THE METABOLISM AND EXCRETION OF THYROXINE AND TRIIODOTHYRONINE IN CHICKENS

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TRIIODOTHYRONINE IN CHICKENS

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CHAPTER I

INTRODUCTION

The thyroid gland synthesizes and secretes two hormones, 3:5:3'5' tetraiodo-l-thyronine (T_4) and 3:5:3' triiodo-l-thyronine (T_3). These hormones are transported by the circulatory system to the peripheral tissues, i.e., their target sites. The primary effects of the thyroid hormones are: (1) to inhibit the release of thyroid-stimulating hormone, (2) to enhance growth and differentiation and (3) to stimulate tissue oxidation. Although the effects of the thyroid hormones are qualitatively similar in both mammalian and avian classes, they produce responses that differ quantitatively between species of the same class and between species of the different classes, mammals and aves.

In mammals, T_3 has two to six times the potency of an equimolar quantity of T_4 as measured by a number of physiological tests which involve: basal metabolic rate, body growth, tissue metabolism and inhibition of goiter (Barker, 8). However, in birds, T_3 has the same potency as T_4 with respect to growth and metabolic rate (77), and less potency in stimulating oxygen consumption in isolated cardiac muscle (78) and in inhibiting goiter in thiouracil-fed chicks (77, 67).

In an attempt to explain the difference in potency between these two hormones in mammals and birds, the following possibilities have been suggested: Tata and Shellabarger (103) indicated that T_3 and T_4 are not bound to the plasma proteins in mammals with equal affinity, whereas in chickens the two hormones are bound equally to the plasma proteins.

According to this observation, protein binding should limit the rate of entry of the two hormones into the cells. A second possible explanation for the difference in potency of the two hormones is that the less potent hormone is degraded and excreted by the liver and kidneys at a greater rate than the more potent one (68, 47). This observation is not supported by the work of Keating and Albert (59) who have found that T_3, the more potent hormone in rats, is excreted and degraded at a greater rate than T_4 in these animals.

In view of the above considerations, an investigation has been conducted into the reasons for the different potencies of T_3 and T_4 in chickens by determining the amount of radioactivity that is excreted following intravenous injection of either T_3 or T_4 labeled with I^{131}. Also there is relatively little information available in birds concerning the radioactive metabolic compounds found in plasma, bile and urine following administration of I^{131}-labeled thyroid hormones.

CHAPTER II

LITERATURE REVIEW

Transport of Thyroid Hormones

The thyroid hormones that have been detected in the plasma of mammals were T_4 (108, 109) and T_3 (42). T_4 was detected in higher concentrations in the plasma than T_3 and was considered to be the primary thyroid hormone in the blood of mammals. The same thyroid hormones have been identified in the plasma of birds (95, 117) and T_4 has also been found in greater concentrations in birds as it is in mammals.

It appeared even from the early work of Trevorrow (115) that the thyroid hormones were associated with the plasma proteins. This investigator observed that this association could not be broken by dialysis or by reagents such as trichloroacetic acid, but was broken by solvents such as acetone or butanol.

Gordon, et al. (38) were among the first investigators to demonstrate that T_4 was transported by the plasma proteins; the alpha globulins and to a lesser extent by the plasma albumin. The alpha globulins are referred to as thyroxine-binding globulin, TBG. Ingbar (53) discussed the emergence of an additional thyroxine-binding component, the thyroxine-binding prealbumin, commonly called TBPA. Collectively TBPA, TBG, and thyroxine-bound albumin may be called thyroxine-binding proteins, TBP.

It has been demonstrated by Larson and Albright (62) that all of the analogues of T_4 studied have less affinity than T_4 for TBG. It has been

shown earlier by Rall, et al. (82) that in euthyroid patients, T_3 has a shorter biological half-life of about two days as compared to seven to eleven days for T_4. It was felt that this difference in half-life between the two hormones was due to their differential affinities for the plasma proteins. Christensen (16), using a dialysis procedure instead of an electrophoretic method, demonstrated that T_3 not only had less affinity for the plasma protein but was about 15 times as concentrated in the free form as was T_4. Since T_3 in mammals is considered to be more potent than T_4 by various investigators (44, 48), the concentration of free hormone apparently determines the rate of its peripheral utilization or excretion. This latter statement may explain an investigation conducted by Myant (72) who observed that the biliary clearance rate (bile/plasma I^{131} concentration ratio x rate of flow of bile) of rats injected with less than 10 micrograms of T_4 was significantly less than the biliary clearance rate of rats receiving 10 μgm. of T_3.

Evidence in support of the view that the relative potency and rate of peripheral utilization of T_3 and T_4 is determined by the binding power of the plasma proteins has been postulated by Tata and Shellabarger (103) in their attempt to explain why T_3 and T_4 are equally potent in chickens (Shellabarger, 94). Tata and Shellbarger (103) have shown that in chicken plasma, analyzed by paper electrophoresis in barbiturate buffer at pH 8.6, T_3 and T_4 were both bound by albumin and were not bound to any extent by other serum proteins. However, according to Newcomer (77), Newcomer and Barrett (78) and Mellen and Wentworth (67), T_4 was more potent in these animals than T_3. In a recent paper, Dubowitz, et al. (19) observed that at physiological pH (pH 7.4) chicken serum and rat serum resembled each other in their binding of T_3 and T_4 more closely than either of them resembles human serum; however, the relative potencies of T_3 and T_4 in rats was similar to that in man but differed from that in

chickens. They concluded that differences in relative potencies of T_3 and T_4 in men, rats, and chickens cannot be explained by differences in the hormone-binding properties of the serum.

Metabolites of Exogenous T_4 and T_3 in Plasma

Although a large amount of research has been conducted on the plasma proteins that bind and transport the thyroid hormones, little research has been conducted on determining the iodinated organic metabolities that may be present in plasma in addition to iodide and the two main thyroid hormones in animals which have been injected with either T_3 or T_4. Gross, et al. (45) injected mature, anesthetized rabbits with I^{131}-labeled T_3 and collected bile and plasma samples at different time intervals. They then subjected these samples to paper chromatography and radioautography to detect radioactive metabolites. They detected in the plasma samples T_3, iodide and a T_3 complex which was readily dissociated by dilute acid. Roche, et al. (93) also detected this unknown T_3 complex in rat plasma and determined that it was a sulfate conjugate of T_3.

Ford, et al. (32) observed in rats and guinea pigs a rapid decrease in the percent of T_3 present in plasma following the intravenous injection of T_3; however, the percent of iodide in the plasma of these animals rose quickly. Ford, et al. (31) observed at the end of two hours only a slight amount of inorganic iodide and approximately 90 percent of the total radioactivity of plasma present as T_4 in guinea pigs. Flock and Bollman (22) noted that in the plasma of rodents previously injected with T_4, only 15 percent of the total activity in plasma at the end of six hours was present as iodide and 77 percent of the plasma radioactivity was T_4.

Using chickens as experimental animals, Wentworth and Mellen (117) injected radioactive iodide and collected plasma 24 hours later. The plasma was extracted, concentrated and subjected to separation with paper

chromatography. These investigators observed that T_4 accounted for 60 percent of the total hormonal radioactivity in plasma and T_3 the other 40 percent.

Although traces of other iodinated metabolites have been identified in the plasma of T_3 or T_4-injected mammals, the major metabolites are the parent thyroid hormone and iodide.

Metabolism and Excretion of Thyroid Hormones by the Liver

The major metabolic pathways of T_3 and T_4 are (a) glucuronide and sulfate conjugation, (b) oxidative deamination and decarboxylation, and (c) deiodination. In general, the types of metabolic transformations which are observed in vitro are similar to those which occur in vivo. In the following review, both the in vivo and in vivo metabolic transformations of the thyroid hormones and their analogues in the liver will be discussed.

The liver is important in the metabolism of the thyroid hormones as shown by the work of Myant (72) and Albert and Keating (1) who demonstrated that the liver of rats rapidly removes intravenously injected T_4. Clayton, et al. (17) gave T_4 both orally and intravenously to cats and noted an increase in the excretion of organic iodine into the bile and feces.

Some of the early work which attempted to determine the nature of the iodinated compounds excreted in bile was conducted by Clayton, et al. (17) and Taurog (105). Clayton and his group (17) extracted the iodinated organic metabolites from cat bile with butanol and concluded that approximately half of the I^{131} in the bile consisted of unchanged T_4. However, Taurog, et al (110) injected a smaller dose of I^{131}-labeled T_4* containing

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I^{131}-labeled T_4 and T_3 will be designated with an asterisk (T_4 and *T_3).

a high specific activity into rats. Bile was collected and the I^{131}-containing compounds in bile were studied by means of filter paper chromatography. They demonstrated that most of the T_4 was present as the glucuronide conjugate although some free T_4 was present in bile. The amount of free T_4 present directly depended on the amount injected according to both Taurog (105) and Myant (72). The glucuronide of T_3 in the bile of rats receiving labeled T_3 has been identified by both Gross, et al. (45) and Roche, et al. (87). More than one glucuronide may be found in the bile of normal animals injected intravenously with either $^*T_3$ or $^*T_4$. Upon hydrolysis, these glucuronides yield the derivatives of the parent compounds. The presence of free triiodothyroacetic acid (T_3A) and conjugated T_3A in the rat bile have been demonstrated by Michel and Etling (69) and Roche, et al. (92). Culp and Rice (18) detected the glucuronide conjugate of I^{131}-labeled tetraiodothyroformic acid in the rat following the injection of the same compound.

The metabolism of T_3A and T_4A in humans was recently examined by Green and Ingbar (39) who found that the acetic acid derivative of T_3 was conjugated and excreted more rapidly into the bile than the conjugate of tetraiodothyroacetic acid (T_4A).

A quantitative difference in the parent compound and its derivatives which were bound as glucuronides was reported by Flock and Bollman (22). They observed in rats which had received radioactive T_4, that six hours later approximately 67 percent of the distribution of I^{131} in the bile of rats was in the glucuronide region. Incubation of the glucuronides with beta glucuronidase yielded T_4, 3:3':5' T_3, 3:3':5' T_3 and T_4A. Flock, et al. (29) also demonstrated that in dogs the acetic acid derivatives of T_4 and T_3 and the propionic acid derivatives of T_4 and T_3 were excreted into the bile chiefly as the glucuronides.

In vitro conjugation of the thyroid hormones has been shown by Isselbacher (56) who demonstrated that a liver microsomal enzyme plus uridine diphosphate glucuronic acid incubated with $*T_4$ gave rise to the glucuronide of T_4. Becker and Prudden (10) also detected the presence of the thyroid hormone glucuronides in perfusate of plasma and bile from an isolated perfused rabbit liver.

The thyroid hormones may also be conjugated as the sulfate. After injecting physiological doses of $*T_3$ into thyroidectomized rats, Roche, et al. (89) found the sulfuric ester of T_3 in the bile of these animals. They used eight different solvents and the appearance of sulfate anions after hydrolysis to identify the sulfate ester. He concluded that a substance he believed to be free T_3 in a collidine solvent was actually the sulfate of T_3. In a later paper, Roche, et al. (92) demonstrated the presence of T_4, T_3 and T_3A bound as the sulfates in the bile of I^{131}-injected rats.

Flock, et al. (29) injected T_4A, tetraiodothyropropionic acid (T_4P), T_3A, and triiodothyropropionic acid (T_3P) into dogs with biliary fistulas and at the end of six hours, small amounts of ethereal sulfate conjugates of 3:3' diiodothyroacetic acid and 3:3' diiodothyropropionic acid were detected in the bile. In an earlier paper Flock, et al. (25) found both T_3 and 3:3' diiodothyronine (T_2) conjugated with sulfate following the injection of T_3. The percent of the iodinated organic metabolites conjugated with sulfate depends on the species of animal use. In dogs injected with $*T_4$, about 18 percent of the radioactivity in bile was found in the sulfate region; however, in rats Flock and Bollman (22) detected only 6 percent conjugated with sulfate.

The role that conjugation plays in the metabolism of the thyroid hormones is not completely clear. It is known that conjugation does not

potentiate hormonal action by giving rise to more active compounds. It was stated by Tata (102) that in this area "conjugation represents a detoxification mechanism and a means of regulating the tissue and circulating levels of thyroid hormone." However, Tapley, et al. (98) and Herz, et al. (50) suggested that glucuronide formation was important for the transfer of the thyroid hormones and their metabolites through tissues or membranes because the glucuronide conjugate was water soluble in contrast to the thyroid hormones which are more soluble in lipids. It was felt by Gross, et al. (45) and Roche and his colleagues (85) that in mammals the sulfate ester of T_4 was the transport form of that hormone. Roche explained that T_4 left the circulatory system at a rapid rate and the free T_4 was conjugated as the sulfate ester by liver and kidneys and was secreted back into the circulatory system in order to enter the cells of the peripheral target.

The second major metabolic pathway of T_4 and T_3 in the liver has been shown to be deamination and/or transamination and decarboxylation. In the search for the peripherally active form of T_4 and T_3, it was discovered by Thibault (111) that the acetic acid analogues had a shorter latent period compared to the thyroid hormones in stimulating the oxygen uptake of kidney and liver slices and of stimulating the metabolic rate in thyroidectomized rats. Barker and Lewis (9) have not, however, been able to obtain the same prompt response. Van Zyl and Engeblricht (116) using T_3A observed an immediate uptake of O_2 in kidney cortex, but observed inhibition later. It has been summarized by Tata (102) that although these analogues may possess biological activity, they were not more active compounds than T_4 or T_3. However, because of this controversy the conversion of T_4 or T_3 to the acetic acid or pyruvic analogues in the peripheral tissue was investigated.

Roche, et al. (88) were among the first investigators to demonstrate analogues of T_3 and T_4 in bile and urine. They collected bile for 24 hours following the injection of 3 mg. of $*T_4$ and of $*T_3$ and observed primarily 3:5:3' triiodothyropyruvic acid and some 3:5:3':5' tetraiiodothyropyruvic acid. These investigators postulated that oxidative deamination was the first stage of the degradation of T_3 and T_4 in the liver. Later Michel and Etling (69) gave a physiological dose of $*T_3$ to thyroidectomized rats and observed a small quantity of T_3A in the bile, but no pyruvic acid analogues. Subsequently, many investigators have detected the presence of the acetic analogues in the bile of animals previously injected with $*T_3$ or $*T_4$. Among these investigators were Flock, et al. (25, 27) who demonstrated the analogues in dog bile. Galton and Pitt-Rivers (36) identified the acetic acid analogues of T_4 and T_3 in the liver of mice, 12-48 hours after the mice had received I^{131} intraperitoneally.

A study of the metabolic degradation of the thyroid hormones to their corresponding acetic acids was begun by Tomita, et al. (113) and Albright, et al. (3). Using isolated mitochondria from the liver and kidney of rats, they observed the conversion of l-$*T_3$ and l-$*T_4$ to their corresponding acetic acid analogues. In an attempt to determine the pathway whereby this conversion takes place, Tomita and Lardy (112) observed that this metabolic pathway required DPN for its activity. Yamamota, et al. (119) reported that T_4 and T_3 were transaminated by enzymes in intact kidney mitochondria. This would imply that the alanine side chain is first transaminated to a pyruvic acid intermediate; decarboxylation would then occur leading to the acetic acid analogue of the parent thyroid hormone. In contrast to the above finding, Nakano and Danowski (74, 75) demonstrated that the pyruvic and acetic acid analogues were formed from the parent hormones in the presence of an l-amino acid oxidase from cobra venom.

These investigators (76) prepared an enzyme from rat kidney mitochondria which they found was not DPN dependent and which appeared to be an oxidative deaminase. This enzyme rapidly converted T_4 or T_3 to its pyruvic acid analogue; oxidative decarboxylation then occurred producing the acetic acid analogues. Regardless of the metabolic pathway present in vivo that gives rise to the biologically active T_3A and T_4A, Tata (102) and Pitt-Rivers (79) observed that the acetic acid derivatives were not as biologically active as T_4 and T_3, and suggested that T_4A and T_3A were merely metabolic products of the thyroid hormones.

Deiodination of thyroid hormones in the hepatic tissue is the most important and most extensively studied metabolic pathway of the thyroid hormones. Labeled thyroid hormones which are synthesized with I^{131} in the phenolic ring can be detected during its metabolic degradation as long as deiodination of the labeled element does not occur. Consequently, most of the work that has been conducted on deiodination requires further study because of the nature of the hormone injected. For example, most investigators examining this third metabolic pathway have used T_4 or T_3 labeled in a prime position which is the most labile position (Stanbury, 97). As a result, when deiodination occurs and includes removal of the labeled element, the fate of the remainder of the molecule is unknown.

Deiodination of labeled thyroid hormones is said to have occurred if: (1) T_3 appears after T_4 administration; (2) T_2 appears after T_3 or T_4 administration; (3) iodide appears in urine or feces following administration in vivo of thyroid hormone; (4) presence of iodide is established by identification in more than one chromatographic solvent (79).

The study of deiodination of the thyroid hormones began after Gross and Pitt-Rivers (42) postulated that T_3 was the peripherally active form of the T_4 and that T_4 was deiodinated to this more active compound. This

postulation was made on the basis of three observations: (1) Gross and Leblond (41) observed the appearance of inorganic iodide in urine, plasma and feces following a subcutaneous injection of radioactive T_4, (2) Gross and Pitt-Rivers (42) discovered T_3 in human plasma, and (3) Asper, et al. (6) demonstrated that T_3 was more potent in man than T_4. After the advancement of this hypothesis, a number of investigators attempted to demonstrate the conversion of T_4 to T_3 in the liver, muscle, kidney and other tissues. Maclagan and Sproutt (65) demonstrated that rat liver homogenates could rapidly deiodinate T_4. Kalant, et al. (57) injected $*T_4$ into thyroidectomized rats and later showed that the liver rapidly accumulated T_4 and that T_3 could be demonstrated in the hepatic tissue in rats.

Similar work was conducted by Hogness, et al. (52) who injected $*T_4$ intravenously into both normal and thyroidectomized rats. Specimen of liver, kidney and muscle were collected at various time intervals and extracts were made and subjected to column chromatography. Although they noted the appearance of T_3 following the injection of T_4, these investigators stated that since only a small amount of T_3 was found, the conversion of T_4 to T_3 was not an important metabolic pathway of T_4. Becker and Prudden (10) perfused isolated rabbit liver with $*T_4$ for 12 hours and found in the perfusate of most samples T_3, iodide, and several other unknown materials (which later proved to be the glucuronides). Flock, et al. (27) observed the appearance of a small amount of glucuronide-bound T_3 in the bile of dogs injected with $*T_4$. Although the amount of T_3 produced by deiodination of T_4 by the dog's liver was small, it was about equal to that produced from T_4 in rat liver (Flock and Bollman, 22).

There are many investigators who do not agree with the postulate made by Pitt-Rivers. Although these investigators have been unable to

demonstrate the appearance of T_3 from T_4, they have noted that the liver is one of the sites of peripheral deiodination of the thyroid hormones. Albright and Larson (2) who earlier were among the foremost advocates of the hypothesis that T_4 is deiodinated to T_3 at the peripheral tissues demonstrated this in human kidney, but were unable to demonstrate conversion of T_4 to T_3 in liver, skeletal muscle, and cardiac muscle. Roche, et al. (84) failed to identify T_3 in the urine or bile of rats given $*T_4$ intraperitoneally. In a more recent investigation, Roche, et al. (87) observed a trace of T_3 conjugated as the sulfate ester in the bile of thyroidectomized rats that had been injected with d-l$*T_4$. Lassiter and Stanbury (63) attempted to confirm the deiodination of T_4 to T_3 in athyreatic humans but were unable to find T_3 in the blood plasma after the injection of $*T_4$. Many investigators now feel that although a small amount of T_3 is undoubtedly produced from T_4 in the liver, T_4 does not have to undergo partial deiodination to T_3 in order to exert its metabolic action.

Although the hypothesis that T_3 is the peripherally active form of T_4 has been rejected, it has been shown by many investigators that the liver has the ability to deiodinate the thyroid hormones. Flock and Bollman (24) observed that T_3 was deiodinated by the liver at a greater rate than was T_4. Flock, et al. (26, 28) have not only demonstrated that the liver is an important organ of deiodination of the thyroid hormones, but they have also demonstrated that deiodination of T_3 and T_4 and excretion of urinary iodide is markedly depressed in hepatectomized animals. A study made by Yamazaki and Slingerland (120) has shown that endogenously $*T_4$ and $*T_3$ incubated with spleen, kidney, or liver slices were partially deiodinated at the end of three hours. No deiodinated intermediates were found; only inorganic iodide and origin material appeared to be present.

They pointed out that some iodinated organic intermediates may have been formed but were quickly deiodinated. Later this work was supported by Lissitzky, et al. (64) who found that thyronine was one of the metabolites resulting from the deiodination of T_4 and T_3 in vitro.

