

FORMATION OF ORGANIC BY-PRODUCTS FOLLOWING CONSUMPTION
OF IODINE DISINFECTED DRINKING WATER

BY

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A dissertation submitted in partial fulfillment of the
requirements for the degree of

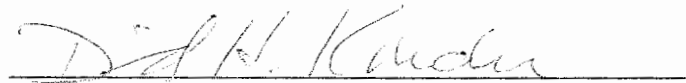
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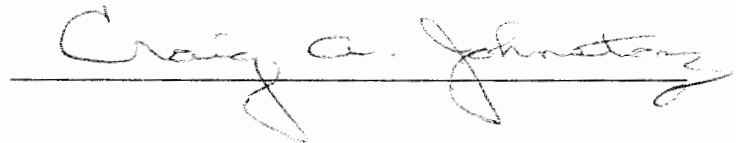
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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of KARLA DENISE THRALL find it satisfactory and recommend that it be accepted.


chair







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OF IODINE DISINFECTED DRINKING WATER

Abstract

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On previous missions, NASA has used an iodine (I_2) residual to disinfect water for drinking purposes. Unlike ingestion of I^- leading to thyroid goiters, the health effects associated with consumption of I_2 are not well established. It has been reported that subchronic administration of I_2 in drinking water significantly increases plasma thyroxine/triiodothyronine (T_4/T_3) ratios in the Sprague-Dawley rat. This project was designed to determine how this increase originates and what implications this has on using I_2 as a water disinfectant.

We show that radioactivity derived from iodine distributes in the rat differently depending on form administered. In particular, more radioactivity distributes to the thyroid in animals treated with $^{125}I^-$ versus $^{125}I_2$. This suggests that radioactivity derived from I_2 exists in a form not sequestered by the thyroid. Furthermore, twice as much I^- is present in plasma of animals treated with I^- compared to I_2 . In blood components, some radioactivity

from animals treated with $^{125}\text{I}_2$ is identified in serum lipids as cholesteryl iodide, providing direct evidence that I_2 reacts with endogenous organic compounds.

In animals receiving 2 mg/Kg I_2 , a 30% increase in plasma T_4 and decrease in T_3 levels occurs shortly after dosing. In an *in vitro* system of intestinal washes I_2 increases the concentration of a chemical that binds to a T_4 antibody. A similar increase in a radioactive product was observed in blood after oral administration of $^{125}\text{I}_2$, but not $^{125}\text{I}^-$.

These studies indicate that I^- and I_2 are not physiologically or toxicologically equivalent. In particular, these data suggest that I_2 reacts with T_4 metabolites within gastrointestinal tract to resynthesize T_4 and elevate blood levels.

These data have some bearing using I_2 as a water disinfectant. Apollo and Skylab astronauts were found to have elevated plasma T_4 levels. It is not clear if this results from consuming iodinated water or from weightlessness. No toxicological effect is associated with elevated T_4 levels. Studies need to be conducted to determine the effect of I_2 .

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Dedication

To Brian and Megan

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Chapter I
INTRODUCTION

Iodine has long been recognized as effective for disinfection of water, but its use for this purpose has been limited primarily to emergency situations (Thomas et al., 1969). For many years iodine has represented a convenient way to disinfect suspicious backcountry water for drinking (Zemlyn et al., 1981). Iodine has been used extensively by the military for emergency disinfection of water (Thomas et al., 1979). Iodine has gained recent interest because of its proposed use in disinfecting recycled water on the Space Station where it will be consumed for periods of up to 180 days. On previous short-term space missions, NASA has used an iodine (I_2) residual of about 2 mg/L (ppm) to disinfect non-recycled water for drinking purposes.

In the closed environment planned for the Space Station Freedom, drinking water will be produced from cabin condensate (NASA, 1987) which has a high probability for bacterial and viral contamination. Therefore some type of a disinfectant process is unavoidable. Iodine is favored as a residual, or secondary, disinfectant over other

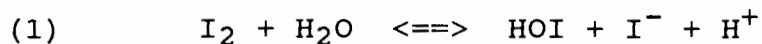
disinfectants because its lower volatility presents less of an inhalation hazard in the closed atmosphere of the Space Station.

One other advantage of iodine disinfection is that trihalomethane (THM) formation is about 70% less with iodine than with chlorine disinfected surface waters (Sauer et al., 1987). However, measurable amounts of THMs are produced, with iodoform (CHI_3 or triiodomethane) representing the main THM that has been detected. The formation of other by-products with iodine has not been extensively investigated.

Iodine is the fourth of five halogens (fluorine, chlorine, bromine, iodine and astatine) in order of increasing atomic weight and decreasing reactivity. Although iodine will react to form iodinated THMs, it is by far the least reactive of the three significant halogen disinfectants (Cl_2 , Br_2 , I_2) in this regard. Gould et al. (1985), reported that at levels similar to those to be expected in normal groundwaters and surface waters, iodine will neither lead to significant levels of iodinated THMs nor modify the overall THM reactions. The authors postulated that steric hindrance associated with the bulky iodine atom retarded formation of these compounds.

As a result of the number of oxidation states available to iodine under ambient conditions, the system of molecular iodine in aqueous solution is known to be very complex.

Molecular iodine reacts with water to form hypoiodous acid according to the generally accepted formula (1):

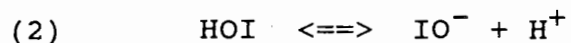


($K = 3 \times 10^{-13} \text{ M}^2$, Palmer and Van Eldik, 1986).

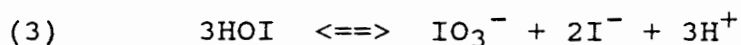
At a pH of 5 approximately 99% of the total iodine residual is present as iodine (I_2) and only 1% as hypoiodous acid (HOI). At pH 7, the two forms are present in equal concentrations, while at pH 8, only 12% is present as iodine and 88% as hypoiodous acid.

The hydrolysis of I_2 to form HOI can occur via two intermediate species, H_2OI^+ and I_2OH^- . Protonated hypoiodous acid is present in very small amounts (Palmer and Van Eldik, 1986) and is not an important species in bulk solution (Dunford and Ralston, 1983). The subsequent dissociation of I_2OH^- to HOI and I^- occurs rapidly, with a rate constant of $1.0 \times 10^3 \text{ s}^{-1}$ (Palmer and Van Eldik, 1986).

