# EVIDENCE OF THYROXINE FORMATION FOLLOWING IODINE ADMINISTRATION IN SPRAGUE-DAWLEY RATS

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*Iodine ($I_2$) has been proposed to be used as a water disinfectant on the manned space station. Previous work has shown that subchronic administration of $I_2$ to Sprague-Dawley rats in drinking water significantly increases plasma thyroxine/triiodothyronine ($T_4/T_3$) levels. This is not observed with iodide ($I^-$) treatment. The present study addresses the possibility that $I_2$ reacts with deiodinated $T_4$ metabolites in the gastrointestinal tract to resynthesize $T_4$. Incubation of diiodothyronine ($T_2$), $T_3$, or reverse $T_3$ with $I_2$ in phosphate-buffered saline resulted in the formation of $T_4$ as measured by radioimmunoassay. Washes from the initial segments of the small intestine of the rat show that substrates are present that react with $I_2$ to produce $T_4$. Single oral doses of $I_2$ to rats produced significant dose-related increases in serum $T_4$ and decreases in $T_3$ concentrations after 2 h. Administration of an equivalent dose of $I^-$ did not alter significantly plasma $T_4$ concentrations. Higher concentrations of a radioactive substance that bound a $T_4$-specific antibody are present in plasma of animals treated with $^{125}I_2$ compared to $^{125}I^-$. These data support the hypothesis that $I_2$ reacts with metabolites of thyroid hormone in the gastrointestinal tract to resynthesize $T_4$ and elevate its levels in blood.*

Sincere appreciation is expressed to Todd T. Sherer for the preliminary studies comparing human thyroid hormone standards supplied with the RIA kits and rat standards prepared in the laboratory. This work has been supported by NASA grant NAG 9-226.

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## INTRODUCTION

On short-term space missions, NASA has used an iodine ($I_2$) concentration of about 15.8 $\mu$mol I/l to maintain the microbiological quality of water for drinking purposes (Janik and Thorstenson, 1986). However, the safety of long-term consumption of elevated $I_2$ has received little study, and the safety of this practice for more extended missions has been questioned.

Much of the concern about $I_2$ in drinking water has focused on the well-established involvement of $I^-$ in thyroid function. The two forms of iodine generally are considered equivalent toxicologically (Haynes and Murad, 1985), ignoring the fact that $I_2$ is in a much higher oxidation state than $I^-$. The reactive nature is important as it will govern whether iodine will produce by-products in drinking water or in the gastrointestinal tract (Dunford and Adeniran, 1988; Dunford and Ralston, 1983; Marks and Strandskov, 1950). Such reactions are well established for other oxidants used in the disinfection of drinking water, such as chlorine (Bull and Kopfler, 1991). However, the complex nature of iodine complicates the potential mechanisms of iodination of organic compounds, which could occur following consumption of iodine-disinfected drinking water.

Previous studies conducted in our laboratory indicate clear differences in the pharmacokinetic behavior of $I^-$ and $I_2$ in the Sprague-Dawley rat (Thrall and Bull, 1990). Experiments in fasted animals involving oral exposure to radiolabeled $I^-$ indicate rapid distribution of radioactivity into the thyroid gland. Although there is no difference in uptake of radioactivity into blood when equivalent doses of iodine or iodide were administered, thyroid uptake of radioactivity is significantly lower for $^{125}I_2$ than for $^{125}I^-$. Since thyroid uptake is specific for $I^-$ (Ingbar, 1985), this finding suggests that iodine exists in different chemical forms in the blood when administered as $I_2$ versus $I^-$.

A subchronic study in rats compared the effects of $I_2$ to equivalent doses of $I^-$ in drinking water for 100 d (Sherer et al., 1991). Although neither form of iodine produced any overt toxic effects, $I_2$ consistently induced an increase in the plasma thyroxine/triiodothyronine ($T_4/T_3$) ratio, whereas $I^-$ did not.

The work presented here was designed to test the hypothesis that $I_2$ reacts with $T_4$ metabolites in the gastrointestinal tract to resynthesize $T_4$ and elevate its levels in blood.

## MATERIALS AND METHODS

### Chemical

The $^{125}I$ as $Na^{125}I$ in 0.1 *N* NaOH (10 mCi/ml, specific activity 17.4 Ci/mg) was purchased from Dupont-NEN Research Products (Boston).

Neutral pH was achieved by the addition of HCl. Oxidation of $^{125}I^-$ to $^{125}I_2$ was accomplished by reacting $Na^{125}I$ with $H_2O_2$ and HCl according to the method described by McAlpine (1945). The yield of $^{125}I_2$ (specific activity 17.4 Ci/mg) was found to be 100% as measured using an isocratic high-performance liquid chromatography (HPLC) system capable of simultaneous ultraviolet (UV) and radioisotope detection ($C_{18}$, 4.6 × 250 mm column, 70 : 30 : 0.1% MeOH : $H_2O$ : $H_3PO_4$, Beckman Instruments, Inc., San Ramon, Calif.).

