{1}{2}} = \frac{0.301}{b^{**}} \quad . \qquad (4)$$

Then,

$$\text{TDS, in ml} = \frac{100}{\text{anti-log } a} \qquad (5)$$

$$\text{TDS, in ml/100 gm b.wt.} = \frac{\text{TDS}}{\text{gm body wt} \div 100}$$

In experiment II, the line of best fit for each slope together with the other statistical constants were

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*Tables of the Exponential Functions e^x, 1961, 4th ed., National Bureau of Standards Applied Mathematics Series 14.

**the numerical, not the algebraic, value of b.

determined by the method of least squares (Li, 1964). Final mathematical treatments were then done as outlined in equations (1) to (5).

Significance of differences between the control and drug-treated groups was obtained by either the student t test (Li, 1964) or by the Mann-Whitney U-test (Siegel, 1956). The latter was used where there was no variance homogeneity.

III. Correction for Recycling in Control Curves

All of the control birds in experiment II showed a break in their T_4 degradation curves somewhere between the 15th and 25th hour following the labelled hormone injection. As the first step in resolving the two slopes, separate regression equations were computed for the 5 plasma radioactivity values obtained during the first 20 hours (Slope I) and the 5 values obtained during the subsequent 25 hours (Slope II).

The first and steeper part of the curves was considered to represent predominantly degradation of ^{131}I-L-thyroxine. The second curve could only be due to recycling of metabolized iodine back through the thyroid. Consequently, in order to obtain the true degradation rate, it was necessary to separate the exponential of Slope II from that of Slope I. To accomplish this the calculated line for Slope II was extrapolated back to zero time. The values for Slope II were subtracted from those of Slope I at several time intervals.

Then,

$$b = \frac{\text{Log } A_t - \text{Log } A_o}{t} \qquad (6)$$

where,

A_t = percent injected dose at time t (hours) and
A_o = percent injected dose at time zero

The constant b obtained in this way most nearly approximates the true degradation rate of the ^{131}I-T4 originally injected.

CHAPTER III

RESULTS

Descending semi-logarithmic curves of very comparable slopes were obtained by both the Weeks chronic infusion technique (1964) and the veni-puncture method of injections and sampling. Technique did not therefore affect the results.

Experiment I

The thiocyanate-injected quail showed a significantly shorter ($p < 0.001$) mean biological half-life than the control birds (Table 1). This suggested that thiocyanate increased the rate of degradation of ^{131}I-L-thyroxine (Fig.2). The concept of a heightened rate of degradation was also indicated by the much higher fractional degradation rates per hour shown by the thiocyanate-treated birds relative to the controls. The iodide resulting from the degraded hormone did not, however, appear to be accounted for by an increased retention of radioiodide by any particular organs or tissues. There was no significant increase or decrease in the radioiodide retention by any particular tissue system among those counted. Retention by the spleen was negligibly small with or without thiocyanate treatment.

TABLE 1.--Effect of thiocyanate on T1/2, TDS and tissue retention in bobwhite quail. Experiment I.

A: T1/2 and TDS

T1/2 or TDS	No. of Birds in Group	Treatment	Group Mean and Standard Error	Probability of Significance by Mann-Whitney U-Test
T1/2 in hours)	16	Control	4.58±0.33	
	5	SCN-injected	2.77±0.16	$p < 0.001$
Fractional Degradation Per Hour	10	Control	0.15±0.01	
	5	SCN-injected	0.22±0.001	$p < 0.001$
% Injected Dose/ml Plasma At Zero Time	10	Control	2.34±0.15	
	5	SCN-injected	5.70±0.97	$p < 0.001$
TDS in ml/ 100gm Body Weight	16	Control	31.36±2.73	
	5	SCN-injected	11.77±2.05	$p < 0.001$

B: Tissue Retention

Tissues: % Injected Dose		State of Group	
Whole Thyroid	10	Control	0.36±0.03
	5	SCN-injected	0.27±0.10
Liver Per gm of Tissue	11	Control	0.38±0.01
	5	SCN-injected	0.34±0.07
Kidney Per gm of Tissue	11	Control	0.56±0.01
	5	SCN-injected	0.48±0.01
Spleen Per gm of Tissue	11	Control	0.29±0.003
	5	SCN-injected	0.16±0.001

