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Constance S. Pittman, ... , Melvin W. Buck, Joseph B. Chambers Jr.

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Research Article

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Urinary Metabolites of ^{14}C -Labeled Thyroxine in Man

CONSTANCE S. PITTMAN, MELVIN W. BUCK, and JOSEPH B. CHAMBERS, JR.

From the Department of Medicine, University of Alabama School of Medicine, Birmingham, Alabama 35233

ABSTRACT Studies were carried out to determine the chemical structures of thyroxine metabolites after total deiodination. Normal subjects were given thyroxine labeled with ^{14}C on the nonphenolic ring and the alanine side chain, 8–11 $\mu\text{g}/\text{day}$ for 10 days. By paper chromatography of fresh urine, six or more ^{14}C -labeled compounds were separated. The ^{14}C -labeled metabolites were concentrated by passing the urine through a nonionic polymeric adsorbent. Two major thyroxine metabolites were identified. The identification was made by three different methods: (a) chromatography, (b) synthesis of derivatives, and (c) recrystallization to constant specific activity. One ^{14}C -labeled metabolite was identified as thyroacetic acid or 4-phenoxy-(4'-hydroxy) phenylacetic acid. Another one was identified as thyronine. Of the total urinary ^{14}C radioactivity, 43.7% was recovered as thyroacetic acid and 19.8% was recovered as thyronine. Approximately one-fifth of each of these metabolites was present in the urine in bound form which released the free metabolites during acid hydrolysis. The average daily excretion of thyroacetic acid was 13.7% of the renal disposal rate of thyroxine, or approximately 7.5 $\mu\text{g}/\text{day}$. The average daily excretion of thyronine was 6.5% of the renal disposal rate of thyroxine or approximately 3.9 $\mu\text{g}/\text{day}$ while the urinary iodide made up 64.7% of the renal disposal rate of thyroxine. Our findings provide the needed proof that the major metabolic pathways of thyroxine remove the iodine atoms by substituting hydrogen for iodine and leave the diphenyl ether nucleus intact.

INTRODUCTION

The biochemical transformation of the iodothyronines may be broadly grouped into deiodination, side-chain alteration, and conjugation. Of these, deiodination has received most of the attention of investigators. Deiodination of thyroxine may be associated with either substitution with hydrogen or aromatic hydroxylation. In

an aromatic hydroxylation reaction the diphenyl ether nucleus of thyroxine may be cleaved and one or both of the phenyl rings may be ruptured. However, in a substitution reaction with hydrogen, the diphenyl ether may remain intact.

Plaskett(1), Roche, Nunez, and Jaquemin (2) and Wynn and Gibbs (3) studied thyroxine metabolites by in vitro systems in which radiothyroxine was incubated with a subcellular preparation of rat liver. After hydrolysis, several double-ringed and single-ringed products, among them 1,4-dihydroquinone and diiodotyrosine, were identified in the media and in the tissue. The radioactive 1,4-dihydroquinone and diiodotyrosine identified were thought to be the degradation products of thyroxine after cleavage of the diphenyl ether nucleus. Lissitzky, Bénévnt, Roques, and Roche (4) identified thyronine as a thyroxine metabolite after total deiodination. The earlier reports of in vivo experiments after the injection of radiothyroxine also described single-ringed products. Nunez, Rappaport, Jacquemin, and Roche (5) identified monoiodotyrosine and diiodotyrosine in hydrolyzed rat tissue. Wynn (6) identified ^{14}C -labeled 1,4-dihydroquinone in the urine of one human subject after the administration of ^{14}C -labeled thyroxine. On the other hand, Pittman and Chambers found that the diphenyl ether link of thyroxine was not cleaved to any significant extent during its metabolism in intact rats (7). More recently Pittman, Read, Chambers, and Nakafuji gave normal men a mixture of two radiothyroxines (8). One was labeled with ^3H on the alanyl side chain and the other with ^{14}C on the phenolic ring. The ratios of phenolic ring to nonphenolic ring in the urine, as indicated by the $^3\text{H}/^{14}\text{C}$ ratios, remained the same as those in the administered thyroxine dose. Therefore, the bulk of thyroxine metabolites was present as diphenyl ethers in urine.

The present study was carried out to elucidate the structures of thyroxine metabolites after total deiodination. A simple method of extracting the thyroxine metabolites from urine was devised. The identification and quantitation of the two major urinary metabolites of thyroxine constitute the substance of the present report.

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METHODS

Radioisotopes. The L-thyroxine used was labeled with ^{14}C on the nonphenolic ring and alanyl side chain, L-[tyrosyl- ^{14}C]-T₄ (SA 135 mCi/mole). It was synthesized with the method of Shiba and Cahnmann by Amersham/Searle, Corp. (Arlington Heights, Ill.) (9). The radiothyroxine was purified by repeated chromatography on filter paper in multiple solvent systems and had a purity of 98% or greater before use.

