The Integrity of the Ether Linkage

during Thyroxine Metabolism in Man

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A B S T R A C T The structures of thyroxine metabolites
after total deiodination bear on the mode of thyroxine
(T_4) action in vivo. The present study was undertaken
to determine the integrity of the ether linkage during
thyroxine metabolism in man. Normal volunteers were
given simultaneous intravenous injections of two thy-
roxines labeled with either ^{14}C or ^3H on the opposite
sides of the ether linkage, D,L-$[\alpha,\beta-^3H]T_4$ and D,L-[phe-
nolic ring-$^{14}C]T_4$. The ratio of alanine side chain to
phenolic ring which was measured as $^3H:^{14}C$ ratio was
found to remain constant in the serum, urine, and feces
during the subsequent 3 wk. The disappearance rates
of the ^3H and ^{14}C radioactivity from blood were similar.
The values of half-life were in the range of 4.2–6.7 days.
51–63% of the ^3H and 50–57% of the ^{14}C doses were
recovered from urine and 13–20% of the ^3H and 15–20%
of the ^{14}C doses were recovered from feces. Chroma-
tography of the urinary metabolites confirmed that the
phenolic ring and the nonphenolic ring including at
least part of the side chain remained linked together.

INTRODUCTION

When thyroxine interacts with the cellular constituents,
certain degradative reactions other than deiodination
may happen to the molecule. Conceivably these reactions
may result in quinone formation of the phenolic ring
and cleavage of the ether linkage. However, there are
very few published reports on the metabolism of the
carbon structure of thyroxine, and the data obtained
from studies of the lower animals are controversial.

Plaskett (1), Roche, Nunez, and Jacquemin (2), and
Wynn and Gibbs (3) incubated preparations of rat liver
with specifically labeled radiothyroxines. Subsequently
they found significant radioactivity in a protein material
which yielded radioactive diiodotyrosine upon hydroly-
sis. These observations suggest that under certain in
vitro conditions, thyroxine may undergo cleavage of its
ether linkage before total deiodination. Furthermore
Wynn and Gibbs (3) and Wynn (4) reported that the
phenolic ring of thyroxine may be recovered from incu-
bated liver microsomes and from the urine of man as
a hydroquinone. However, a contrary conclusion was
advanced by other investigators. From similar incuba-
tion studies of rat liver, thyronine was observed to be
one of the thyroxine metabolites by Lizzitzky, Benevent,
Roques, and Roche (5). Dunn and Werner (6) admin-
istered to patients a radiothyroxine labeled with ^{131}I
on the nonphenolic ring. Subsequently, from the serum
of these patients these investigators found no radio-
active diiodotyrosine although they did observe non-
radioactive diiodotyrosine. The chromatographic studies
of the urinary metabolites of ^{14}C-labeled thyroxine from
our laboratory (7) also indicated that at least in the
living rat the majority of degraded thyroxine is ex-
creted in the urine as intact diphenyl ethers.

The purpose of this study was to investigate the me-
tabolism of the deiodinated metabolite(s) of thyroxine
and the integrity of the ether linkage of thyroxine in nor-
mal man during thyroxine metabolism. In this study two
specifically labeled thyroxines labeled with either ^3H or
^{14}C on the opposite sides of the ether were administered
simultaneously to each of the experimental subjects.
The ratio of alanine side chain to phenolic ring was
measured as $^3H:^{14}C$ ratio in the blood, urine, and feces
during the subsequent 3–4 wk. The thesis was that if
hydroquinone and diiodotyrosine were the major thyrox-
ine products, the metabolic pathways and the rates of
elimination of these compounds would probably be dif-

ferent in a living host. In this instance the $^{3}H:^{14}C$ ratios of the thyroxine products in the urine of our experimental subjects would be different from the $^{3}H:^{14}C$ ratios of the administered radiothyroxines. On the other hand, if some diphenyl ethers were the major thyroxine products, then the $^{3}H:^{14}C$ ratios of the experimental subjects must necessarily remain unchanged from the $^{3}H:^{14}C$ ratios of their respective radiothyroxine doses. It was not our purpose to extend the already extensive literature on the metabolism of ^{131}I-labeled thyroxine in man, although a tracer amount of ^{131}I-labeled thyroxine was also administered to each subject as a monitoring device.

METHODS

Materials. D,L-thyroxine labeled with ^{3}H substituted on the alpha and beta carbons of the alanine side chain (D,L-$[\alpha,\beta$-$^{3}H]T_4$) was purchased from EURATOM, Brussels, Belgium. It was prepared by the laboratory of Dr. J. Nunez according to his published method (8) and it had a specific activity of 120 mCi/mmole.