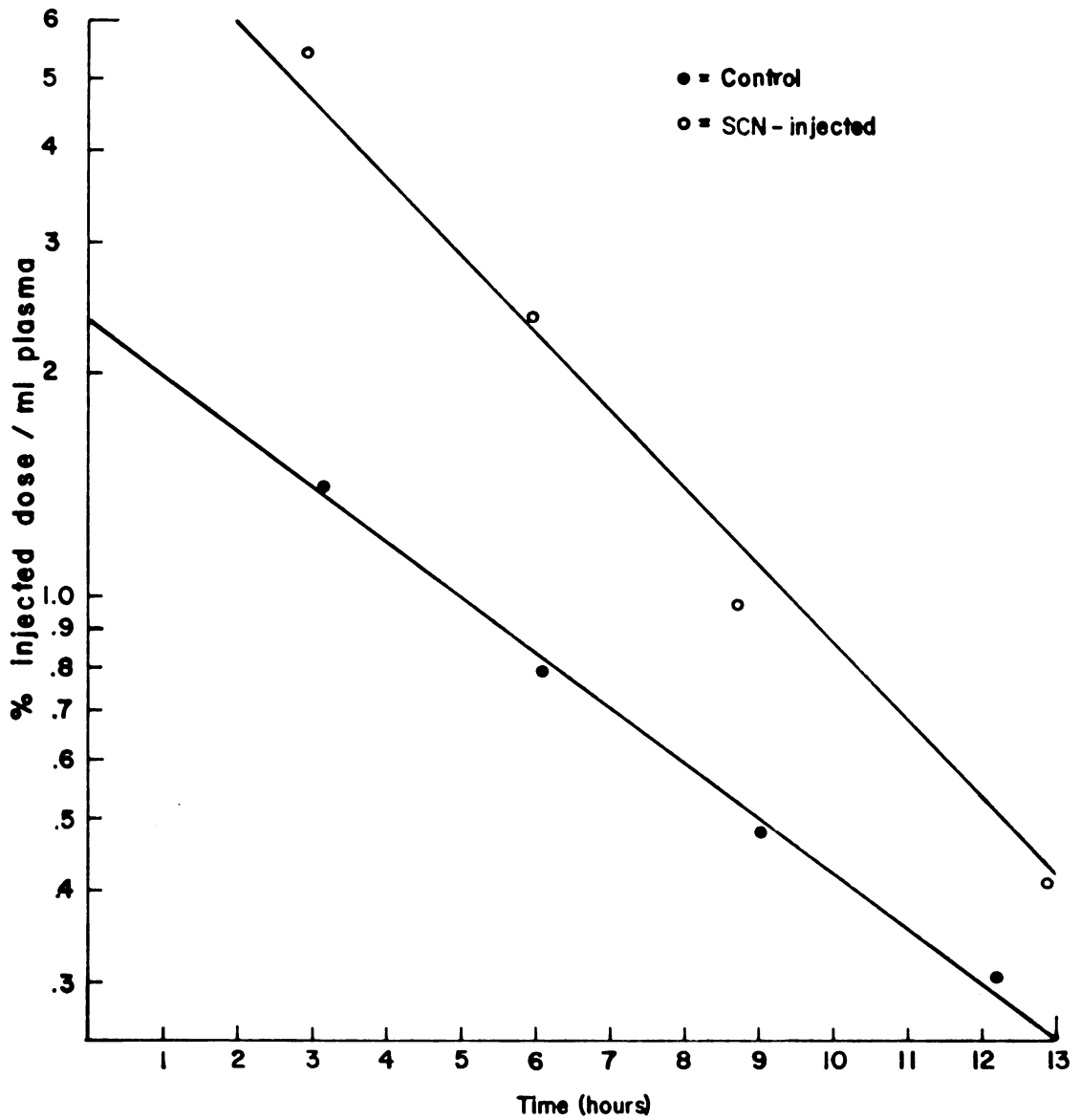

Fig. 2. Representative ^{131}I-T_4 degradation curves for control and thiocyanate-treated bobwhite quail in experiment I.

Thyroxine distribution space was drastically reduced by thiocyanate injection ($p < 0.001$).* Mean TDS values in the thiocyanate-treated group were a little over one-third of mean control values ($p < 0.001$).* Such a drastic reduction had to mean something. It could not, however, be explained on the basis of the retention data for this experiment (Tables 1 and 3).

Experiment II

A break in the control curves always occurred somewhere between the 15th and the 25th hour (Fig. 3). The first and the second curves were significantly different from one another ($p < 0.001$).* The two curves in the tapazole group of birds were not significantly different. However, "eyeballing" the points clearly showed them to fall into two curves in three out of the five birds. Biological half-lives were calculated from the slopes of the first curve, since these were the shortest t1/2 values that could be obtained from this group of birds. Clearly, t1/2 values obtained either from the second curve or from the single curve fitted onto all ten points would be even longer than those obtained from the first curve. Mean t1/2 and fractional degradation values were obtained by calculation from corrected slopes of the control birds, slopes of the thiocyanate-treated and slopes of the first curve of the

*By Mann-Whitney U-test.

tapazole-treated birds. Table 2 summarizes the statistical constants used to obtain the results shown here. The t1/2 values so obtained for the control birds were essentially the same as those for the thiocyanate-treated birds. Control mean t1/2 was found to be 8.58 $\pm$ 1.36 hours and mean t1/2 for thiocyanate-treated birds was found to be 7.50 $\pm$ 0.76 hours. Mean fractional degradation rates per hour were found to be 8.46 and 8.90 percent for the control and thiocyanate-treated birds, respectively. This finding was rather startling because the data of the first experiment has revealed shorter t1/2 and increased degradation rates for the thiocyanate-injected birds relative to controls. But there, of course, extrathyroidal recycling of iodine had not been accounted for. Biological half-lives and fractional degradation rates in percentages per hour calculated from the first curve of the tapazole-treated birds turned out to be 31.74 $\pm$ 6.69 hours and 2.1 percent per hour, respectively. Tapazole, thus, seems to significantly reduce the rate of degradation of ^{131}I-L-thyroxine in bobwhite quail.

Retention by Tissue Systems and Whole Body

A massive 23.65 percent of the injected dose of radioiodide was retained by control thyroid glands 45 hours after injection of ^{131}I-T4. This compares with 0.48 percent in the thiocyanate-treated and 0.15 percent in the tapazole-injected group of birds. These are all group mean

TABLE 2.--Statistical constants for T_4 Degradation Curves. Experiment II.