### Animals

Male Sprague-Dawley rats were purchased from Laboratory Animal Resource Center (Washington State University, Pullman, Wash.) or from Simonsen Laboratories, Inc. (Gilroy, Calif.). Animals were fed Purina Rodent Chow and water ad libitum.

### Radioimmunoassay

Thyroid hormones were measured using Amerlex radioimmunoassay (RIA) kits (Amersham, Corp., Arlington, Ill.). The antibody in the Amerlex $T_4$ RIA kit was found to cross-react with $T_3$ by approximately 8%. The antibody in the Amerlex $T_3$ RIA kit was found to cross-react with $T_4$ by 0.3% by weight. Negligible (<0.03%) cross-reactivity was found with the thyroid hormone analogs $T_2$ or $rT_3$ with either kit. The human standards supplied with these kits were used to construct the standard curves, as previous studies conducted in our laboratory detected no differences between these standards and rat standards prepared in our laboratory according to the method of Stringer and Wynford-Thomas (1982).

### Iodination of Thyroxine Metabolites

The $T_4$ (15.4 nmol/l) and $T_4$ precursors $T_3$ (4.0 nmol/l), 3,3′,5′-triiodothyronine ($rT_3$) (18.5 nmol/l), 3,5-diiodothyronine ($T_2$) (1.9 nmol/l), and 3-iodotyrosine (MIT) (17.9 nmol/l) were added to phosphate-buffered saline (PBS) (50 $\mu$l) and $I_2$ or $I^-$ (1581 nmol I/l) and allowed to react for 60 min. Pretreatments with hypochlorous acid (HOCl) were conducted under the same conditions. In this case $I_2$ or $I^-$ additions were made immediately following addition of HOCl. The $T_4$ levels were then measured in the solution by RIA. A standard curve was constructed using known concentrations of $T_4$ dissolved in 0.8% saline. The $T_4$ assay had measured sensitivity to a concentration of 0.26 nmol/l, calculated as the concentration that is two standard deviations above a series of blank determinations.

### Detection of Thyroxine Precursors in the Small Intestine

Animals weighed approximately 180–220 g and had food removed from the cage 18 h prior to initiation of an experiment. Thirty naive

animals were sacrificed by a lethal ip injection of Ketaset (ketamine HCl, Fort Dodge, Iowa), followed by excision of the heart. A 5-cm section of the small intestine, beginning at the pyloric sphincter, was dissected out and flushed with 2 ml 0.8% saline into an ice-cold test tube. Aliquots of this intestinal wash (50 $\mu$l) were added to $I_2$ or $I^-$ (15.8, 158, or 1581 nmol I/l, final concentrations) and allowed to react for up to 2 h. The $T_4$ levels were measured in the wash at each time period using the RIA kit as described previously. A standard curve was constructed using known concentrations of $T_4$ dissolved in 0.8% saline. To determine if the conditions were appropriate for reaction with $T_3$, $rT_3$, and $T_2$, these metabolites were added to 50 $\mu$l of isolated intestinal wash with $H_2O$, $I_2$, or $I^-$ (1581 nmol I/l) and allowed to react for 60 min, and $T_4$ was production assayed by RIA.

### Effects of Iodine and Iodide Treatment on Plasma Thyroxine and Triiodothyronine Levels

Animals weighed approximately 100–120 g and had food removed from their cage 18 h prior to beginning the experiment. Thirty-five rats were randomly assigned to 5 treatment groups (7 animals/dose) and 10 to 2 treatment groups (5 animals/dose). Animals received a single dose of $I_2$ or $I^-$ by gavage using distilled water as the vehicle. The total doses of iodine were 0, 5.9, 15.8, and 47.4 $\mu$mol I/kg body weight. Blood (500 $\mu$l) was collected from the tail vein immediately prior to treatment and at 2 h following treatment. Total plasma $T_3$ and $T_4$ levels were determined in duplicate using Amerlex RIA kits.

In a second experiment, the ability of $I_2$ in drinking water to increase plasma $T_4/T_3$ ratios was studied. In this experiment animals weighed approximately 80–100 g prior to treatment, and were provided Purina Rodent Chow ad libitum. Sixty rats were randomly assigned to 10 treatment groups (6 animals/dose). Rats were maintained on distilled water containing $I_2$ or $I^-$ at dose levels of 0, 23.7, 79.1, 237, and 791 $\mu$mol I/l of drinking water for 7 d. Blood (500 $\mu$l) was collected from the tail vein on d 0 and 7 of treatment, and total plasma $T_3$ and $T_4$ levels were determined as previously described.