On the other hand, a group of investigators have identified 3:5 diiodotyrosine as a product of deiodination of T_4. Plaskett (80) used T_4 labeled with I^{131} in 3' or 5' position and incubated this hormone with rat liver homogenates. By chromatographing the incubation mixture, he noted that T_4 was apparently metabolized to a compound which contained iodine atoms in the 3:5 positions, but from which 3':5' iodine atoms were absent. This same investigator (81) later demonstrated that this unknown compound was 3:5 diiodotyrosine. He further demonstrated that deiodination of the alpha ring of the thyronine molecule was slight, whereas I^{131} atoms in either 3' or 5' position on the beta ring were released rapidly as inorganic iodide. This work was confirmed with rat liver microsomes by Wynn and Gibbs (118) who further stated that 3:5 diiodotyrosine was an end product of this type of degradation and that 3:5:3' T_3, and 3:5 diiodothyronine were not intermediates in these reactions. These findings indicated that rupture of the diphenyl ring occurred during the metabolism of T_4. A review by Stanbury (97) who discussed the physical chemistry of the iodine atoms in the phenol rings of iodinated tyrosines and thyronines supported the above mentioned work. Stanbury stated that "a major factor in the chemical reactivity of any of these aromatic iodine containing compounds is the easy ionizability of the hydroxyl group. The linkage of the iodothyronines is not ionized and, accordingly, there is more stability of the 3 and 5 positions. Thus one might expect the 3 and 5 positions to be more stable than the corresponding prime positions."

Although diiodotyrosine may be a degradation product of T_4 in the

liver of mammals, it has seldom been demonstrated in vivo. One explanation for this observation was the fact that most investigators used T_4 labeled exogenously with I^{131} in the prime positions; consequently, once rupture of the diphenyl linkage or diiodination of the radioactively-labeled beta ring occurs, the fate of the molecule or of the alpha ring is unknown.

Although it appears from Stanbury's review (97) that the iodine atoms in the alpha ring were more stable than those in the beta ring, some investigators have found no evidence of it in vivo using endogenously labeled T_4. Flock, et al. (27) found that in the bile of dogs injected with T_4 there was more 3:3':5' T_3 (reverse T_3) than 3:5:3' T_3 both in the free and conjugated form. It may be concluded from this work that partial deiodination proceeds more readily from position 5 than 5'. In this same paper these investigators recognized that 3:3' T_2 was found as a sulfoconjugate which was hydrolysable with Mylase P which contains a phenol sulfatase. D-*T_4, a compound with less calorigenic activity than l-T_4, was administered to dogs with biliary fistulas (Flock, 23). The metabolism and excretion of this compound by the liver was essentially similar to that observed with l-*T_4 except that deiodination of iodine from the 3 and 5 positions occurred, rapidly leading to the formation of reverse T_3 and 3:3' T_2. Reverse T_3 and 3:3' T_2 according to Money, et al. (71) were metabolically inactive in calorigenic and antigoitrogenic assays; therefore, the formation of these two metabolites represent an important mechanism in the inactivation of T_4 by the liver.

Flock, et al. (25) collected bile from dogs injected with *T_3 and observed the following radioactively labeled compounds: T_3 in the free state, iodide, and conjugated metabolites. These conjugates consisted of T_3 bound as the glucuronide and T_3 and 3:3' T_2 bound as the sulfoconjugates.

They speculated that 3' monoiodothyronine might also be present. They noted again that deiodination had occurred in the 5 position on the alpha ring, but they pointed out that using T_3 labeled in the 3' position they were unaware of the fate of the hormone when the I^{131} in the 3' position was removed.

Metabolism of Thyroid Hormones by Renal Tissue

The metabolism of the thyroid hormones by the kidneys has been studied by many investigators. Keating and Albert (58) stated that when radioiodine was administered to myxedematous patients that within three days, 98 percent of the radioiodine was excreted into the urine as inorganic iodide. Therefore, it appears that the kidneys compete with the thyroid gland for inorganic iodide. Further work with the renal metabolism of the thyroid hormones revealed that with the intravenous injection of iodothyronines labeled with I^{131} most of the labeled inorganic iodide appeared as free inorganic iodide. However, 5-10 percent of urinary iodide was in an iodinated organic form which Keating and Albert (58) believed was diiodotyrosine. Roche, et al. (88) administered a large dose of $*T_3$ and $*T_4$ (2-3 mgm.) to rats and detected iodide and pyruvic acid analogues of T_4 and T_3 but no diiodotyrosine. In general, less than 10 percent of the urinary iodine compounds represented organic compounds. These iodinated compounds or compound appeared to be the parent hormone in normal dogs and rats (Flock, et al., 22, 28), although small amounts of the glucuronides of T_4 and T_3 have been observed in the urine of rats and dogs (Flock, et al., 26 and Taurog, 105).

The position of the labeled iodine atoms on the thyroid hormone molecule greatly affects the rate of renal excretion of radioactivity. According to Roche and Michel (84), the 3' or 5' position on the beta ring

was more labile than those in position 3 or 5. Therefore, T_4 or T_3 labeled with radioiodine in the prime positions appeared to be deiodinated and the iodide excreted into the urine at a more rapid rate than endogenously-labeled thyroid hormones. In dogs radioactive iodide from the metabolism of $^*T_3$ appeared in the urine more rapidly than radioactive iodide from $^*T_4$ (Flock, et al. 28). Using albino rats, Keating and Albert (59) demonstrated that the total percent of I^{131} execreted by the kidneys was 2.5 percent/hour for $^*T_3$ compared with 1.4 percent/hour for $^*T_4$.

Although deiodination is one metabolic transformation of the thyroid hormones by the kidneys, this tissue may metabolize the thyroid hormones by any one of three possible pathways of degradation. Albright, et al. (5) observed that $^*T_4$ in kidney slices was deiodinated and that chromatography of the extract revealed iodide, T_4 and T_3. Etling and Barker (20, 21) observed rapid deiodination of $^*T_4$ by rat kidney cortex, but in contrast to the above work these investigators found no T_3 in the extract. T_4, T_3 and their acetic and propionic acid analogues were incubated with kidney slices, and according to Yamazaki and Slingerland (120), the only product of deiodination detected was iodide. Most investigators have demonstrated only inorganic iodide as the principal metabolite when $^*T_4$ was deiodinated; however, using $^*T_3$ other metabolites appeared besides iodide from the metabolic deiodination of T_3. Roche, et al. (90) injected $^*T_3$ intraperitoneally into thyroidectomized rats; three hours later the kidneys were removed and extracts made and chromatographed. These investigators observed T_3A and 3:3' T_2 in addition to T_3 and iodide in the renal tissue. When these investigators (91) injected 3:3' T_2, they observed the parent hormone, iodide and 3:3' diiodothyroacetic acid (3:3' T_2A). In a similar experiment, 3:3':5' T_3 was injected and in addition to the injected hormone, iodide, 3:3':5' triiodothyroacetic acid, and

3:3' T_2 were found in the kidney tissue. They concluded that the deiodination process of these substances (3:3' T_2 and 3:3'5' T_3) appeared to be relatively unspecific.

The second of these three metabolic pathways is deamination and decarboxylation of the alanine side chain. As mentioned in the preceding paragraph, Roche, et al. (86) demonstrated in vivo the metabolism of T_3 to its acetic acid analogue. The results of this work were supported by Galton and Pitt-Rivers (36) who demonstrated the acetic acid analogues of T_4 and T_3 in the kidneys and liver of mice given large doses of I^{131}. They estimated that these two compounds accounted for 0.1 percent of the total radioactivity in the tissues. Tomita, et al. (114) demonstrated that the mitochondrial fraction of rat kidney converts *T_4 and *T_3 to their respective acetic acid analogues. That rat kidney mitochondria have the ability to deaminate and decarboxylate the alanine side chain of T_3 and T_4 has been verified and studied carefully by Nakana and Danowski (76).

The third metabolic pathway, conjugation of the thyroid hormones in renal tissue, was first observed by Taurog, et al. (110) who demonstrated that the kidney in addition to the liver was capable of forming the glucuroconjugates of T_4 and T_3. This observation has been verified by Galton and Pitt-Rivers (35), Etling and Barker (21) and by Braverman and Ingbar (15). It is probable that renal tissue was able to conjugate the thyroid hormones and their analogues as the sulfate ester since in the dehepatized dog, a small amount of T_3 and 3:3' T_2 conjugated as the sulfate ester has been found in the urine (Flock, et al., 25). From this work, it appears that renal tissue has the ability to metabolize the thyroid hormones by all three pathways. Of the three, deiodination, conjugation and deamination, the latter pathway in the renal tissue appears to be the most prominent (Galton and Pitt-Rivers, 36 and Roche, et al., 91).

Metabolism of Thyroid Hormones by Extra-renal and Extra-hepatic Tissues

Skeletal muscle from thyroidectomized rats (Roche, et al, 86) and skeletal muscle from normal rabbits (Gross, et al., 45) injected with $*T_3$ was found to contain $*T_3$ and inorganic iodide, although 3:3' T_2 was identified only in rat skeletal muscle. Gross and colleagues further demonstrated that tissues from the brain and the anterior pituitary did not metabolize the thyroid hormones. However, work by Tata (99) revealed that both skeletal muscle and brain tissue could deiodinate the thyroid hormones, but only deamination could be carried out by the brain tissue. Later Tata, et al. (104) demonstrated that brain tissue from rat, chick, and dog presumably possessed enzymatic systems capable of deaminating and of deiodinating the thyroid hormones.

According to Tata (99), deiodination was the only metabolic transformation of T_4 and T_3 which could take place in skeletal muscle. This investigator has succeeded in isolating this deiodinating enzyme and found that its activity depended on the presence of T_4 since its activity increased in parallel to O_2 consumption in thyroidectomized animals receiving l-T_4 (Tata, 101). Further proof of the latter statement was the observation by Tata (101) and Ingbar and Frienkel (54) that hormonal deiodination of T_4 increased in hyperthyroidism and decreased in hypothyrodism.

The physiological significance of deiodination in vivo is not clearly known but Tata (101) has suggested some possibilities: (a) deiodination of thyroid hormones may be a non-specific manifestation of cellular metabolism, (b) it may be specifically linked to the action of thyroid hormones on cellular metabolism, (c) it may be a mechanism for the deactivation of the thyroid hormones, (d) it may help in the formation of

more active compounds principally the conversion of T_4 to T_3, and (e) it may be a mechanism for regulating the enzyme content of the body.

CHAPTER III

MATERIALS AND METHODS

The experimental procedures used in this work were designed to compare the rate of excretion and peripheral metabolism of T_4 and T_3 in chickens in order to provide possible explanation(s) for the observed differences in potencies of these hormones.

Experimental Chickens

Six to ten-month old White Leghorn cockerels weighing four to five pounds were used throughout these experiments. These animals were housed together in a partially-open, frame house. They were fed ad libitum on a standard commercial ration and had free access to fresh water.

I^{131}-labeled T_4 and T_3

Radioactively labeled T_4 and 3:5:3' T_3 prepared with I^{131} in the prime positions,* and having a specific activity of 29-40 mc. per mgm. were used in this experiment.

The compounds were diluted upon receipt with 1.0 percent bovine albumin to reduce radiodecomposition and refrigerated. Galton and Ingbar (34) reported measurable success in reducing the spontaneous liberation of I^{131} from these compounds by diluting with 1.0 percent human serum albumin.

On the day of arrival, a portion of the material was subjected to

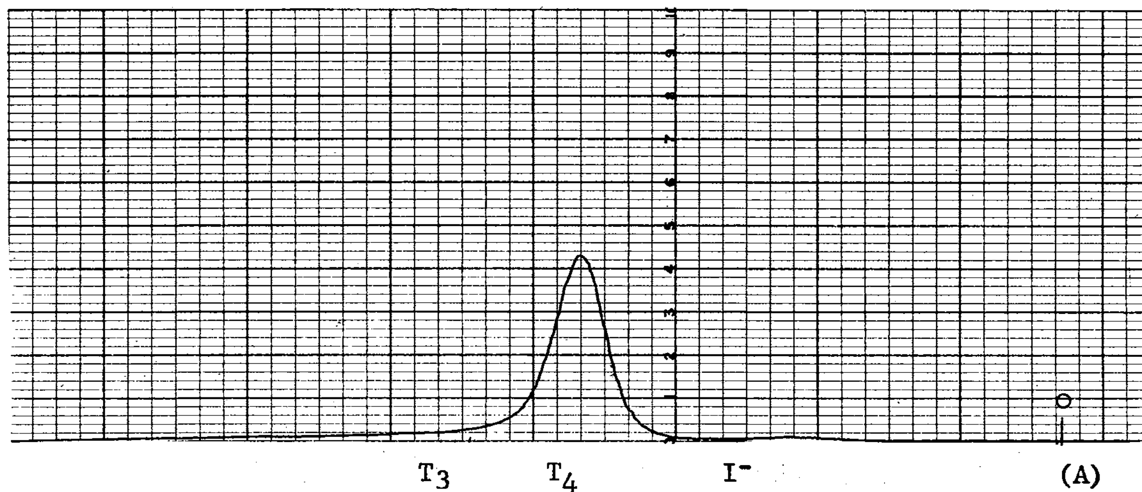
*Abbott Laboratories, Oak Ridge, Tennessee.

paper electrophoresis and to paper chromatography in order to estimate the amount of I^{131} and radioactive contaminants present in the propylene glycol solutions. Before spotting the material on paper, a small amount of 1.0 percent bovine albumin dissolved in physiological saline was spotted and dried at the origin since Tata (100) reported that this protein had a stabilizing effect on the radioactively-labeled molecule.

In Figure 1, typical scanned chromatogram records of the commercial I^{131}-labeled thyroid hormones are shown. When the commercially labeled T_4 and T_3 were examined by single-dimension chromatography, radioactive iodide was found to be the chief breakdown product of $*T_3$ and $*T_4$. In Table I, the average percents of the hormone and iodide were found to be 88.3 and 86.7 percent for $*T_4$ and $*T_3$ while iodide was 8.8 percent and 1.4 percent respectively in $*T4$ and $*T_3$ propylene glycol solutions. Origin material accounted for only a small percentage of the radioactivity in these commercial thyroid hormones. T_2 was believed to have been present as an impurity in the T_3 source and it was estimated that about 10 percent of the radioactivity in the $*T_3$ propylene glycol solution was due to this impurity. However, Abbott Laboratories* stated that they did not believe this compound to be present in the commercial $*T_3$ source because T_3 was labeled only in the prime position and the iodine in the 5 position on the phenol ring was very stable. The organic solvent, dioxane, when improperly distilled, could produce various artifacts. Since dioxane was used extensively in this work, it should be pointed out that certain artifacts may have been produced which were not produced by the experimental animals.

The commercially labeled I^{131} thyroid hormones were not used after 12 days had elapsed from receipt of the compounds or when iodide contamination exceeded 20 percent of the total radioactivity.

*Personal communication.



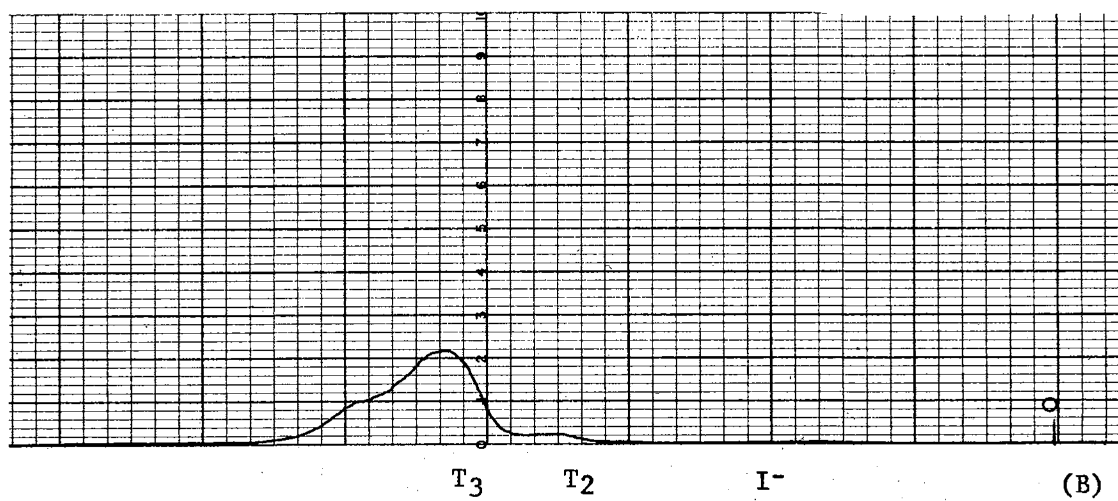


Figure 1. Scanned Chromatogram Records of the Solution of T_4 (A) and T_3 (B) as Obtained from Abbott Laboratories, Oak Ridge, Tennessee. Solvent: butanol, dioxane, 2N ammonium hydroxide 4:1:2. O=Origin. Solvent front not shown.

TABLE I

THE PERCENT DISTRIBUTION OF RADIOACTIVITY IN COMMERCIAL I^{131} COMPOUNDS*

Solvent	Hormone	No. of Estimations	Origin	I^-	T_4	T_3	T_2	T_3A
Butanol-4	T_4	6	0.3**	8.8	88.3	2.5	---	---
Dioxane-1			±0.2	±6.3	± 6.4	±2.2		
$2NNH_4OH$-2								
	T_3	3	0.3	1.4	---	86.7	9.8	1.7
			±0.2	±0.4		± 2.2	±4.4	±2.9

*Time after date of shipping when chromatographic separations were started was 1-8 days for both hormones.

**Mean ± standard deviation.

Preparation of Animals

In order to collect samples of bile, urine and plasma, all birds were treated in the following manner. Each bird was anesthetized with sodium pentobarbitol at the start of the experiment. A smaller amount of the anesthetic was usually given midway through the experiment. This was usually enough to keep the bird anesthetized for the entire experiment. Following the administration of the anesthetic, the birds were placed in a dorsal recumbent position and secured to a V-shaped holding board.

Bile was collected by inserting polyethelene cannulae into both the posterior and anterior bile ducts. During preliminary experiments with these animals, it was noted that if only the anterior duct was cannulated, less bile was collected; whereas, if both were cannulated, a larger volume of bile was collected during the experiment. Urine was collected by cannulating the ureters approximately one inch from the cloaca. Once the cannulae were secure, $*T_4$ or $*T_3$ was administered by single intravenous injection in doses of approximately 100-200 μc. Bile and urine were collected continuously for four hours in graduated 15 ml. centrifuge tubes which were kept in a beaker of crushed ice.

Urine and bile were not collected from all animals. Instead 40 birds were randomly and evenly placed in four groups; Group I consisted of birds from which bile alone was collected, bile and urine were collected from Group II, Group III consisted of birds from which only urine was collected, and Group IV was the control group, i.e., bile and urine were not collected from this group although they were sham-operated. Each group was further divided so that T_4 was administered to five birds in each group and T_3 injected into the remaining birds in each group. Plasma was collected from all birds in each group regardless of the hormone

injected. At 1, 2, and 3 hours the cock's comb was lanced and blood collected in two heparinized capillary tubes which were centrifuged for two minutes. At the end of four hours approximately 5 ml. of blood was collected by cardiac puncture with a heparinized syringe, and plasma was collected by centrifugation. The experiment was then terminated and all collected samples were refrigerated for further use.

Determination of the Percent of I^{131} in Urine and Bile

The percent of I^{131} in urine and bile per administered dose was calculated by measuring the radioactivity in a 0.1 ml. of urine or bile in a well-type scintillation counter. The counts per minute were calculated for the total volume of bile or urine collected during the four hour period. A known volume, 25 lambda, of the commercially prepared $*T_4$ or $*T_3$ was diluted 100 times. By counting a 0.1 ml. of the diluted sample and calculating the radioactivity or counts per minute in the 25 lambda sample, the total counts per minute could be calculated for the dose of I^{131} injected. The percent radioactivity in the sample was calculated as follows:

$$\frac{\text{counts/minute in bile or urine sample}}{\text{counts/minute in sample } T_4 \text{ or } T_3 \text{ injected}} \times 100$$

Extraction Procedure

The method reported by Flock, et al. (27) for the extraction of urine, bile, and plasma was used in this work. This extraction procedure consisted of placing 1 ml. of bile, urine, and plasma in a 50 ml. centrifuge tube. Four ml. of methanol:acetone (1:1 v/v) mixture was added. The centrifuge tube was stoppered and shaken vigorously for 30 seconds; approximately three minutes later the tube was reshaken and centrifuged.

The supernatant containing the thyroid hormones was removed and the precipitant in the centrifuge tube was washed with 2 ml. of metanol: acetone, shaken and recentrifuged. The second supernatant was pooled with the first and the solution then dried with reduced pressure at 30° C. All supernatant samples collected from plasma were dried over a nitrogen atmosphere. By this procedure, approximately 90 percent of the I^{131} was recovered in a protein-free residue which was suitable for chromatography.

Chromatographic Procedures

In order to obtain an estimate of the percentage of radioactive T_4 or T_3 and their metabolites in bile, urine, and plasma, both paper chromatography and paper electrophoresis were used. Ascending, one-dimensional chromatography was used throughout the experiments except for an occasional two-dimensional chromatogram for purposes explained later in this section.

Of the several solvents available, three were used for single-dimensional chromatography. The first of these solvents was butanol saturated with 2N ammonium hydroxide. This solvent has been used extensively by Roche and Michel (85) and was prepared following the directions given by Barker (7). This solvent was prepared by thoroughly equilibrating 250 ml. of n-butanol and 250 ml. of 2N ammonium hydroxide in a separatory funnel. The upper butanol layer was placed in a chromatography jar and the lower aqueous layer in a small beaker inside the same jar.