D,L-thyroxine uniformly labeled with ^{14}C in the phenolic ring (D,L-$[\phi$-$^{14}C]T_4$) was synthesized by the former Nuclear Research Chemicals, Inc., Orlando, Fla. ^{14}C-labeled 3,5-diiodo-4-hyroxyphenylpyruvic acid was coupled with D,L-3,5-diiodotyrosine to form D,L-$[\phi$-$^{14}C]T_4$ by the method of Meltzer and Stanaback (9) as modified by Shiba and

Cahnmann (10). This radiothyroxine had a specific activity of 21 mCi/mmole.

D,L-thyroxine labeled with ^{14}C in the beta position of the alanine side chain (D,L-$[\beta$-$^{14}C]T_4$) was also prepared by the former Nuclear Research Chemicals, Inc. It was synthesized with [COOH-^{14}C]-p-carboxyanisol which had been formed from p-bromo-methoxybenzene and $^{14}CO_2$ during a Grignard reaction. After halogenation, the ^{14}C-labeled carboxyanisol was allowed to react with an ethyl ester of acetamidomalonic acid to form D,L-3,5-diiodotyrosine after hydrolysis. The D,L-3,5-diiodotyrosine prepared by this method was labeled in the beta carbon. It was then coupled with 3,5-diiodo-4-hydroxyphenylpyruvic acid to give D,L-$[\beta$-$^{14}C]T_4$ according to the methods mentioned before (9, 10). The D,L-$[\beta$-$^{14}C]T_4$ had a specific activity of 20 mCi/mmole.

L-thyroxine labeled with ^{131}I on the 3' and 5' positions (L-$[3',5'$-$^{131}I]T_4$) with a specific activity in the range of 21-33 Ci/mmole was purchased from Abbott Laboratories, North Chicago, Ill.

The radiothyroxines were repurified by paper chromatography immediately before the preparation of dose. The positions of their various radioisotopic labeling are shown in Fig. 1.

Subjects. The subjects were normal volunteers. They were one female (M. B.) and three males in the age range of 21-25 yr who were selected for their negative history of thyroid diseases, good general health, normal protein-bound iodine, and normal 24 hr uptake of radioiodine. The thyroid function tests were performed at both the beginning and the end of each study. Throughout the experiment the subjects were given a routine hospital diet and kept on the Clinical Research Unit of the University of Alabama Medical Center, Birmingham, Ala. Two of the four subjects, G. N. and J. T., were given Lugol's solution, 3 drops three times daily, while M. B. and G. B. were not given any medication to block the thyroidal recirculation of ^{131}I.

Three subjects were chosen for the experiment, J. T., G. N., and G. B., to whom a mixture of D,L-$[\alpha,\beta$-$^{3}H]T_4$ and D,L-$[\phi$-$^{14}C]T_4$ was given. One subject (M. B.) was chosen to test the stability of the ^{3}H labels on D,L-$[\alpha,\beta$-$^{3}H]T_4$. To this last subject a mixture of D,L-$[\alpha,\beta$-$^{3}H]T_4$ and D,L-$[\beta$-$^{14}C]T_4$ was given.

The radiothyroxine dose was prepared by dissolving the desired amounts of purified ^{3}H- and ^{14}C-labeled thyroxines in a small amount of 5% ammonia in ethanol and diluting to 20 ml with 0.9% NaCl. The pH of the solution was adjusted to 7.4 with 0.1 N HCl. Then the solution was passed through a Millipore filter (Swinnex × 25) and was handled with sterile technique from that point on. Lastly, enough of a sterile solution of human albumin was added to constitute 1% of the mixture as well as a tracer amount of L-$[3',5'$-$^{131}I]T_4$ (0.02-0.12 µg or 0.15-6.5 µCi/day) was added to the dose as a monitoring device. The dose was administered to each subject intravenously every day for 6-7 days. The total amounts of thyroxine received by these subjects were 31-114 µg/day as listed in Table I.

From the start of an experiment, blood, total urine, and feces were collected from the subjects every day for the following 30-36 days. However, the samples collected beyond the 3rd wk contained very low radioactivities and they were not included in the calculation of data.