### Demonstration of Iodine Incorporation into Thyroxine

Rats were purchased from Simonsen Laboratories, Inc., and weighed approximately 120–150 g prior to use. Animals had food removed from their cage 18 h prior to administration of 15.8 $\mu$mol I/kg body weight of either $I_2$ or $I^-$ (4 animals per treatment) containing tracer amounts (50 $\mu$Ci) or $^{125}I$ by gavage. Two hours after dosing, blood (5 ml) was drawn from the caudal vena cava under ketamine anesthesia, and plasma was separated. Duplicate radioactive plasma samples (50 $\mu$l) were incubated with $T_3$- or $T_4$-specific antibodies (500 $\mu$l) available in the Amerlex RIA kit for 1 h at 37°C. Radioactivity associated with the anti-

body was measured in an LKB gamma counter. To test for nonspecific binding, increasing amounts of nonradioactive $T_4$ or $T_3$ were used to displace radioactivity specifically bound to the antibodies. The difference in the binding in the presence and absence of excess $T_4$ or $T_3$ was taken to represent specific binding.

### Statistical Analyses

A one-way analysis of variance (ANOVA) was used to test for statistical differences among treatment groups. Groups with $p$ values $\leq .05$ (ANOVA) were subjected to two-sample $t$-test or Tukey's multiple range test, as noted.

## RESULTS

The addition of iodine to solutions of $T_3$, $rT_3$, or $T_2$ in phosphate-buffered saline (pH 7.4) resulted in increases of $T_4$ as measured by RIA (Table 1). On a molar basis, $I_2$ converted 73, 69, and 51% of $T_3$, $rT_3$, or $T_2$, respectively, to $T_4$. Iodide ($I^-$) in equivalent concentrations did not result in increased concentrations of $T_4$ under identical conditions. These data confirm that the nonenzymatic iodination of $T_3$, $rT_3$, or $T_2$ with $I_2$ is chemically possible at a respectable yield, as similarly reported by Dunford and Adeniran (1988). MIT does not produce $T_4$ in this system, ruling out interference of monocyclic compounds with $T_4$ determinations.

Addition of iodine to isolated intestinal washes also resulted in measurable increases in $T_4$ (Fig. 1*a*). The rate of $T_4$ production was propor-

**TABLE 1.** Levels in PBS Solution Following Treatment with $I^-$ or $I_2$ and Metabolite Supplementation

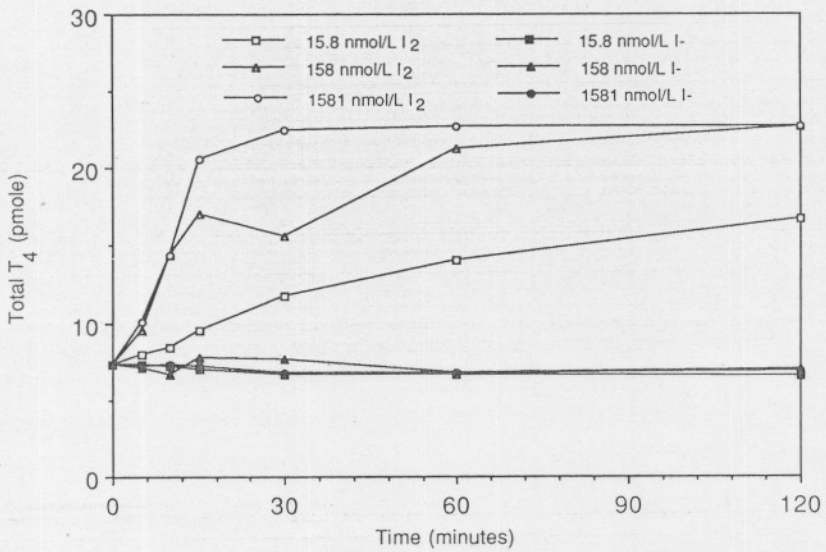
| | Treatment | | |
|---|---|---|---|
| Addition[a] | None | $I_2$ | $I^-$ |
| $T_4$ | 16.91 ± 1.16[b] | 15.93 ± 1.42 | 14.34 ± 1.08 |
| $T_3$ | — | 1.92 ± 0.51[c] | 0 ± 0.08 |
| $rT_3$ | — | 0.88 ± 0.19[c] | 0.12 ± 0.04 |
| $T_2$ | — | 0.90 ± 0.13[c] | 0 ± 0.03 |
| MIT | — | 0 ± 0.06 | 0 ± 0.04 |

*Note.* $T_4$ levels were measured in phosphate-buffered saline (PBS) solution using Amerlex RIA kit 60 min following addition of 1581 nmol I/l.

[a]At 15.4 nmol/l $T_4$, 4.0 nmol/l $T_3$, 1.5 nmol/l $rT_3$, 1.9 nmol/l $T_2$, and 17.9 nmol/l MIT.

[b]Values expressed as average total concentration in PBS solution in nmol/l ± SEM of $n = 4$ samples in duplicate.

[c]Value differs significantly from equivalent treatment with $I^-$ by two-sample $t$-test ($p < .05$).