After primary dissolution of I_2 , a complex set of secondary reactions is thought to occur. HOI dissociates in water (2):

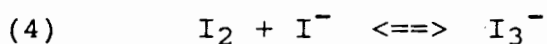


($K_d = 4.5 \times 10^{-13}$ to 2.0×10^{-11} at 25°C) (Janik and Thorstenson, 1986). Consequently, significant dissociation of hypiodous acid occurs only at high pH, and is not important in water disinfection (NAS, 1980). At water distillation temperatures and pressures, HOI may undergo further decomposition (3):



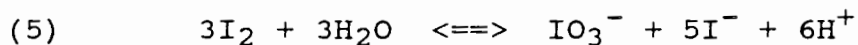
(K_d unknown). Neither the iodate nor iodide ion possesses disinfecting ability (Marks and Strandkov, 1950).

Iodine can react with iodide according to the equation (4):



($K_r = 1.4 \times 10^{-3}$ at 25°C). Iodine, HOI and iodide can form triiodide in water, however this has not been detected in iodinated potable water at standard temperature, pressure and neutral pH (Black et al., 1965).

At neutral pH, such as would occur in disinfected water, the hydrolysis of molecular iodine to iodate ion becomes important in the absence of added iodide (5):



competing to lower the concentration of molecular iodine and as a result, the concentration of hypiodous acid.

The complex chemistry of iodine complicates the mechanism of iodination of organic compounds which could occur following consumption of iodine disinfected drinking water. The negatively charged species I_3^- is not an electrophile and is thus ineffective as a disinfectant. The same considerations appear to apply to I_2OH^- . Thus, the potential reagents are reduced to two, I_2 and HOI.

The toxicological data on the use of iodine is inadequate for establishing its safe use as a drinking water disinfectant. The long-term consumption of elevated I_2 has received little study. Most studies have concentrated on iodide (I^-) and iodate (IO_3^-) (Mahmoud et al., 1986; Savoie et al., 1975), the forms of iodine in the diet, ignoring the fact that I_2 is the chemical species used in water disinfection. Dietary studies on iodide or iodate cannot determine if the use of I_2 has any toxicological effects, at concentrations used for drinking water disinfection.

The most extensive study of I_2 in humans involved inmates of three prisons in Lowell, Florida, where the water systems were iodinated for 5 years (1 mg/L). It was reported that certain parameters of thyroid function, including a decrease in ^{131}I uptake by the thyroid gland and

an increase in serum protein-bound iodine concentrations, were altered by the use of this water (Freund et al., 1966). While the most comprehensive study available, it is limited by a select population, the absence of nonendocrine physiologic measurements, and a loss of subjects due to release of inmates (Thomas et al., 1969).

Recently, Sherer et al. (1991), compared the subchronic effects of iodine and iodide in drinking water on male and female Sprague-Dawley rats. They found that neither I^- or I_2 produced any significant alterations in hematological or clinical chemistry parameters at concentrations up to 100 mg/L. Examination of brain, liver, kidney, heart and testes revealed no signs of gross pathology. The thyroid glands in male rats were enlarged at the higher doses of I^- although they appeared normal histologically. This effect was not observed in male rats treated with I_2 or in female rats with either treatment.

In the same study, animals receiving I_2 had an increase in thyroxine/triiodothyronine (T_4/T_3) ratios measured at days 10 and 100 of treatment. Alteration in the ratio depended on a significant increase in T_4 levels as well as a decrease in T_3 levels. This was not observed in animals treated with I^- .

Iodine is essential for normal thyroid physiology, where it is incorporated into amino acids leading to the

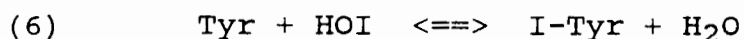
synthesis of thyroid hormones (Haynes and Murad, 1985). The thyroid contains the largest pool of iodine in the body, under normal circumstances approximately 8000 ug, most of which is in the form of iodinated amino acids in thyroglobulin. Normally this pool of iodine turns over slowly (roughly 1% per day) (Ingbar, 1985).

Iodine is well-absorbed from the gastrointestinal tract primarily as iodide (Gushurst et al., 1984). Absorption occurs quickly, although the specific sites and mechanisms are unknown. Once into the blood, iodide is rapidly distributed throughout the body. Iodide is specifically accumulated in blood, thyroid, gastric, salivary and breast alveolar cells (Haynes and Murad, 1985).

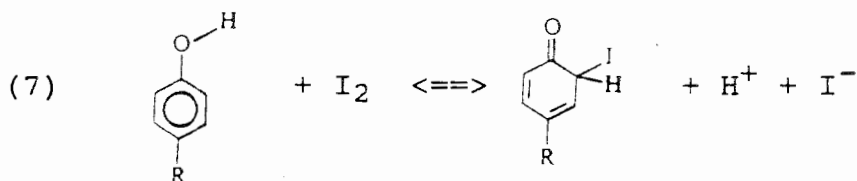
Iodide is taken up by the thyroid gland by an active process (Bastomsky, 1974). Sodium and probably other anions are passively carried into thyroid cells during the iodide transport process. Intracellular sodium is then actively excreted back into the blood via a cellular adenosine triphosphatase (ATPase) mediated sodium pump (Bastomsky, 1974). Chloride passively follows sodium, resulting in an apparent ATPase-mediated, iodide-chloride exchange mechanism (Berkowitz, 1981). Under normal circumstances the concentration of thyroidal iodide is on the order of 20-50 times higher than plasma iodide.

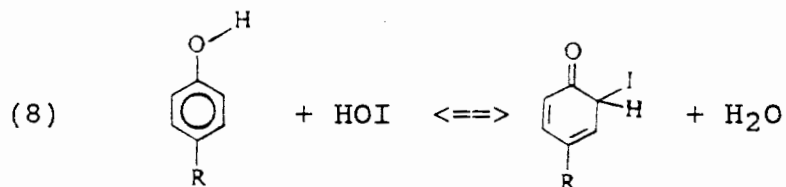
Once transported into the thyroid, iodide enters into a series of reactions that ultimately lead to the synthesis of the thyroid hormones T_3 and T_4 (Taurog, 1978). The first of these reactions involves oxidation of iodide by a thyroid peroxidase-catalyzed reaction (for which H_2O_2 serves as a substrate), which in turn iodinates tyrosyl residues of the intrathyroidal colloidal thyroglobulin to form mono- and diiodotyrosines (MIT and DIT, respectively) (Morrison and Schonbaum, 1976).

There are three plausible mechanisms for the iodination of tyrosine (Tyr). Mechanism I (6), occurs in solutions of neutral and higher pH, and is therefore unlikely to take place in the acidic environment of the gastrointestinal tract following consumption of iodine disinfected drinking water.

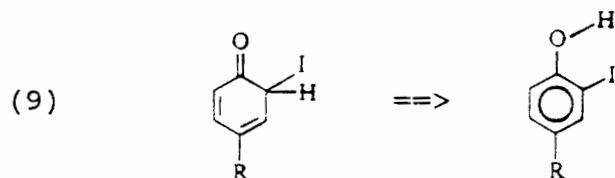


Mechanisms II (7), and III (8) involve the formation of an iodinated quinoid intermediate in a reversible reaction of iodine with the ortho position of the phenolic ring.