Bird Group and Slope		Slopes	Correlation Coefficient	Y Intercept	No. of Points
Control, 1:	Slope I	-0.02300	-0.98723	2.305	5
	II	-0.01636	-0.98645	1.816	5
Corrected		-0.05321		0.489	5
Control, 2:	Slope I	-0.01953	-0.97475	1.789	5
	II	-0.00805	-0.71586	0.9643	5
Corrected		-0.03518		0.825	5
Control, 3:	Slope I	-0.01883	-0.97624	2.358	5
	II	-0.00929	-0.93089	1.695	5
Corrected		-0.04949		0.663	5
Control, 4:	Slope I	-0.02279	-0.98259	2.551	5
	II	-0.00815	-0.88662	1.083	5
Corrected		-0.03203		1.468	5
Control, 5:	Slope I	-0.01365	-0.97374	2.314	5
	II	-0.00703	-0.88606	1.499	5
Corrected		-0.02277		0.815	5
SCN-Injected:	S_1	-0.04710	-0.99591	4.379	10
	S_2	-0.05141	-0.99487	3.833	6*
	S_3	-0.03354	-0.98241	3.942	10
	S_4	-0.06337	-0.98840	2.858	6*
	S_5	-0.04106	-0.99576	3.175	10
Tap.-Injected:					
T_1	Slope I	-0.00114	-0.99112	3.171	5
T_2	I	-0.01879	-0.97589	3.921	5
T_3	I	-0.00651	-0.85811	2.355	5
T_4	I	-0.01154	-0.86189	1.959	5
T_5	I	-0.00779	-0.99346	2.837	5
T_1	II	0.00128	0.59099	1.561	5
T_2	II	-0.00611	-0.95778	1.965	5
T_3	II	-0.00156	-0.45591	1.756	5
T_4	II	-0.00978	-0.97647	1.788	5
T_5	II	0.00533	0.83543	1.303	5

*These birds died after the sixth blood sample was taken.

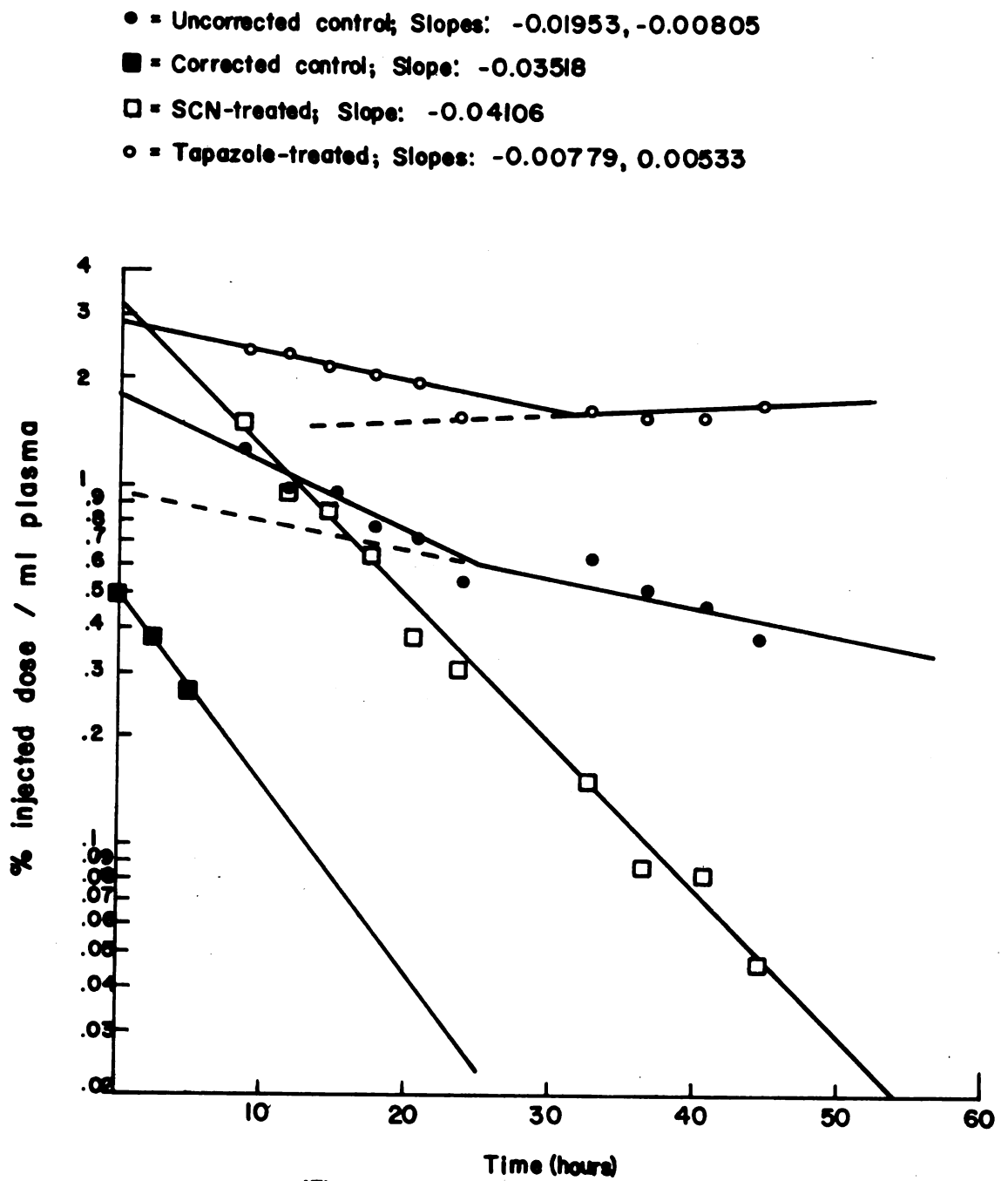

Fig. 3. Representative ^{131}I-T_4 degradation curves for control, corrected control, thiocyanate- and tapazole-treated bobwhite quail.

TABLE 3.--Slopes, T1/2, Degradation Rates and Thyroxine Distribution Space. Experiment II.