Radioactivity assay. Immediately after collection, the serum, urine, and feces were measured for ^{131}I radioactivity. The ^{131}I radioactivity was measured by a well scintillation counter (Nuclear-Chicago, Corporation, Des Plaines, Ill.). The serum was measured in 1.0-ml aliquots

D,L-(ϕ -^{14}C) T_4

D,L-(β—^{14}C) T_4

D,L-(α,β—^{3}H) T_4
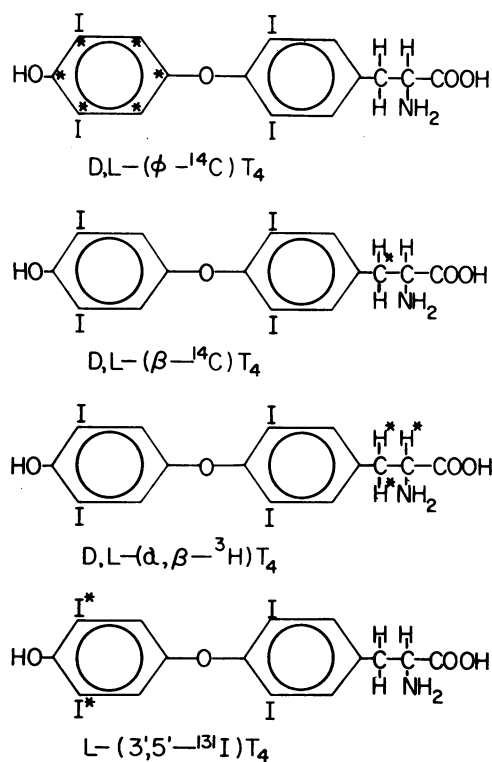
L-(3',5'—^{131}I) T_4

FIGURE 1 The radiothyroxines. The * indicates the location of the radioisotopic label.

and the urine was measured in 3.0-ml aliquots. The feces were first solubilized in water and weighed before measurement of ^{131}I radioactivity in 2.0-g aliquots. All samples were counted in duplicates.

The serum, urine, and feces were then frozen and stored for approximately 15 half-lives of ^{131}I before they were measured for ^{14}C and ^{3}H radioactivities simultaneously in a liquid scintillation counter (Nuclear-Chicago, Corporation, Des Plaines, Ill.). Quenching was corrected by using toluenes labeled with either ^{14}C or ^{3}H as the internal standards (Nuclear Chicago, Corporation, Des Plaines, Ill.). Each sample was counted in triplicate and was counted until the counting error was less than 3%. For the assay of ^{14}C and ^{3}H radioactivities, the serum was measured in 0.5-ml aliquots. A serum aliquot was digested in 3.0 ml of NCS^{TM} solubilizer (Amersham/Searle, Des Plaines, Ill.) at room temperature and then was counted in a toluene scintillator (0.5% 2,5-diphenyloxazole (PPO), 0.03% p-bis[2-(5-phenyloxazolyl)] benzene (POPOP). The urine radioactivity was measured in 1.0 ml of urine aliquots. Each urine aliquot was first decolorized with 0.2 ml of 30% H_2O_2 before the aliquot was counted in a dioxane scintillator (1% PPO, 0.05% POPOP, and 5% naphthalene). The radioactivity of the feces was measured in 4.0-g aliquots of a solubilized specimen. Each fecal aliquot was first extracted twice with butanol which was constituted to a final volume of 15 ml. Then 1.0-ml aliquots of the butanol extract were counted in triplicates in a toluene scintillator. The butanol extract contained approximately 90% of the total fecal radioactivity (11). The counting efficiencies of ^{14}C in the serum, urine, and feces were approximately 54, 53, and 39%, respectively. The corresponding values of ^{3}H assay were approximately 18, 7, and 5%.

Chromatography. A freshly collected urine sample was concentrated by lyophilization to approximately 5% of its original volume. An aliquot of the concentrate was applied directly to Whatman paper No. 1 or No. 3 (10–50 μl/cm) and developed in an ascending system in butanol:ethanol:2 N ammonia (5:1:2 BEA). After drying, a tinfoil strip was used to cover a portion of the chromatogram along its entire length to shield off the radioactivity of ^{3}H and ^{14}C. Then a radioautogram that differentiates the ^{131}I radioactivity from the ^{3}H and ^{14}C radioactivities was made from the chromatogram with Kodak no-screen X-ray film.