Removal of the ortho-hydrogen (9) is likely the rate limiting step (Baldas et al., 1981).



Kinetic data cannot offer a clear distinction between Mechanisms I, II or III because the proposed mechanisms predict the same factors in the denominator of the rate law (Dunford and Adeniran, 1988).

The formation of MIT and DIT residues in thyroglobulin is followed by the synthesis of the thyroid hormones, T_3 and T_4 . The most commonly held view is that the synthesis of T_4 and T_3 requires the coupling of two iodotyrosines, both of which are initially held in a peptide bond within the thyroglobulin molecule (Ingbar, 1985). Coupling of two peptide-bound iodotyrosines requires disruption of the peptide bonds holding the iodotyrosyl group that yields the beta ring of the thyronine nucleus. This requires substantial changes in the tertiary structure of thyroglobulin.

T_4 and T_3 enter the blood after their liberation from thyroglobulin by proteolytic cleavage within the follicular cell (Wollman, et al., 1964). Hydrolysis is facilitated by reduction of disulfide bonds in thyroglobulin catalyzed by a transhydrogenase that utilizes reduced glutathione (GSH).

Upon entering the blood T_4 and T_3 are bound firmly but reversibly to several serum proteins (Robbins et al., 1978). Electrophoretic studies have disclosed two plasma proteins to which T_4 is mainly associated, a T_4 -binding inter-alpha globulin (TBG) and a T_4 -binding prealbumin (TBPA). In man, T_4 and T_3 are primarily bound to TBG but T_3 is also bound to a small extent, by albumin (Berson, 1954).

It is generally accepted that the important thyroid hormone for cells is T_3 (Engler and Burger, 1984). The cellular supply of T_3 depends largely on conversion of T_4 to T_3 by an enzyme 5'-deiodinase, which exists in two forms (Edmonds, 1987). Studies of the metabolism of T_4 have shown that iodine atoms from both the phenolic and tyrosyl rings of this molecule are removed at a similar rate. T_3 is formed when deiodination occurs first in the phenolic ring, (outer ring deiodination, or ORDase), and 3,3',5'-triiodothyronine (reverse T_3 or rT_3) is formed when deiodination occurs first in the tyrosyl ring (inner ring deiodination, or IRDase) (Surks and Oppenheimer, 1971).

Both T_3 and rT_3 can be further deiodinated to diiodothyronine ($3,3'$ - T_2 , $3',5'$ - T_2 or $3,5'$ T_2).

In rat liver both ORDase and IRDase activities are associated with the microsomal fraction and cosediment with marker enzymes of the endoplasmic reticulum. Studies provide evidence that the iodothyronine-deiodinating enzymes in corresponding tissues of humans and rats are similar (Visser et al., 1988). Different enzymes have been detected in other tissues, such as rat kidney, brain, pituitary, brown adipose tissue, skin and placenta (Leonard and Rosenberg, 1980; Visser et al., 1982; 1983; Leonard et al., 1983; Kaplan and Shaw, 1984; Huang et al., 1985).

About 35% of the T_4 secreted in normal man is deiodinated to yield T_3 , and about 40% is deiodinated to yield rT_3 (Surks et al., 1973). With a normal T_4 production rate of 90 ug/day (Chopra, et al., 1978), approximately 26 ug of T_3 and 30 ug of rT_3 are produced by peripheral deiodinations. The synthesis and release of T_3 and T_4 is regulated by circulating pituitary thyroid stimulating hormone (TSH) which is in turn under hypothalamic control (Haynes and Murad, 1985). The hypothalamus exerts a dominant stimulatory influence on TSH secretion through the release of thyroid releasing hormone (TRH) into the hypothalamic-hypophyseal portal system (Murad and Haynes,

1985). T_4 inhibits pituitary TSH production, creating a negative feedback loop (Larsen and Silva, 1981).

In geographical regions having extreme deficiencies in iodine, a high incidence of endemic cretinism is detected at birth. The child is dwarfed, mentally retarded, the heart rate is slow and body temperature may be low (Ingbar, 1985). Alternatively, excessive secretion of thyroid hormones, termed hyperthyroidism, is characterized by thyrotoxicosis and ophthalmopathy.

Thus, the increased thyroxine levels in the Sprague-Dawley rat induced by I_2 and the lack of a similar effect with I^- indicates that this increase depends on the reactivity of I_2 . It was first postulated that this increase may be due to the formation of iodinated by-products in the gastrointestinal tract. To pursue this, studies were conducted to determine if the pharmacokinetics of I_2 and I^- are consistent with the formation of organic by-products.

Second, two alternative hypotheses were pursued to explain the elevation of the T_4/T_3 ratio observed in previous studies with iodine treatments:

- 1) Iodine, or a reaction product of iodine, is capable of inhibiting the 5'-deiodinase enzyme responsible for the conversion of T_4 to T_3 .

- 2) Iodine reacts with thyroxine (T_4) metabolites in the gastrointestinal tract to resynthesize T_4 and elevate its levels in blood.

Chapter II

**Differences in the Distribution of Iodine and Iodide in the
Sprague-Dawley Rat**Abstract

Use of iodine as a drinking water disinfectant for extended space flight raises concerns about potential chronic effects on health. A key question is whether the chemical form of iodine might play a role. To address this question the influence chemical form has on the uptake and distribution of radioiodine was studied in fed and fasted rats. Following oral administration of $^{125}\text{I}_2$ or $^{125}\text{I}^-$, blood ^{125}I levels were maximal at 2 hours and reached similar concentrations in fed animals receiving $^{125}\text{I}^-$ and fasted animals receiving either $^{125}\text{I}_2$ or $^{125}\text{I}^-$. However, when $^{125}\text{I}_2$ was administered to fed animals the initial levels of ^{125}I into blood were significantly lower than the other treatments. The half-life of elimination of ^{125}I from the blood appeared independent of the form of iodine administered. The initial distribution of ^{125}I to the thyroid depended sharply on chemical form, being greater when iodide was administered than iodine regardless of whether animals were fed or fasted. In fed animals administered I_2 , this may largely be explained by the

increased retention of ^{125}I in the stomach contents. In fasted animals, both stomach content and blood levels of ^{125}I were similar whether I_2 or I^- was administered. Since thyroid uptake of iodine is specific for I^- , this suggests that the form of iodine in the blood was different in animals administered I_2 . This notion was further supported by the finding that pretreatment of animals with varying concentrations of I^- in drinking water was four times as effective in suppressing the uptake of a test dose of $^{125}\text{I}^-$ than pretreatment with equivalent concentrations of I_2 .