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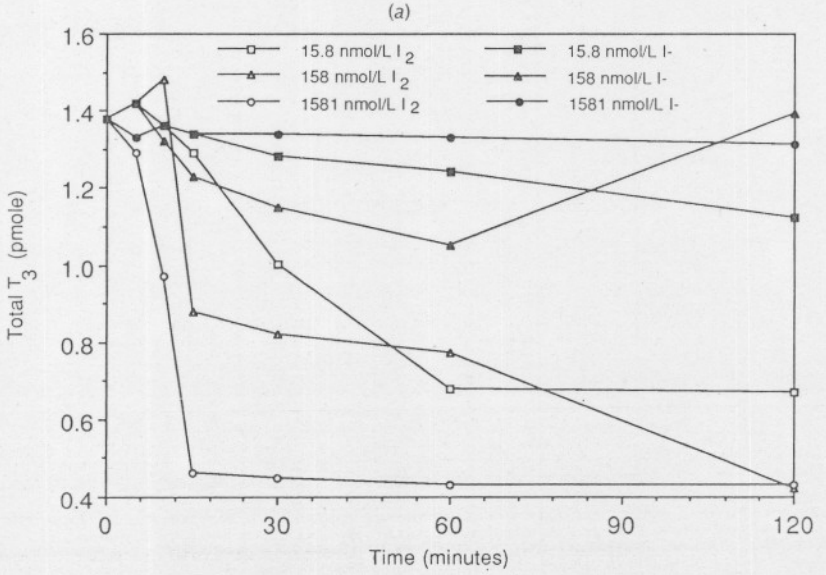


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**FIGURE 1.** Change in (*a*) $T_4$ and (*b*) $T_3$ levels in intestinal wash with time following iodine treatment. Naive animals were sacrificed and the upper intestine was dissected out and flushed with 0.8% saline. Wash (50 $\mu$l) was added to $I_2$ or $I^-$ (in excess) and allowed to react for various times before the initiation of the $T_4$ RIA assay as described in the Materials and Methods section. Values expressed as concentration of $T_4$ or $T_3$, in pmol, corrected to the total concentration in isolated wash (2 ml). Each point represents four samples in duplicate.

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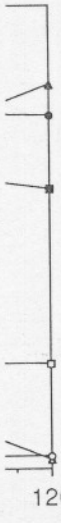

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tional to $I_2$ concentration. A maximum $T_4$ yield resulted from $I_2$ addition at a concentration of 158 nmol I/l. The $I_2$ reacts to produce a total of approximately 14.2 pmol $T_4$ from precursors available in a 5-cm section of the small intestine. Equivalent concentrations of $I^-$ did not increase $T_4$ levels.

The total amount of $T_3$ decreased in isolated intestinal wash following the addition of $I_2$ at various concentrations (Fig. 1*b*). The $I^-$ did not produce reproducible changes in $T_3$ in intestinal wash at equivalent concentrations. The rate and extent of $T_3$ loss were dependent on increasing concentrations of $I_2$. However, the decrease in $T_3$ was too small to account for the increase in $T_4$ (0.92 pmol loss of $T_3$ versus 14.2 pmol increase in $T_4$).

Inclusion of $T_3$, $rT_3$, or $T_2$ in the intestinal wash indicated recoveries as $T_4$ comparable to those obtained in PBS (Table 2). The addition of 15.4 pmol $T_4$ to $I_2$ produces an apparent 18.5 pmol increase in $T_4$ levels, a greater than 100% recovery of added $T_4$, although within the range of analytical variability. The $I_2$ increased $T_4$ concentrations when added to $T_3$-, $rT_3$-, or $T_2$-spiked intestinal washes by values ranging from 52 to 67% on a molar basis. As with other conditions, $I^-$ addition does not change $T_4$ levels.

To demonstrate that increases in $T_4$ were due to iodination of $T_4$

**TABLE 2.** Production of $T_4$ from $T_4$ Metabolites in Intestinal Wash Following Treatment with Iodine

| | Treatment | | |
|---|---|---|---|
| | | Net increase above control with treatment[a] | |
| Addition | Control | $I^-$ | $I_2$ |
| Control | 11.1 ± 2.8[b] | 1.0 ± 0.6 | 11.6 ± 1.0[c] |
| $T_4$ (15.4 pmol) | 25.5 ± 6.7 | 7.6 ± 2.3 | 18.5 ± 2.4[c] |
| $T_3$ (15.4 pmol) | 11.5 ± 2.7 | 2.6 ± 1.0 | 21.1 ± 1.2[c] |
| $rT_3$ (15.4 pmol) | 12.5 ± 3.2 | 0.1 ± 0.0 | 24.8 ± 2.4[c] |
| $T_2$ (19.1 pmol) | 12.0 ± 2.6 | −1.3 ± 1.5 | 20.0 ± 2.1[c] |

*Note.* Wash (50 μl) was collected from the duodenum was added to the indicated substrate along with $H_2O$, $I_2$, or $I^-$ (1581 nmol/l, final concentration) and allowed to react for 60 min prior to determination of $T_4$ levels by an Amerlex RIA kit.