RESULTS

^{3}H : ^{14}C ratios. In the experimental group each subject received a mixture of D,L-[α,β-^{3}H]T$_4$ and D,L-[ϕ-^{14}C]T$_4$. The ratio of alanine side chain to phenolic ring in the body fluids was measured as the ratio of ^{3}H radioactivity to ^{14}C radioactivity. The ^{3}H : ^{14}C ratios of the radiothyroxine doses and of the serum, urine, and feces samples are presented in Table I. G. B. was the first experimental subject admitted to the present study. The amount of ^{3}H and ^{14}C radioactivity administered to him was low. The counting statistics of his samples collected beyond the 2nd wk were unsatisfactory. This difficulty was partly overcome in the later subjects admitted to our study to whom the ^{3}H and ^{14}C doses administered were doubled. The differences between the dose and sample ratios in each subject, including G. B., were not statistically significant ($P > 0.5$). The data were compatible with the hypothesis that the radioactive metabolites of thyroxine as well as the parent hormone present in these body fluids contain both the phenolic and the nonphenolic rings.

In order to validate our assumption that in these experimental subjects the ^{3}H : ^{14}C ratio did represent the ratio of alanine side chain to phenolic ring, it was necessary for us to demonstrate the stability of the ^{3}H label on the alanine side chain. Therefore, a mixture of D,L-[α,β-^{3}H]T$_4$ and D,L-[β-^{14}C]T$_4$ was given to one subject. M. B. In this subject both ^{3}H and ^{14}C were labeled in the same area of the thyroxine molecule. The stability of the ^{3}H label was demonstrated by the unchanging ^{3}H : ^{14}C ratios in the body fluids of subjects M. B. over a period of 3 wk.

Serum disappearance rates. The biological half-life ($t_{\frac{1}{2}}$) of the serum radioactivity was obtained from the linear regression of time on the logarithm of serum

TABLE I

The Mean ^{3}H:^{14}C Ratios of the Daily Collections of the Blood, Urine, and Feces Obtained during the First 3 wk after the Administration of the Radiothyroxines

Patients	Radiothyroxines	Dose		^{3}H:^{14}C	Mean ^{3}H:^{14}C ratio ±SD		
					Serum	Urine	Feces
		$\mu Ci/day$	$\mu g/day$				
J. T.	D,L — [α, β — ^{3}H]T$_4$	3.91	25.30	5.43	5.66	5.55	5.02
	D,L — [ϕ — ^{14}C]T$_4$	0.72	26.57		±0.52	±0.68	±0.77
G. N.	D,L — [α, β — ^{3}H]T$_4$	5.58	36.10	5.08	6.63	6.54	5.49
	D,L — [ϕ — ^{14}C]T$_4$	1.10	40.59		±0.89	±0.98	±0.38
G. B.	D,L — [α, β — ^{3}H]T$_4$	2.35	15.20	5.52	8.28	6.70	4.38
	D,L — [ϕ — ^{14}C]T$_4$	0.43	15.87		±2.86	±1.06	±1.05
M. B.	D,L — [α, β — ^{3}H]T$_4$	8.23	53.25	5.27	4.86	5.21	5.89
	D,L — [β — ^{14}C]T$_4$	1.56	60.68		±0.34	±0.69	±0.78

The Ether Linkage of Thyroxine 375

radioactivity as depicted in the upmost charts in Figs. 2–4. In the experimental subject, J. T., the half-lives of ³H and ¹⁴C radioactivities were 6.66 and 6.10 days. The corresponding half-lives in the second experimental subject, G. N., were 5.16 and 5.15 days, and in the third experimental subject, G. B., were 5.85 and 4.21 days. In subject M. B., the half-lives of ³H and ¹⁴C radioactivities were, respectively, 7.82 and 7.19 days. Therefore, regardless of the location of the isotopic labeling on the T₄ molecule, the half-lives of ³H and ¹⁴C in the same subject showed no significant difference. In all instances the correlation coefficient of Pearson and Lee was greater than 0.9.

Two of the subjects, M. B. and G. B., received no medication to prevent thyroidal recirculation of ¹³¹I. The half-lives of ¹³¹I in these subjects were 8.76 and 5.27

days. The other two subjects, J. T. and G. N., did receive Lugol's solution throughout the experiments. Reduction of the thyroidal recirculation of ¹³¹I resulted in a closer correlation of the half-lives of all three radioisotopes, ³H, ¹⁴C, and ¹³¹I, in J. T. and G. N., whose half-lives of ¹³¹I radioactivity in blood were 6.27 and 5.97 days, respectively. The [3'5'-¹³¹I]T₄ used in this study was an L-isomer while all the ³H- and ¹⁴C-labeled radiothyroxines were D,L-racemic mixtures. It is interesting to note that irrespective of the optical configurations, in neither J. T. nor G. N. were there any significant differences between the half-lives of ¹³¹I and those of ³H and ¹⁴C.