Groups and States	Slopes and Standard Errors (Sy)	T1/2 and Standard Errors (in hours)	Fractional Degradation in Percent per Hour and Standard Errors	TDS in ml/100 gm body weight. For all 5 Birds of each Group
Control:				
Slope I	-0.01956 ± 0.00173	15.75 ± 1.66		22.63 ± 1.6869
Slope II	-0.00977 ± 0.00173	33.95 ± 4.14		
	Slope I vs. Slope II = **p < 0.01			
Corrected	-0.03853 ± 0.01131	8.58 ± 1.36	8.46 ± 1.8888	
Thiocyanate-Injected	-0.04056 ± 0.00408 (for the 3 surviving birds) **p > 0.50*	7.50 ± 0.76 (for 3 quail) **p > 0.50	8.90 ± 0.8217 **p > 0.50*	15.23 ± 1.0321 **p < 0.01*
Tapazole-Treated	-0.01115 ± 0.00287 (for 4 birds)	31.74 ± 6.69	2.08 ± 0.6715	20.09 ± 2.3276
Slope I	**p < 0.02*	**p < 0.02*	**p < 0.02*	**p > 0.50*

*Significance of difference between this and corrected control group.

**Probability of significance estimated by the Mann-Whitney-U-test.

values. Both thiocyanate and tapazole were, therefore, seen to drastically and significantly reduce thyroidal retention ($p = 0.004$ in each case relative to control). Thiocyanate depressed the retention of radioiodine in all major systems of the body except the gastrointestinal tract. Retention by the gastrointestinal tract is significantly increased ($p < 0.01$) while that by the quail muscle is significantly decreased ($p < 0.02$) following thiocyanate treatment. Tapazole significantly increased liver retention. The retention of most other tissues was also increased by tapazole administration. Since the first experiment had indicated that quail spleens retained a negligibly small amount of the radioiodine injected into the bird, spleen retentions were not taken into account in this experiment.

Table 4A and Fig. 4 show body retentions expressed as percent injected dose and including thyroidal retentions. Comparisons were drawn, between the total body retentions (including thyroidal retention) of radioiodine in the control, thiocyanate-treated and tapazole-treated groups of birds. Here it was found that the ^{131}I retention in the thiocyanate group was only 0.13 times that of the control value ($p < 0.01$). Tapazole treatment resulted in a radioiodine retention of 0.81 times the control value ($p > 0.5$). Comparison of body retentions of the groups,

TABLE 4A.--Tissue Retentions Expressed as Percent Injected Dose.

Bird Group and Number	Total Thyroid	Total Liver	Total Kidney	Total Muscle	Washed G.I. Tract	Total Body Retention	
						Without Thyroid	With Thyroid
Control							
1	10.36	0.80	0.34	13.68	1.50	16.32	26.68
2	20.11	0.82	0.43	7.80	2.74	11.79	31.90
3	33.96	1.48	0.54	0.71	5.19	7.92	41.88
4	25.83	1.02	0.35	7.75	3.19	13.02	38.85
5	28.01	1.75	0.51	14.76	3.77	20.79	48.80
Group Mean ($\bar{X}$)	23.65	1.17	0.43	8.94	3.42	13.96	37.6 $\pm$3.85
SCN-Injected							
1	0.26	0.18	0.08	1.30	1.65	3.21	3.47
2	0.25	0.65	0.25	3.45	3.02	7.37	7.62
3	0.14	0.32	0.18	2.30	3.76	6.56	6.70
4	0.60	0.27	0.10	2.40	1.38	4.15	4.75
5	1.14	0.08	0.03	0.69	0.35	1.15	2.29
Group Mean ($\bar{X}$)	0.48	0.30	0.13	2.03	2.03	4.49	4.96 $\pm$0.99
		**p < 0.01*					
Tapazole-Injected							
1	0.22	3.30	1.14	22.80	6.07	33.04	33.26
2	0.26	1.87	0.59	15.73	5.31	23.50	23.76
3	0.09	2.92	1.28	22.35	5.53	32.08	32.17
4	0.09	1.41	0.54	10.40	2.42	14.77	14.86
5	0.10	4.78	1.07	37.24	5.33	48.42	48.52
Group Mean ($\bar{X}$)	0.15	2.80	0.92	21.70	4.93	30.35	30.52 $\pm$ 5.59
		**p > 0.50*					

*Probability of significance of difference compared to controls.

**By Student t-test.

TABLE 4B.--Tissue Retentions as Percent of Total or Extrathyroidal Radioiodine.

Tissue Systems	Groups of Birds	Retention in Percent of Total (for thyroids) or Extrathyroids Radioiodine					Group Means, Standard Errors and Probability of Significance Compared to Controls
		1	2	3	4	5	
Thyroids	Control	38.82	63.02	63.45	66.48	57.39	57.83 ± 1.57
	SCN-injected	7.48	3.28	2.09	12.64	49.73	15.04 ± 28.04* p = 0.008
	Tapazole-injected	0.66	1.09	0.27	0.60	0.20	0.56 ± 1.5905* p = 0.004
							SCN-vs Tap-group: *p = 0.004
Liver	Control	4.92	6.97	8.26	7.81	8.40	7.27 ± 0.64
	SCN-injected	5.66	8.80	4.88	6.43	6.77	6.50 ± 0.66* p = 0.210
	Tapazole-injected	9.17	7.95	9.10	9.54	9.87	9.12 ± 0.33* p = 0.016
							SCN-vs Tap-group: *p = 0.008
Kidney	Control	2.05	3.64	3.00	2.68	2.47	2.76 ± 0.27
	SCN-injected	2.40	3.44	2.71	2.36	2.86	2.75 ± 0.20* p = 0.500
	Trapazole-injected	3.45	2.51	3.97	3.64	2.20	3.15 ± 0.34* p = 0.210
							SCN-vs Tap-group: *p = 0.210
Muscle	Control	83.80	66.11	59.76	59.52	70.98	68.03 ± 4.48
	SCN-injected	40.46	46.81	35.08	57.88	60.16	48.07 ± 1.38* p = 0.016
	Tapazole-injected	69.01	66.94	69.67	70.40	76.91	70.58 ± 1.68* p = 0.50
							SCN-vs Tap-group: *p = 0.004
G.I. Tract	Control	9.21	23.26	28.96	29.97	18.13	21.90 ± 3.82
	SCN-injected	51.44	40.92	57.32	33.30	30.21	42.63 ± 5.19* p = 0.004
	Tapazole-injected	18.36	22.58	17.24	16.40	11.00	17.11 ± 1.86* p = 0.115
							SCN-vs Tap-group: *p = 0.004

*Probability estimated by the Mann-Whitney-U-test.