For injection, the radiothyroxine was dissolved with a small amount of 0.1 N NaOH and ethanol. It was then diluted with NaCl 0.9% to the desired concentration and its final pH adjusted to 7.4. After passing the solution through a Millipore filter (Swinnex \times 25 Millipore Corp., Bedford, Mass.), sterile human albumin was added to it to make a 1% solution.

Subjects. Throughout the experiment the eight normal male volunteers were housed in the Clinical Research Unit of the University of Alabama Medical Center. They were all free of thyroid disorders. L-[Tyrosyl- ^{14}C]-T₄, 8-11 μg , was administered to the subjects intravenously every day for 10 days. Urine collections were obtained from these subjects until 2 wk past the last injection of radiothyroxine. Because of acute urinary tract infection, the urine collection of subject D. G. was terminated on the 18th day of experiment. Several of the urine collections from J. H. were incomplete. His data were eliminated from Fig. 1. All urine samples were stored in frozen state until use.

Extraction procedure. The ^{14}C -labeled metabolites of thyroxine were extracted from urine by a nonionic polymeric adsorbent, Amberlite XAD-2 (Mesh 20-50, Mallinckrodt Chemical Works, St. Louis, Mo.). After washing with distilled water, the resin was made into a slurry with water (1:4), its pH adjusted to 5.0 and then stored at 4°C until use.

A mixture of 1 vol of the Amberlite slurry and 5 vol of urine was placed in a large beaker and stirred continuously for 1 hr at room temperature. The supernate was decanted and the resin was washed with 5 vol of water three times. Then the adsorbed ^{14}C -labeled compounds were eluted from the resin by vigorously stirring the washed resin with 5 vol of methanol for 10 min. The methanol solution was clean enough to be applied to paper for chromatography directly or it was concentrated by lyophilization after its pH was adjusted to 7.0. The recovery from the methanol solution was 80-85% of the total urinary ^{14}C radioactivity.

Paper chromatography. The samples were applied to Whatman No. 3 paper and developed in the following solvent systems: (a) *n*-butanol: ethanol: 2 N ammonia (5:1:2, BEA).¹ (b) *n*-butanol: acetic acid: water (78:5:7, BAW). (c) Hexane: *t*-amyl alcohol: ammonia (1:5:6, HTAA). (d) Collidine: water (1:3, CW). Both ascending and descending systems were used. The fresh urine was concentrated by lyophilization before being applied to paper for chromatography. The extracted ^{14}C radioactive metabolites were purified by repeated one-dimensional chromatography, first in BEA once and then in BAW twice. For identification, a purified preparation of a radioactive metabolite was added into an ammoniacal methanol solution of a known reference compound. A portion of the mixture was assayed for radioactivity. The mixture in known amounts was applied to pa-

¹ Abbreviations used in this paper: BAW, *n*-butanol: acetic acid: water; BEA, *n*-butanol: ethanol: 2 N ammonia; CW, collidine: water; HTAA, hexane: *t*-amyl alcohol: ammonia.

pers and chromatographed in four different solvent systems. The area of the carrier was visualized under ultraviolet light and the area was cut into 0.5 \times 2.5-cm strips. The ^{14}C radioactivity of the paper strips was assayed in a dioxane scintillator (1% 2,5-diphenyloxazole (PPO), 0.05% 1,4-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP), 5% naphthalene and 16.7% 2-ethoxyethanol) by a liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.).

The reference compounds for chromatography included thyroxine, 3,5,3'-triiodothyronine, 3,5,3',5'-tetraiodothyroacetic acid, 3,5,3'-triiodothyroacetic acid, and thyronine which were purchased commercially. 3,3'- and 3,5'-diiodothyronines and their respective acetic acid derivatives, 3-monoiodothyronine and 3-monoiodothyroacetic acid were provided by Dr. Henry H. Freeman of the Warner-Lambert Co., Morris Plains, N. J. The standard of thyroacetic acid was synthesized by Dr. Roy Gigg (National Institute of Medical Research, Mill Hill, London, England) and generously given to us through the courtesy of Dr. Rosalind Pitt-Rivers. It was prepared by the method of Petit and Buu-Hoi (10) and had a melting point of 192-194°C.

Synthesis of derivatives. The methyl and butyl esters were prepared by the method of Brenner and Huber (11). A 25% solution of HCl in either methanol or butanol was prepared. A known amount of a purified radioactive metabolite and 10.0 mg of a reference compound were dissolved in 1.0 ml of the desired acid alcohol. The mixture was incubated at 70°C for 2 hr. The reaction mixture was then applied to paper for chromatography in multiple solvent systems. The unreacted standard and its ester were visualized by ultraviolet light on the chromatograms. Each spot was cut out and assayed for ^{14}C radioactivity.