Introduction

Iodine has long been recognized as an effective disinfectant of drinking water, but its use for this purpose has previously been limited primarily to emergency situations by the military (Thomas et al, 1969, 1979). On early relatively short-term, space missions, NASA has used a nominal iodine (I_2) residual of about 2 mg/L to disinfect non-recycled water for drinking purposes (Janik and Thorstenson, 1986). Now NASA is contemplating more extended space missions (90-180 days), raising the question whether iodine can be safely used for these longer periods.

Prior studies of the toxicology of iodine have concentrated on iodide and iodate, the forms of iodine in the diet, ignoring the fact that I_2 is the chemical species

used in water disinfection (Morgan and Karpen, 1953). From research on other halogen disinfectants it is clear that these compounds may be indirectly toxic by producing toxic by-products (Bull, 1986). For example, the chlorination of drinking water results in the formation of trihalomethanes (THMs). Four THMs (chloroform, dibromochloromethane, bromodichloromethane and bromoform) have been shown to produce cancer in laboratory animals (NCI, 1976; Dunnick et al, 1985; NTP, 1986; NTP, 1989). Therefore, dietary studies on iodide or iodate are not necessarily applicable to estimating hazards of I_2 used for drinking water disinfection.

The most extensive study of I_2 in humans involved every 5th inmate of 3 prisons in Lowell, Florida, where the water systems were iodinated for five years. As expected, certain parameters of thyroid function, including decreased ^{131}I uptake by the thyroid gland and an increase in serum protein-bound iodine concentrations, were altered by the use of this water (Freund et al, 1966). While the most extensive study available, it is limited by a select population, the absence of non-endocrine physiologic measurements, and a loss of subjects due to release of inmates (Thomas et al, 1969). No studies in experimental animals on repeated low-level exposures to I_2 have been reported.

Based on the above, we suggest that I_2 may induce unique toxicological effects based on the reactions it will participate in within the gastrointestinal tract. As a first test of this hypothesis, we have examined the pharmacokinetic behavior of I_2 and I^- in the rat.

Materials and Methods

Chemicals. ^{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, MA). Neutral pH was achieved by the addition of HCl. The oxidation of $^{125}I^-$ to $^{125}I_2$ was achieved by reacting $Na^{125}I$ with H_2O_2 and HCl according to the method described by McAlpine (1945). The purity of $^{125}I_2$ (specific activity 17.4 Ci/mg), was found to be 100% as measured using an isocratic HPLC system capable of simultaneous UV and radioisotope detection (C_{18} 4.6 X 250 mm column, 70:30:0.1% MeOH:H₂O:H₃PO₄, Beckman Instruments, Inc., San Ramon, CA).

Comparison of the absorption and distribution of radioactive iodine and iodide. Male Sprague-Dawley rats were purchased from Laboratory Animal Resource Center (Washington State University, Pullman, WA). These animals, approximately 70-95 days old, weighed 300-425 g prior to

iodine exposure, and were individually housed in metabolism cages. Fed animals were provided Purina Rodent Chow and water *ad libitum*. Fasted animals had their food removed from the cage 18 hours prior to dosing. Rats were similarly treated with carrier-free $^{125}\text{I}^-$ or $^{125}\text{I}_2$ in 1.0 ml of water, as a single oral dose. Thirty-six rats were randomly assigned to six treatment groups (I^- -fed, 2 I^- -fasted groups, I_2 -fed and 2 I_2 -fasted groups). Animals from one of the I^- -fasted and I_2 -fasted groups were sacrificed at one hour following dosing and the remaining four groups at 2 hours. Lethal injection of Ketamine HCl (Bristol Laboratories, Syracuse, NY) was the method of sacrifice in all experiments. Blood (250 ul), thyroid, the stomach and selected organs were collected to determine content of radioactive iodine using a LKB gamma counter (Model 1282). The radioactivity in 250 ul of blood was normalized to total blood volume using the reference value of 6 ml blood/100 g body weight (Waynforth, 1980). The stomach contents were separated, and the stomach washed and the wash collected. Radioactivity for each was determined separately (i.e., stomach wash, stomach contents and stomach wall).

Comparative pharmacokinetics of orally administered iodine and iodide in fasted and non-fasted rats. Twenty-four male Sprague-Dawley rats were purchased from Laboratory

Animals Resource Center (Washington State University, Pullman, WA). These animals, approximately 70-95 days old, weighed 300-425 g prior to treatment and were randomly assigned to four treatment groups, two of which were fasted (fasted animals) and two which were not (fed animals). Fed animals were provided Purina Rodent Chow and water ad libitum. Fasted animals had their food removed from the cage 18 hours prior to dosing and were provided Purina Rodent Chow and water ad libitum immediately after dosing. All rats were given a single oral dose of carrier-free $^{125}\text{I}^-$ or $^{125}\text{I}_2$ in 1.0 ml of water. Blood (250 ul) was collected from the tail vein at 1, 2, 4, 8, 24, 48 and 72 hours following dosing and radioactivity determined as outlined in the previous experiment. Urine and feces were separately collected at 24, 48 and 72 hours and radioactivity determined. At 72 hours the animals were sacrificed by lethal injection (Ketamine HCl, Bristol Laboratories, Syracuse, NY) and the thyroids were excised and radioactivity determined.

Relative effects of iodine and iodide pretreatment on radioiodide uptake by the thyroid. Male Sprague-Dawley rats were temporarily unavailable from Washington State University, therefore they were purchased from Simonsen Laboratories, Inc., (Gilroy, CA). These animals,

approximately 60-77 days of age, weighed 275-350 g prior to treatment and were provided Purina Rodent Chow and water ad libitum. One hundred six rats were randomly assigned to 11 treatment groups (9 animals/compound/dose and 16 control animals receiving distilled water). Rats were maintained on distilled water containing either I_2 or I^- (in the form of NaI) at dose levels of 1, 3, 10, 30 and 100 mg of iodine/L of drinking water for the period of 1 week. At the end of one week all animals received a single oral dose of radioiodide ($^{125}I^-$) in 1.0 ml of water one hour prior to sacrifice, at which time thyroids were excised and radioactivity determined using a LKB gamma counter (Model 1282).

Statistical Analyses. Results are expressed as percent of administered dose as a means of normalizing data. Statistical analyses were performed using a two sample t-test for the first two experiments, and a two-way ANOVA for the experiment on the effects on iodide uptake. Significant differences in the two-way ANOVA were determined using two sample t-tests and Least Significant Differences. The level of significance was set at 0.05.