[a]Difference between solutions containing iodine (columns 2 and 3) and control solutions (column 1).

[b]Values expressed as average amount of $T_4$ in isolated intestinal wash (in vitro system) in pmol ± SEM of $n = 6$ samples in duplicate.

[c]The difference between treated washes and their corresponding control (column 1) was statistically significant by paired *t*-test at $p < .05$.

metabolites rather than releasing a complexed form of $T_4$, the intestinal washes were pretreated with hypochlorous acid (HOCl). HOCl acts as both an oxidant and a halogenating agent. Treatment of intestinal washes with 1906 nmol HOCl/l did not produce a compound that interacted with the $T_4$ antibody. When this treatment preceded the addition of $I_2$, the formation of $T_4$ was completely blocked (Table 3). The inhibition of iodination by HOCl occurred with an apparent $K_i$ of 324 nmol HOCl/l, a concentration that is more than twice the molar concentration of $I_2$ (158 nmol I/l) required to yield maximal amounts of $T_4$ in intestinal washes (Fig. 1*a*). Measurement of $T_4$ in solutions containing 1906 nmol HOCl/l spiked with $T_4$ indicates that the $T_4$ antibody was not altered by the HOCl treatment.

Single doses of iodine administered by gavage produced a dose-related increase in plasma $T_4$ levels (Fig. 2*a*) to a maximum at 15.8 $\mu$mol I/kg. Plasma $T_3$ levels (Fig. 2*b*) reached a minimum at the same dose level. No further increase in $T_4$ nor decrease in $T_3$ is observed at higher dose levels. In contrast, no significant effect in plasma $T_4$ and $T_3$ levels was observed in animals receiving equivalent doses of $I^-$.

Figure 3 depicts the effect on plasma $T_4/T_3$ levels in rats following administration of $I_2$ or $I^-$ in drinking water for 7 d. Iodine ($I_2$) treatment results in a dose-related increase in the plasma $T_4/T_3$ ratio, which is significant at the 791 $\mu$mol I/l dose level. This change results from a significant decrease in $T_3$ levels as well as an increase in $T_4$ levels, relative to controls (inset, Fig. 3). In contrast, a dose-related decrease in the plasma $T_4/T_3$ ratio, significant at the 791 $\mu$mol I/l dose level, was observed in animals treated with equivalent doses of $I^-$.

The amount of radioactivity in plasma that binds to antibodies specific for $T_4$ and $T_3$ following 15.8 $\mu$mol I/kg oral doses of $^{125}I_2$ or $^{125}I^-$ is

**TABLE 3.** Production of $T_4$ in Intestinal Wash Following Treatment with Iodine and HOCl

| | Treatment | | |
|---|---|---|---|
| Addition[a] | None | $I_2$ | $I^-$ |
| None | 11.1 ± 2.8 | 22.7 ± 2.6[b] | 12.1 ± 4.0 |
| HOCl (38.1 nmol/l) | 10.2 ± 3.9 | 19.1 ± 3.0[b] | 10.6 ± 2.4 |
| HOCl (190 nmol/l) | 11.1 ± 2.7 | 18.0 ± 3.1[b] | 11.6 ± 2.8 |
| HOCl (1906 nmol/l) | 8.1 ± 4.2 | 12.2 ± 2.8 | 10.3 ± 2.8 |
| HOCl + $T_4$[a] | 24.0 ± 5.5 | 23.0 ± 6.7 | 24.2 ± 4.0 |

*Note.* Values expressed as average total concentration in isolated intestinal wash (in vitro system) in pmol ± SEM of $n = 6$ samples in duplicate.

[a]For 1906 nmol HOCl/l and 12.9 pmol $T_4$ added simultaneously.

[b]$T_4$ concentration in intestinal wash treated with $I_2$ differs significantly from control (column 1), $p < .05$ by two-sample *t*-test.