Recrystallization. A purified preparation of a radioactive metabolite and 1.0 mg of a known reference compound were placed into a small glass tube. The mixture was dissolved in 1.0 ml of hot 0.1 N Na₂CO₃. A 25 μl portion of this solution was removed for assay of ^{14}C radioactivity. A second 25 μl portion was added into 4.0 ml of absolute ethanol. The concentration of the reference compound in this alcoholic solution was measured with a spectrophotometer (Beckman DU Beckman Instruments, Inc., Palo Alto, Calif.).

To the original Na₂CO₃ solution two drops of glacial acetic acid were added. The precipitate was harvested by removal of the supernatant fluid and was washed three times with cold water. The harvested precipitate was redissolved in 1.0 ml of hot 0.1 N Na₂CO₃. Again 25- μl portions were removed for assays before acidification with glacial acetic acid. This procedure of recrystallization and sampling was repeated until the precipitates recovered were too low for spectrophotometric measurement.

Acid hydrolysis. A sample of purified metabolite was dissolved in small amounts of distilled water and its pH was adjusted to 1.2 with 2 N HCl. The acidified solution was allowed to stand at room temperature for 1 hr. Its pH was brought back to 7.0. Then it was concentrated by lyophilization for chromatography.

RESULTS

Eight normal men were given L-[tyrosyl- ^{14}C]-T₄ in small intravenous doses, 8-11 μg /day for 10 days. During the 3 wk of study, 41.0 \pm 7.4% (mean \pm SD) of the administered ^{14}C dose was recovered from urine with a range from 32 to 54% as shown in Fig. 1. The ^{14}C -labeled metabolites were found to be very soluble in *n*-butanol and

more soluble in this solvent than in other common solvents such as ethanol, ethyl ether, and ethyl acetate. In six experiments in which fresh urine was extracted with equal volume of *n*-butanol, 60.2±5.4% of the total ¹⁴C radioactivity was recovered in the butanol phase. When the urine was saturated with (NH₄)₂SO₄ more than 80% of the ¹⁴C activity was extractable into butanol at neutral pH.

Altogether six or more radioactive bands could be distinguished on the chromatogram of fresh urine depending on the solvent systems. Two histograms of chromatographed fresh urine are shown in Fig. 2. When chromatographed in butanol:ethanol:ammonia system (BEA), most of the urinary ¹⁴C radioactivity was located in three bands: A (R_f 0.42), B (R_f 0.21), and C (R_f 0.81). The results from 10 studies showed 21.6±1.2% of the total urinary ¹⁴C activity was recovered in B band, 59.2±1.5% in A band, and 6.3±0.9% in C band. As seen in Fig. 3, this distribution pattern of ¹⁴C radioactivity among the different bands of thyroxine metabolites remained remarkably constant in the urine collected on 4 consecutive days from one normal subject, and in four urine collections from four different subjects.

When the A band was eluted and rechromatographed in butanol:acetic acid:water (BAW), it further resolved into two different compounds, A₁ and A₂. As shown in Fig. 4, A₁ and A₂ were chromatographed in four different solvent systems along with known reference compounds and were found to have chromatographic characteristics similar to thyroacetic acid [4-phenoxy-(4'-hydroxy) phenylacetic acid] and thyronine, respectively. For further identification, methyl esters of metabolites A₁ and A₂ were synthesized. A large quantity of the thyroxine metabolites was obtained with relative ease by passing urine through Amberlite resin. More

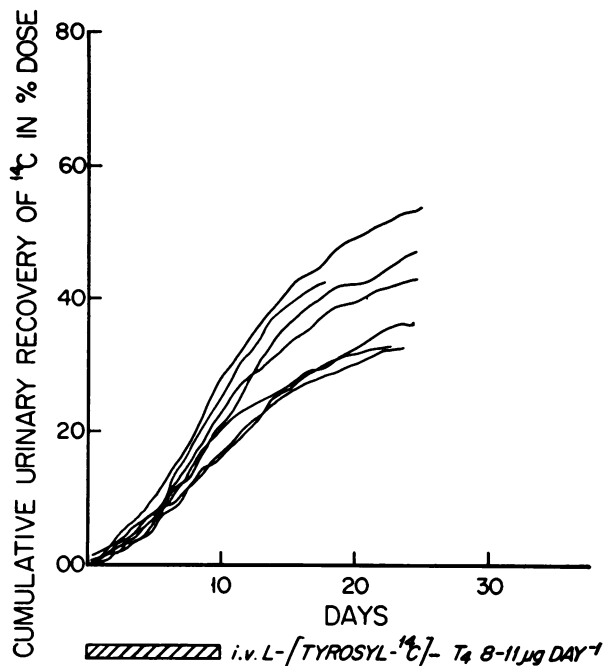


FIGURE 1 Cumulative urinary recovery of ¹⁴C after intravenous L-[tyrosyl-¹⁴C]-T₄ in seven subjects.

than 90% of the ¹⁴C activity contained in A and B bands was recovered in the methanol extract of the washed Amberlite resin, while the ¹⁴C activity contained in band C was largely lost to the resin. Table I contains the data of metabolite A₁ and metabolite A₂ isolated separately from the urine of J. T. and J. McD. Each sample was treated with acid methanol. The reaction of A₁ proceeded to near complete esterification, while the reaction of A₂ was only 50-60% complete. The known methyl ester of thyroacetic acid and the methyl ester of A₁ showed the