Results

The uptake and distribution of ^{125}I administered as iodide (I^-) or iodine (I_2) in fasted and fed animals is presented in Table 1. Differences in the distribution of ^{125}I to the thyroid and skin depended on chemical form. No significant differences in the distribution of ^{125}I between I^- and I_2 were observed in the remaining tissues collected, including kidneys, spleen, liver, blood, salivary glands, small intestine and large intestine. (Data not shown). A summation of recovered radioactivity in tissues collected accounts for 80-90% of administered dose. The remaining 10-20% is unaccounted for, perhaps due to localization in tissues not collected or lost in expired air.

At 2 hours $^{125}\text{I}_2$ distributed to the thyroid to only 66 and 75% of that observed with equivalent doses of $^{125}\text{I}^-$ in fed and fasted animals, respectively (Table 1). Uptake at this time still represents the initial rates of uptake since radioiodine uptake into the thyroid was linear over the first two hours in fasted animals.

Distribution of $^{125}\text{I}^-$ to the skin of fasted animals was slightly greater than for fed animals. This was not observed in animals administered $^{125}\text{I}_2$ at 2 hours post dosing. The greater retention of $^{125}\text{I}^-$ in the stomach contents of fed animals compared to fasted might have

accounted for this, except that even greater amounts of $^{125}\text{I}_2$ were retained in the stomach contents of fed animals without apparent effect on the distribution of ^{125}I to the skin. Therefore, the distributional differences of the two forms of iodine cannot be simply attributed to greater retention of radioisotope in the stomach or its contents.

The uptake of $^{125}\text{I}_2$ and $^{125}\text{I}^-$ into, and their elimination from the blood is presented in Figure 1. In all dose groups, the maximum concentration of radioactivity in the blood occurred at 2 hours following administration. The peak of $^{125}\text{I}_2$ concentration in fed animals is reduced by approximately 40% at this time relative to the other groups. The half-life of elimination of radioactivity from the blood was found to be 26.5 hours for I_2 -fasted animals, 30.3 hours for I_2 fed animals, 35.3 hours for I^- -fasted animals, and 29.9 hours for I^- -fed animals (data not shown). There was no statistically significant differences between half-life of elimination as measured by one-way ANOVA.

The urinary elimination data (Table 2), suggest that over the entire 72 hour period equivalent amounts of ^{125}I are eliminated within fed or fasted animal groups, regardless of form administered. However, at 48 and 72 hours there is a small, but statistically significant smaller amount of radioactivity eliminated in the urine from fed animals administered I_2 compared to I^- . Fecal

elimination was essentially equivalent over the entire 72 hour period, regardless of form administered.

Figure 2 shows the suppression of thyroid uptake of a test dose of carrier-free $^{125}\text{I}^-$ in animals pretreated with I^- or I_2 in their drinking water at various concentrations. It required approximately 4 times the concentration of I_2 than I^- in the drinking water during the pretreatment phase to suppress $^{125}\text{I}^-$ uptake to an equivalent extent (within the range of 1 and 4 mg/L). At 100 mg/L both forms of iodine maximally suppressed $^{125}\text{I}^-$ uptake. Drinking water consumption was not affected by either I^- or I_2 treatments, therefore, concentration is a dependable measure of dose. These data suggest that the effective dose of I^- reaching the thyroid when I^- is used in the pretreatment phase is considerably higher than when I_2 is administered at equivalent pretreatment doses.

Discussion

The experiments presented indicate clear differences in the behavior of I^- and I_2 in the body. These data lead us to question the view that iodide and iodine are essentially interchangeable (Haynes and Murad, 1985) in their toxicological properties at doses that would be encountered in drinking water disinfected with iodine. There are four observations that are key to this conclusion: (1) Fed

animals initially retained a larger percentage of ^{125}I derived from I_2 in their gastric contents than with I^- at 2 hours. (2) In the fasted state, the uptake of I_2 and I^- into the thyroid differed significantly despite the absence of differences in uptake into blood. (3) I_2 pretreatments were significantly less effective in suppressing uptake of radioiodide into the thyroid gland than I^- pretreatments. (4) Total urinary excretion of ^{125}I over a 72 hour period when administered as I_2 and I^- was basically equivalent, indicating that total absorption was essentially identical. Yet, the retention of ^{125}I by the thyroid between the two forms is substantially different in both fed and fasted animals. Each of these points are discussed below in turn.

At 2 hours, substantially higher concentrations of radioactivity are retained in the stomach wall and contents of fed animals administered $^{125}\text{I}_2$ compared to $^{125}\text{I}^-$. This suggests that I_2 reacted with the components of the diet present such that it retarded ^{125}I absorption. It is notable that uptake of $^{125}\text{I}_2$ into the thyroid was affected to a greater degree than peripheral distribution as measured in the skin.

The initial uptake of ^{125}I into the blood in fasted animals does not differ significantly between I^- and I_2 . This indicates that, at 2 hours, the smaller uptake of radioactivity into the thyroids in fasted animals

administered $^{125}\text{I}_2$ cannot be related to a lower concentration of ^{125}I in blood. Thus it is probably related to a difference in chemical form within the blood stream.

Thyroid uptake of iodine is specific for I^- (Ingbar, 1985). Therefore, the fact that pretreatment with I_2 was significantly less effective in suppressing uptake of $^{125}\text{I}^-$ into the thyroid gland than I^- pretreatments supports the findings that I_2 probably exists in a different chemical form within the blood stream.

Total urinary and fecal elimination of radioactivity over the entire 72 hour period was basically equivalent indicating that overall absorption was similar in both $^{125}\text{I}^-$ and $^{125}\text{I}_2$ treated animals. However, the retention of ^{125}I in the thyroid was greater at 72 hours in both fed and fasted animals treated with $^{125}\text{I}^-$ compared to $^{125}\text{I}_2$. This further supports the notion that a significant fraction of radioactivity in the blood of animals administered $^{125}\text{I}_2$ is present in a form other than I^- for an extended period of time.

There appears to be some discrepancy between the thyroid uptake in animals treated with distilled water in the pretreatment study (Figure 2), and fasted animals dosed with $^{125}\text{I}^-$ and sacrificed at 1 hr (Table 1). Several explanations could account for these differences; animals were received from 2 different sources (Laboratory Animal

Resource Center and Simonsen), the animals were slightly younger in the pretreatment study (60-77 days old versus 70-95 days old), and the diet lot had changed. None of these possibilities have been investigated, but it is difficult to see how they would alter the main conclusions of the study.

By-products are formed in the gastrointestinal tract after chlorine is administered orally (Mink et al, 1983). Many of the by-products result from the chlorination of endogenous compounds such as amino acids (Pereira et al, 1973), peptides (Ayotte and Gray, 1985), proteins (Scully et al, 1985), lipids (Ghanbari et al, 1982) and purine and pyrimidine bases (Hoyano et al, 1973). Although less reactive than chlorine, iodine is probably still capable of reacting with some of these same compounds in the gastrointestinal tract to form a variety of iodinated compounds. Iodine is known to react with histidine, tyrosyl and sulfhydryl moieties. Harrington et al, (1985) have documented that chlorine dioxide activates I^- in the diet to a species (presumably I_2) that is capable of iodinating organic material in the gastrointestinal tract.