Radioactivity recoveries. The radioactivity recovery was calculated from the urinary and fecal radioactivities collected over 3 wk and was expressed as per cent of a
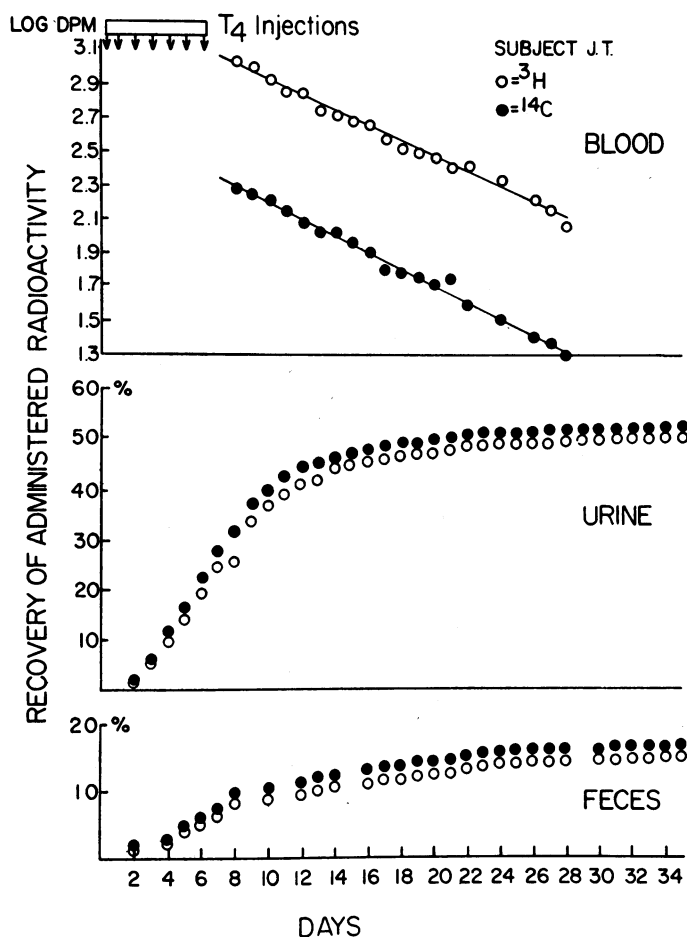

FIGURE 2 The metabolism of simultaneously injected radiothyroxines. Each subject received a mixture of radiothyroxines labeled on the opposite sides of the ether linkage of thyroxine, D,L-[α,β-³H]T₄ (○) and D,L-[phenolic ring-¹⁴C]T₄ (●). The radiothyroxine dose was given by daily intravenous injections in equally divided doses for 6–7 days.

given dose. The cumulative recoveries from urine and feces of the three experimental subjects are depicted in the lower two charts of Figs. 2–4. The recoveries of subject M.B. are depicted in the lower two charts of Fig. 4. In all the subjects 51–63% of the ^{3}H and 50–57% of the ^{14}C doses were recovered from the urine. In the same subjects 13–20% of the ^{3}H doses and 15–20% of the ^{14}C doses were recovered from the feces. Therefore, the total recovery of ^{3}H or ^{14}C in every subject was better than 63%. Although not observed in subjects J. T. and M. B., the urinary recoveries of subjects G. B. and G. N. showed a small but consistent increase in the [3]H:[14]C ratio with time.