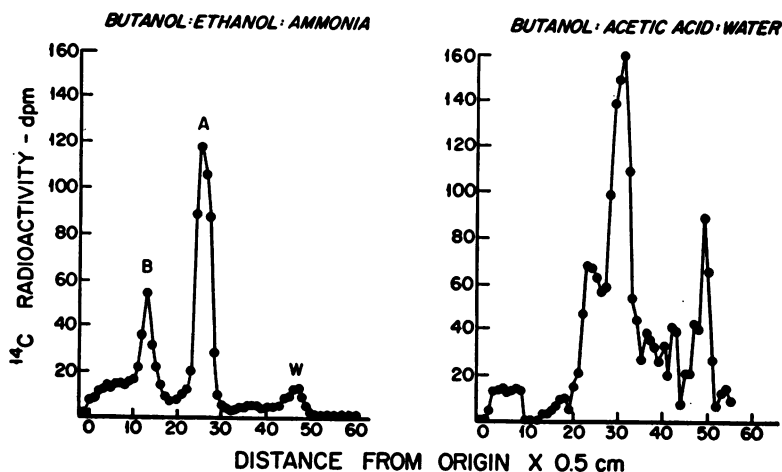


FIGURE 2 Histograms of urine chromatogram after intravenous L-[tyrosyl-¹⁴C]-T₄.

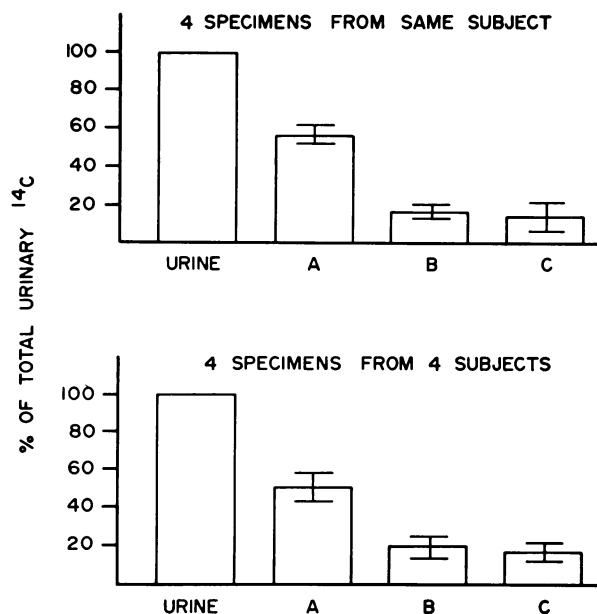


FIGURE 3 Distributions of urinary ¹⁴C after intravenous L-[tyrosyl-¹⁴C]-T₄. The upper bar graph shows the ¹⁴C distribution (mean \pm SD) in four urine collections from a single subject. The lower bar graph shows the ¹⁴C distribution in four urine collections from four different subjects. See text for further details. (Chromatography in the butanol: ethanol: ammonia system.)

same chromatographic characteristics. The known methyl ester of thyronine and the methyl ester of A₂ showed the same chromatographic characteristics.

Next, the solubility characteristics of A₁ and A₂ were studied. Purified samples of metabolite A₁ were repeatedly recrystallized along with known thyroacetic acid in hot 0.1 N Na₂CO₃ solution. The specific activity of the precipitates was determined by spectrophotometric measurement and assay of ¹⁴C activity. Fig. 5 shows the spectrophotometric characteristics of our reference thyroacetic acid. As shown in Fig. 6, during repeated precipitation the mixtures of metabolite A₁ and thyroacetic acid exhibited constant specific activity, suggesting that A₁ and thyroacetic acid have similar solubility characteristics. As a comparison, the metabolite A₁ was also recrystallized along with a known L-thyronine. The specific activity of two mixture of A₁ and thyronine fell progressively with each recrystallization.

In Fig. 7 reference samples of thyronine and thyroacetic acid were separately recrystallized with a purified sample of metabolite A₂ in exactly the same manner. During repeated recrystallization the precipitates from the mixtures of thyronine and metabolite A₂ showed constant specific activity while that from the mixtures of thyroacetic acid and metabolite A₂ did not. These findings suggest that thyronine and metabolite A₂ had similar

solubility characteristics. In total, the metabolites A₁ and A₂ isolated separately from three subjects were studied. The results from all three recrystallization experiments were similar.