The second solvent used in this work was butanol, dioxane and 2N ammonium hydroxide in a 4:1:2 ratio. This solvent has been used extensively by Flock, et al. (27, 29) in a 4:1:5 ratio and by Nakano (73) in a 4:1:2 ratio. It was used in this work because of its ability to

resolve metabolites into narrow bands. Barker (7) stated this solvent had no advantages over butanol saturated with 2N ammonium hydroxide because the dioxane required rigorous purification and he noted little improvement by his use of it.

The third solvent, collidine and 3N ammonium hydroxide (100:35), was used to estimate the glucuronides present in bile. This solvent has two disadvantages which make it unfit for obtaining a quanitative estimate of T_4 or of T_3: (1) the redistillation of collidine before each use and (2) the breakdown of the thyronines (Taurog, et al., 110).

A fourth solvent used only for two-dimensional chromatography was tertiary amyl alcohol saturated with 2N ammonium hydroxide. This solvent has been used by Roche, et al. (91) and Flock, et al. (27) for separating the acetic acid derivatives of T_4 and T_3. This solvent was prepared by equilibrating 100 ml. of tertiary amyl alcohol, 80 ml. of water, and 20 ml. of concentrated ammonium hydroxide in a separatory funnel. The upper organic phase was placed in the jar and the lower aqueous layer was placed in a small beaker inside the tank. This latter step was an important factor in obtaining adequate separation of the iodinated compounds used in this experiment.

Two rectangular, Pyrex[R] chromatography jars measuring 53 cm. x 29 cm. x 8 cm. served as containers for the collidine and butanol saturated with 2N ammonium hydroxide solvents. A third and larger Pyrex[R] chromatography jar measuring 61 cm. x 30 cm. x 30 cm. served as container for the butanol, dioxane, ammonium hydroxide solvent. Large sheets of Whatman No. 1 MM filter paper covered three sides of the jars and were saturated with the developing solvent. This was found to aid separation of the compounds since it provided for more complete saturation of the air inside the jars and reduced the evaporation of the solvent from the chromatographic strips.

It was desirable to use fresh solvent for each run. This was especially true for the collidine solvent, but for the other two solvents not more than three runs were made without changing the solvent. A small amount of thiouracil (1 mgm. per 100 ml. of solvent) was added to each batch of solvent to prevent spurious oxidation of the iodide during chromatography (Galton and Pitt-Rivers, 36). Stopcock grease was used on the lid to produce an air-tight seal and prevent solvent evaporation from the container.

The dried extract obtained from the extraction procedures was dissolved in 0.5 ml. of 1:1 ethanol:0.5N ammonium hydroxide solution. Five to ten µl. of this dissolved extract were applied at the origin of a 1.5 by 18-25 inch strip of Whatman No. 3 MM filter paper. It was desirable to apply the sample in a line 20 mm. in length and not more than 5 mm. wide in order to obtain adequate separation of the various compounds in the sample. In the cases of urine and plasma, approximately 25 µl. could be added at the origin; however, only 5-10 µl. of bile could be spotted because of bile pigments which caused inadequate separation and distortion of the bands if present in a higher concentration.

On all chromatograms, 50-100 µgm. of unlabeled carrier was spotted with the experimental material; however, only two or three different carriers were spotted on any one chromatogram. After spotting the strips they were immediately placed in the chromatography jars to reduce the spontaneous deiodination of I^{131}-labeled thyroid hormones and derivatives on filter paper which has been reported by Taurog (106, 107). Rf values of the various iodinated compounds in the two solvent systems: (1) butanol, dioxane, and 2N ammonium hydroxide and (2) butanol saturated with 2N ammonium hydroxide are shown in Table II.

TABLE II

R_f VALUES FROM THE PRESENT INVESTIGATIONS OF VARIOUS IODINATED DERIVATIVES IN THREE DIFFERENT SOLVENT SYSTEMS

Compound	Collidine 3N Ammonium Hydroxide 100:35	Butanol Saturated 2N Ammonium Hydroxide	Butanol, Dioxane 2N Ammonium Hydroxide 4:1:2
T_4	0.56*	0.34	0.60
T_4A	----	0.45	0.72
T_3	0.65	0.54	0.76
T_3A	----	0.63	0.81
3:3':5'T_3	----	----	0.57
3:3'T_2	----	----	0.60
I^-	0.68	0.19	0.43
Glucuronides	0.10**	0.07	0.33

*Mean R_f of unlabeled carrier compound from five randomly selected, single-dimensional chromatograms developed in the given solvent.
**Mean R_f of labeled glucuronides calculated from five scanned chromatogram records.

Detection of Radioactivity

The distribution of radioactivity on the single-dimensional chromatograms was determined with a continuously recording chromatogram scanner* before staining. The carrier compounds were then located on the chromatograms with the proper stain or by ultra-violet light. All radioactive areas were marked off on the chromatograms, cut out, and counted in a well-type scintillation counter.** All areas were counted for a period of time that exceeded 10 times the background. The background was subtracted and the percent of radioactivity in each peak was calculated.

For the preparation of radioautograms, an aliquot of bile, plasma, or urine containing approximately thirty thousand counts per minute was applied near one corner of a 9 x 11 inch sheet of Whatman No. 3 MM filter

*Atomic Accessories Inc.
**Baird Atomic

paper. This sheet was rolled into a cylindrical form, stapled, and placed in a cylindrical, 18 inch high Pyrex[R] jar. The sheet was first developed by butanol, dioxane and 2N ammonium hydroxide solvent for 20 hours and then in tertiary amyl alcohol for 36 hours.

Radioautograms were made from both two-dimensional and single-dimensional chromatograms. In preparing the latter, aliquots of the respective samples were spotted at the origin on tapered Whatman No. 3 MM filter paper since it was found that as the compounds migrated they did not spread out as readily as on the 1.5 inch strips, i.e., each compound was confined to a relatively narrow band. Using this tapered paper as recommended by Albright (4), it was possible to separate two compounds of close R_f into two separate and distinguishable bands. In all cases, the samples on tapered Whatman paper were developed in butanol, dioxane and 2N ammonium hydroxide solvent.

Radioautographs were prepared by placing the chromatograms next to Kodak "no-screen" x-ray film for a period of time varying from six days to three weeks depending on the radioactivity added to the chromatograms. The films were developed by placing them in Kodak x-ray developer until the radioactive areas turned black, but never longer than four minutes. The films were fixed in Kodak x-ray fixer for eight minutes after which they were rinsed in water and sponge dried. The films were superimposed on the stained chromatogram and the radioactive area located and identified by the known carrier added to the chromatogram. Not all samples of bile, urine and plasma were radioautographed; only random samples were selected for radioautography.

Staining of Carriers

The chromatograms were stained with one of the following reagents:

(A) Palladium chloride (12): this reagent is specific for iodide and was prepared by adding 100 mgm. of the reagent to 100 ml. of water and allowing this to stand overnight before use.

(B) Pauly reagent (13): 5.0 ml. of 1.0 percent sulfanilamide in 10 percent hydrochloric acid and 1.0 ml. of a 5 percent sodium nitrite were mixed thoroughly in a 50 ml. graduated cylinder. N-butanol was added to the 50 ml. mark and the mixture shaken and allowed to stand for four minutes. The butanol was decanted and the chromatograms were dipped in this solution. After drying, they were dipped in 50 percent saturated sodium carbonate and dried. The tyrosine and thyronine derivatives gave characteristic orange and red colors with this reagent.

(C) Ceric sulfate-arsenious acid reagent (11): this stain is sensitive to concentrations of iodide and thyroid hormones as low as 0.04 ugm. A liter of 2 percent ceric sulfate in 1N sulfuric acid was prepared; a second solution of arsenious trioxide and 7 gm. sodium hydroxide in 500 ml. of water was prepared and then adjusted with sulfuric acid to pH 7. To this second solution, 42 ml. of sulfuric acid was then added and the resulting solution was diluted with water to 1 liter. These two reagents were stable when stored for as long as six months. Just before use the stain was prepared with one part each of water, ceric sulfate solution and arsenious acid solution. This stain was sprayed on as evenly as possible and allowed to dry partially. The chromatogram was then sprayed with 1.0 percent aniline in acetone solution. The presence of iodide and the thyronines was noted by white spots on a blue background.

(D) Ultraviolent Light: another method of locating the carrier compounds, especially the thyronines, was carried out by passing the chromatogram over an ultraviolet light. The thyronines would "quench" the illumination and appear as dark blue bands. The advantage of this method

was the speed with which the carriers could be located. The disadvantages of this method in locating carriers were decreased sensitivity, i.e., carriers had to be present in concentration of 50-100 μgm. before they could be seen, and some molecular agitation may have occurred under the ultraviolet light resulting in deiodination of the compound.

Electrophoresis

Electrophoresis of nonextracted bile, urine and plasma on Whatman No. 3 MM filter paper was carried out in order to obtain an accurate estimate of the iodide present in the three samples.

Five paper strips 1.5 inches by 22 inches containing the samples to be subjected to electrophoresis were placed so that the origin was near the negative pole and the metabolites would move towards the positive pole. The buffer used for this experiment was a 0.05M solution of ammonium carbonate pH 8.6. This buffer has been used extensively by Roche, et al. (89) who used it to separate the metabolic derivatives of T_4 and T_3. The paper strips were lightly moistened with the buffer by means of a small-bore 5 ml. pipette. The electrophoresis equipment with the paper strips were placed in a refrigerator so that the temperature could be held at 11°C $\pm$ 1°C. A potential of 400 volts and a current of 8-10 milliamps was applied across the strips for four hours. The strips were then removed, dried, and iodide located with palladium chloride.

The plasma samples which were collected at 1, 2, 3, and 4 hours were spotted at the origin together with the carrier iodide. Approximately 25 μl. of unextracted plasma could be spotted at the origin without difficulty. Additional plasma made the origin material increasingly insoluble in the buffer and consequently interferred with ionic migration.

Hydrolysis of the Conjugated I^{131} Compounds

Radioactive compounds suspected of being conjugates of T_3 and T_4 were hydrolysed with either beta glucuronidase* or with Mylase P,** which contains a phenol sulfatase.

In order to hydrolyze the conjugated metabolites, they had to be separated from the free thyronines in bile. Portions of the 50 percent alkaline ethanolic solution were spotted on five strips of Whatman No. 3 MM filter paper. These strips were developed for 20-24 hours in butanol, dioxane and 2N ammonium hydroxide and the radioactive regions on the strips were located with a strip counter. In this solvent system the glucuronides were located in the region with an R_f of 0.22-0.40. The region with R_f 0.40-0.50 included the metabolites conjugated as sulfate ester and free iodide. As suggested by Taurog, et al. (110), the sections of the filter paper strip corresponding to unknown peaks were cut off, tapered to a point at one end, and eluted with 0.02N ammonium hydroxide. The elutions were carried out in a cylindrical jar with a tight-fitting lid. It required approximately three hours to collect 80-90 percent of the radioactivity from the filter paper sections. Eluates containing the glucuronide conjugates were taken to dryness under reduced pressure and redissolved in 0.5 ml. of 0.2M phosphate buffer at pH 6.5. Two mgm. of beta glucuronidase were added to half of the solution; the remaining portion served as a control.

Column Chromatography

Because of the large concentration of bile pigments in chicken bile, only 10 ul. or less of bile could be subjected to paper chromatography.

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*Sigma Chemical Company
**Nutritional Biochemical Corporation

Any amount greater than 10 µl. resulted in distortion of the developing bands. Due to the small amount of radioactivity in 10 µl. (30,000 c/m), an estimate of the distribution of I^{131} in chicken bile was difficult to obtain using two-dimensional chromatography (Flock, et al., 5). For this reason, single-dimensional chromatography was used to obtain the percent distribution of radioactive metabolites in bile.

Another procedure, column chromatography, was used to a limited extent. Column chromatography (Flock, et al., 27) was of special value because of greater sample capacity. Following the procedure as outlined by Braasch, et al. (14) and Gross and Pitt-Rivers (43), a kieselguhr column was prepared by first heating the kieselguhr for four hours at 400°C. After cooling, the powder was mixed with 6N hydrochloric acid. Twenty-four hours later the acid was decanted and the kieselguhr washed well and dried. Glass columns with an internal diameter of 13 mm. and fitted with a stopcock were used. The lower part of the kieselguhr column was prepared by mixing 6 gm. of kielselguhr with 4.8 ml. of 0.5N sodium hydroxide saturated with 25 percent chloroform in n-butanol (v/v). Twenty-five ml. percent chloroform in n-butanol (v/v) saturated with 0.5N sodium hydroxide was then added until a thin slurry was obtained. The mixture was degassed. Glass wool was used at the tapered portion of the column. The degassed mixture was then added and packed in the column to a height of 15 cm. The dried extract of bile was dissolved in 0.5 ml. of 0.5N sodium hydroxide and was added to the column. The mixture was allowed just to penetrate the column and approximately 0.3 gm. of Gooch crucible asbestos was next packed on top of this first layer of kieselguhr. Another layer of kieselguhr (5 gm. of kieselguhr mixed with 4 ml. of base saturated with 20.0 percent chloroform in n-butanol) was then added on top of the column.

The effluent from the column was collected in 3.0 ml. fractions with a fraction collector.* The rate of flow of the solvent was adjusted so that 3 ml. was collected about every 12 minutes. The radioactivity in each tube was determined by counting each tube in a well-type scintillation counter. Peaks were identified by plotting count rate per tube against the tube number. Those tubes containing any given peak were pooled and dried under reduced pressure at 30°C. The dried eluant was dissolved in 50 percent ethanolic 0.5N sodium hydroxide solution and spotted on filter paper strips. The radioactive compounds were identified with appropriate carriers or, in the case of the conjugated metabolites, with specific hydrolytic enzymes, beta glucuronidase and Mylase-P.

*Micro Specialities Chemical Company

CHAPTER IV

RESULTS AND DISCUSSION

The Percent of Dose of I^{131} in Total Volumes of Bile and Urine Collected From Birds Injected with I^{131}-labeled T_3 or T_4

In order to determine which hormone was degraded and excreted at the greater rate, the percent of I^{131} excreted in four hours into urine and bile from $*T_4$-injected birds was compared with that from urine and bile respectively collected from birds injected with $*T_3$ (Table III). These comparisons were made within three groups of birds according to pattern of excretory product or products which were collected: Group I (bile), Group II (bile and urine), and Group III (urine). In Group I, an average of 6.0 ml. and 4.9 ml. of bile was collected respectively from five birds injected with $*T_4$ and five birds injected with $*T_3$. An average of 9.7 percent of the injected radioactivity was excreted into the bile of $*T_4$-injected birds in Group I, compared with 11.6 percent for the $*T_3$-injected birds in the same Group. The differences between the means for the volume of bile and I^{131} content in bile from birds injected with $*T_3$ and $*T_4$ were not significantly different at the 0.10 level of probability.

In Group II the average volumes of bile and urine collected from five birds injected with $*T_4$ were 6.1 ml. and 21.4 ml. respectively, while 4.1 ml. of bile and 52.2 ml. of urine were collected from birds injected with $*T_3$. The amount of bile collected from birds injected with $*T_4$ was significantly greater ($P < 0.10$) than the mean volume of bile from birds injected with $*T_3$. There was no significant difference between the

TABLE III

RADIOACTIVITY AND VOLUME OF BILE AND URINE COLLECTED FOR FOUR HOURS AFTER THE INJECTION OF I^{131}-T_4 OR I^{131}-T_3

Group	Hormone Injected	No. of Birds	Volume (ml.) Bile	P*	Volume (ml.) Urine	P	I^{131} Content (percent of dose) Bile	P	I^{131} Content (percent of dose) Urine	P
I	T_4	5	6.0** ±2.1	P>0.10	---		9.7 ±3.0	P>0.10	---	
	T_3	5	4.9 ±1.6		---		11.6 ±3.6		---	
II	T_4	5	6.1 ±2.0	P<0.10	21.4 ±8.9	P>0.10	7.8 ±4.0	P<0.10	3.1 ±1.4	P<0.10
	T_3	5	4.1 ±0.6		52.2 ±37.6		14.3 ±2.6		8.2 ±5.5	
III	T_4	5	---		15.8 ±9.3	P<0.005	---		2.1 ±0.8	P<0.01
	T_3	4	---		70.3 ±17.4		---		9.4 ±4.6	

*The differences between the means within each Group were tested for significance with "Student's" t-test. When P 0.10 the differences between the means are considered statistically significant, i.e., that there are two population means instead of one.
**Mean ± standard deviation.

volumes of urine excreted by the two subgroups of birds. The birds injected with $*T_4$ excreted 7.8 percent of the radioactivity into the bile and 3.1 percent into the urine, while $*T_3$-injected birds excreted an average of 14.3 percent into the bile and 8.2 percent of the total injected radioactivity into the urine. In Group II the mean percent of radioactivity excreted into bile and urine by $*T_3$-injected birds was significantly greater ($P < 0.10$) than the percent of I^{131} excreted by the $*T_4$-injected birds. In Group III those birds injected with $*T_3$ excreted a significantly greater volume of bile and a significantly greater percent of the radioactivity was excreted into the urine by these birds than those birds injected with $*T_4$ (Table III).

The results that have been obtained show that birds injected with $*T_4$ excreted a greater volume of bile than birds injected with $*T_3$. Little significance was attached to this difference although Gans and McEntee (37) observed that the thyroid hormones decreased bile flow in dogs. Their observation cannot be satisfactorily verified in this work since no bile was collected from chickens that were not injected with $*T_3$ or $*T_4$. Birds injected with $*T_3$ excreted less bile and a greater volume of urine in four hours than the birds injected with $*T_4$. Approximately 60 ml. of urine was excreted in four hours by the birds injected with $*T_3$ compared with 20 ml. of urine excreted in four hours by $*T_4$-injected birds. When these values were used to calculate urine volume excreted in 24 hours, $*T_4$-injected birds excreted 120 ml. and $*T_3$-injected birds excreted 360 ml. in a 24 hour period. According to Hester, et al. (51), previous investigators calculated urine flow for a short period of time, and found that mature chickens excreted as much as 500 ml. of urine in a 24 hour period. This estimate has been rejected by Hester, et al. (51) because mature birds consume less water than this per day and these investigators by

exteriorizing the ureters estimated that only 90 ml. $\pm$ 30 of urine was formed in 24 hours.

Since in this work no urine was collected from birds not injected with either $*T_3$ or $*T_4$, it was difficult to determine how the thyroid hormones affected urine formation although it appeared that $*T_3$ produced diuresis when compared to birds injected with $*T_4$. This problem requires further investigation concerning the average amount of urine excreted by mature birds per day and the action of physiological doses of T_3 or T_4 on urine flow before any conclusions may be derived.

The results from this work indicate that birds injected with $*T_3$ excreted a greater percent of the radioactivity into the bile and urine than did the birds injected with $*T_4$ in a four hour period, and that the main route of excretion was via the bile and not the urine. When these results were compared with mammals as to the route and rate of radioactivity excreted from injected $*T_3$ and $*T_4$, it was noted that birds and rodents were similar. Keating and Albert (59) observed in the rat that of the total $*T_3$-injected, 55 percent of the radioactivity was excreted into the urine as compared with only 36 percent when $*T_4$ was injected. A similar observation was made by Hatfield, et al. (46) in rats. These investigators observed that during a 72 hour period, 50 percent of the radioactivity of $*T_3$ or $*T_4$ was excreted into the urine and the remainder into the bile. Lang and Premachandra (61) observed that the percent of radioactivity excreted by $*T_3$-injected rats was 16.6 percent and that of $*T_4$-injected rats only 7.9 percent at the end of a four hour collection period. In the dog, $*T_3$ and $*T_4$ were excreted more rapidly in urine than in bile while the radioactivity of T_3 labeled with I^{131} was excreted more rapidly than $*T_4$ (Flock, et al., 25, 27). Although no literature was located which was related to the rate of excretion of $*T_3$ to $*T_4$ in man, Keating

and Albert (58) observed that little radioactivity from T_4 was excreted into the feces and that the major pathway of excretion of $*T_4$ was in the urine. In general, urinary elimination of the thyroid hormones exceed biliary elimination in all mammals which have been studied with the exception of the rodents.

In conclusion, it appears that birds and rodents resemble each other more in the route of thyroidal excretion than either of them resembles men or dogs. The latter statement has been supported by the work of Frey and Albert (33) who observed that day-old chicks, injected subcuteneously with $*T_4$, excreted this hormone slowly. The greatest amount of radioactivity was located in the stomach, liver and intestines with the least in the kidneys. They postulated that the major route of excretion was the gastro-intestinal tract and noted that their findings were similar to previous work on the rat.

The Percent of Iodinated Organic Compounds in Chicken Plasma at Various Time Intervals After Injection of T_3 or T_4 Labeled with I^{131}

In order to investigate the observation (49) that the biological half-lives of T_3 and T_4 were equal in chicken plasma, the percent of T_3 and T_4 was determined in plasma for four hours following the injection of $*T_3$ or $*T_4$. In the experimental design, birds were divided into four primary groups: Group I (bile collected), Group II (bile and urine collected), Group III (urine collected) and Group IV (sham-operated). Each group of birds was further subdivided into birds injected with either $*T_4$ or $*T_3$. Plasma was collected at various time intervals and subjected to electrophoresis which separated inorganic iodide from the iodinated organic compounds (Figures 2 and 3).