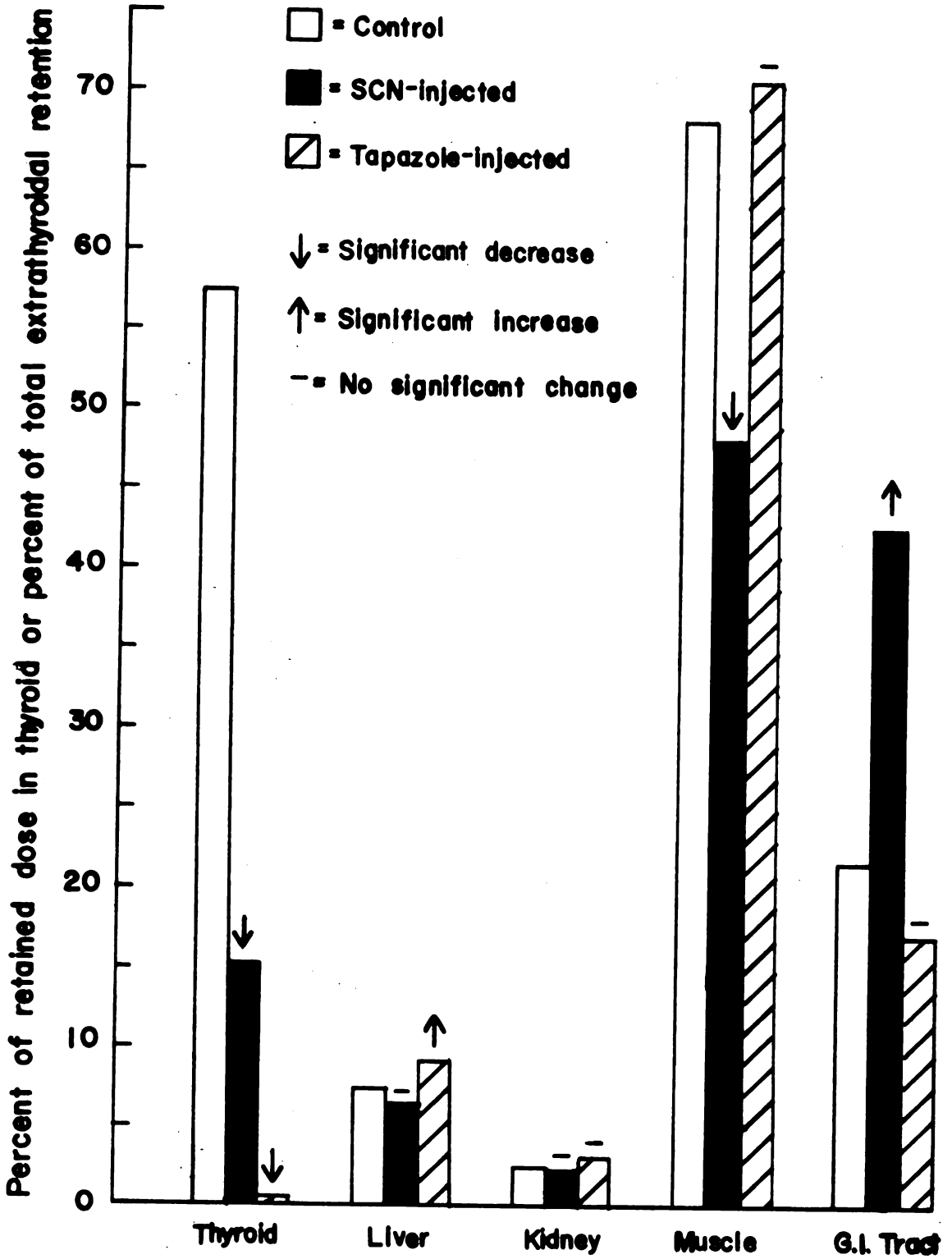

Fig. 4. Effect of thiocyanate and tapazole on percent of total body radioiodine located in the thyroid and total of extrathyroidal radioiodine located in the Liver, Kidney, Muscles and Gastrointestinal Tract.

excluding their thyroidal retentions yielded different results. Thiocyanate treatment then resulted in a retention value that was 0.32 times that of the control group. Tapazole yielded a retention value 2.17 times the control value. The massive retention of radioiodine by the control thyroids was responsible for differing results of the comparisons. A comparison in which thyroidal retentions were not permitted to occlude the relative group retentions would probably more nearly reflect the true situation. Such a comparison would show thiocyanate to drastically reduce, and tapazole to greatly increase body retention of radioiodine. The low plasma level of radioiodine in the thiocyanate-injected and persistently high levels in the tapazole-injected birds would appear to bear out the same concepts.

TDS and Blood Level of Radioiodine

Thiocyanate treatment depressed thyroxine distribution space. The tapazole-treatment compared to the state of other groups did not appear to significantly affect thyroxine distribution space in bobwhite quail. There was a much more rapid decline in the level of radioactivity in the blood of the thiocyanate-injected group of birds relative to control levels. Tapazole-injected birds showed a very persistently high level of radioactivity in their blood through the entire period of sampling.