Lastly, purified material from B band was subjected to acid hydrolysis. The material obtained separately from subjects J. S. and J. T. was used. After incubation at pH 1.2 for 30 min, the B band material resolved into four distinctive bands of ¹⁴C radioactivity on the chromatograms developed in BEA. Approximately 72.8% of the total ¹⁴C radioactivity was located in the midzone (R_f 0.51) which was then eluted and rechromatographed in multiple solvent systems along with reference A₁, A₂, thyroacetic acid, and thyronine. Approximately 34.6% of the ¹⁴C activity contained in the midzone material consisted of metabolite A₁ and 19.5% of the ¹⁴C activity consisted of metabolite A₂. The material from B band was also treated with glucuronidase. However, the enzyme failed to hydrolyze the B band material in our preliminary study. Our limited source of B band material prevented our further study at this moment.

DISCUSSION

After the administration of ¹⁴C-labeled thyroxine in subreplacement amounts, the cumulative recovery of ¹⁴C in the urine was approximately 41% of the total given dose 2 wk after the last intravenous injection. This recovery was comparable with that reported by West, Simons, Gortatowski, and Kumagai (12) and that reported by us in other studies (8).

TABLE I
The Chromatographic Characteristics (R_f Values) of Thyroxine Metabolites and Their Methyl Esters

Compounds	Solvent systems		
	BEA	BAW	HTAA
Thyroacetic acid	0.53 \pm 0.05	0.89 \pm 0.02	0.36 \pm 0.03
Thyronine	0.48 \pm 0.02	0.57 \pm 0.01	0.29 \pm 0.07
A ₁	0.58 \pm 0.06	0.87 \pm 0.01	0.35 \pm 0.02
A ₂	0.49 \pm 0.03	0.59 \pm 0.02	0.26 \pm 0.05
Methyl esters of:			
Thyroacetic acid	0.91 \pm 0.02	0.92 \pm 0.01	0.89 \pm 0.04
Thyronine	0.85 \pm 0.01	0.70 \pm 0.01	0.87 \pm 0.02
A ₁	0.89 \pm 0.03	0.89 \pm 0.03	0.88 \pm 0.03
A ₂	0.88 \pm 0.01	0.68 \pm 0.01	0.87 \pm 0.01

The ¹⁴C-labeled metabolites A₁ and A₂ were purified by repeated chromatography. The methyl esters were synthesized by allowing the compounds to react with acidified methanol for 2 hr at 70°C. Each R_f value represents the average \pm SD of four to six studies.

BEA, butanol:ethanol:ammonia; BAW, butanol:acetic acid: water; HTAA, hexane:*t*-amyl alcohol:ammonia.

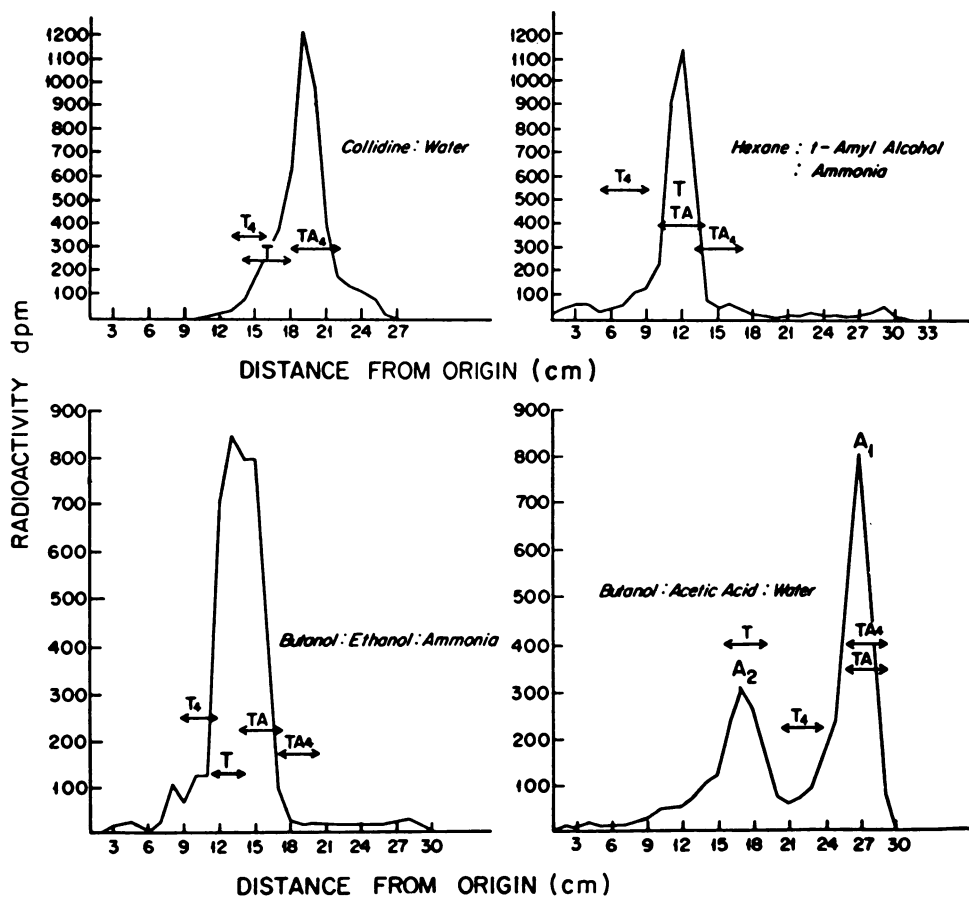


FIGURE 4 Identification of band A by paper chromatography. A preparation of band A was added into a methanol solution of thyroacetic acid. The mixture was chromatographed in four solvent systems. The reference compounds were visualized under ultraviolet light. Each chromatogram was cut into 2.5×0.5 -cm strips and assayed for ^{14}C radioactivity. T₄, thyroxine; TA₄, 3,5,3',5'-tetraiodothyroacetic acid; TA, thyroacetic acid; T, thyronine.