A quantitative estimate of the iodide and the iodinated organic

TABLE IV

PERCENT DISTRIBUTION OF I^{131} IN PLASMA SAMPLES COLLECTED FOUR HOURS AFTER INJECTION OF I^{131}-T_4 OR I^{131}-T_3

Group	Hormone Injected	No. of Birds	I^-	Iodinated Organic Compounds
I (bile)	T_4	5	16.5* ± 5.8	83.5[a]** ± 5.8
	T_3	5	31.0 ± 9.6	68.9[b] ± 9.6
II (bile and urine)	T_4	5	42.7 ± 8.9	57.3[bc] ± 8.9
	T_3	5	69.0 ±10.2	31.0[e] ±10.2
III (urine)	T_4	5	52.1 ±16.8	47.8[cd] ±16.8
	T_3	4	60.9 ± 3.8	39.0[de] ± 3.8
IV (sham operated)	T_4	5	12.2 ± 2.2	87.7[a] ± 2.2
	T_3	5	49.8 ± 9.8	50.1[cd] ± 9.8

*Mean ± Standard Deviation

**Duncan's multiple range test of the differences between the means of the Iodinated Organic Compounds. Values with the same letter are not significantly different from each other at the 5 percent level of probability. For example the iodinated organic compounds in T_3-injected birds in Group II and III are not significantly different.

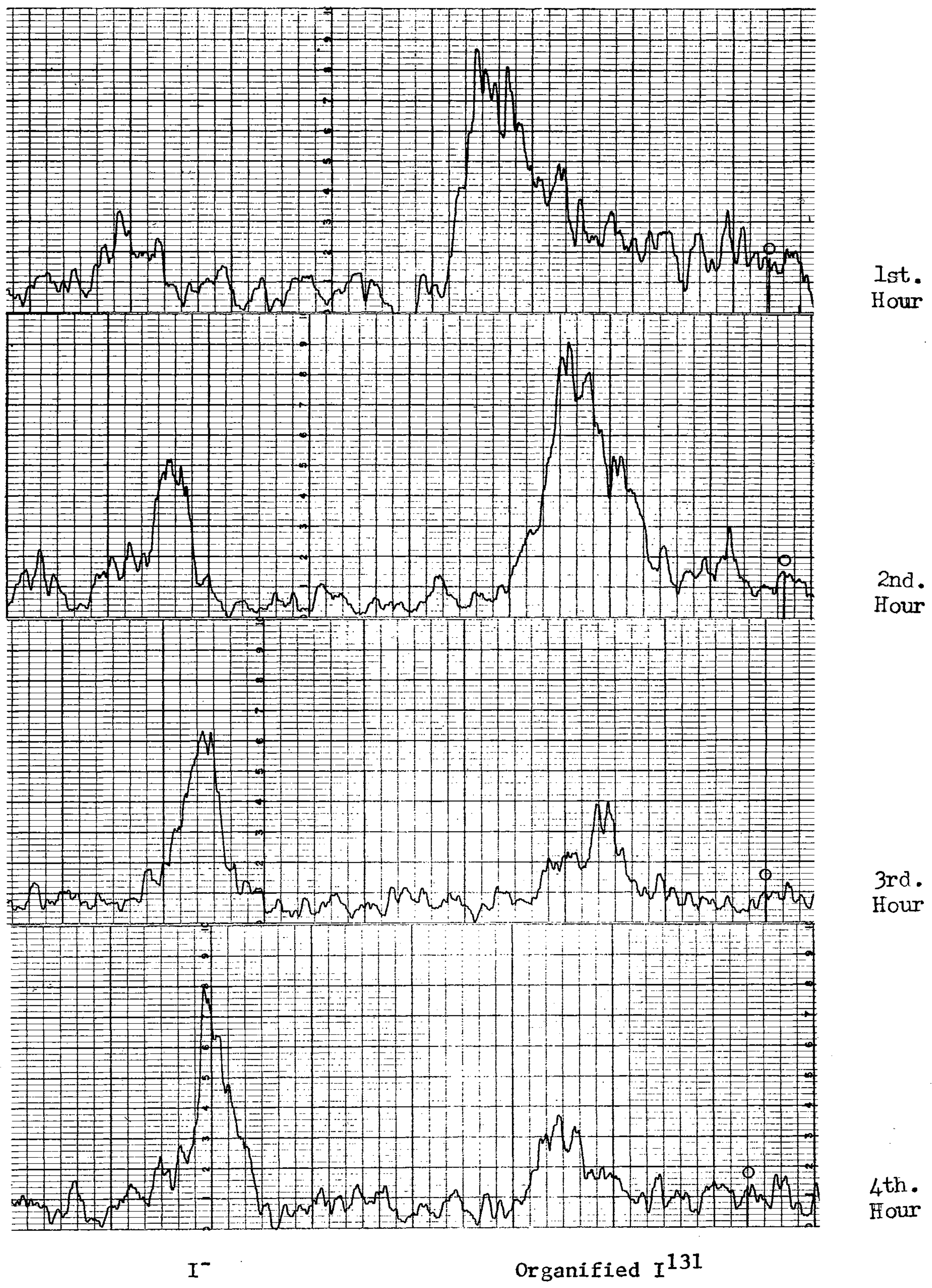


Figure 2. Electrophoretograms Showing Distribution of I^{131} in Plasma at Different Time Periods after Injection of $*T_3$ into Chickens. O=Origin.

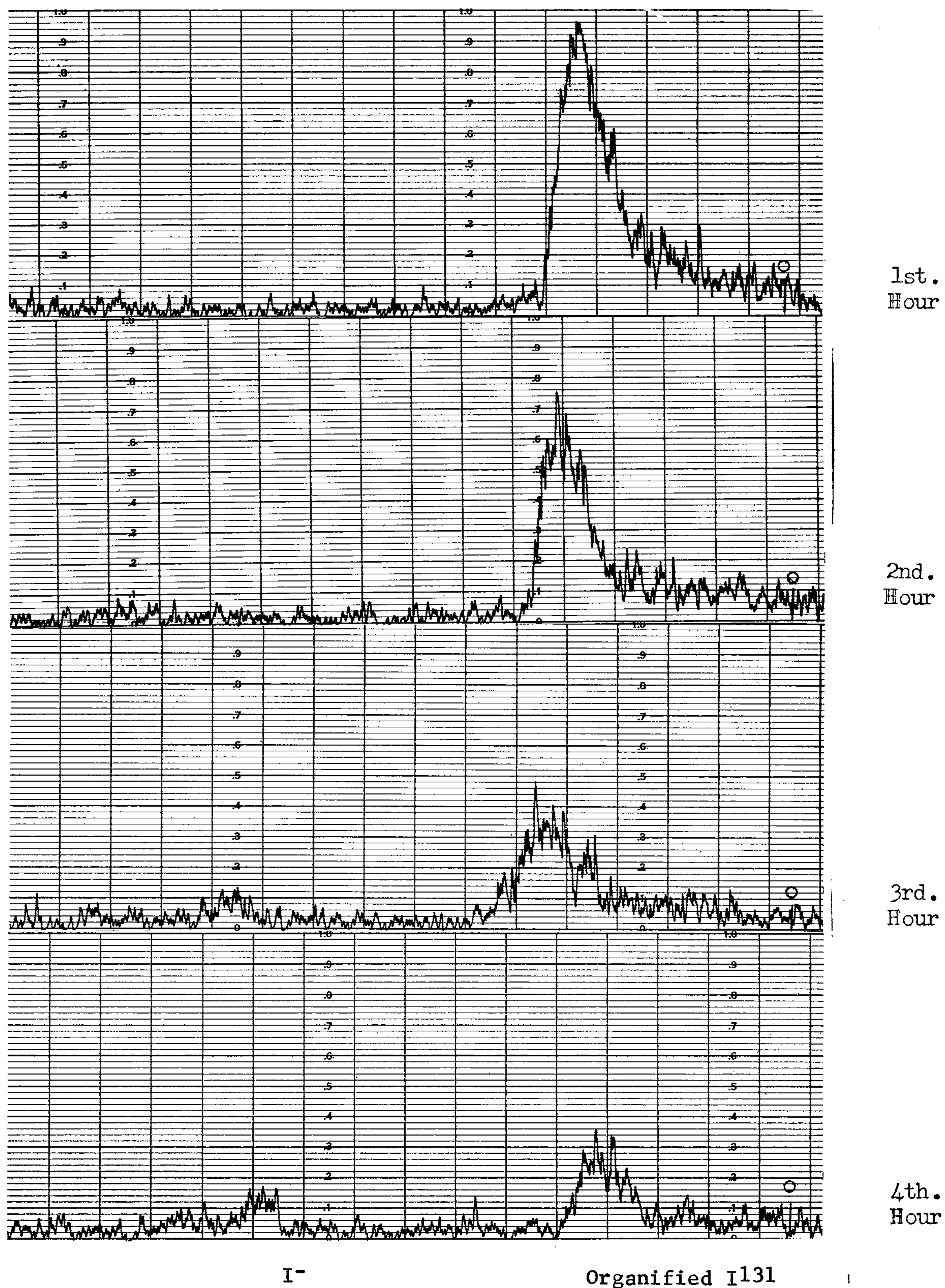


Figure 3. Electrophoretograms Showing Distribution of I^{131} in Plasma at Different Time Periods after Injection of $*T_4$ into Chickens. O=Origin.

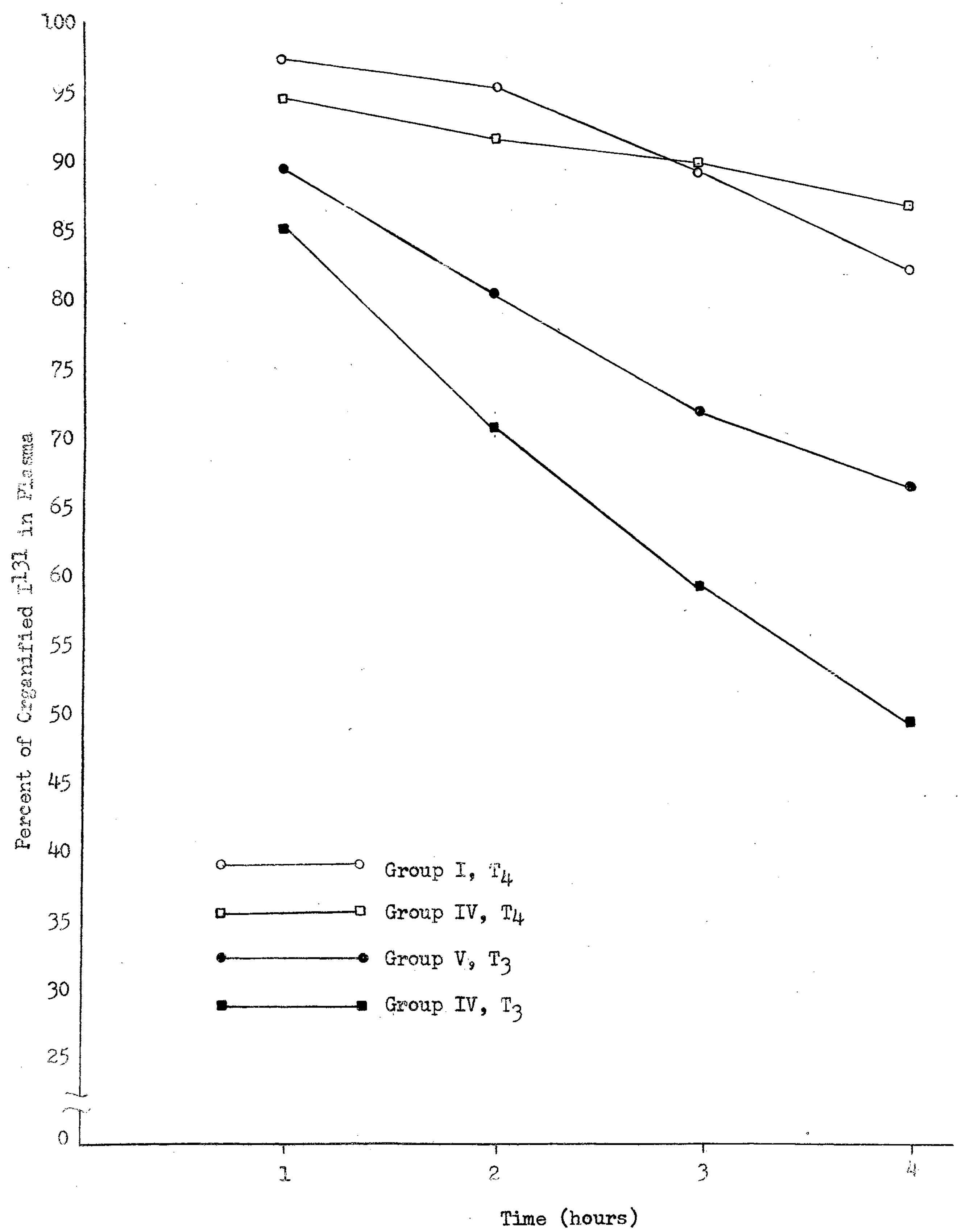


Figure 4. Percent of Organified I^{131} in Plasma at Different Time Intervals after Intravenous Injection of I^{131}-Labeled T_3 or T_4 into Two Groups of Chickens.

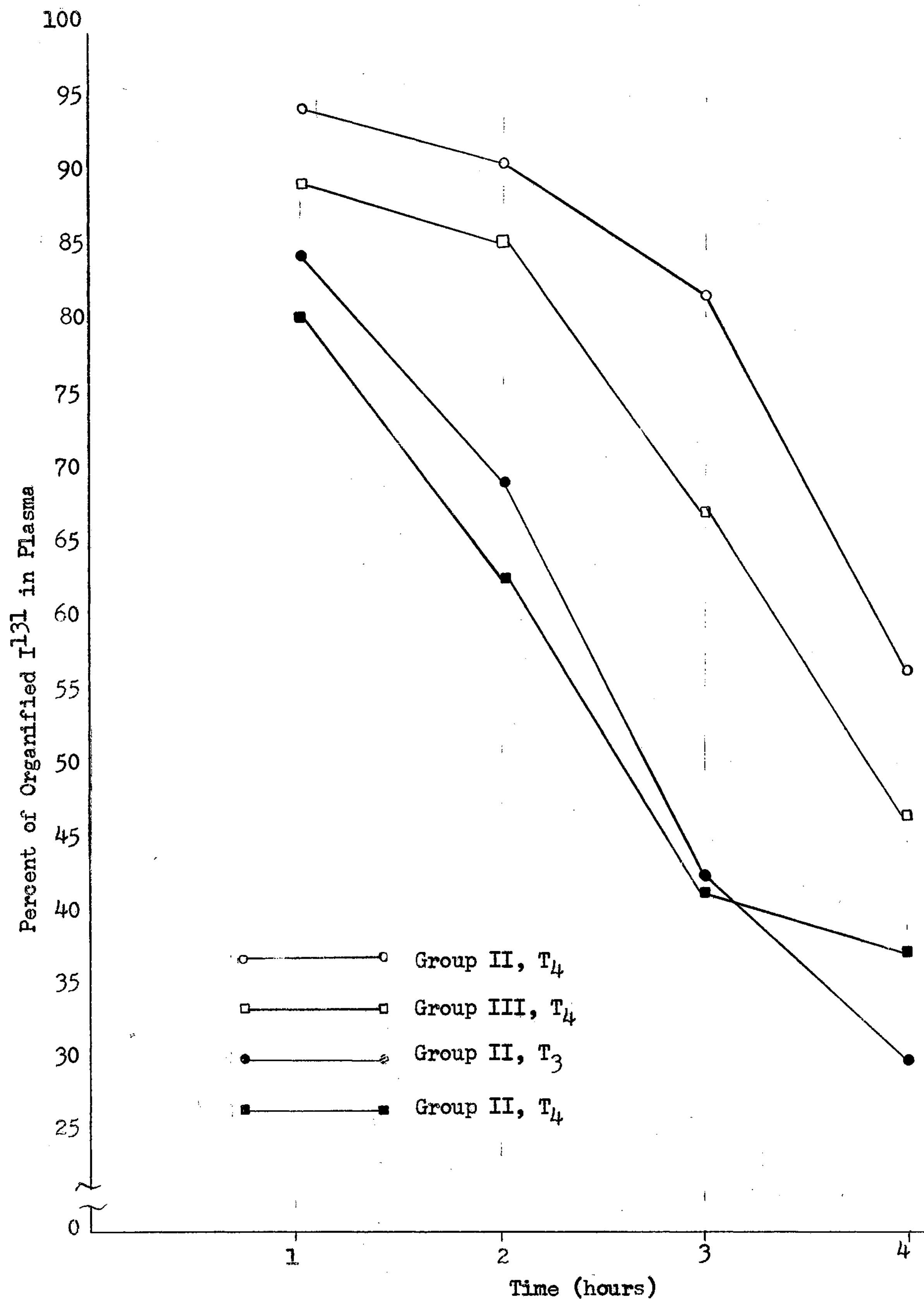


Figure 5. Percent of Organified I^{131} in Plasma at Different Time Intervals after Intravenous Injection of I^{131}-Labeled T_3 or T_4 into Two Groups of Chickens.

compounds present on the electrophoretic strips was made and expressed as a percent of total radioactivity on the strip. In general, the percent of iodinated organic compounds in the plasma decreased with time in all birds injected with either $*T_3$ or $*T_4$ and the percent of iodinated organic compounds in the plasma of birds injected with $*T_3$ decreased at a more rapid rate than the same parameter in $*T_4$-injected birds (Figures 4 and 5). For each group the mean percent of iodinated organic compounds in plasma collected four hours after the injection of $*T_3$ or $*T_4$ was calculated and subjected to an analysis of variance. Duncan's multiple range test was utilized to locate any significant differences in the mean percent of the iodinated organic compounds in the plasma collected from birds in all groups injected with either $*T_3$ or $*T_4$ (Table IV).

From this statistical analysis, it was observed that the mean percent of iodinated organic compounds in the plasma of birds injected with $*T_3$ at four hours was significantly less ($P<0.05$) than the same parameter in $*T_4$-injected birds in all groups with the exception of Group III. There was a significant decrease ($P<0.05$) in the mean percent of iodinated organic compounds in the plasma of birds injected with $*T_4$ in Groups II and III compared with plasma from birds in Groups I and IV that were also injected with $*T_4$. In Group I the plasma from birds injected with $*T_3$ contained a significantly greater percent of iodinated organic compounds when compared with plasma collected from $*T_3$-injected birds of Groups II, III, and IV. It appeared that the collection of urine resulted in a significant decrease in the percent of iodinated organic compounds; however, in Group IV the mean percent of iodinated organic compounds was not significantly different from the same parameter in the plasma of birds in Group III injected with $*T_3$.

In the plasma of birds injected with $*T_3$ or $*T_4$, the iodinated

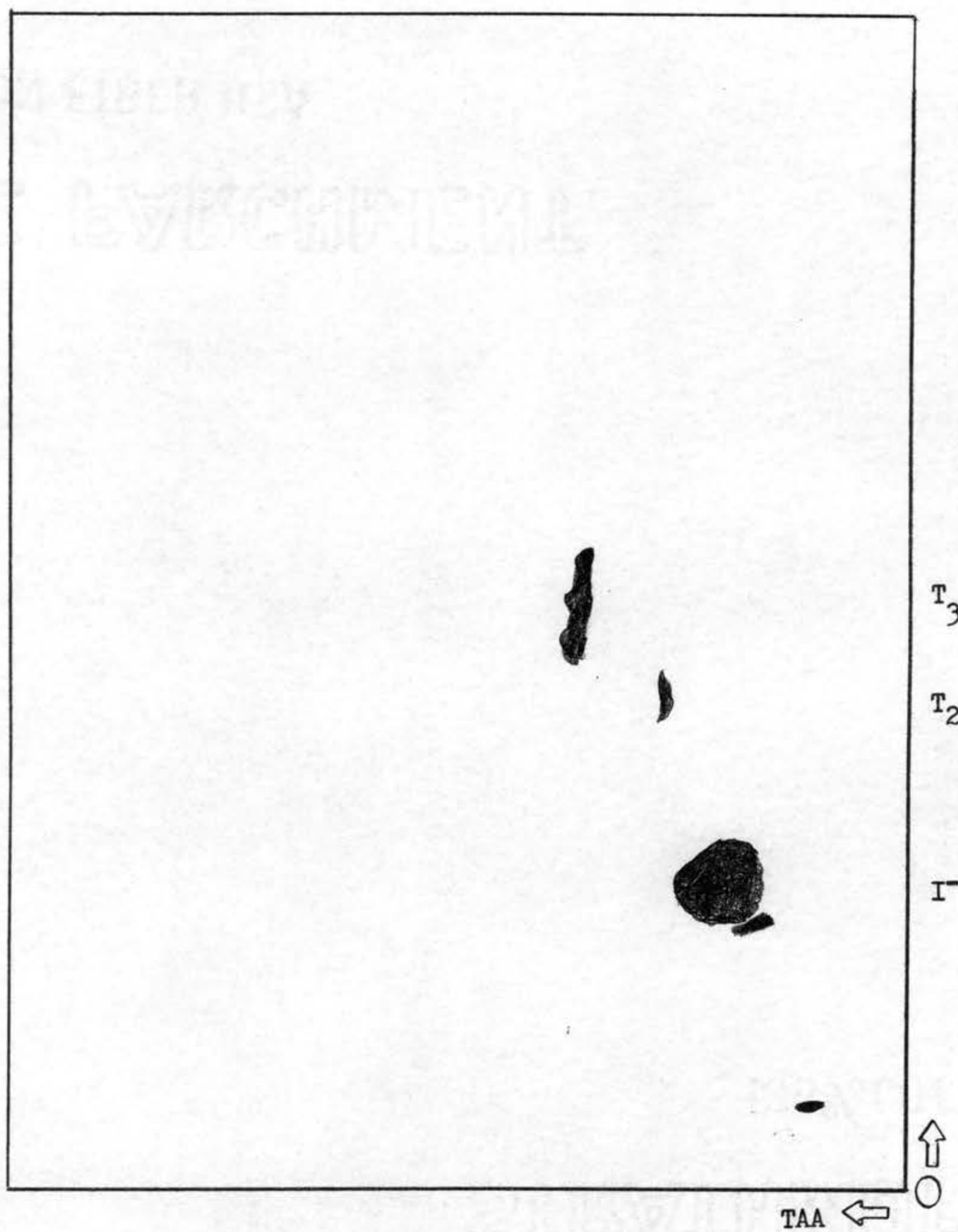


Figure 6. Radioautograph of Two-Dimensional Chromatogram from Plasma of $*T_3$-Injected Chickens. Solvents: butanol, dioxane, 2N ammonium hydroxide (4:1:2) and tertiary amyl alcohol saturated with 2N ammonium hydroxide.