CHAPTER IV

DISCUSSION

Effects of Thiocyanate Treatment

Biological Half-lives and Degradation Experiments

A mean biological half-life of 4.58 hours was obtained for ^{131}I-L-thyroxine in the control birds of the first experiment. Control birds of the second experiment yielded a mean of 7.81 hours for t1/2. The first value of t1/2 obtained agreed rather well with the value of 4.60 hours reported for the bobwhite quail by Singh, Reineke and Ringer, (1968) and reasonably well with the value of 4.9 hours reported by Heninger and Newcomer (1964) for the chicken. All these values differ considerably from the corrected control values of bobwhite quail in the second experiment of this study. The fact that control values in this study were corrected for recycling definitely made them more accurate. This does not, however, explain why the t1/2 values should be higher than those of the first experiment and those reported by the above-named workers. Correction for recycling would tend to give steeper degradation curves and so should result in shorter t1/2 values than those that had been earlier reported. The difference due to the time of first blood sampling and the total duration of the sampling period would seem to offer some clues.

In the first experiment of this study, as well as in the study by Singh, Reineke and Ringer (1968) sampling was started about three hours after the injection of labelled hormone into the bird. Blood sampling continued for 12 hours following radioiodine-labelled thyroxine injection. It was noted that the first point of the curve, which was fitted onto the resulting four points, usually indicated a higher percentage of the injected dose in the plasma sample than would have been obtained if the curve were fitted to the last three points. This suggested that the sample taken three hours after radioactive thyroxine injection was possibly taken too early to have allowed a complete and even mixing of the injected hormone. There was also the possibility that the point in question belonged to an earlier degradation curve that is not confounded by recycling of ^{131}I. The second experiment circumvents this possible problem since the first blood sample was taken some nine hours after radioactive hormone injection. Besides, a 12-hour duration of blood sampling did not yield enough points to give reliable enough curves. Such workers like Hendrich and Turner (1966), who took blood samples over a 40-hour period, have reported a longer t1/2 value for chicken (11.4 hours) in summer temperatures. In this case, the t1/2 value was not corrected for recycling. The writer believes that if the value reported by Hendrich and Turner (1966) was corrected for

recycling, it would come very close to the control t1/2 values reported in this study.

Another possible reason for obtaining such widely varying t1/2 values in the two experiments of this study could be the lack of acclimation of the birds to their new environment in the second experiment. These birds were transferred from the relatively dark colony cages of the University poultry pens to individual cages in the bright atmosphere of a normal laboratory. The birds neither fed nor drank as often as they normally would during the course of the second experiment, perhaps because they had not become acclimated to their changed and strange environment. However, since these control values were the only ones known to the author to have been corrected for recycling, they would seem to be the nearest approximation to the true biological half-life of ^{131}I-L-thyroxine in bobwhite quail yet reported under the given experimental conditions.

Extrathyroidal Effects of Thiocyanate

In the two main experiments of this study, thiocyanate appeared to increase degradation rates when compared to control degradation rates uncorrected for recycling. In fact, Yamada (1967) reported that anions like perchlorate, and probably thiocyanate, increased tissue utilization of thyroxine by displacing the hormone

from its binding proteins in the plasma and so making the hormone more available to peripheral tissues. This increased tissue utilization would show up as decreased biological half-lives. On the basis of the data corrected for recycling in this study, thiocyanate did not significantly increase degradation rates. Mean values of degradation rates for control and thiocyanate-treated groups of birds turned out to be 8.46 and 8.90 percent, respectively. Thiocyanate did not seem to have increased the availability of thyroxine to the tissues since tissue retentions were actually decreased. In fact, total body counts were decreased by about 70 percent. Among individual tissues, only the retention of the gastrointestinal tract was significantly increased. Muscle retention was decreased by about the same percentage as the increased retention by the gastrointestinal tract. Muscle retention was decreased by 19.96 percent of total extrathyroidal retention, whereas gastrointestinal retention increased by 20.73 percent. So the total body retention remained depressed and did not reflect a greater availability of hormone to the tissues (see Table 4B). The increased retention by the gastrointestinal tract would suggest that a large part of the radioiodine was excreted. Total body retention counts would back up this concept (Table 4A). Whether the apparent rapid rate of excretion in the thiocyanate-treated birds is a primary effect of thiocyanate,

or whether rapidity of excretion merely shows the accumulation of radioiodide in the plasma, cannot be determined from this data. Muscles form the bulk of the body weight of all animals. A significant depression of the retention of radioiodide by the quail muscle would, therefore, show up either as a fall in thyroxine distribution space (TDS) or a more rapid fall in blood radioactivity, or both. These two facets were observed to have considerably decreased in this study. The TDS in ml per 100 gm body weight of the thiocyanate-treated birds was, therefore, significantly less than that of control birds ($p < 0.01$). Comparisons between the tissue retentions in the thiocyanate-injected and control group of birds were, however, confounded by the recycling of freshly synthesized ^{131}I-T4 through the tissues as well as through the thyroids of the two groups of birds. In this regard the tissue retentions of the thiocyanate-injected group of birds were probably closer to the true retention of quail tissue without the influence of recycling.