The ^{14}C -labeled products of thyroxine in urine were highly soluble in butanol. By saturating the urine with $(\text{NH}_4)_2\text{SO}_4$, nearly 80% of the ^{14}C radioactivity was extractable by butanol at neutral pH. These solubility characteristics suggest that in man, the bulk of thyroxine metabolites was excreted by the kidney in a free rather than conjugated form. At least in the instances of thyroacetic acid and thyronine, which make up more than half of the deiodinated thyroxine products in urine, they were observed to be present in a ratio of free to bound forms of 4:1. In the present study, thyroacetic acid and thyronine were freed from their bound forms after acid hydrolysis. Limitation of our supply of ^{14}C -labeled thyroxine metabolites prevented us from carrying out extensive enzymic hydrolysis of the material contained in band B. Therefore, the chemical nature of these derivatives of thyroxine products was not determined. However, animal studies by Pittman and Shimizu (13) had shown previously that after the administration of ^{14}C -la-

beled thyroxine to rats, the urine chromatograms also contained a B band which changed its chromatographic characteristics after its incubation with glucuronidase. These results suggest that some of the material contained in B band was conjugated as a glucuronide. These same investigators also observed a species difference in the distribution pattern of free and bound thyroxine metabolites in urine. In rats nearly two-thirds of the urinary ^{14}C activity was located in B band, while in man only one-fifth of the urinary ^{14}C activity was located in B band.

The ^{14}C radioactivity in the urine of our subjects was found to be a mixture of many thyroxine products which showed rather similar chromatographic characteristics in the alkaline chromatographic systems. Some of the thyroxine products were found in very small amounts in the urine, which made detailed studies of their chemical nature very difficult. In the present study, we were able to separate two urinary fractions of thyroxine me-

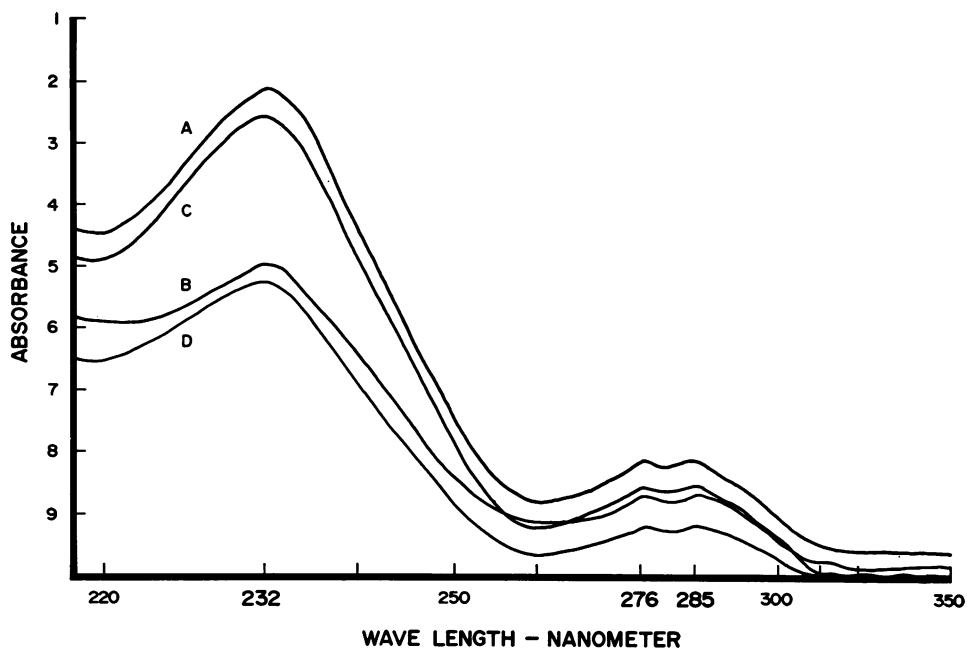


FIGURE 5 The ultraviolet absorption spectra of the following compounds were measured in ethanol solution 4.01×10^{-5} mole/liter at pH 5.0 with a ratio recording spectrophotometer (Beckman DK 2). λ max = 232, 276, and 285 nmeters. (A) Reference thyroacetic acid; (B) A_1 and thyroacetic acid at the end of recrystallization; (C) Reference thyronine; (D) A_2 and thyronine at the end of recrystallization.

tabolites, band A and band B. By their chromatographic characteristics, chemical and physical characteristics, the labeled compounds in band A were found to be composed of free thyroacetic acid and thyronine. Band B contained three or more different compounds from which free thyroacetic acid and thyronine were released during acid hydrolysis. Of the total urinary ^{14}C radioactivity, approximately 35.2% was identified as free thyroacetic acid, 8.5% was identified as a derivative of thyroacetic acid, 15.2% was identified as free thyronine, and 4.6% as a derivative of thyronine.