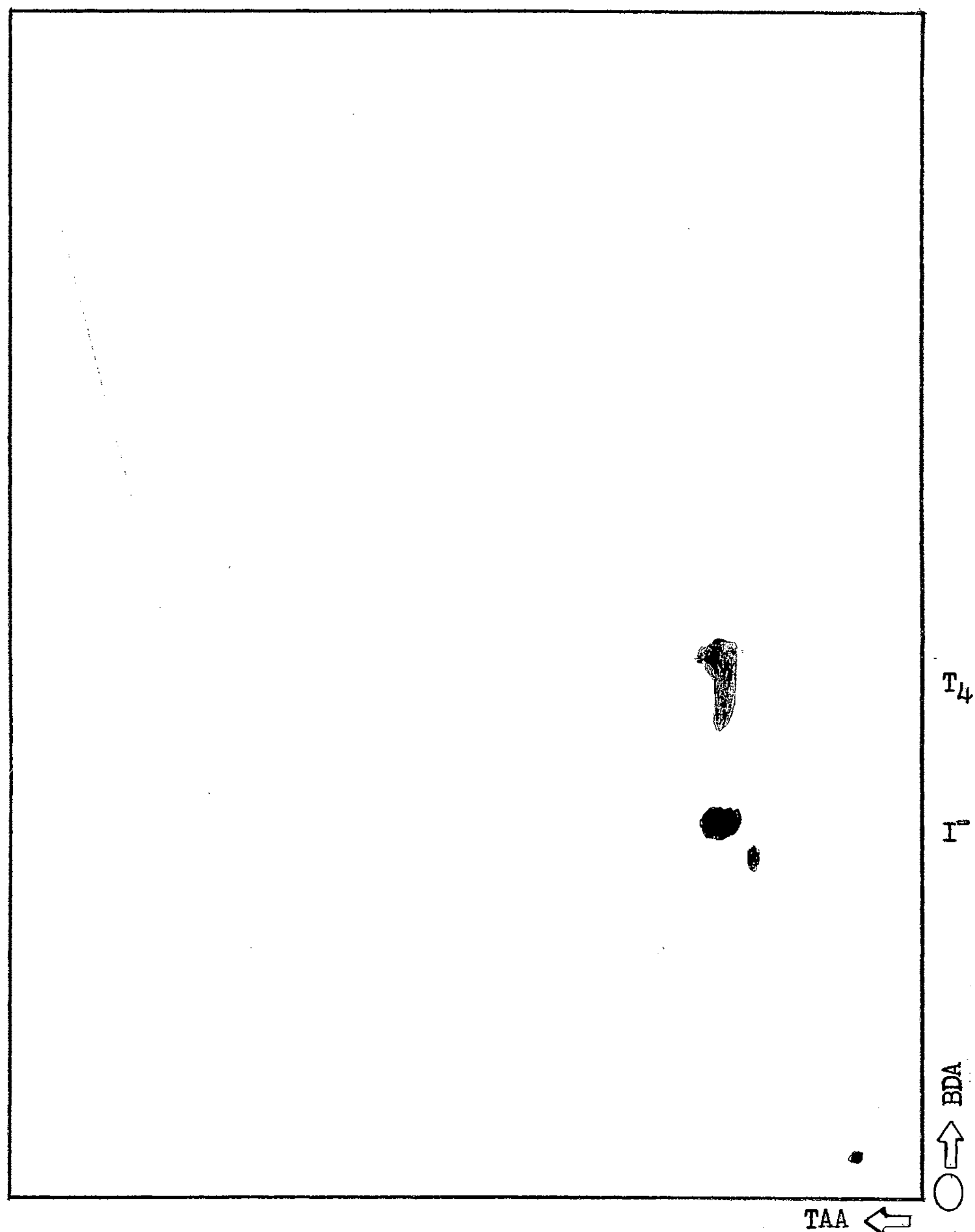


Figure 7. Radioautograph of Two-Dimensional Chromatogram from Plasma of $*T_4$-Injected Chickens. Solvents: butanol, dioxane, 2N ammonium hydroxide (4:1:2) and tertiary amyl alcohol saturated with 2N ammonium hydroxide.

organic compounds consisted primarily of the injected hormone, $*T_3$ or $*T_4$, although other organic compounds besides the thyroid hormones were observed (Figures 5 and 6). Traces of T_2 were identified in the plasma of birds injected with $*T_3$ and a trace of a possible conjugated metabolite was seen in the plasma of birds injected with either $*T_3$ or $*T_4$.

Binding of thyroid hormones with plasma protein will first be considered in discussing the observation that the percent of organified I^{131} in the plasma of birds injected with $*T_3$ decreased at a significantly greater rate than the percent of organified I^{131} in the plasma of birds injected with $*T_4$. Tata and Shellabarger (96, 103) observed that the two thyroid hormones were bound with equal affinity to the plasma proteins and disappeared from the whole body at a similar rate. Heninger, (49) using a different technique, observed that T_3 was not bound to chicken plasma proteins as firmly as T_4 was bound. However, in support of Tata and Shellabarger's work, he found no difference in the half-lives of the two hormones in chicken plasma. Dubowitz, et al. (19) found that the binding of T_3 to the plasma protein of chickens at physiological pH was less than the binding of T_4 at the same pH. The latter observation does not support Tata and Shellabarger's hypothesis (96, 103), that T_3 to T_4 potency was due to the speed at which the hormone leaves the circulatory system, which was dependent on plasma protein binding of the hormones. In mammals, T_3 was not bound as firmly as T_4 to the plasma proteins, therefore T_3 left the circulatory system at a greater rate than T_4. The results obtained from this work indicated that the percent of T_3 in chicken plasma decreased at a greater rate than T_4 in a four hour period. Consequently, the observations made by Heninger (49) and by Dubowitz, et al. (19) that T_3 was not bound as firmly to the plasma proteins as T_4 in birds are partially supported by the present work.

No published information was found concerning the distribution of I^{131} in the plasma of birds that had been injected with either thyroid hormone. The only work found was the observation made by Wentworth and Mellen (117) who noted that T_4 constituted 60 percent and T_3 40 percent of the total hormonal radioactivity in the plasma of I^{131}-injected birds. These differences between T_4 and T_3 were not significant at the 10 percent level of probability.

The second observation, that with the collection of urine the hormonal radioactivity in plasma was significantly less than when urine was not collected, is difficult to interpret. The volume of urine collected from $^*T_4$-injected birds was normal according to Hester, et al. (51) but was high for $^*T_3$-injected birds. The only explanation offered for the reduction in the relative percent of $^*T_3$ and $^*T_4$ in the plasma of the birds in Groups II and III was the loss of water from the intravascular system. In the birds of Groups I and IV in which urine was not collected, water was presumably reabsorbed from the urine in the cloaca and the urine was concentrated as in the normal bird.

The Distribution of I^{131} in Urine

The urine collected from birds in Group II and Group III was subjected without extraction to electrophoresis. When the electrophoretic strips were scanned, only iodide appeared to be present. However, when the urine was extracted, concentrated and developed on chromatographic strips in butanol saturated with 2N ammonium hydroxide, traces of iodinated organic compounds were present. The percent of iodide and these compounds can be seen in Table V. A trace of glucuronide conjugate was present in urine from both Group II and Group III birds. The conjugated metabolite accounted for approximately 6.0 percent of the radioactivity in the urine

TABLE V

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF URINE COLLECTED IN FOUR HOURS AFTER BIRDS WERE INJECTED WITH I^{131}-T_4 OR I^{131}-T_3
(Chromatograms developed in butanol saturated with 2N ammonium hydroxide.)

Compounds	Group II			Group III		
	T_4 Injected	T_3 Injected	P	T_4 Injected	T_3 Injected	P
Origin + Glucuronides	2.1** ±2.4	5.0 ±8.0	P>0.10	6.2 ±6.1	6.0 ±4.0	P>0.10
Parent Hormone	0.4 ±0.7	3.0 ±3.1	P>0.10	3.0 ±4.6	2.5 ±3.1	P>0.10
I^-	97.3 ± 2.6	91.8 ± 6.8	P>0.10	90.7 ± 6.8	91.4 ± 3.5	P>0.10

*The differences between the means within each Group were tested for significance with "Students" t-test. No significant differences were found between the means at the 0.10 level of probability, i.e., there are not two population means.
**Mean ± standard deviation of five estimations for each compound.

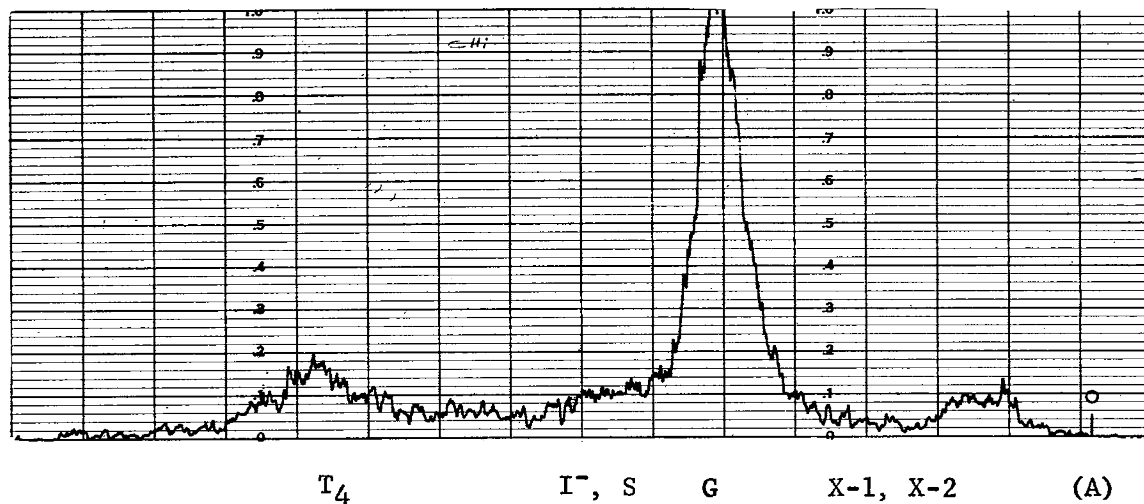
from birds in Group III injected with either $*T_3$ or $*T_4$. The parent hormone represented as T_4 or T_3 accounted for a trace of the radioactivity in urine. The major metabolite present in the urine of Groups II and III accounted for 90 percent or more of the radioactivity in the urine of both $*T_3$ and $*T_4$-injected birds. There was no significant difference between the mean percent of these metabolites in birds injected with $*T_3$ or $*T_4$; however, $*T_3$-injected birds excreted a greater volume of urine and a greater percent of radioactive iodide during a four hour period.

The distribution of iodide and iodinated organic metabolites in urine following the intravenous injection of $*T_3$ or $*T_4$ in birds, Table V, agreed with the percent distribution of these metabolites found in mammals. In man, Keating and Albert (58) observed that 95.0 percent of radioactivity in urine was inorganic iodide and 5.0 percent was an iodinated organic compound or compounds following intravenous injection of inorganic I^{131}. Flock, et al. (22, 28) observed in rats and dogs injected with either $*T_4$ or $*T_3$ that the major metabolite in urine was iodide with a trace of the parent hormone and glucuronide-conjugated metabolites. Therefore, it appears that iodide is the major metabolite in the urine of birds injected with I^{131} labeled thyroid hormones as it is in mammals.

Metabolites of Thyroid Hormones and Their Distribution in Chicken Bile

Since the liver plays an important role in mammals in the degradation and excretion of the thyroid hormone according to Pitt-Rivers and Tata (79), an investigation was undertaken to identify and determine the percent distribution of the hepatic metabolic products of T_3 and T_4 found in chicken bile.

Bile was collected for four hours after the intravenous injection of



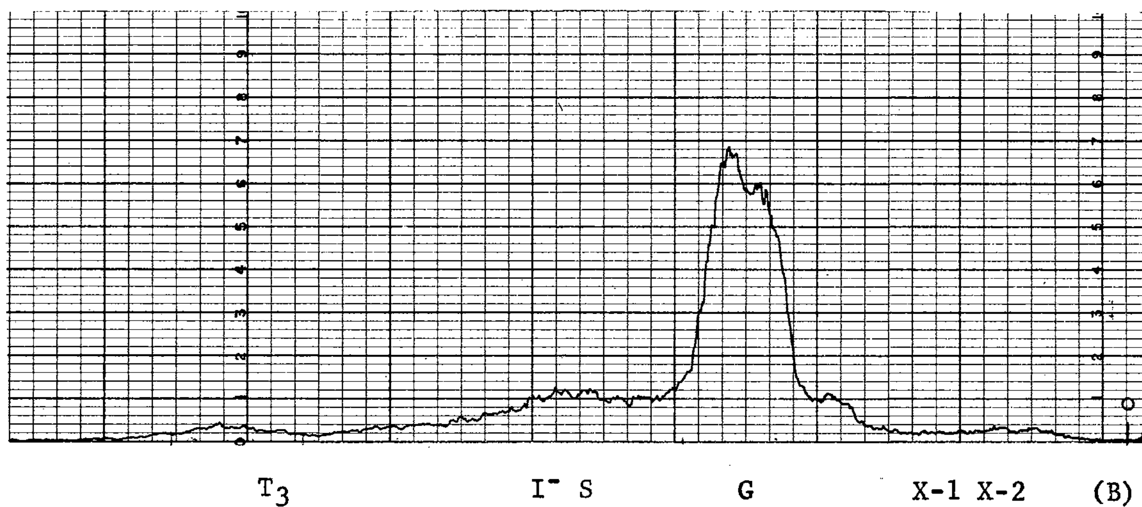


Figure 8. Scanned Chromatogram Records of Bile Collected for Four Hours after Intravenous Injection of $*T_4$ (A) and $*T_3$ (B). Solvent: butanol, dioxane, 2N ammonium hydroxide. O=Origin, X-1 and X-2=Unknowns, G=Glucuronides, and S=Sulfates.

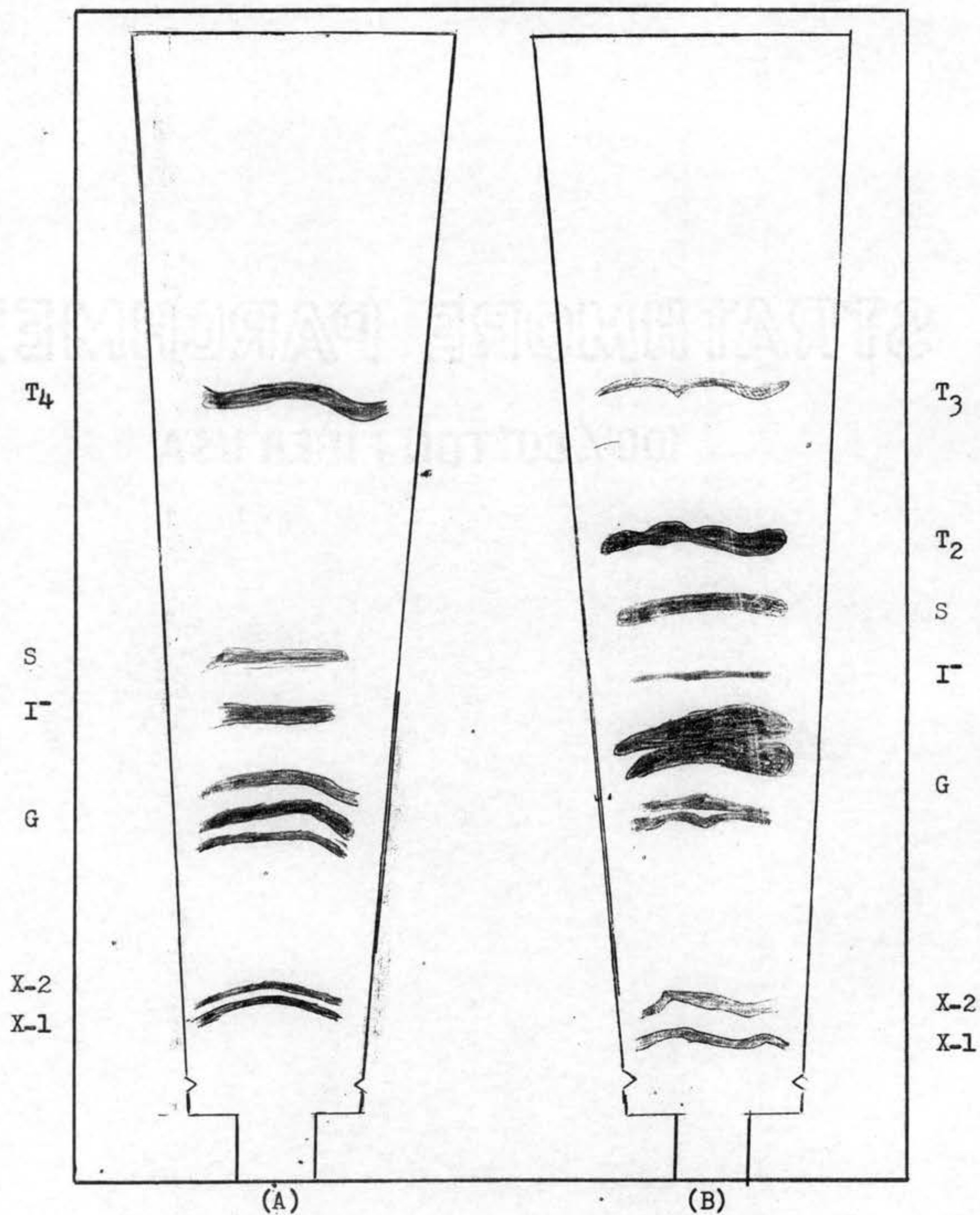


Figure 9. Radioautographs of Chromatograms of Bile Collected for Four Hours After Chickens were Injected with I^{131}-Labeled T_4 (A) or T_3 (B). Solvent: butanol, dioxane, 2N ammonium hydroxide (4:1:2).

$*T_3$ or $*T_4$. The iodinated products were then extracted and subjected to chromatographic analysis in three different solvent systems: butanol, dioxane, 2N ammonium hydroxide, butanol saturated with 2N ammonium hydroxide and collidine with 3N ammonium hydroxide. A scanned chromatogram record of bile from birds injected with $*T_3$ and $*T_4$ may be seen in Figure 8. Most of the radioactive peaks in Figure 8 have been identified with single or two-dimensional chromatography with known carrier compounds and by hydrolysis of the conjugated compounds with either beta glucuronidase or Mylase P (Figures 9, 10, 11, 12). Under the conditions used for detection of the radioactive metabolites, as many as ten radioactive metabolites were seen in the bile of birds injected with $*T_3$ (Figure 9). Only six metabolites were observed on the radioautograms of bile from $*T_4$-injected birds; however, three additional metabolites were identified with two-dimensional chromatography.

Near the origin of the chromatograms of bile collected from birds injected with $*T_3$ or $*T_4$, two radioactive unknown compounds were observed. These products were called Unknown 1 (X-1) and Unknown 2 (X-2) because when they were incubated with beta glucuronidase or Mylase P, they did not hydrolyze although they were partially deiodinated during hydrolysis to inorganic iodide. The conjugated glucuronides migrated just in front of Unknowns 1 and 2. In birds injected with $*T_4$, the glucuronides were hydrolyzed (Figure 10) and appeared as conjugates of T_4, T_3, reverse T_3 (3:3':5' T_3) and the acetic acid derivative of T_4, (T_4A). In the bile of $*T_3$-injected birds, the glucuronides were hydrolyzed to the iodinated compounds T_3, 3:3' T_2, T_3A and an unknown which was not identified (Figure 11).