A total of 59–62% of the ^{131}I was recovered from all the subjects. Approximately 49–51% of the ^{131}I dose was recovered from the urine and 10–12% from the feces. The distribution of the ^{131}I excretion agrees with the published results of Ingbar and Freinkel (12) and it strongly indicates that the sample collections in our patients were reasonably complete. In three out of four subjects the fecal recoveries of ^{14}C and ^{3}H were nearly twice the fecal recovery of [131]I. Therefore, it is likely that some deiodinated metabolite(s) of thyroxine was present in the feces.
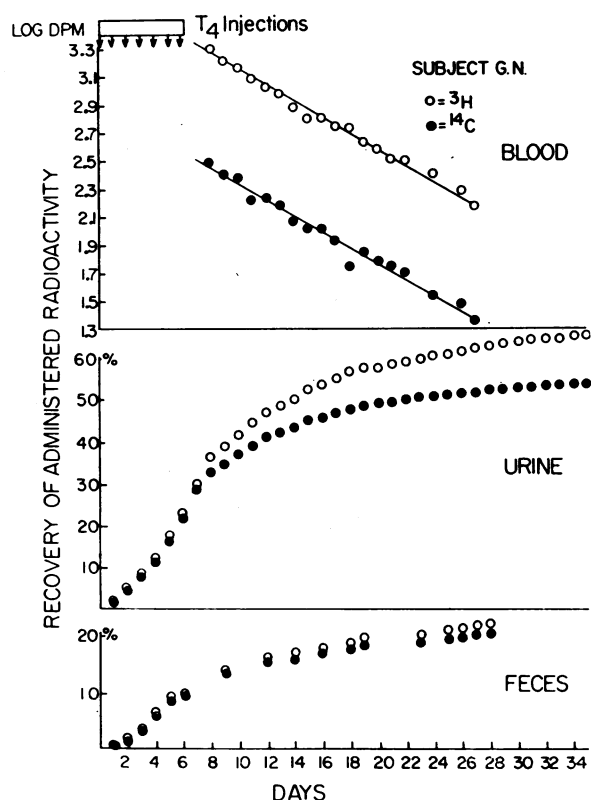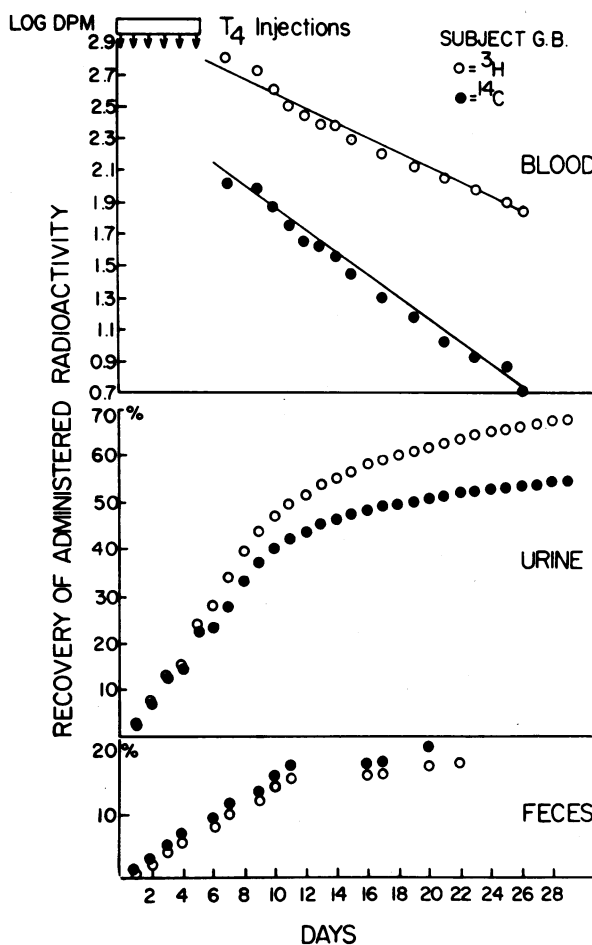

FIGURE 3   See legend for Fig. 2.

FIGURE 4   See legend for Fig. 2.

Chromatography. The amount of ^{14}C we were allowed to administer to our subjects was too low to carry out satisfactory chromatography, although extensive attempts were made. [131]I activity was not demonstrable in any of the ^{14}C-labeled metabolites from urine. The metabolites of all four subjects showed similar chromatographical characteristics and suggested that these metabolites contained components from both the phenolic and the nonphenolic rings. The exact identities of these metabolites were not established.

DISCUSSION

Early in vitro studies (1–4) showed that cleavage of the ether linkage is a very plausible pathway of thyroxine degradation that leads to the production of derivatives of quinone and iodinated tyrosines and possibly other smaller fragments. The turnover rate of hydroquinone appears quite rapid in man. Wynn (4) recovered 40% of a given dose in 10 hr. On the other hand, iodinated tyrosines must join the vast pool of free tyro-

sines after deiodination and become available for protein and hormone syntheses and other reactions. Therefore, the urinary eliminations of these greatly different compounds in man are probably different. If thyroxine is degraded exclusively through ether cleavage, then the urinary excretions of the phenolic and nonphenolic rings of thyroxine are likely to be different. On the other hand, if thyroxine remains intact or if it is degraded primarily through a pathway that leaves the ether linkage intact, then the ratio of the phenolic ring to the nonphenolic ring (or alanine side chain) can be expected to remain constant in all body compartments.