The finding of an increased excretion following thiocyanate treatment reported by Yamada (1967) is confirmed by the data in this study. Apparently, it is because there is such a huge disposal of radioiodine through the gastrointestinal tract that a significant increase in the tract retention of radioactive material is noticed. Yamada had surmised that thiocyanate probably displaced

hormones from the plasma binding proteins and, like other compounds that had been observed to have the same effects, concurrently depressed gastrointestinal reabsorption of radioiodine. Such a compound was 2-4-dinitrophenol. In this study, gastrointestinal reabsorption did not appear to have been depressed. It is entirely possible that since no report known to the writer had up to now corrected for recycling through the thyroid, untenable comparisons and explanations would inadvertently be advanced in the interpretation of observed differences between control and thiocyanate-treated birds. It is also possible, though highly improbable, that rats (on which Yamada worked) were different from bobwhite quail in responding to thiocyanate treatment with a decrease in reabsorption of radioiodine by their gastrointestinal tract. It is perhaps, more likely that the method of calculation of tissue retentions would obscure the real picture as was discovered in this experiment. This, along with the fact that thyroidal recycling of I^{131} in control birds was not accounted for, would leave both the results and their interpretations entirely open to question.

The most important effect of thiocyanate on thyroid hormone metabolism is evidently thyroidal--the blocking of the thyroid iodide trap (Vanderlaan and Bissel, 1946a; Franklin, et al. 1944). Such observed extrathyroidal effects like a decreased PBI or an increased output of

radioiodine by excretion (Yamada, 1967) might be secondary effects resulting either from a blockage in thyroidal trapping of iodide or from the ability of thiocyanate to flush out iodide from the thyroid (Vanderlaan, 1947). This would drastically and rapidly boost blood levels of radioiodide followed just as drastically and rapidly by a depletion of radioiodine from the blood along the concentration gradients between blood and/or urine and fecal levels of radioiodine. The exact mechanism is very unclear, and it is more difficult to explain the fact that blood concentrations of radioiodine subsequently fell so much lower. Since the thyroids are specialized for the uptake of iodine, it is entirely possible that a block of this principal method of salvaging iodine would result in a net loss of iodine to the entire body, especially if such a block were concurrent with a decrease in muscle retention. Whatever the exact simultaneously occurring extrathyroidal processes the drug might trigger off, there can be no doubt that the use of thiocyanate to correct for recycling will have an immense impact on the accuracy of thyroxine degradation as well as secretion rates.

Tissue Retentions and Thyroxine Distribution Space Following Thiocyanate Injection

As mentioned above, thiocyanate drastically reduced whole body retention of radioiodide to only 0.32 times of normal body retention. The increased gastrointestinal

retention observed was almost exactly matched by a decreased muscle retention, leaving the net total body retention very much reduced. Thyroidal retention was drastically and significantly reduced ($p < 0.01$). This finding confirms reports by Vanderlaan and Vanderlaan (1947).

It might be asked: Why was a decrease in thyroidal retention of radioiodine not clearly detectable in the first experiment of this study? Thyroid retentions were measured twelve hours following labelled thyroxine treatment in the first experiment. The second experiment suggested that degradation remained the major process taking place in quail until 15-25 hours following labelled hormone injection. It was at this time that breaks in control curves were observed. Since there did not appear to be as much recycling occurring before as after the fifteenth hour, only very little radioiodide would be retained by the thyroids if these were counted before the fifteenth hour. This was the case in the thyroids of the first experiment. In the second experiment, the thyroids were counted 45 hours following the administration of ^{131}I-L-T4. These thyroids were observed to retain a high percentage of the injected dose in the control birds. The effects of thiocyanate, therefore, showed up in the second experiment because recycling which would have become considerable from the 15th hour on, assumed increasingly greater magnitude until it almost completely masked degradation at the end of the experiment.

Effects of Tapazole on Thyroxine Metabolism

Biological Half-lives and Degradation Rates

Mean degradation rates of 8.46 and 2.1 percent/hour were obtained for the control and the tapazole-injected group of birds, respectively. Mean biological half-lives of 8.58 and 31.74 hours for the corresponding degradation rates above were also obtained. Tapazole, therefore, seems to significantly impair hormone degradation or some reaction in the chain that constitutes thyroxine metabolism. Such a finding has also been reported for thiouracils by Escobar and Escobar (1961); Hogness, et al. (1954); Jones and Van Middlesworth (1960); Hershman and Van Middlesworth (1962). Tanabe, et al. (1965), while reporting the same findings for the thiouracils, insisted that tapazole lacked such extrathyroidal effects. As was pointed out in the literature review, the argument for such a conclusion is very unclear and the evidence definitely conflicting. Yamada (1967) reported that methimazole administration raised the protein-bound iodine (PBI) level of rat blood. The very persistently high levels of radioiodine in the blood of the tapazole-treated quail would seem to support Yamada's findings. It seemed justifiable to interpret the greatly decreased degradation rates and high level of radioactivity in the blood of tapazole-treated birds as

indicative of some impairment in the peripheral metabolism of thyroxine. Tanabe, et al. (1965) conceded that tapazole had similar effects to the thiouracils with regard to their interference in normal thyroid hormone synthesis, thyroidal iodide output and the production of substantially similar thyroxine secretion rates. Degradation is an indication of secretion rate as was pointed out in the literature review. Whatever similarities might be evident between tapazole and thiouracils regarding secretion rates of thyroxine should, therefore, correspond to their effects regarding degradation of the same hormone. There might be a difference in the extent of the effects of the two types of drugs. Such differences could very well give different interpretations employing different methods of calculations and under varying experimental conditions.

Most thyroid hormone degradation has been reported to occur in the liver (Flock and Bollman, 1959). Van Arsdel and Williams (1956), therefore, suggest that the decrease in the peripheral degradation of thyroid hormone following goitrogen treatment was probably due to a localized effect of these drugs on liver reactions. The fact of a significantly increased liver retention of radioiodine following tapazole treatment would tend to support the above suggestions. It is possible that earlier studies did not detect a significant increase in liver retentions because such studies probably calculated tissue retentions

in terms of total body retentions. When this method of calculation was used in this study, the picture was confused because of the massive retentions by control thyroids. When comparisons were made between liver tissue retentions in terms of percentages of total extrathyroidal retention, the picture became much clearer.