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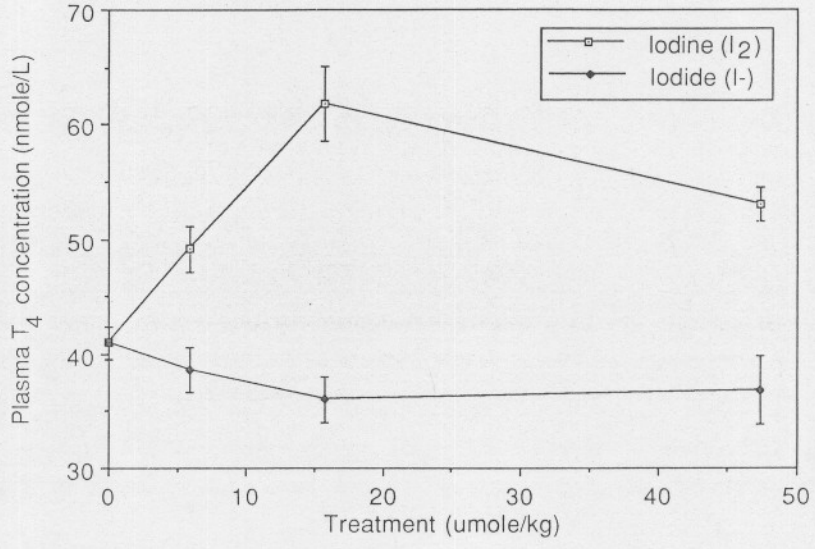


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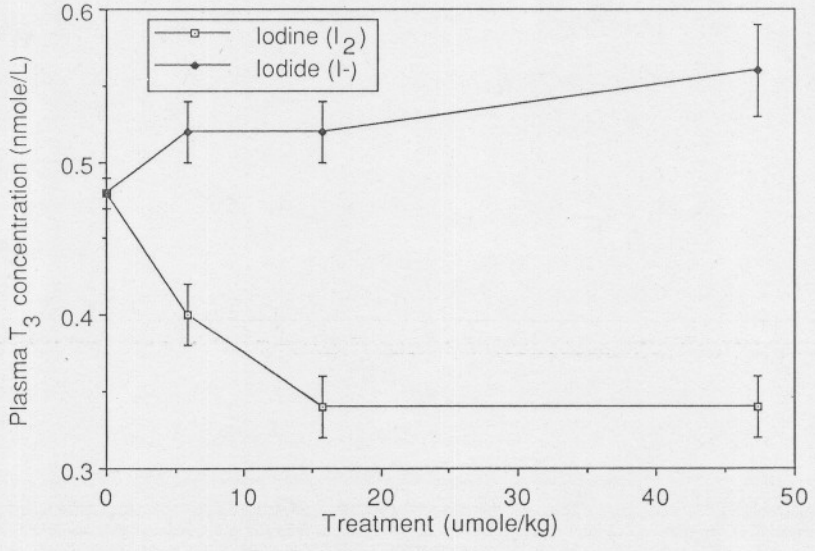


(*b*)

**FIGURE 2.** Change in plasma (*a*) $T_4$ and (*b*) $T_3$ levels following $I_2$ or $I^-$ treatment. Animals were treated with various doses of $I_2$ or $I^-$ as a single oral dose. Blood (500 $\mu$l) was collected from the tail vein immediately prior to treatment and at 2 h following treatment. Total plasma $T_4$ and $T_3$ levels were determined in duplicate using RIA kits as described in the Materials and Methods section. Each point represents the mean and standard error of the mean (SEM) of at least five animals.