In our study the experimental subjects were given a mixture of radiothyroxines simultaneously. The ^3H and ^{14}C were labeled on the opposite sides of the ether linkage of thyroxine. The ratio of the phenolic ring to the nonphenolic ring was measured by the $^3H:^{14}C$ ratios in the blood, urine, and feces. Our results showed that during a period of 3 wk after the administration of the radiothyroxines, the average of the daily $^3H:^{14}C$ ratios of the blood, urine, and feces showed no significant difference from the $^3H:^{14}C$ ratio of the radiothyroxine

FIGURE 5 The stability of the ^3H label. This subject received a mixture of radiothyroxine, each labeled on the alanine side chain D,L-[α,β-^3H]T$_4$ (O) and D,L-[β-^{14}C]T$_4$ (●)

dose in each subject $(P > 0.5)$. This conclusion was based on the data obtained from samples that represented over $\frac{2}{3}$ of the given radioactive dose.

It is known that after the administration of ^{131}I-labeled thyroxine the major constituent of the serum and fecal radioactivities is thyroxine itself. In the urine the ^{131}I is present largely in the form of inorganic iodide. The results of the present study show that the disappearance rates of ^3H, ^{14}C, and ^{131}I from serum were very close within the same individual. This close relationship was best demonstrated in subjects J. T. and G. N. to whom Lugol's solution was administered to prevent thyroidal recirculation of ^{131}I. Unchanged thyroxine is likely the major constituent of the ^3H, ^{14}C, and ^{131}I radioactivities in serum. Any thyroxine metabolite derived from either deiodination or cleavage of the diphenyl ether was present in too low a concentration in the blood to allow its detection by our method. This agrees with the experience of Dunn and Werner (6). More recently at the 1969 annual meeting of the Endocrine Society in New York City, Braverman, Ingbar, and Sterling showed in a preliminary report that a significant amount of ^{131}I-labeled triiodothyronine may be derived peripherally from ^{131}I-labeled thyroxine. However, due to the fast turnover rate of the triiodothyronine pool, the ^{131}I triiodothyronine was only found in minute amounts in the serum.

West, Simons, Gortatowski, and Kumagai (13) observed that after the simultaneous administration of L-[3',5'-^{131}I]T$_4$ and L-[phenolic ring-^{14}C]T$_4$ to man the disappearance rates of ^{131}I and ^{14}C radioactivity from plasma were essentially identical with a t½ of 6.8 days. Our present findings agree with their observation; although our earlier studies in rats (11, 14), to which the various radiothyroxines were not given simultaneously, had shown these same ^{14}C- and ^3H-labeled thyroxines to have slower disappearance rates from L-[3',5'-^{131}I]-T$_4$. West et al. (13) also observed that in the dog the disappearance rate of ^{14}C was much faster than that of ^{131}I. Perhaps there are species difference in the handling of inorganic iodide and the deiodinated thyroxine product(s).

The naturally occurring isomer of thyroxine was found by Harrington and Salter (15) to be L-thyroxine, and this was found to be many times more active than the D-isomer in vivo. Other studies, in which the two isomers of thyroxine were not administered simultaneously into the same experimental subject, found that the metabolic disposal of D-thyroxine was much faster than its L-isomer (16, 17). The early work of Sterling, Lashof, and Man (18) had shown that after a single injection of L-[3',5'-^{131}I]T$_4$, the biological half-life of serum radioactivity in euthyroid subjects varied with a range of 5–10 days. Therefore, unless the test compounds
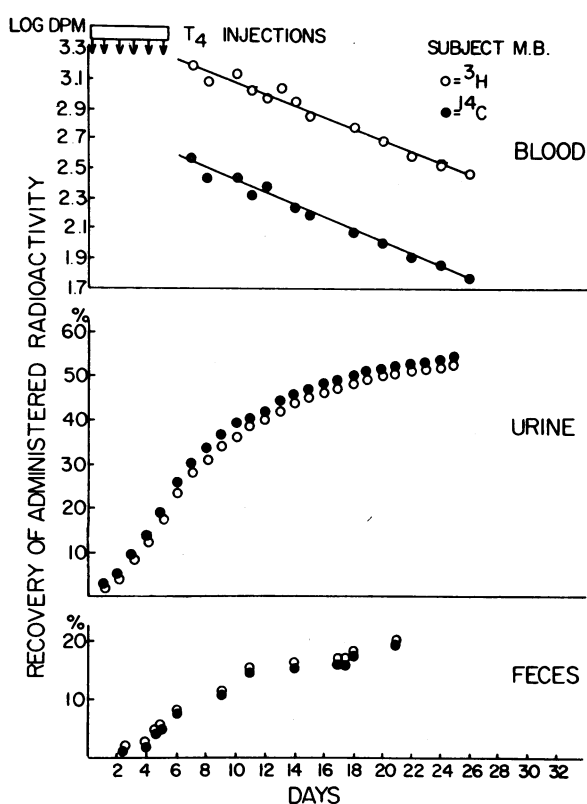
are administered to a subject simultaneously, any meaningful comparison of the usual metabolic parameters such as the biological half-life is difficult.