In the five subjects in whom T_4 kinetics had been studied previously, the average disposal rate of thyroxine was found to be $82.4 \mu\text{g/day}$ (14). Of this amount of thyroxine, two-thirds or $54.9 \mu\text{g/day}$ was disposed through the renal route. Therefore, it can be estimated that in the five subjects studied, the average daily excretion of thyroacetic acid was $7.5 \mu\text{g}$ or 13.7% of the renal disposal rate of thyroxine products, including iodide and iodinated metabolites. The average urinary excretion of thyronine was $3.59 \mu\text{g/day}$ or 6.53% of the total thyroxine products in the urine. Since iodine in both organic and inorganic forms constituted only 64.7% of the thyroxine disposed through the kidney, there remained 15–20% of the thyroxine products of which the chemical nature was unknown.

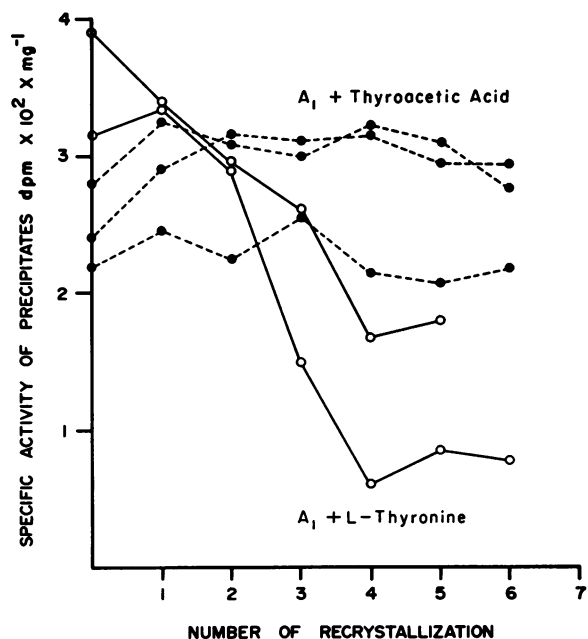


FIGURE 6 The solubility characteristics of A_1 . Purified samples of A_1 obtained from three subjects were recrystallized repeatedly from a solution of 0.1 N Na_2CO_3 . The specific activities of mixtures of L-thyronine and A_1 are represented by (○). The specific activities of mixtures of thyroacetic acid and A_1 are represented by (●).

Our preliminary study showed that after intravenous administration of radioiodine-labeled thyroxine to normal man, approximately 10% of the radioiodine in urine represented organic iodine. Several labeled compounds were observed on urine chromatograms. One of them had the chromatographic characteristics similar to that of thyroxine itself. Earlier studies in rats and dogs by other investigators have also detected free thyroid hormones, their glucuronides, and pyruvic acid analogues in the urine (15, 16). The urinary contents of these compounds increased when the normal enterohepatic circulation of thyroid hormones was interrupted in the animals.

After incubation of thyroxine with liver microsomes, Wynn and Gibbs observed diiodotyrosine, 1,4-dihydroquinone, and three other thyroxine products which retained parts of both phenyl rings and the side chain (3). Wynn also observed a derivative of 1,4-dihydroquinone in the urine of a human subject after intravenous administration of ^{14}C -labeled thyroxine. However, the conclusion of this study was obscured by the fact that the same subject was also given ^{14}C -labeled 1,4-dihydroquinone just before the study (6). Contrary to the findings of these same investigators we did not find any diiodotyrosine or 1,4-dihydroquinone among the major thyroxine products in normal urine, but our findings did

not exclude the presence of small amounts of some single-ringed compounds.

The results of present study are in complete agreement with our earlier study in intact rats (7) and in normal human subjects (8) that the bulk of deiodinated thyroxine products in urine retains moieties from both phenyl rings as well as parts of the alanyl side chain. These results agree with the report by Lissitsky and coworkers (4) that thyronine was found to be a thyroxine metabolite after incubation of radiothyroxine with a liver preparation. Our findings that thyroacetic acid and thyronine alone made up 63.5% of the ^{14}C -labeled thyroxine metabolites in urine provide support for our thesis, namely that the major metabolic pathways of thyroxine remove the iodine atoms from both the phenolic and nonphenolic rings by substituting hydrogen for iodine and leave the diphenyl ether linkage intact.