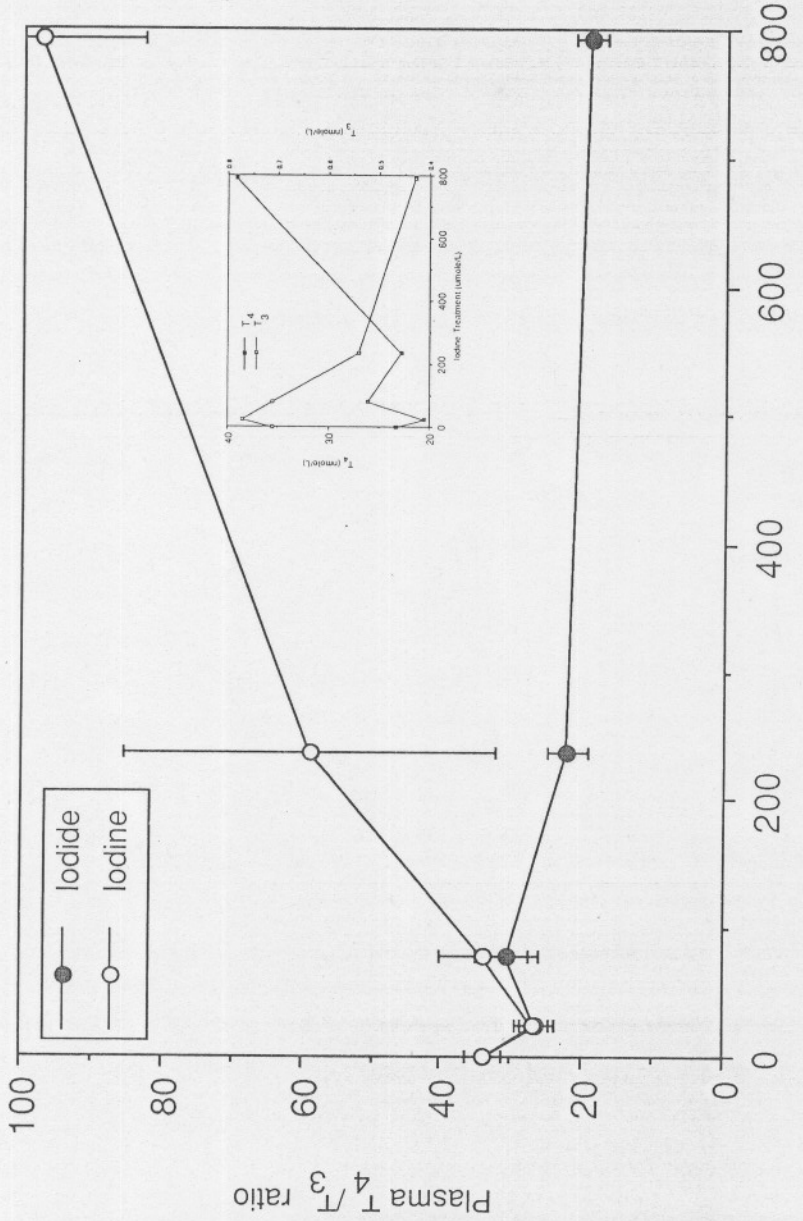


**FIGURE 3.** Effect of $I_2$ treatment for 7 d on plasma thyroid hormone levels. Animals were maintained on water containing 0, 23.7, 79.1, 237, or 791 μmol I/l for 14 d. Blood (500 μl) was collected on d 0 and 7, and plasma $T_4$ and $T_3$ levels were measured in duplicate using RIA kits as described in the Materials and Methods section. Inset shows that plasma $T_4$ increases with time and $T_3$ decreases with time in animals treated with $I_2$. Each point represents the mean of $n = 6$ animals.

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shown in Table 4. Twice as much radioactivity associates with both the $T_4$ and $T_3$ antibodies in animals treated with $^{125}I_2$ compared to animals treated with $^{125}I^-$ (1563 vs. 703 and 783 vs. 433 cpm, respectively). In addition, three times the radioactivity derived from $^{125}I_2$-treated animals is displaceable (and therefore considered to represent specific binding) from either the $T_4$ or $T_3$ antibody by excess nonradioactive $T_4$ or $T_3$, respectively. If it is assumed that only one $^{125}I$ molecule was incorporated into each $T_3$ molecule to form a molecule of $T_4$, then the 1209 cpm bound to the $T_4$ antibody represents 23.5 nmol $T_4$/l plasma formed following $^{125}I_2$ administration (specific activity $3.84 \times 10^{-11}$ pCi/molecule I). Incubating the antibody complex with increasing concentrations of cold $T_4$ to displace $^{125}I$-$T_4$ indicated that approximately 32.2 nmol/l of $T_4$ had been formed and adsorbed. This indicates that somewhat more than one $^{125}I$ was bound per molecule of $T_4$, suggesting some binding might occur to $T_2$ as well as $T_3$ and $rT_3$. Nonspecific binding, the radioactivity not displaceable from the antibody by the addition of excess nonradioactive $T_4$ or $T_3$, was found to be similar regardless of treatment.

## DISCUSSION

The experimental results of this study strongly support the hypothesis that iodine reacts with $T_4$ metabolites in the gastrointestinal tract to resynthesize $T_4$ and elevate its levels in blood. This is supported by the following evidence: (1) $I_2$ produces $T_4$ as measured by RIA when it is added to solutions of $T_3$, $T_2$, or $rT_3$ in vitro. (2) Substances that react with $I_2$ to form $T_4$ are present in the small intestine of the rat. (3) A single oral dose of $I_2$ significantly increases plasma $T_4$ levels in vivo within a 2-h

**TABLE 4.** Amount of $^{125}I$ in Plasma That Binds to Antibodies Specific for $T_4$ and $T_3$ Following Oral Treatment with 15.8 $\mu$mol I/kg as Iodine or Iodide

| Oral treatment | Associated with antibody[a] | Displaced from antibody | Nonspecific binding[b] |
|---|---|---|---|
| $T_4$ | | | |
| $^{125}I_2$ | 1563 ± 42 | 1209 ± 31 | 351 ± 23 |
| $^{125}I^-$ | 703 ± 51 | 397 ± 47 | 310 ± 26 |
| $T_3$ | | | |
| $^{125}I_2$ | 783 ± 39 | 491 ± 21 | 296 ± 14 |
| $^{125}I^-$ | 433 ± 17 | 153 ± 5 | 283 ± 4 |

[a]Average ± SEM of four animals in duplicate. All values expressed as CPM/ml plasma.

[b]Nonspecific binding represents the amount of radioactivity associated with the $T_4$ or $T_3$ antibody that is not displaceable with the addition of excess nonradioactive $T_4$ or $T_3$, respectively.