Based on results in this paper, we propose that distributional differences between I_2 and I^- are the result of reactions of I_2 with endogenous organic chemicals in the gastrointestinal tract. These reactions would be expected to render I_2 less available for uptake into the thyroid,

which actively transports I^- , in an energy-requiring process, from the blood into the thyroid gland (Ingbar, 1985). Further work is necessary to isolate and identify iodinated by-products produced in the gastrointestinal tract and to determine whether they present an unacceptable risk to humans consuming iodinated drinking water.

Table 1
 Uptake and Distribution of ^{125}I in Fed and Fasted Animals
 with Time

	Iodide (I^-)	Iodine (I_2)	P (I^- vs. I_2)
<u>Thyroid</u>			
Fasted (1 hr)	2.4 \pm 0.5 ^a	1.8 \pm 0.3	0.29
Fasted (2 hr)	5.6 \pm 0.5	3.7 \pm 0.3	0.01
Fed (2 hr)	5.7 \pm 0.5	4.3 \pm 0.6	0.02
<u>Skin</u>			
Fasted (2 hr)	20.5 \pm 1.1	17.5 \pm 2.2	0.02
Fed (2 hr)	15.9 \pm 1.0*	17.4 \pm 1.7	0.40
<u>Stomach Wall</u>			
Fasted (1 hr)	1.2 \pm 0.1	2.4 \pm 0.2	0.001
Fasted (2 hr)	4.5 \pm 1.3	6.8 \pm 1.2	0.23
Fed (2 hr)	2.4 \pm 0.5	3.8 \pm 0.5	0.09
<u>Stomach Contents</u>			
Fasted (1 hr)	43.6 \pm 2.1	47.3 \pm 2.2	0.27
Fasted (2 hr)	14.5 \pm 1.9	17.4 \pm 1.8	0.32
Fed (2 hr)	24.7 \pm 3.1*	38.4 \pm 4.3**	0.03

^aValues measured as percentage of administered dose, \pm S.E.M. of 6 animals.

*Indicates that the amount of ^{125}I in the indicated compartment is significantly different from that observed with equivalent doses administered in fasted animals and measured at equivalent times with $P < 0.05$ and ** $P < 0.01$ by two sample t-test.

Figure 1: Uptake into and elimination from total blood volume of ^{125}I depending on chemical form. Animals were administered a single oral dose of $^{125}\text{I}^-$ or $^{125}\text{I}_2$ and blood collected at intervals and normalized to total blood volume. Each experimental point represents the mean of 6 animals. Fed animals administered $^{125}\text{I}_2$ were found to have blood levels of radioactivity significantly depressed compared to other treatment groups ($P < 0.05$).