Inorganic iodide was present in the bile from birds injected with either $*T_3$ or $*T_4$. Another conjugated metabolite was identified which

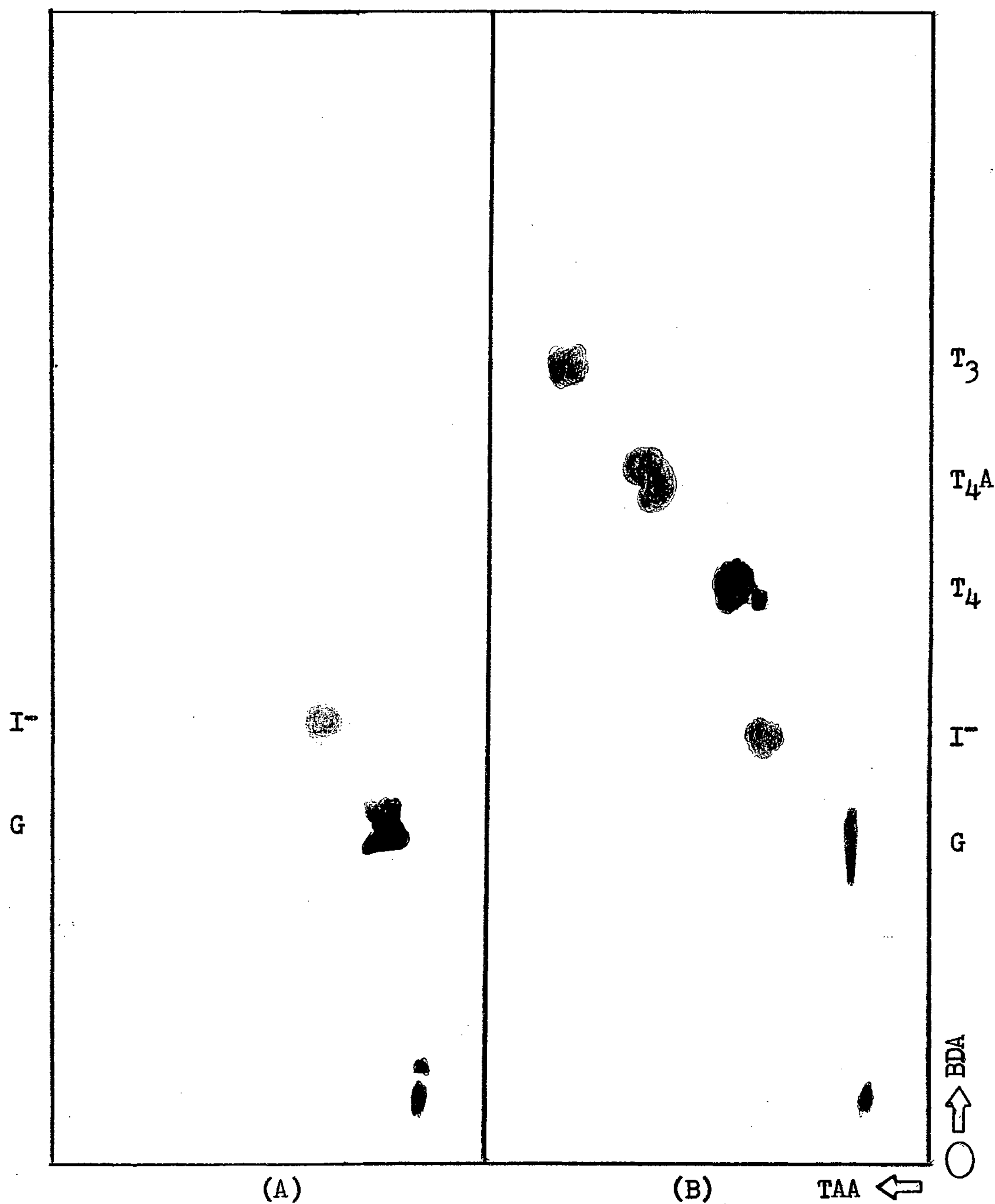


Figure 10. Radioautographs of Two-Dimensional Chromatograms of Eluted Glucuronides from Bile of *T_4-Injected Chickens. (A) Control Glucuronides (B) Hydrolysis of Glucuronoconjugates with Beta Glucuronidase. Solvents: butanol, dioxane, 2N ammonium hydroxide (4:1:2) and tertiary amyl alcohol saturated with 2N ammonium hydroxide.

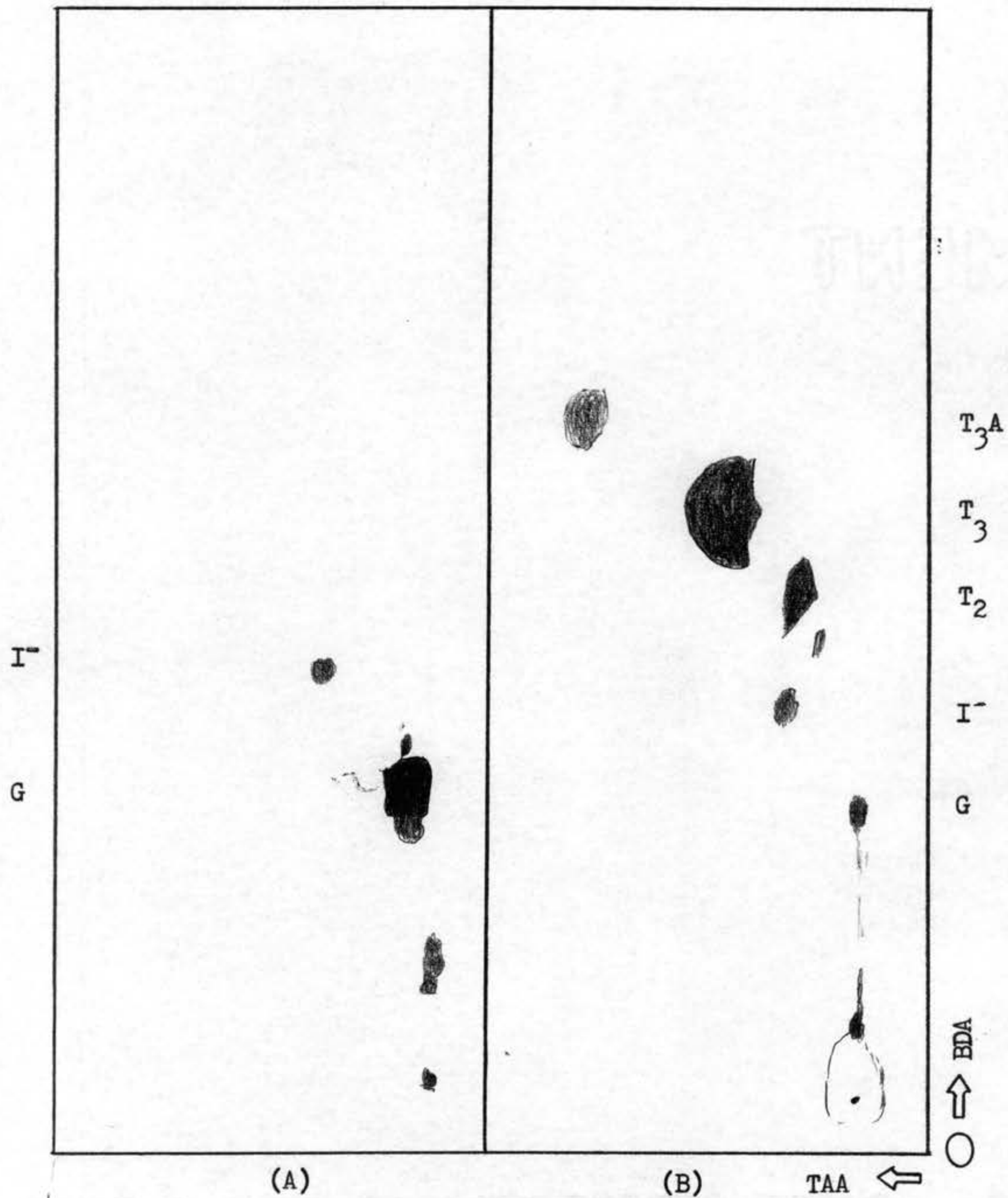


Figure 11. Radioautographs of Two-Dimensional Chromatograms of Eluted Glucuronides from Bile of $*T_3$-Injected Chickens. (A) Control Glucuronides (B) Hydrolysis of Glucuronoconjugates with Beta Glucuronidase. Solvents: butanol, dioxane, 2N ammonium hydroxide (4:1:2) and tertiary amyl alcohol saturated with 2N ammonium hydroxide.

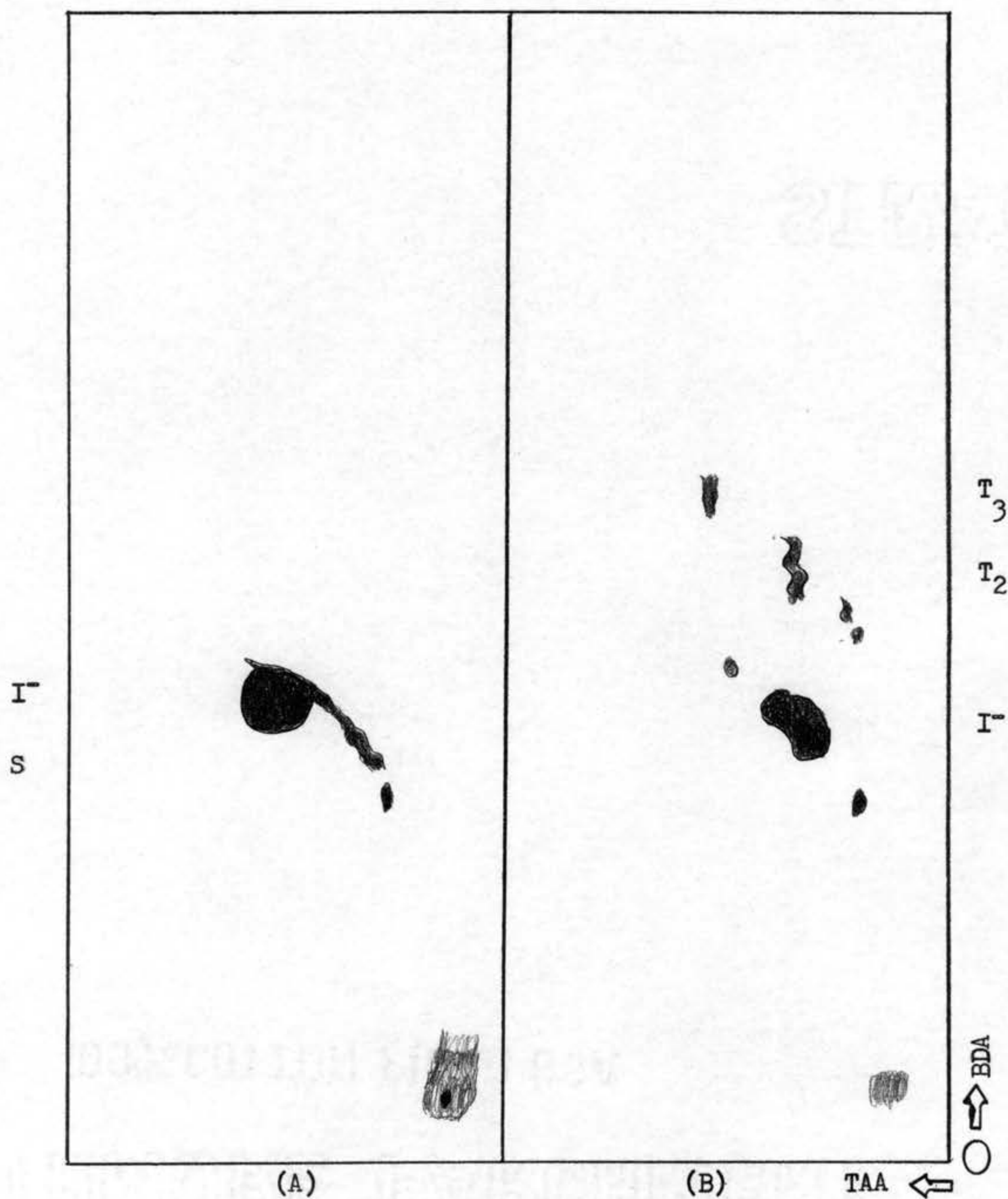
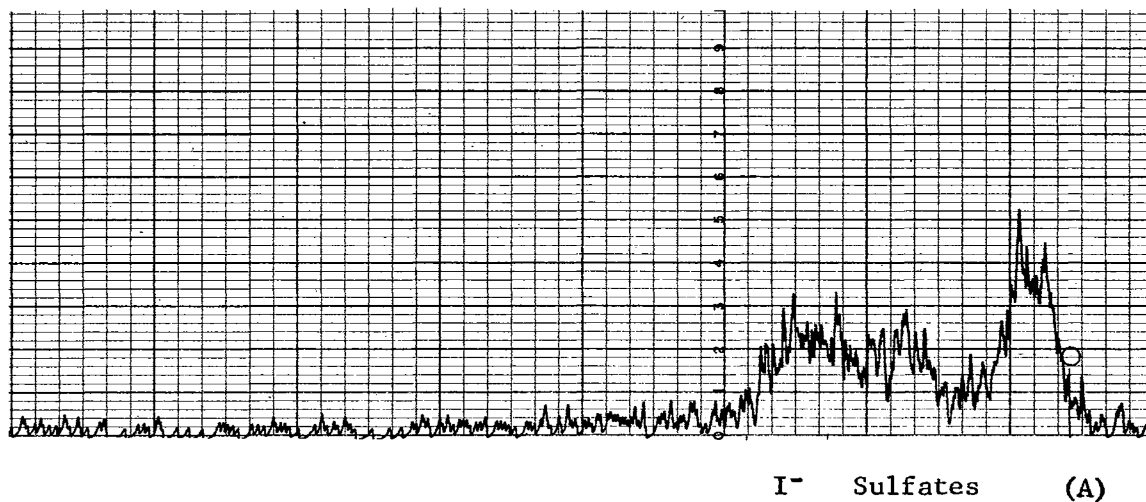


Figure 12. Radioautographs of Two-Dimensional Chromatographs of Eluted Sulfates from Bile of *T_3-Injected Chickens. (A) Control Sulfates (B) Hydrolysis of Sulfates with Mylase P. Solvents: butanol, dioxane, 2N ammonium hydroxide (4:1:2) and tertiary amyl alcohol saturated with 2N ammonium hydroxide.



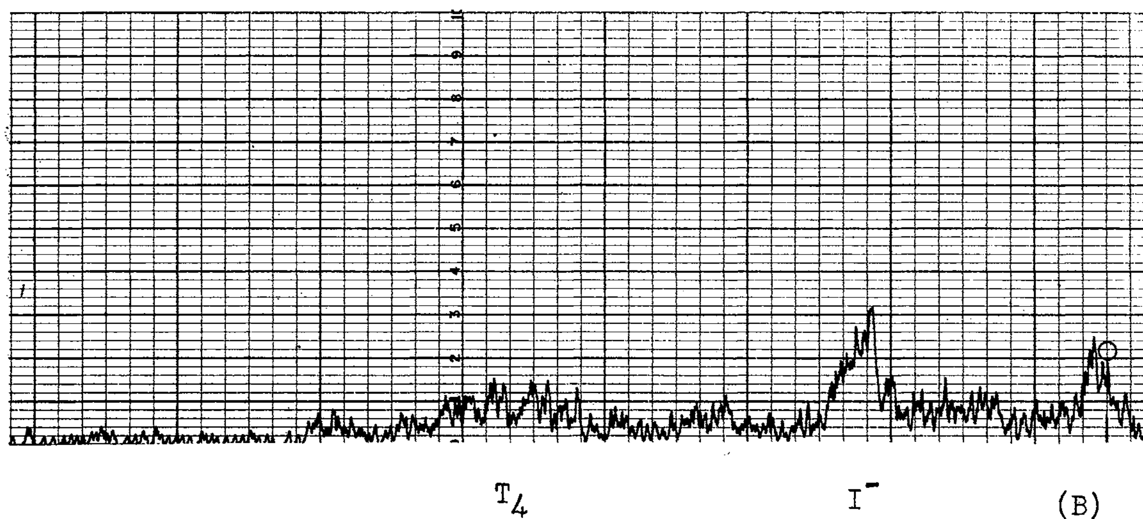


Figure 13. Scanned Chromatogram Record of Sulfate Peak from the Column Chromatogram of Bile from $^*T_4$-Injected Chicken. (A) Control Sulfates (B) Hydrolysis of Sulfates with Mylase P. Solvent: butanol saturated with 2N ammonium hydroxide. O=Origin.

migrated with inorganic iodide in the solvent system; butanol, dioxane and 2N ammonium hydroxide. This conjugate when hydrolyzed with Mylase P appeared as T_3 and T_2 in *T_3-injected birds (Figure 12) and when the conjugate from birds injected with *T_4 was hydrolyzed, the hydrolytic products were T_4 and possibly a trace of reverse T_3 (Figure 13). Since Mylase P is specific for hydrolyzing the sulfate ester of a phenol group, this conjugate was probably a sulfate-bound metabolite. In the same solvent system (butanol, dioxane, and 2N ammonium hydroxide), a free thyronine was identified migrating just in front of inorganic iodide and the sulfate region. In the bile of birds injected with either *T_4 or *T_3, these thyronines corresponded to reverse T_3 or T_2 respectively, as identified with known markers using two-dimensional chromatography.

The parent hormone was identified in the bile of birds injected with *T_4 or *T_3 with known markers of T_3 and T_4. Only traces of free T_3A and T_4A were present in the bile of *T_3 and *T_4-injected birds. In this solvent (butanol, dioxane and 2N ammonium hydroxide), the acetic acid derivatives migrated just ahead of the parent hormones.

The percent distribution of the iodinated metabolites in the bile of birds injected with *T_3 or *T_4 has been estimated with three different chromatographic solvents. The results of these estimations may be seen in Tables VI, VII, and VIII. By comparing the percent distribution of the iodinated metabolites in these three solvents, a large variation may be seen between each metabolite in the same group of animals. These variations in the estimates of the same metabolite in different solvents occur for two reasons: (1) Single-dimensional chromatography of bile adequately separated only two or three of the iodinated metabolites. Consequently, the percent that was calculated for a single metabolite may have included a fraction of the adjacent metabolites. (2) The percent of a

TABLE VI

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE COLLECTED IN FOUR HOURS AFTER BIRDS WERE INJECTED WITH I^{131}-LABELED T_4 OR T_3
(Chromatograms developed in butanol, dioxane and 2N ammonium hydroxide.)

Compounds	Group I			Group II		
	T_4 Injected	T_3 Injected	P	T_4 Injected	T_3 Injected	P
Unknown 1 + Unknown 2	16.3** ± 2.2	7.8 ±2.9	P<0.025	17.1 ± 5.1	7.1 ±1.8	P<0.025
Glucuronides	48.5 ± 5.9	56.3 ±11.7	P>0.10	58.8 ± 7.9	65.8 ± 5.8	P>0.10
I^- + Sulfates	10.7 ± 5.0	15.8 ± 4.9	P>0.10	12.9 ± 2.6	14.8 ± 7.1	P>0.10
3:3':5'T_3	8.0 ±3.4	----		1.9 ±0.4	----	
3:3'T_2	----	10.1 ± 3.4		----	5.8 ±2.9	
T_4	14.2 ± 3.0	---	P<0.025	7.6 ±2.1	---	P<0.025
T_3	Trace	6.8 ±3.5		Trace	2.4 ±1.1	
T_4A	2.1 ±1.6	---	P>0.10	1.4 ±0.5	---	P>0.10
T_3A	---	3.0 ±1.3		---	0.7 ±0.4	

*The differences between the means within each Group were tested for significance with "Students" t-test. When P<0.10 the differences between the means are considered statistically significant, i.e., there are two population means instead of one.
**Mean ± standard deviation of five estimations for each compound.

TABLE VII

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE COLLECTED IN FOUR HOURS AFTER BIRDS WERE INJECTED WITH I^{131}-LABELED T_4 OR T_3
(Chromatograms developed in collidine and 3N ammonium hydroxide)

	Group I			Group II		
Compound	T_4 Injected	T_3 Injected	P*	T_4 Injected	T_3 Injected	P
Origin	14.5** ± 2.6	8.1 ±7.5	P>0.10	12.9 ± 3.4	6.1 ±3.8	P<0.05
Glucuronide 1	50.3 ± 7.5	61.4 ± 6.0	P<0.05	42.1 ±11.0	63.1 ± 4.4	P<0.05
Glucuronide 2	10.0 ± 4.7	8.7 ±3.2	P>0.10	17.3 ±11.0	6.8 ±0.8	P<0.10
Sulfates	---	9.6 ±3.2		----	14.9 ± 3.7	
I^- + Thyronines, et al.	---	12.1 ± 4.0		----	6.7 ±2.6	
I^-, Sulfates + Thyronines, et al.	26.3 ± 9.7	---		27.4 ± 7.3	---	

*The differences between the means within each Group were tested for significance with "Students" t-test. When P<0.10 the differences between the means are considered statistically significant, i.e., there are two population means instead of one.
**Mean ± standard deviation of five estimations for each compound.