Treatment (umole I/L)

**FIGURE 3.** Effect of $I_2$ treatment for 7 d on plasma thyroid hormone levels. Animals were maintained on water containing 0, 23.7, 79.1, 237, or 791 $\mu$mol I/l for 14 d. Blood (500 $\mu$l) was collected on d 0 and 7, and plasma $T_4$ and $T_3$ levels were measured in duplicate using RIA kits as described in the Materials and Methods section. Inset shows that plasma $T_4$ increases with time and $T_3$ decreases with time in animals treated with $I_2$. Each point represents the mean of $n = 6$ animals.

period. (4) A radioactive product was detected in the plasma of animals treated orally with $^{125}I_2$ that bound antibodies specific for $T_4$ and $T_3$. This was substantially in excess of labeling seen with $^{125}I^-$, providing evidence that a direct reaction of iodine is responsible for increased $T_4$ levels.

Reactions with metabolites present in the gastrointestinal tract appear sufficient to account for a large fraction of the increase in plasma $T_4$ levels. In vivo, a single oral dose of $I_2$ produced a 14.2 nmol/l increase in plasma $T_4$ levels (average of 49.3 pmol increase in total plasma volume). In a preliminary study, a 106.8 pmol oral dose of $T_4$ produced a 9.0 nmol/l, or 74.8 pmol, increase in total plasma $T_4$. Based on this we calculate that 70.4 pmol $T_4$ was likely formed in the gastrointestinal tract. In vitro experiments using intestinal wash from the first 5 cm of the small intestine indicate that enough $T_4$ metabolites are available to produce 14.2 pmol $T_4$. Therefore, 20% of the increase in plasma $T_4$ levels following $I_2$ administration may be accounted for by the iodination of $T_4$ metabolites in the small segments of the intestine that were utilized for these experiments. Presumably, examination of the entire small intestine may account for the remaining increase.

It is not surprising to find measurable amounts of thyroid hormones in the gastrointestinal tract, where substantial amounts of endogenous thyroid hormones reach the intestine via the bile and are apparently largely reabsorbed (DiStefano et al., 1988). It has been proposed that the gastrointestinal tract may serve as a storage reservoir for $T_3$, where the concentration of $T_3$ may be 25 times higher than plasma levels (Galton, 1975). It does not seem likely that these increases in $T_4$ could have resulted from $I_2$-induced hydrolysis of glucuro- or sulfo-conjugated metabolites of $T_4$, because there was no induction of such an increase with HOCl treatments. In addition, it was shown that the majority of the thyroid hormones entering the gut in the rat are unconjugated (DiStefano et al., 1988).

Although the rat is a useful model for study of thyroid function in humans, some species differences do exist. In humans $T_4$ is carried primarily by thyroxine-binding globulin (TBG) (Berson and Yalow, 1954), rather than by albumin as in the rat. The binding affinity of TBG is approximately 1000 times higher than the binding affinity of albumin (Rall, 1976). Because hormone binding generally is accompanied by slower metabolic degradation of the hormone, the turnover of $T_4$ in the rat occurs at a faster rate than in humans. The biological half-time of $T_4$ in human plasma (5–9 d) (Sterling and Lazarus, 1977) is longer than in rat plasma (12–24 h) (Harland and Orr, 1969; Larsen and Frumess, 1977).

Based on the lower turnover of $T_4$ in humans, we would predict that repeated doses of $I_2$ would be more effective in increasing plasma $T_4$ concentrations than single doses. This is because the concentration of metabolites in the intestine at any given moment in time should be

lower than in the rat, and thus limit $T_4$ production. On the other hand, the slower turnover of $T_4$ in humans should allow greater increases in $T_4$ concentrations with repeated doses.

These data have some bearing on the use of $I_2$ as a drinking water disinfectant. With 7-d drinking water exposure, the steady-state levels of thyroid hormones are not increased until animals are exposed to $I_2$ at a concentration of approximately 79 $\mu$mol I/kg/d. On the other hand, the increase in $T_4/T_3$ ratios seen with an acute bolus dose of $I_2$ occurs with doses of 15.8 $\mu$mol I/kg. Thus, a higher daily dose is required to produce an effect when the concentration of $I_2$ in the gastrointestinal tract is introduced throughout the day, reflecting the relatively rapid turnover of $T_4$ in the rat.

Astronauts returning from Apollo and Skylab space missions were found to have elevated $T_4$ levels (Leach et al., 1975; Leach, 1977; Leach and Rambaut, 1977). It has been assumed previously that this is a result of the physiological adaptation to microgravity. However, poor control over iodine concentrations in drinking water occurred on some space flights, with levels reaching an estimated 79–119 $\mu$mol/l (Compton and Benson, 1983), rather than the intended concentration of 16 $\mu$mol/l. The data presented here suggest that the elevated $T_4$ levels in these astronauts may have resulted from direct iodination of $T_4$ metabolites. It is not presently clear whether these changes have any adverse impact on normal physiological function.

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