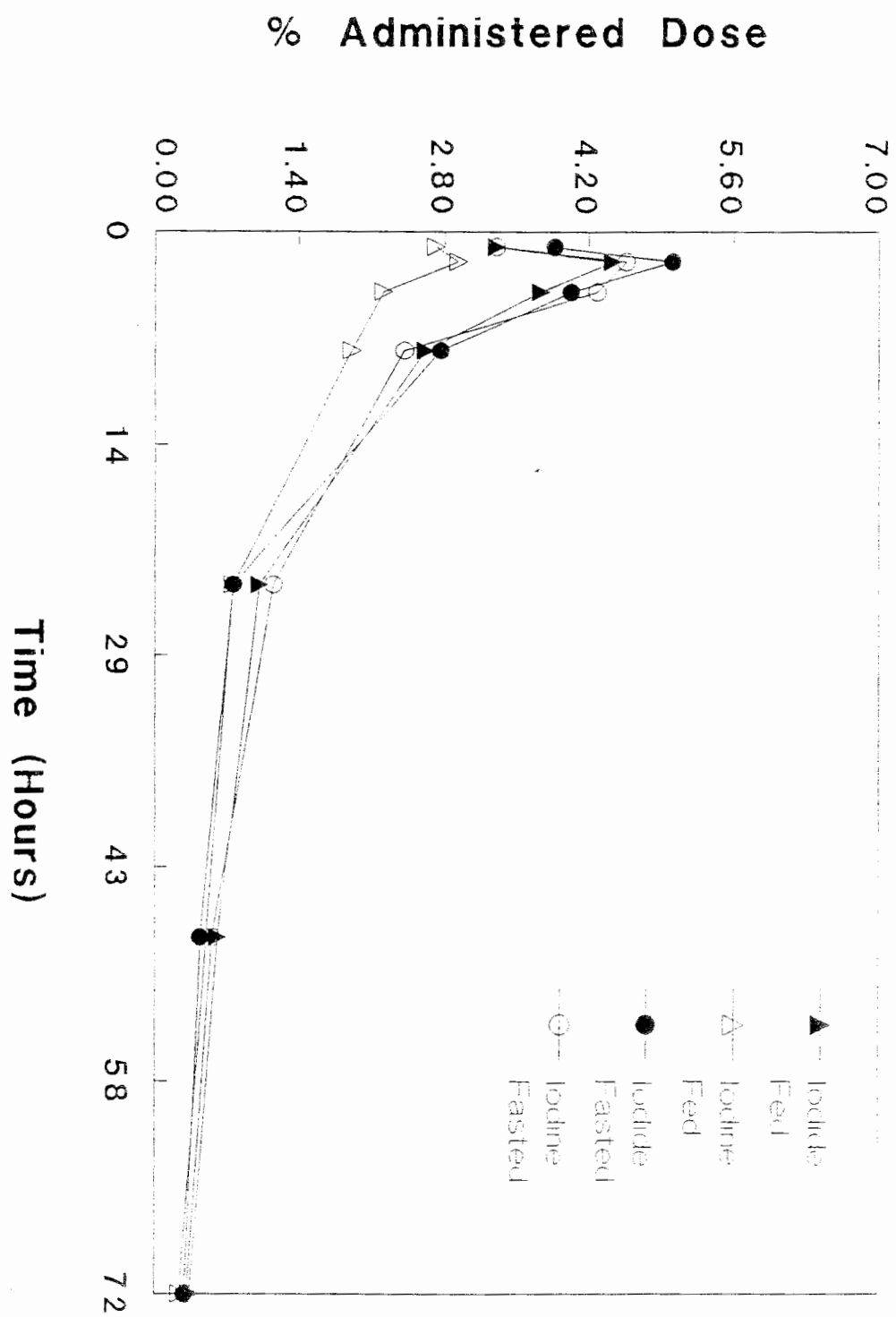


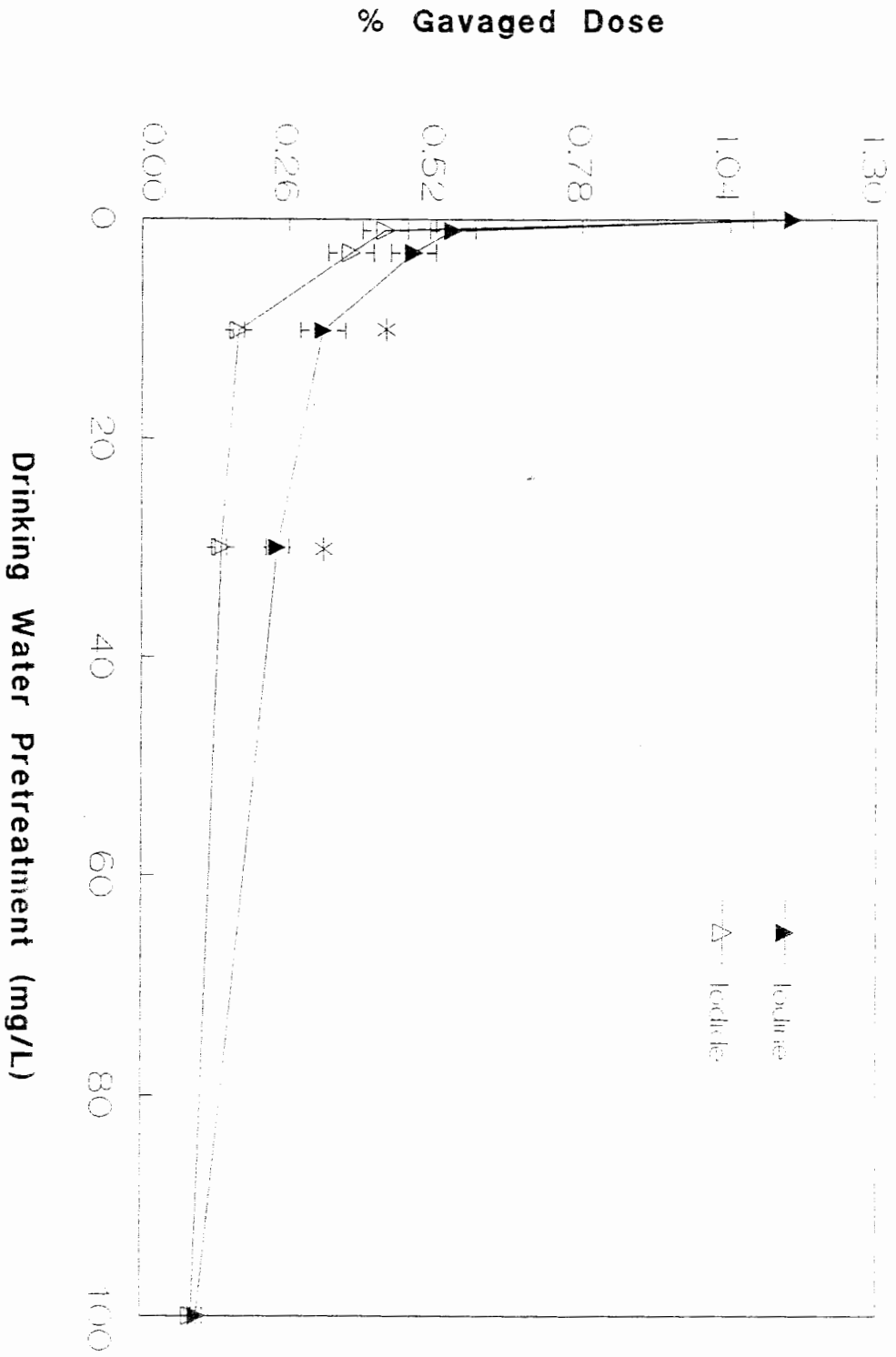
Table 2
 Elimination of ^{125}I and its Retention in Thyroid Gland after
 72 Hours

	Fasted I^-	Fasted I_2	Fed I^-	Fed I_2
<u>Urine</u>				
24 hrs	28.6 \pm 5.6 ^a	27.3 \pm 4.5	21.8 \pm 3.3	17.5 \pm 2.6
48 hrs	4.5 \pm 0.9	5.0 \pm 0.7	5.0 \pm 0.6	3.4 \pm 0.4*
72 hrs	1.6 \pm 0.3	1.5 \pm 0.2	2.0 \pm 0.2	1.4 \pm 0.2*
Total	34.7 \pm 5.7	33.8 \pm 4.8	28.8 \pm 3.8	22.3 \pm 2.8
<u>Feces</u>				
24 hrs	4.0 \pm 0.7	4.8 \pm 1.0	2.1 \pm 0.4	2.0 \pm 0.4
48 hrs	2.4 \pm 0.3	2.3 \pm 0.5	2.5 \pm 0.2	2.3 \pm 0.5
72 hrs	1.7 \pm 0.2	1.8 \pm 0.3	2.1 \pm 0.2	2.0 \pm 0.4
Total	8.2 \pm 1.0	8.6 \pm 1.7	6.7 \pm 0.5	6.3 \pm 1.1
<u>Thyroid</u>				
72 hrs	6.7 \pm 1.2	4.7 \pm 0.4	8.0 \pm 1.0	4.8 \pm 0.4*

^aValues measured as percentage of administered dose, \pm S.E.M. of 6 animals.

*Indicates that the amount of ^{125}I in the indicated compartment is significantly different from that observed with equivalent doses of tracer administered as I^- at $P < 0.05$ by two sample t-test.

Figure 2: Comparison of the ability of iodine and iodide in drinking water to suppress radioiodide ($^{125}\text{I}^-$) uptake by the thyroid gland of male rats. Animals were pretreated for 7 days with varying doses of I^- or I_2 . At the end of 7 days all animals received a single oral dose of ^{125}I one hour prior to sacrifice. Each treatment point represents the mean of 9 animals and 16 control animals. *Values are significantly different from corresponding iodide treatment ($P < 0.05$).



Chapter III

**Distribution of Radioactivity Derived from Iodine
into Blood Components Differs Depending
on Chemical Form Administered**

Abstract

It has been previously reported that radioactivity derived from iodine distributes differently in the Sprague-Dawley rat depending on chemical form administered. In the present communication we report the differential distribution of iodine (I_2) and iodide (I^-) into blood components. Twice as much radioiodine is in the form of iodide (I^-) in the plasma of animals treated with $^{125}I^-$ compared to $^{125}I_2$ -treated rats. No I_2 could be detected in the plasma. With an increase in dose, increasing amounts of radioactivity derived from $^{125}I_2$ -treated animals distributes to whole blood compared to equivalent doses of $^{125}I^-$, reaching a maxima at a dose of 2 mg/Kg. Most of the radioactivity derived from I_2 associates with serum proteins and lipids, in particular with albumin and cholesteryl iodide. This data supports earlier conclusions that iodine exists in different chemical forms within the blood compartment when administered as I_2 compared to I^- .