TABLE VIII

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE COLLECTED IN FOUR HOURS AFTER BIRDS WERE INJECTED WITH I^{131}-LABELED T_4 OR T_3
(Chromatograms developed in butanol saturated with 2N ammonium hydroxide)

Compound	Group I		Group II	
	T_4 Injected	T_3 Injected	T_4 Injected	T_3 Injected
Origin	16.8* ± 4.6	---	17.8 ± 2.6	---
Origin + Glucuronides	---- ----	68.3 ±7.4	----	66.3 ± 8.4
Glucuronides	50.9 ±11.5	----	48.4 ± 4.1	----
I^- + Sulfates	10.8 ± 5.1	----	16.0 ±2.5	----
3:3':5'T_3	4.5 ±3.3	----	3.6 ±0.9	----
3:3'T_2	---	8.7 ±2.7	---	7.5 ±1.9
T_4	15.0 ± 0.7	Trace	12.1 ± 1.0	Trace
T_3	Trace	6.6 ±4.0	Trace	4.9 ±1.5
T_4A	1.7 ±1.1	---	1.9 ±0.4	---
T_3A	---	Trace	---	---

* Mean ± standard deviation of five estimations for each compound.

given iodinated metabolite in bile was calculated from the total radioactivity of all metabolites identified on chromatographic strips, therefore, subtle, insignificant changes in a few of the metabolites could cause a given metabolite to vary considerably from solvent to solvent.

The unknown iodinated compounds, X-1 and X-2, in the bile collected from $*T_4$-injected birds contained approximately 16 percent of the total radioactivity in bile (Tables VI, VII, VIII). The same unknowns, X-1 and X-2, accounted for approximately 7 percent of the total radioactivity in the bile of birds injected with $*T_3$ (Tables VI, VII). The difference between the two means was significant at the 0.025 level of probability.

Most of the radioactivity in the bile of birds injected with $*T_3$ and $*T_4$ appeared in the glucuronide regions of the chromatograms. When the two solvents containing butanol were used (Tables VI, VIII), roughly 50 percent of the radioactivity of the bile of $*T_4$-injected birds appeared to be the glucuronide conjugated compounds. In the bile of $*T_3$-injected birds, the glucuronides accounted for 60 percent of the radioactivity in the bile from these animals. The difference between the mean percent of glucuronide-bound metabolites in the bile of birds injected with either $*T_4$ or $*T_3$ was not significantly different ($P>0.10$). When collidine and 3N ammonium hydroxide was used, the glucuronide region was more discretely separated and a more accurate estimate of the glucuronide region was thought to have been obtained. With this solvent, the glucuronide region in the bile of $*T_4$-injected birds was approximately 60 percent, that of $*T_3$-injected birds 70 percent. The difference between the means was statistically significant, indicating that close to 10 percent more of the iodinated metabolites in the bile of birds injected with $*T_3$ or $*T_4$ was bound and secreted into the bile by the liver as the glucuronide.

A reasonably accurate estimate of iodide in the bile of $*T_4$ and $*T_3$-

TABLE IX

ELECTROPHORETIC DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE COLLECTED IN FOUR HOURS FROM BIRDS INJECTED WITH I^{131}-LABELED T_4 OR T_3

Compounds	Group I: T_4 Injected	Group I: T_3 Injected	P*	Group II: T_4 Injected	Group II: T_3 Injected	P
I^-	1.1** ± 0.4	1.6 ± 1.5	P>0.10	4.5 ± 2.1	2.0 ± 1.8	P>0.10
Iodinated Organic Compounds	98.8 ± 0.4	98.3 ± 1.5	P>0.10	95.5 ± 2.1	97.9 ± 1.8	P>0.10

*The differences between the means within each Group were tested for significance with "Students" t-test. No significant differences were found between the means at the 0.10 level of probability, i.e., there are not two population means.

**Mean ± standard deviation of five estimations for each compound.

injected birds was obtained by means of electrophoresis (Table IX). In Group I, the percent distribution of iodide in bile from $*T_4$ and $*T_3$-injected birds was approximately 1.5 percent. In Group II, iodide accounted for approximately 3 percent of the total radioactivity in the bile of birds injected with $*T_4$ or $*T_3$. There was no significant difference between the means for percent iodide in bile from birds injected with $*T_3$ or $*T_4$.

A calculation of the percent of thyronines conjugated as the sulfate ester was not satisfactorily obtained with the three solvents used in this work. In the butanol, dioxane, and 2N ammonium hydroxide solvent system, iodide and reverse T_3 or T_2 were not completely separated from the sulfate conjugates. In butanol saturated with 2N ammonium hydroxide, the conjugated sulfates seemed to be incompletely separated from iodide and the glucuronides. In collidine and 3N ammonium hydroxide, the sulfates were found with the thyronines. Consequently, the values that were found for sulfate-bound metabolites were unreliable.

Reverse T_3 was identified in the bile of $*T_4$-injected birds. The relative percent distribution of this hormone in the free form accounted for 8.0 percent of the radioactivity in the bile of Group I birds, while in Group II only 1.9 percent of the radioactivity was reverse T_3 (Table VI). This hormone in another solvent (Table VIII) was calculated to contain approximately 4 percent of the radioactivity in Groups I and II. It appears from Table VI that the collection of urine (Group II) in some way lowered the percent of reverse T_3 in bile. The latter statement was also true for all the free thyronines in the bile of $*T_4$-injected birds. For example, from the birds of Group I, free T_4 and T_4A were calculated to contain 14.2 and 2.1 percent respectively, while in Group II, T_4 was 7.6 and T_4A was 1.4 percent. The separation of T_4 was altered by changing

the chromatographic solvent; consequently, the percent distribution of T_4 in the bile of Group II birds also varied (Table VIII).

The percent distribution of T_2 in the bile of *T_3-injected birds was 10.1 percent in Group I and 5.8 percent in Group II (Table VI). Using the butanol saturated with 2N ammonium hydroxide solvent system, the percentages of T_2 in the same bile in Group I was 8.7 and 7.5 percent in Group II (Table VIII). In the bile of birds injected with *T_3, the free parent hormone accounted for approximately 6.7 percent of the total radioactivity in Group I and approximately 3.5 percent in Group II (Tables VI, VIII).

An estimation of the free acetic acid derivative of T_3 revealed only a trace of this derivative in the bile of *T_3-injected birds although in Table VI, 3.0 percent was recorded for Group I birds. On many of the radioautograms prepared from two-dimensional chromatograms of bile, no T_3A was seen; however, the x-ray film was exposed for two to three weeks which may have been too short a time period to detect the derivative.

In Tables X and XI, the relative percent of iodinated organic compounds were estimated both before and after hydrolysis with beta glucuronidase. The control samples in *T_3 or *T_4-injected birds of Groups I and II contained X-1 and X-2, glucuronide region, iodide and sulfate, and a trace of thyronine metabolites. The control sample was obtained by eluating all iodinated compounds that had an R_f less than fifty from chromatograms containing bile and developed in the butanol, dioxane, 2N ammonium hydroxide solvent system. When a portion of the control eluate was incubated with beta glucuronidase, three or four hydrolytic products appeared.

The unidentified iodinated compounds, X-1 and X-2, initially appeared to be glucuronides because they were partially hydrolyzed by beta

TABLE X

DISTRIBUTION OF I^{131} FOLLOWING A SECONDARY SEPARATION AND HYDROLSIS WITH BETA GLUCURONIDASE OF THAT FRACTION OF BILE HAVING R_f 0-50 IN AN INITIAL CHROMATOGRAPHIC SEPARATION WITH THE SAME SOLVENT

(Group I)

Hormone Injected	Control			Hydrolytic Products		
	Metabolites	Percent* Mean	±S.D.**	Metabolites	Percent Mean	±S.D.
Thyroxine (3)***	X-1 and X-2	22.1	± 7.8	X-1 and X-2	13.7	± 5.3
	Glucuronides (G Region)	60.6	±11.6	Unhydrolysed Glucuronides	10.7	± 4.7
	I^- + Sulfates	9.5	± 3.8	I^- + Sulfates	17.8	± 4.2
	3:3':5'T_3	6.0	± 2.7	3:3':5'T_3	17.4	±10.1
	T_4	Trace		T_4	26.2	± 6.5
				T_4A	8.2	± 3.6
				T_3	4.4	± 5.1
3:5:3' Triiodothyronine (2)	X-1 and X-2	15.4	± 8.4	X-1 and X-2	8.4	± 3.0
	Glucuronide-1	18.9	± 1.5	Unhydrolysed Glucuronide-1	3.5	± 1.4
	Glucuronide-2	58.4	±12.1	Unhydrolysed Glucuronide-2	10.5	± 2.6
	I^- + Sulfates	3.3	± 1.3	I^- + Sulfate	5.7	± 3.5
	3:3':5'T_3	----	----	3:3':5'T_3	4.8	± 1.4
	3:3' T_2	Trace		3:3' T_2	14.9	± 2.1
	T_3	Trace		T_3	49.7	± 6.0
				T_3A	2.1	± 0.2

*Percent of the total radioactivity on the chromatograms developed in butanol, dioxane, and 2N ammonium hydroxide.

**Standard deviation.

***Number of estimations.

TABLE XI

DISTRIBUTION OF I^{131} FOLLOWING A SECONDARY SEPARATION AND HYDROLYSIS WITH BETA GLUCURONIDASE OF THAT FRACTION OF BILE HAVING R_f 0-50 IN AN INITIAL CHROMATOGRAPHIC SEPARATION WITH THE SAME SOLVENT

(Group I)

	Control			Hydrolytic Products		
Hormone Injected	Metabolites	Percent* Mean	±S.D.**	Metabolites	Percent Mean	±S.D.
Thyroxine (3)***	X-1 and X-2	22.3	±12.7	X-1 and X-2	18.6	±2.6
	Glucuronides (G Region)	63.0	±18.2	Unhydrolyzed Glucuronide	18.8	±5.5
	I^- + Sulfates	11.3	± 2.6	I^- + Sulfate	15.7	±3.3
	3:3':5'T_3	1.5	± 1.7	3:3':5'T_3	4.4	±2.7
	T_4	Trace		T_4	30.7	±4.1
				T_4A	8.4	±3.4
				T_3	3.0	±2.6
3:5:3' Triiodo-thyronine (2)	X-1 and X-2	13.8	± 5.5	X-1 and X-2	6.1	±0.6
	Glucuronide-1	18.8	± 1.5	Unhydrolyzed Glucuronide-1	3.8	±1.5
	Glucuronide-2	54.5	±16.4	Unhydrolyzed Glucuronide-2	5.9	±2.9
	I^- + Sulfates	9.6	±10.8	I^- + Sulfate	11.3	±3.7
	X-3	2.9	± 2.4	X-3	3.2	±4.6
	3:3' T_2	Trace		3:3' T_2	16.1	±3.2
	T_3	Trace		T_3	49.9	±1.7
				T_3A	3.3	±2.7

*Percent of the total radioactivity on the chromatograms developed in butanol, dioxane, and 2N ammonium hydroxide.
**Standard deviation.
***Number of estimations.

glucuronidase in all samples regardless of the thyroid hormone injected (Tables X, XI). However, when the unknowns, X-1 and X-2 were hydrolyzed with beta glucuronidase in preliminary experiments, the only new metabolite that arose was inorganic iodide. An increase in the percent of sulfate conjugates and iodide in the control sample that was hydrolyzed with beta glucuronidase may have been due to the partial degradation of X-1 and X-2 to iodide. It was impossible to hydrolyze the glucuronides completely during the incubation period, consequently, the unhydrolyzed glucuronides amounted to 8-18 percent in the sample incubated with beta glucuronidase. The control sample accounted for 60-75 percent of the total radioactivity on the chromatograms which indicated that 65-75 percent of the glucuronides were hydrolyzed.

In $*T_4$-injected birds in both Groups I and II, the values of the relative percent distribution of the labeled, hydrolytic products were as follows: T_4 accounted for approximately 30 percent of the total radioactivity in the hydrolyzed sample, compared with only a trace of T_4 in the control sample. Values of 17.4 percent were found for reverse T_3 in Group I (Table X) in contrast to 4.4 percent in Group II. In both groups, T_4A and T_3 accounted for about 8 percent and 3.5 percent respectively of the total radioactivity in the hydrolyzed samples.

T_3 was the major hydrolytic product after hydrolysis of the glucuronide component of bile in birds injected with $*T_3$ (Groups I and II). Approximately 50 percent of the radioactivity in the hydrolyzed sample consisted of labeled T_3. About 16 percent of the radioactivity in the hydrolyzed samples of Groups I and II was due to the labeled T_2. The remaining hydrolytic products, T_3A and an unidentified compound (Unknown 3), contained 2-4 percent of the radioactivity of the hydrolyzed sample.

It is necessary to be cautious in assigning a physiological role to

any slight differences in the thyronine metabolites which were present in low concentrations or which may have been artifacts produced by chromatographic solvents.

The metabolic compounds of T_4 or T_3 which were found in the bile from birds injected with $*T_4$ or $*T_3$ were qualitatively similar to the metabolic compounds identified in the rat by Flock and Bollman (22) and Roche, et al. (92). In general, the glucuronide conjugates of the thyronines are the major metabolites excreted in the bile by birds and also by albino rats. In $*T_4$-injected birds and rats, the glucuronides are T_4, reverse T_3, T_3, and T_4A. The percent of these conjugated metabolites in bile was similar in both birds and rodents. Birds injected with $*T_3$ excreted into the bile glucuronides of T_3 and T_2 which were qualitatively the same in thyroidectomized rats (Roche and Michel, 36). Thyronines conjugated as the sulfate ester were excreted by rats (Roche, et al., 89) and were also demonstrated in birds. Marked differences in the percent of conjugated I^{131} compounds were noted between dogs and chickens. According to Flock, et al. (25, 27) less of the radioactive metabolites were bound as glucuronides and more of the metabolites were bound as the sulfate conjugate in the dog than in the rat.

No metabolites were identified in chicken bile that were not commonly found in rat bile following the injection of $*T_3$ or $*T_4$, although some iodinated compounds were present in chicken bile that have not been identified. One difference between the iodinated compound in chicken bile to rat bile was the greater percent of inorganic iodide (about 5 percent) in the bile of rats that were injected with $*T_4$ (Flock and Bollman, 22).

General Considerations

It has been established by the work of many investigators (Barker, 8)

that T_3 was more potent than an equimolar quantity of T_4 when assayed by a wide variety of tests in mammals. T_3 in contrast to T_4 was weakly bound to the plasma proteins and T_3 has a shorter half-life than T_4 in mammals. In mammals T_3 was excreted and deiodinated at a greater rate than T_4 (30, 47, 68); however, there was no difference in the deiodination of the two hormones when incubated with isolated mammalian tissue. According to Pitt-Rivers and Tata (79) the higher biological activity of T_3 was related to its higher rate of metabolism and excretion, these in turn were related to the weaker binding of T_3 to the plasma proteins.

In birds, T_3 was not more potent and in some physiological tests less potent than T_4 (77, 117). The two thyroid hormones, T_3 and T_4 were bound equally to the plasma proteins and had similar half-lives in the bird (40, 103). Therefore an analogous conclusion from mammals was applied to birds that T_4 and T_3 may be deiodinated and excreted at similar rates in these animals. However, the results obtained from the present work show that $^*T_3$ when injected into birds was metabolized and excreted at a greater rate than $^*T_4$ during a four hour period, which may indicate a lesser binding ability of T_3 to the plasma proteins in birds. Other investigators (19, 40) have shown that the two thyroid hormones were not equally bound to the plasma proteins in chickens, which may be reflected in the present work by the observation that during a four hour period the percent of T_3 in chicken plasma decreased at a greater rate than the percent of T_4. It is of interest to note that T_3 which is equal to or less potent than T_4 in birds was excreted at a greater rate than T_4. Therefore, a possible explanation for the difference in potency in the thyroid hormones may be the different rate the two hormones are excreted. The latter statement is in direct contrast to the situation in mammals where other reasons are responsible for the difference in potency between the two hormones.

An investigation of the metabolic pathways in birds injected with $*T_3$ or $*T_4$ indicated that conjugation and deiodination were the primary ways in which both T_3 and T_4 were metabolized and excreted into bile and urine. The third metabolic pathway, deamination and decarboxylation, was of little significance in the metabolism of $*T_3$ in birds since little T_3A was identified in the bile of $*T_3$-injected birds; however, in $*T_4$-injected birds approximately 10 percent of the radioactivity in bile was the biologically active derivative T_4A. This difference in the amount of biologically active acetic acid derivatives formed from T_3 and T_4 in the liver could conceivably result in T_4 being the more active hormone in the liver. This suggestion as a solution for the problem of potency differences in birds obviously requires further investigation in the liver and in other organs as well.

CHAPTER V

SUMMARY AND CONCLUSIONS

This problem was undertaken (1) to examine the major pathways that exist in birds for the excretion of the thyroid hormones and to identify and determine the relative distribution of the iodinated metabolites of $*T_4$ and $*T_3$ in bile, urine and plasma, and (2) to correlate the above findings with the observation that T_3 was equal to or less potent than T_4 in birds.

Four hours after the injection of I^{131}-labeled T_4, 7.8 percent and 3.1 percent of the radioactivity per injected dose was excreted into the bile and urine respectively. In $*T_3$-injected birds 14.3 percent of the radioactivity was excreted into the bile and 9.5 percent into the urine during the same period of time.

Approximately 90-95 percent of the iodinated metabolic products that were identified in the total volumes of urine from $*T_3$ or $*T_4$-injected birds was iodide. The remaining 5-10 percent consisted of a trace of the hormone that was injected (I^{131}-labeled T_3 or T_4) and an iodinated organic compound which was tentatively identified as a conjugated metabolite. After chromatographic and radioautographic analysis the iodinated organic compounds in the bile were identified and the percent distribution of the compounds was calculated. Approximately 55 percent of the radioactivity in the bile collected for four hours from $*T_4$-injected birds was conjugated as a group of glucuronides. Other iodinated organic compounds found in the bile of $*T_4$-injected birds were: Unknowns, X-1 and X-2, (16.5

percent), iodide plus sulfate conjugates (11 percent), 3:3':5' T_3 (2-8 percent), T_4 (11 percent), T_4A (2 percent) and a trace of T_3. Several iodinated organic compounds were also recognized in the bile of *T_3-injected birds. Approximately 60 percent of the radioactivity in bile was the glucuronide conjugates. Other iodinated organic compounds found in bile of *T_3-injected birds were: iodide and sulfate (15 percent), Unknowns, X-1 and X-2 (7.5 percent), 3:3' T_2 (8 percent), T_3 (4 percent) and T_3A (2 percent).

Following hydrolysis of the glucuronides from the bile of *T_3-injected birds, three or more iodinated organic compounds appeared which were: T_3, 3:3' T_2 and a trace of T_3A. Thyroxine, T_4A, T_3 and 3:3':5' T_3 were identified following the hydrolysis of the glucuronides from the bile of *T_4-injected birds. The sulfate conjugates from the bile of *T_4-injected birds were hydrolyzed to T_4 and a trace of 3:3':5' T_3 while T_3 and 3:3' T_2 were identified as a sulfate ester in the sulfate conjugates found in the bile of *T_3-injected birds.

Plasma was collected at 1, 2, 3, and 4 hours following the injection of *T_3 or *T_4 into birds. The percent of radioactivity due to inorganic iodide and to iodinated organic compounds present in plasma was calculated after electrophoretic analysis. As determined after chromatographic analysis, the iodinated organic compound was primarily the same thyroid hormone as the one that was injected. The percent of the radioactivity in the iodinated organic compounds in the plasma of *T_3-injected birds decreased at a significantly more rapid rate than the same parameter in the plasma of *T_4-injected birds. These comparisons were made within three groups of birds according to the pattern of excretory product or products collected and compared to sham-operated birds which were injected with *T_3 or *T_4. At the end of four hours the percent of the iodinated organic

compounds in the plasma of $*T_4$-injected birds which had been sham-operated was 87.7 percent. In the same group of birds injected with $*T_3$ the same parameter measured 50.1 percent. When urine was collected during the four hour period, the percent of the iodinated organic compounds in plasma was 47.8 for $*T_4$-injected birds and for $*T_3$-injected birds, 39.0 percent.

The results of these experiments indicate that (1) T_3 is excreted into the bile and urine at a more rapid rate than T_4; (2) the thyroid hormones are metabolized by similar pathways in the liver and presumably in other peripheral organs; (3) the metabolic products that appear in both bile and urine are similar both quantitatively and qualitatively to results obtained by other investigators in rats; (4) the percent of T_3 in plasma decreases at a greater rate during a four hour period than the percent of T_4 which is in contrast to the equal disappearance rates of T_4 and T_3 in chicken plasma as observed by other investigators; and (5) a significantly greater percent of T_4A was identified in the bile of $*T_4$-injected birds compared to a trace of T_3A identified in the bile of $*T_3$-injected birds. These differences in the rate of excretion, deiodination and deamination of T_3 compared to T_4 could affect the extent to which the two hormones exert their metabolic effects in birds.