The concept that goitrogens had extrathyroidal effects has been reported from a slightly different aspect. Stasilli, et al. (1960); Barnett and Gassner (1951); Andik, et al. (1949), Barker, et al. (1949) and Mellen (1958) have all reported that goitrogens significantly depressed the increase in metabolic rate that would normally follow the exogenous administration of thyroxine. So the evidence for the extrathyroidal effects of goitrogens is overwhelming even if that for the effects of tapazole might be fragmentary and perhaps conflicting. The use of goitrogens in methods for estimating thyroxine secretion rates would, therefore, be very questionable if not totally unacceptable unless such extrathyroidal effects of goitrogens were accounted for.

Tissue Retentions

Aside from the drastic reduction of thyroidal retention, tapazole did not significantly decrease the retention of radioiodide by any other major tissue system. The significant increase in liver retention following tapazole treatment has already been discussed. Retentions of

radioiodide by the gastrointestinal tracts of tapazole-treated birds were lower (though not significantly) than those of control quail. It could be reasoned that this tended to indicate a decreased excretion of radioiodide through the gastrointestinal route. Total body retention was greatly increased compared to that of the controls. This would tend to support the suggestion that excretion is drastically reduced. This increase in total body retention agrees very well with the report by Jones and Van Middlesworth (1960) that rats fed with propylthiouracil accumulated radioiodine in their bodies. Jagiello and McKenzie (1960) also observed that propylthiouracil increased the level of PBI to twice normal levels in rats. Hershman and Van Middlesworth (1962), in reporting much the same observation, suggested that goitrogens probably inhibited deiodination by becoming incorporated into RNA. This would diminish the synthesis of deiodinase which would reduce peripheral degradation, specifically, deiodination. Alternatively, the two workers suggested that goitrogens reduced peripheral metabolism of thyroid hormones by increasing the avidity of plasma proteins to bind thyroid hormones. This would be the opposite hypothesis to Yamada's "hormone-displacement" concept. The data in this study could not confirm either hypothesis, but it certainly suggested an interference in the extrathyroidal metabolism of ^{131}I-L-T4. This could be due to a decrease

in the rate of excretion of radioiodide or to a local effect of tapazole on metabolic reactions of thyroid hormones that normally occurred in the liver of bobwhite quail. The idea of interference has been abundantly confirmed by Escobar and Escobar (1961) who reported a decreased deiodination of ^{131}I-L-T4 in rats treated with thiouracils to half of the control rate.

Tapazole drastically reduced the retention of radioiodide by thyroid glands. This concept confirmed reports by Albert and Tenney (1951) and Grosvener (1963) that goitrogens quickly accelerated the rate of thyroidal iodide release. Astwood (1944-45) reported that an increase in the amount of antithyroid substance given to rats resulted in an increased depletion of iodine from the thyroid gland. The avidity of the thyroid gland for iodide, however, increased with increasing amounts of antithyroid substance. Thus, even though the thyroid glands lost more iodide, the total capacity of the gland for iodide could be increased to 30-fold (Vanderlaan and Vanderlaan, 1947). If determinations of thyroid iodide were made several hours after thiouracil treatment, the drug would appear to prevent uptake of iodide since at that time the uptaken iodide would have already been flushed out (Vanderlaan and Bissel, 1946). The data in this study, thus, confirmed earlier reports about the effect of goitrogens on thyroidal retention of iodide since our determinations were made about 45 hours after tapazole treatment.

CHAPTER V

SUMMARY AND CONCLUSION

1. Experiments were conducted to determine the rate of degradation of ^{131}I-labelled thyroxine in bobwhite quail.
2. Contrary to results reported by earlier workers, it was found that in control birds with intact thyroids there were two distinct slopes in the radioactivity of plasma samples collected during the 45 hours following labelled hormone injection.
3. Tissue distribution studies showed high accumulation of radioiodine in the thyroid glands of control birds. This suggested that the decrease in the slope of plasma radioactivity in later sampling periods must be due to recycling of the radioactive label through the thyroid.
4. A technique for correction of recycling of iodine was applied in these studies. A modification of this technique could be useful for the estimation of more accurate thyroxine secretion rates. By applying the correction for recycling of iodine, much more reliable degradation rates for ^{131}I-L-T4 than have been earlier reported were obtained. Such degradation rates corrected for recycling showed that thiocyanate had no substantial effect on the degradation rate of labelled thyroxine. Corrected control degradation rates

averaged 8.46 $\pm$ 1.89 hours whereas the degradation rate for the thiocyanate-injected group of birds averaged 8.90 $\pm$ 0.82 hours.

5. Thiocyanate treatment appeared to increase the excretion of radioiodine since an increased gastrointestinal as well as a decreased total body retention was observed following thiocyanate injection. Thyroxine distribution space was also significantly lower than that in control birds. However, the recycling of radioactive hormone through control tissues confounded the validity of comparisons between control birds and those treated with thiocyanate where recycling was blocked. From results to date it is believed that it would be necessary to block thyroidal recycling of ^{131}I by the use of thiocyanate or a similar anion to obtain valid results for ^{131}I degradation and distribution space.
6. Tapazole drastically and significantly decreased the amount of label retained by thyroid glands and significantly decreased thyroxine degradation as compared to corrected control and thiocyanate-treated birds.
7. It is concluded that tapazole has extrathyroidal effects. Such effects appear to be primarily on the liver reactions of thyroid hormone metabolism since liver retention of radioiodine was significantly increased by tapazole treatment.

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