ACKNOWLEDGMENTS

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REFERENCES

1. Plaskett, L. G. 1961. Studies on the degradation of thyroid hormones *in vitro* with compounds labeled in either ring. *Biochem. J.* **78**: 652.
2. Roche, J., J. Nunez, and C. Jacquemin. 1962. Nature des produits de la désiodation des hormones thyroïdiennes marquées simultanément par le tritium et l'iode radioactif. *Biochim. Biophys. Acta.* **64**: 475.
3. Wynn, J., and R. Gibbs. 1962. Thyroxine degradation. II. Products of thyroxine degradation by rat liver microsomes. *J. Biol. Chem.* **237**: 3499.
4. Lissitzky, S., M. T. Bénévent, M. Roques, and J. Roche. 1958. Caractérisation de la thyronine comme produit de la désiodation de la thyroxine par des compes de foi de rat. *C. R. Seances Soc. Biol. Fil.* **152**: 1490.
5. Nunez, J., L. Rappaport, C. Jacquemin, and J. Roche. 1964. Deiodination *in vivo* de ^3H - et ^{14}C -L-thyroxine. *C. R. Seances Soc. Biol. Fil.* **158**: 12.
6. Wynn, J. 1963. The major human pathway of thyroxine catabolism. *Clin. Res.* **11**: 232.
7. Pittman, C. S., and J. B. Chambers, Jr. 1969. Carbon structure of thyroxine metabolites in urine. *Endocrinology.* **84**: 705.
8. Pittman, C. S., V. H. Read, J. B. Chambers, Jr., and H. Nakafuji. 1970. The integrity of the ether linkage during thyroxine metabolism in man. *J. Clin. Invest.* **49**: 373.
9. Shiba, T., and H. T. Cahnmann. 1962. Synthesis of specifically iodine 131 - and carbon 14 -labeled thyroxine. *J. Org. Chem.* **27**: 1773.

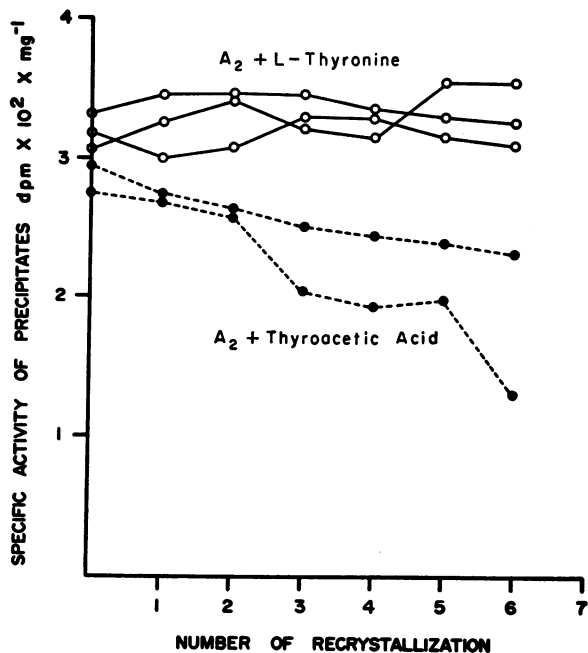


FIGURE 7 The solubility characteristics of A_2 . Purified samples of A_2 obtained from three subjects were recrystallized repeatedly from a solution of 0.1 N Na_2CO_3 . The specific activities of mixtures of L-thyronine and A_2 are represented by (O). The specific activities of mixtures of thyroacetic acid and A_2 are represented by (●).

10. Petit, L., and N. P. Buu-Hoi. 1961. A synthesis of thyronamine and its lower homolog. *J. Org. Chem.* **26**: 3832.
11. Brenner, M., and W. Huber. 1953. Herstellung von alpha-amino Saureestern durch alkoholyse der methyl ester. *Helv. Chim. Acta.* **36**: 1109.
12. West, C. D., E. L. Simons, M. J. Gortatowski, and L. F. Kumagai. 1963. The metabolism of ring-labeled L-thyroxine-¹⁴C *in vivo*. *J. Clin. Invest.* **42**: 1134.
13. Pittman, C. S., and C. Shimizu. 1966. Metabolism of ¹²⁵I and ¹⁴C-labeled thyroxines. *Endocrinology.* **79**: 1109.
14. Pittman, C. S., J. B. Chambers, Jr., and V. H. Read. 1971. The extrathyroidal conversion rate of thyroxine to triiodothyronine in normal man. *J. Clin. Invest.* **50**: 1187.
15. Roche, J., R. Michel, and J. R. Tata. 1954. Sur la nature des combinaisons iodees excretés par la foie et le rein après administration de L-thyroxine et de L-3:5:3'-triiodothyronine. *Biochim. Biophys. Acta.* **15**: 500.
16. Flock, E. V., J. L. Bollman, J. H. Grindlay, and B. F. McKenzie, 1957. Metabolites of radioactive L-thyroxine and L-triiodothyronine. *Endocrinology.* **61**: 461.