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A P P E N D I X

RADIOACTIVITY AND VOLUME OF BILE FROM BIRDS INJECTED WITH EITHER I^{131}-T_4 OR I^{131}-T_3

GROUP I

	I^{131}-T_4		I^{131}-T_3	
Bird No.	Volume (ml.)	I^{131} (percent of dose)	Volume (ml.)	I^{131} (percent of dose)
1	7.2	7.79	4.1	11.67
2	5.6	14.98	5.4	6.24
3	5.5	8.71	3.5	11.62
4	8.8	9.36	7.5	12.58
5	3.1	7.66	4.1	16.26
Mean	6.0	9.70	4.9	11.67
S.D.*	2.1	3.03	1.6	3.58

RADIOACTIVITY AND VOLUME OF URINE FROM BIRDS INJECTED WITH EITHER I^{131}-T_4 OR I^{131}-T_3

GROUP III

	I^{131}-T_4		I^{131}-T_3	
Bird No.	Volume (ml.)	I^{131} (percent of dose)	Volume (ml.)	I^{131} (percent of dose)
11	20.2	2.02	48.7	5.75
12	28.4	3.66	90.0	7.67
13	12.7	1.41	76.5	16.26
14	3.2	1.90	66.0	8.22
15	14.6	1.90	----	----
Mean	15.8	2.18	70.3	9.48
S.D.	9.3	0.86	17.4	4.65

*Standard Deviation

RADIOACTIVITY AND VOLUME OF BILE AND URINE FROM BIRDS

INJECTED WITH I^{131}-T_4

GROUP II

	Bile		Urine	
Bird No.	Volume (ml.)	I^{131} (percent of dose)	Volume (ml.)	I^{131} (percent of dose)
6	4.1	8.25	29.7	3.57
7	6.8	5.14	31.0	3.30
8	6.8	14.58	20.7	3.31
9	3.9	4.35	14.6	4.65
10	8.7	6.82	11.1	0.83
Mean	6.1	7.83	21.4	3.13
S.D.*	2.0	4.00	8.9	1.40

RADIOACTIVITY AND VOLUME OF BILE AND URINE FROM BIRDS

INJECTED WITH I^{131}-T_3

GROUP II

	Bile		Urine	
Bird No.	Volume (ml.)	I^{131} (percent of dose)	Volume (ml.)	I^{131} (percent of dose)
6	3.7	14.30	110.0	15.53
7	3.6	12.05	36.9	5.12
8	5.0	12.35	34.8	2.22
9	3.7	14.30	66.5	6.14
10	4.4	18.67	12.7	12.20
Mean	4.1	14.33	52.2	8.20
S.D.	0.6	2.64	37.6	5.50

*Standard Deviation

PERCENT OF I^{131}-T_4 IN PLASMA AT VARIOUS TIMES

AFTER INJECTION OF I^{131}-T_4

GROUP I (Bile and Plasma Collected)

Bird No.	Time (Hours)			
	1.0	2.0	3.0	4.0
1	97.29	92.43	90.29	82.50
2	96.65	95.42	90.27	87.07
3	98.75	96.87	80.43	73.98
4	95.25	96.55	96.83	88.82
5	98.48	96.43	89.04	85.15
Mean	97.28	95.54	89.37	83.50
S.D.*	1.52	1.82	5.86	6.87

PERCENT OF I^{131}-T_3 IN PLASMA AT VARIOUS TIMES

AFTER INJECTION OF I^{131}-T_3

GROUP I (Bile and Plasma Collected)

Bird No.	Time (Hours)			
	1.0	2.0	3.0	4.0
1	90.98	79.04	75.04	75.60
2	97.73	95.93	78.69	75.11
3	85.13	73.58	65.65	53.92
4	87.73	92.05	73.44	65.00
5	87.48	72.13	73.15	74.28
Mean	89.81	82.54	73.19	68.98
S.D.	4.89	10.81	4.75	11.89

*Standard Deviation

PERCENT OF I^{131}-T_4 IN PLASMA AT VARIOUS TIMES

AFTER INJECTION OF I^{131}-T_4

GROUP II (Bile, Urine and Plasma Collected)

Bird No.	Time (Hours) 1.0	2.0	3.0	4.0
6	97.49	93.37	84.41	59.64
7	97.20	88.43	83.97	54.86
8	90.72	83.37	63.26	43.05
9	97.47	99.47	92.97	63.10
10	92.73	91.46	85.95	65.85
Mean	95.12	91.22	82.11	57.30
S.D.*	3.18	5.96	11.44	8.96

PERCENT OF I^{131}-T_3 IN PLASMA AT VARIOUS TIMES

AFTER INJECTION OF I^{131}-T_3

GROUP II (Bile, Urine and Plasma Collected)

Bird No.	Time (Hours) 1.0	2.0	3.0	4.0
6	75.32	50.23	42.87	19.60
7	85.63	68.46	29.27	27.81
8	84.84	72.51	44.65	29.25
9	94.89	74.88	51.30	30.91
10	89.25	84.58	53.45	47.47
Mean	85.99	70.13	44.30	31.00
S.D.	7.16	12.16	9.50	10.17

* Standard Deviation

PERCENT OF I^{131}-T_4 IN PLASMA AT VARIOUS TIMES

AFTER INJECTION OF I^{131}-T_4

GROUP III (Urine and Plasma Collected)

Bird	Time (Hours)			
No.	1.0	2.0	3.0	4.0
11	97.85	89.13	50.24	36.60
12	91.29	88.26	80.83	32.45
13	88.84	84.72	65.48	38.41
14	89.24	88.54	80.23	69.82
15	86.63	82.29	63.21	61.78
Mean	90.77	86.59	68.00	47.81
S.D.*	4.34	2.95	12.84	16.81

PERCENT OF I^{131}-T_3 IN PLASMA AT VARIOUS TIMES

AFTER INJECTION OF I^{131}-T_3

GROUP III (Urine and Plasma Collected)

Bird	Time (Hours)			
No.	1.0	2.0	3.0	4.0
11	83.16	67.88	33.89	36.08
12	83.94	64.35	49.51	43.23
13	71.34	65.62	56.20	41.28
14	86.47	59.12	32.20	35.62
15	-----	-----	-----	-----
Mean	81.23	64.24	42.95	39.05
S.D.	6.74	3.71	11.81	3.78

* Standard Deviation

PERCENT OF I^{131}-T_4 IN PLASMA AT VARIOUS TIMES

AFTER INJECTION OF I^{131}-T_4

GROUP IV (Plasma Collected)

Bird No.	Time (Hours) 1.0	2.0	3.0	4.0
16	97.60	96.74	93.58	89.76
17	95.59	94.75	89.90	90.35
18	93.58	90.96	88.15	87.10
19	96.86	93.80	89.88	85.17
20	94.81	91.45	89.12	86.53
Mean	95.69	93.54	90.12	87.78
S.D.*	1.60	2.39	2.06	2.20

PERCENT OF I^{131}-T_3 IN PLASMA AT VARIOUS TIMES

AFTER INJECTION OF I^{131}-T_3

GROUP IV (Plasma Collected)

Bird No.	Time (Hours) 1.0	2.0	3.0	4.0
16	93.17	78.90	63.27	54.00
17	75.69	51.90	43.14	37.50
18	84.50	68.17	59.10	43.47
19	90.44	83.13	80.08	62.27
20	86.05	81.95	56.28	53.37
Mean	85.97	72.81	60.37	50.12
S.D.	6.70	13.09	13.34	9.70

* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF URINE FROM BIRDS INJECTED WITH I^{131}-T_4

Group No.	Bird No.	Percent Origin + Glucuronides	T_4	I^-
II	6	0.00	0.00	100.00
	7	2.01	0.07	97.29
	8	0.00	0.00	100.00
	9	5.76	0.00	94.24
	10	2.87	1.69	95.44
	Mean	2.13	0.48	97.39
	S.D.*	2.38	0.74	2.62
III	11	2.50	0.00	97.50
	12	15.42	0.00	84.58
	13	3.94	4.98	91.27
	14	0.00	10.35	89.65
	15	9.14	0.00	90.86
	Mean	6.20	3.07	90.77
	S.D.	6.14	4.61	6.80

Solvent: butanol saturated with 2N ammonium hydroxide

* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF URINE FROM BIRDS INJECTED WITH I^{131}-T_3

Group No.	Bird No.	Percent		
		Origin + Glucuronides	T_3	I^-
II	6	19.44	0.00	80.56
	7	1.42	7.09	91.49
	8	1.57	0.00	98.43
	9	2.36	4.13	93.51
	10	0.57	4.23	95.20
	Mean	5.07	3.09	91.84
	S.D.*	8.06	3.06	6.80
III	11	4.33	0.00	95.67
	12	3.32	7.56	89.12
	13	11.08	0.90	88.02
	14	5.60	1.56	92.84
	15	----	----	----
	Mean	6.08	2.51	91.41
	S.D.	4.05	3.15	3.51

Solvent: butanol saturated with 2N ammonium hydroxide

* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE FROM BIRDS INJECTED WITH EITHER I^{131}-T_4 OR I^{131}-T_3

Group No.	Bird No.	T_4		T_3	
		I^-	Iodinated Organic Compounds	I^-	Iodinated Organic Compounds
I	1	1.15	98.85	4.22	95.88
	2	1.05	98.95	0.82	99.18
	3	1.17	98.93	1.63	98.37
	4	1.90	98.10	0.81	99.19
	5	0.70	99.30	0.80	99.20
	Mean	1.19	98.81	1.66	98.36
	S.D.*	0.44	0.19	1.47	1.47
II	6	3.80	96.20	1.67	98.33
	7	6.18	93.82	5.26	94.74
	8	2.18	97.82	0.93	99.07
	9	3.08	96.92	1.35	98.65
	10	7.24	92.76	0.92	99.08
	Mean	4.50	95.50	2.03	97.97
	S.D.	2.09	2.09	1.83	1.83

Electrophoretic Separation

* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE FROM BIRDS INJECTED WITH I^{131}-T_4

Group No.	Bird No.	Percent					
		Unknown 1 + Unknown 2	Glucuronides	I + Sulfates	3:3':5'T_3	T_4	T_4A
I	1	14.05	45.24	8.10	12.93	18.81	0.87
	2	17.79	58.60	4.36	4.06	13.86	1.33
	3	19.30	46.71	9.34	7.85	15.45	1.34
	4	15.73	48.34	15.41	5.71	12.49	2.32
	5	14.71	43.63	16.33	9.79	10.73	4.81
	Mean	16.32	48.50	10.71	8.07	14.27	2.13
	S.D.*	2.19	5.91	5.07	3.47	3.08	1.59
	6	20.98	54.66	15.19	1.85	6.23	1.09
	7	15.11	61.08	10.20	1.90	9.35	2.35
	8	22.95	50.23	13.88	1.62	10.27	1.05
	9	16.98	57.05	15.42	1.75	7.31	1.49
	10	9.87	71.15	10.02	2.76	5.07	1.13
	Mean	17.18	58.83	12.94	1.97	7.65	1.42
	S.D.	5.14	7.93	2.65	0.45	2.15	0.55

Solvent: butanol, dioxane, and 2N ammonium hydroxide.
* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE FROM BIRDS INJECTED WITH I^{131}-T_3

Group No.	Bird No.	Percent: Unknown 1 + Unknown 2	Glucuronides	I^- + Sulfates	3:3'T_2	T_3	T_3A
I	1	6.08	67.04	10.90	10.05	3.67	2.26
	2	5.95	69.75	10.07	6.88	5.71	1.63
	3	11.86	46.56	18.29	14.86	5.67	2.75
	4	5.33	52.58	20.00	5.66	12.93	3.49
	5	9.90	45.77	19.85	13.39	6.03	5.06
	Mean	7.82	56.34	15.82	10.17	6.80	3.04
	S.D.*	2.87	11.70	4.93	3.98	3.55	1.32
	6	7.36	66.30	13.98	9.48	2.35	0.53
	7	6.83	74.92	10.50	5.72	1.25	0.78
	8	21.78	64.85	6.31	5.07	1.66	0.33
	9	5.39	64.83	19.18	7.29	2.86	0.45
	10	9.96	58.54	24.45	1.64	3.91	1.50
	Mean	7.10	65.88	14.88	5.84	2.40	0.72
	S.D.	1.77	5.87	7.10	2.90	1.08	0.46

Solvent: butanol, dioxane, and 2N ammonium hydroxide.
* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE FROM BIRDS INJECTED WITH I^{131}-T_4

Group No.	Bird No.	Percent					
		Origin	Glucuronides	I^- + Sulfates	3:3':5'T_3	T_4	T_4A
I	1	12.23	60.74	8.14	2.07	15.32	0.50
	2	13.09	60.05	9.73	1.56	14.93	0.64
	3	23.58	32.24	18.33	9.49	13.80	2.56
	4	19.79	51.38	4.95	6.07	15.55	2.26
	5	14.54	50.48	13.21	3.43	15.48	2.87
	Mean	16.85	50.99	10.87	4.52	15.01	1.76
	S.D.*	4.65	11.50	5.12	3.28	0.72	1.11
II	6	16.95	53.60	12.88	2.32	12.36	1.89
	7	18.56	49.06	14.96	3.58	12.10	1.74
	8	16.99	45.97	19.03	3.20	12.87	1.94
	9	21.91	42.74	18.20	4.12	10.46	2.57
	10	14.76	50.78	15.30	4.84	12.96	1.36
	Mean	17.83	48.43	16.07	3.61	12.15	1.90
	S.D.	2.62	4.12	2.52	0.95	1.01	0.44

Solvent: butanol saturated with 2N ammonium hydroxide.
* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE FROM BIRDS INJECTED WITH I^{131}-T_3

Group No.	Bird No.	Percent			
		Origin + Glucuronides	I^- + Sulfates	$3:3'T_2$	T_3
I	1	75.78	13.30	5.94	4.98
	2	72.37	14.47	8.08	5.06
	3	71.51	16.72	6.80	4.97
	4	57.12	16.79	12.22	13.87
	5	64.72	19.83	10.91	4.53
	Mean	68.30	16.22	8.79	6.68
	S.D.*	7.43	2.37	2.68	4.02
II	6	73.52	14.32	8.45	3.71
	7	73.67	14.20	6.81	5.32
	8	62.20	23.66	8.46	5.67
	9	59.25	29.42	4.57	6.77
	10	62.99	24.59	9.35	3.09
	Mean	66.33	21.23	7.53	4.91
	S.D.	8.42	6.73	1.89	1.50

Solvent: butanol saturated with 2N ammonium hydroxide.
* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE FROM BIRDS INJECTED WITH I^{131}-T_4

Group No.	Bird No.	Percent			
		Origin	Glucuronide -1	Glucuronide-2	I^-, Sulfates Thyromines, et al.
I	1	17.72	48.86	6.68	33.14
	2	14.92	42.67	4.55	37.85
	3	14.44	43.90	17.51	24.14
	4	10.43	57.18	8.51	23.88
	5	14.98	59.05	13.24	12.72
	Mean	14.50	50.33	10.09	26.34
	S.D.*	2.61	7.50	4.69	9.68
II	6	10.28	46.81	7.86	35.04
	7	15.36	53.64	14.56	16.44
	8	11.94	24.63	35.27	28.16
	9	17.62	39.16	9.83	33.39
	10	9.61	46.34	19.36	24.69
	Mean	12.96	42.12	17.38	27.48
	S.D.	3.42	11.03	11.06	7.39

Solvent: collidine and 3N ammonium hydroxide.
* Standard Deviation

DISTRIBUTION OF I^{131} IN TOTAL VOLUME OF BILE FROM BIRDS INJECTED WITH I^{131}-T_3

Group No.	Bird No.	Percent				
		Origin	Glucuronide-1	Glucuronide-2	Sulfates	I^- + Thyronines, et al.
I	1	3.79	65.00	7.69	4.33	19.19
	2	2.83	65.86	8.95	12.16	10.20
	3	11.44	53.11	13.86	10.76	10.82
	4	2.60	65.63	8.35	12.08	11.33
	5	19.84	57.39	4.90	8.69	9.18
	Mean	8.10	61.40	8.75	9.60	12.14
	S.D.*	7.51	6.03	3.25	3.26	4.02
II	6	11.95	59.26	7.15	13.91	7.74
	7	4.95	69.63	6.02	19.39	2.69
	8	2.61	58.78	7.92	10.42	5.98
	9	3.45	63.22	5.87	17.91	9.55
	10	7.77	64.61	7.05	12.93	7.64
	Mean	6.15	63.10	6.80	14.91	6.72
	S.D.	3.75	4.42	0.85	3.68	2.58

Solvent: collidine and 3N ammonium hydroxide.
*Standard Deviation

DISTRIBUTION OF I^{131} FOLLOWING A SECONDARY SEPARATION OF THAT FRACTION OF BILE HAVING R_f 0-40 IN AN INITIAL CHROMATOGRAPHIC SEPARATION WITH THE SAME SOLVENT (I^{131}-T_4 Injected Birds)

Group No.	Bird No.	Percent: Unknown 1 + Unknown 2	Glucuronides	I^- + Sulfates	Unknown 3	T_4
I	1	21.52	61.13	6.60	9.19	1.56
	3	16.42	71.96	8.21	4.36	0.85
	5	30.27	48.73	13.91	4.46	2.63
	Mean	22.14	60.60	9.57	6.00	1.68
	S.D.*	7.84	11.62	3.84	2.76	0.89
II	6	15.81	70.07	11.62	0.00	2.50
	8	14.10	75.20	8.50	1.31	0.89
	9	37.05	43.82	13.82	3.40	2.47
	Mean	22.32	63.03	11.31	1.57	1.95
	S.D.	12.79	18.26	2.67	1.72	0.92

Solvent: butanol, dioxane, and 2N ammonium hydroxide.
*Standard Deviation

DISTRIBUTION OF I^{131} FOLLOWING A SECONDARY SEPARATION OF THAT FRACTION OF BILE HAVING R_f 0-40 IN AN INITIAL CHROMATOGRAPHIC SEPARATION WITH THE SAME SOLVENT (I^{131}-T_3 Injected Birds)

Group No.	Bird No.	Percent: Unknown 1 + Unknown 2	Glucuronide-1	Glucuronide-2	I^- + Sulfates	Unknown 3	T_3
I	1	21.48	20.04	49.86	4.26	4.36	--**
	2	9.50	17.81	67.05	2.34	2.44	--
	Mean	15.49	18.93	58.45	3.30	3.40	
	S.D.*	8.47	1.58	12.15	1.36	1.32	
II	11	9.87	17.80	66.12	4.68	1.28	
	15	17.74	19.98	42.91	14.61	4.67	
	Mean	13.81	18.89	54.51	9.64	2.97	
	S.D.	5.57	1.54	16.41	10.85	2.40	

Solvent: butanol, dioxane, and 2N ammonium hydroxide.
*Standard Deviation
**Trace

DISTRIBUTION OF I^{131} FOLLOWING A SECONDARY SEPARATION AND HYDROLYSIS WITH BETA GLUCURONIDASE OF THAT FRACTION OF BILE HAVING R_f 0-40 IN AN INITIAL CHROMATOGRAPHIC SEPARATION WITH THE SAME SOLVENT
(I^{131}-T_4 Injected Birds)

Group No.	Bird No.	Percent						
		Unknown 1 + Unknown 2	Unhydrolysed Glucuronides	I^- + Sulfates	3:3':5'T_3	T_4	T_4A	T_3
I	3	19.33	6.67	16.05	28.91	24.26	4.15	0.62
	4	13.22	15.93	22.69	13.76	20.87	11.07	2.47
	5	8.63	9.77	14.88	9.55	33.56	9.55	10.38
	Mean	13.73	10.79	17.87	17.41	26.23	8.26	4.49
	S.D.*	5.37	4.71	4.21	10.18	6.57	3.64	5.18
II	6	15.74	24.34	12.10	1.36	29.13	12.23	5.10
	8	20.68	13.25	18.52	6.39	35.54	5.61	0.00
	9	19.62	18.90	16.71	5.65	27.71	7.41	4.00
	Mean	18.68	18.83	15.78	4.47	30.79	8.42	3.03
	S.D.	2.60	5.54	3.31	2.72	4.17	3.42	2.68

Solvent: butanol, dioxane and 2N ammonium hydroxide.
*Standard Deviation

DISTRIBUTION OF I^{131} FOLLOWING A SECONDARY SEPARATION AND HYDROLYSIS WITH BETA GLUCURONIDASE OF THAT FRACTION OF BILE HAVING R_f 0-40 IN AN INITIAL CHROMATOGRAPHIC SEPARATION WITH THE SAME SOLVENT
(I^{131}-T_3 Injected Birds)

		Percent							
Group No.	Bird No.	Unknown 1 + Unknown 2	Unhydrolysed Glucuronide-1	Unhydrolysed Glucuronide-2	I^- + Sulfate	Unknown 3	3:3'T_2	T_3	T_3A
I	1	6.27	2.54	12.44	3.26	5.79	13.35	54.05	2.30
	2	10.61	4.62	8.73	8.32	3.80	16.45	45.53	1.94
	Mean	8.44	3.58	10.59	13.95	4.80	14.90	49.79	2.21
	S.D.*	3.07	1.47	2.62	8.65	1.41	2.15	6.02	0.20
II	11	6.59	4.94	8.00	13.95	0.00	13.83	51.22	1.47
	15	5.65	2.80	3.88	8.65	6.52	18.43	48.76	5.30
	Mean	6.12	3.87	5.94	11.30	3.26	16.13	49.99	3.38
	S.D.	0.66	1.51	2.91	3.74	4.61	3.25	1.74	2.70

Solvent: butanol, dioxane and 2N ammonium hydroxide.
*Standard Deviation

VITA

Max Olivo Hutchins

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Doctor of Philodophy

Thesis: THE METABOLISM AND EXCRETION OF THYROXINE AND TRIIODOTHYRONINE IN CHICKENS

Major Field: Physiology

Biographical Sketch:

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