In our study the ^{131}I-labeled thyroxine used was a L-isomer while the [14]C- and ^{3}H-labeled thyroxines used were mixtures of the D-L-isomers. The proportions of each optical form in our radiothyroxines were not known. It is interesting to note that in subjects G. N. and J. T. the half-lives of ^{131}I, ^{14}C, and ^{3}H radioactivities in serum showed very little difference. In addition, the urinary excretion rates as well as the urinary recoveries of these three isotopes were very close over a period spanning 3 wk. Since the conclusion of the present study we have administered a pure L-isomer of thyroxine labeled with ^{14}C in the nonphenolic ring and alanine side chain to two normal subjects in similar fashion as described here. The ^{14}C disappearance rates from serum were found to be 6.14 and 6.30 days. The ^{14}C recoveries from urine 10 days after completion of thyroxine administration were 39.5 and 42.8%. These values were similar to those obtained from our D,L-thyroxines. The urinary metabolites were also found identical to those reported in the present study. Therefore, our findings suggest that either the metabolic handling of the D and L isomers of thyroxine is more similar in man than the results from the earlier studies suggested or the amounts of D-isomer contained in the radiothyroxines used in the present study were very small.

Our total recoveries of the ^{3}H and ^{14}C radioactivities were approximately ⅔ of the given doses 2 wk after the administration of the entire radiothyroxine doses. This recovery is comparable to the recovery reported by Ingbar and Freinkel in their study of ^{131}I-labeled thyroxine (12). However, the fecal excretion of ^{14}C and ^{3}H were consistently higher than the fecal recovery of ^{131}I in all our subjects and nearly twice the recovery of ^{131}I in three out of four subjects. These findings suggest that in addition to intact thyroxine, the fecal ^{14}C and ^{3}H radioactivities were also constituted by some deiodinated thyroxine metabolite(s). Either the host or the intestinal flora could have given rise to such deiodinated metabolite(s). In the latter instance, the inorganic iodide might have been reabsorbed while the deiodinated metabolite remained in the gut. The present study did not attempt to recover ^{14}C activity from the expired air. West and colleagues (13) reported that after the administration of [φ-^{14}C]T₄ to man the ^{14}C recovery from expired air was extremely small.

After the administration of tracer amounts of ^{131}I-labeled thyroxine to man, the major constituent of the urinary radioactivity has been found to be inorganic iodide, although thyroxine as well as iodinated tyrosines have also been observed during some earlier investigations (19,20) when pharmacologic doses of radiothyroxine were employed. Our present study showed that in man the major excretory route of degraded thyroxine is via the kidney. This holds true for the inorganic iodide as well as for the deiodinated product(s). In two subjects, G. B. and G. N., the [3]H:[14]C ratios of urine showed a consistent but statistically insignificant increase with time, although this increase was not apparent in the urinary ratios of J. T. and M. B. Such a trend of increasing [3]H:[14]C ratios in the urine can be explained by a small metabolic shunt that degrades thyroxine either by cleavage of the diphenyl ether or by oxidative deamination of the alanine side chain. However, if the ^{3}H-labeled fragments from the side chain produced by such shunts were reutilized and hence metabolized more slowly, the [3]H:[14]C ratios in the urine ought to show a decrease rather than an increase with time. The authors are inclined to view the discrepancies of urinary recoveries between ^{3}H and ^{14}C as more apparent than real due to the large volume factor and the low ^{3}H counting efficiency (6–7%) of urine.

Finally, our chromatographical results showed that the urine contained three or more ^{14}C-labeled metabolites. The chromatographical characteristics of these metabolites were entirely similar whether they were derived from a mixture of radiothyroxines labeled on the alanine side chain and the phenolic ring or from a mixture of radiothyroxines labeled on the alanine side alone. Therefoer, each metabolite must contain structural components derived from both the phenolic ring and the nonphenolic ring linked by an intact ether bridge. This finding agrees with our earlier observation in the rat urine after the administration of ^{14}C-labeled thyroxines (7). In conclusion, our method did not allow us to define precisely the size of the pathway of ether cleavage. Nevertheless, our data of [3]H:[14]C ratio, radioisotope kinetics, and radioactivity recoveries and our data of chromatography are most consistent with the thesis that in man the major metabolic pathway of thyroxine leaves the diphenyl ether structure intact.

ACKNOWLEDGMENTS

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