Introduction

We have reported that the pharmacokinetic behavior of iodine differs when it is administered as I_2 versus I^- in the Sprague-Dawley rat (Thrall and Bull, 1990). In particular, we found substantially higher quantities of radioactivity in the thyroid gland shortly after dosing in both fed and fasted animals treated orally with $^{125}I^-$ compared to animals treated with $^{125}I_2$. In fed animals treated with I_2 , this can at least partially be explained by the greater retention of radioactivity in the stomach contents. However, in fasted animals the radioactivity in the stomach and stomach contents is essentially equivalent regardless of treatment. Since thyroid uptake is specific for I^- (Ingbar, 1985), these findings suggest that iodine at least partially exists in a different chemical form not sequestered by the thyroid in animals treated with I_2 versus I^- -treated animals.

In the present study, we report that the distribution of radioactivity into blood components depends on chemical form of radioiodine administered.

Methods and Materials

^{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, MA). Neutral pH was achieved by the

addition of HCl. Oxidation of $^{125}\text{I}^-$ to $^{125}\text{I}_2$ was achieved by reacting Na^{125}I with H_2O_2 and HCl according to the method described by McAlpine (1945). The purity of $^{125}\text{I}_2$ (specific activity 17.4 Ci/mg), was found to be 100% as measured using an isocratic HPLC system capable of simultaneous UV and radioisotope detection (C_{18} , 4.6 X 250-mm column, 70:30:0.1% $\text{MeOH}:\text{H}_2\text{O}:\text{H}_3\text{PO}_4$, Beckman Instruments, Inc., San Ramon, CA).

Determination of iodinated amino acids. Animals weighing approximately 150-200 g had their food removed from the cage 18 hours prior to administration of equivalent doses of I_2 or I^- containing tracer amounts (50 uCi) of ^{125}I by gavage. The total dose of iodine in both cases was 6.0 mg/kg body weight. Two hours following administration of the radiolabeled iodine, blood (5 ml) was drawn from the caudal vena cava under Ketamine HCl (Fort Dodge, IA) anesthesia, and plasma separated by centrifugation. Plasma was treated with twice the volume of acetonitrile to precipitate proteins. The supernatant was concentrated to a volume of 1 ml under a stream of N_2 gas and injected (100 ul) into an isocratic HPLC system capable of simultaneous UV and radioisotope detection (CN 4.6 X 250-mm column, 10:90:0.1% $\text{MeOH}:\text{H}_2\text{O}:\text{H}_3\text{PO}_4$, Beckman Instruments, Inc., San Ramon, CA). Fractions were collected every 12 seconds and counted for radioactivity using a LKB gamma counter (Model 1282).

Iodinated amino acid standards were purchased from Sigma Chemical Company (St. Louis, MO).

Distribution of radiolabel into blood components. Rats weighing approximately 200-250 g were fasted overnight. The animals were administered increasing doses of I_2 or I^- containing tracer amounts (25 uCi) of ^{125}I by oral gavage. The total doses of iodine were carrier-free ^{125}I , 0.75, 2.0 and 6.0 mg/kg body weight. Two hours following administration of the radiolabel, blood was drawn via the caudal vena cava under Ketamine HCl (Fort Dodge, IA) anesthesia. Compartments examined were whole blood, serum, packed cells, serum proteins, serum albumin, serum globulin and serum lipids. The methods for separating blood components have been previously described (Annino, 1964; Bennett, 1964). Briefly, Na_2SO_3 was added to serum to precipitate proteins. Protein was mixed with ether to extract albumin from globulin. Lipids were extracted by mixing serum with EtOH:Ether (2:1). Lipids were further separated into classes by thin layer chromatography (TLC) on Silica 60 plates (American Scientific) using hexane:ether:acetic acid (90:10:1) as the mobile phase. Plates were sprayed with 2-chlorofluorescein and visualized by UV light. Spots were scraped and counted for radioactivity using an LKB gamma counter.

Results

The radiochromatogram derived from plasma of $^{125}\text{I}_2$ -treated animals differs significantly from the radiochromatograms of $^{125}\text{I}_2$ or $^{125}\text{I}^-$ in water (Figure 1a). Figure 1b shows a radioactive peak eluting just after the void volume of the column that is common to both I^- - and I_2 -treated animals. The peak is of similar magnitude with both forms of iodine. This peak remains unidentified. It was shown that mono- or di-iodotyrosine, iodohistidine, iodocytosine, T_4 , T_3 , rT_3 , T_2 or iodophenylalanine do not chromatograph in this region. The second peak in the chromatogram coelutes with iodide in water and is presumed to represent free iodide in solution. No peak was observed in plasma from I_2 -treated animals that corresponds to I_2 in water.

Comparison of the chromatograms (Figures 1a and b), suggests that a portion of iodine in I_2 -treated animals is converted to iodide. However, this is a significantly smaller fraction of the radioactivity when the radioiodine is administered as I_2 versus I^- . It also represents a smaller fraction of the radioactivity found in blood as determined by measuring the ratio of the CPM of the two peaks (Table 1).

In an experiment to quantitatively determine the distribution of iodine into blood components, it is found that four to five times the radioactivity administered as

$^{125}\text{I}_2$ distributes to whole blood compared to that observed with equivalent doses of $^{125}\text{I}^-$ (9.3 and 1.7 $\mu\text{Eq I}$, respectively, at the 2 mg/Kg dose level) (Figure 2). However, the distribution of radioactivity derived from $^{125}\text{I}_2$ to blood is not linear with dose, but reaches a maximum at doses of 2 mg/Kg. Higher doses produce no further increase in the amount of radioactivity in whole blood.

Unlike the distribution of radioactivity to whole blood, the distribution of radioactivity to blood cells is nearly linear with dose and continues to increase with increasing dose, regardless of whether I_2 or I^- has been administered (Figure 3). Nevertheless, the quantity of label that associates with blood cells in animals treated with $^{125}\text{I}_2$ is significantly higher than when the animal is treated with $^{125}\text{I}^-$ at all dose levels.

A substantial fraction of radioactivity in plasma following either treatment appears to associate with lipids (Figure 4). As in whole blood, the distribution of label in this fraction reaches a maxima at doses of 2 mg/Kg and does not increase further with increasing dose. The radioactivity that associates with serum lipids is significantly higher in animals treated with $^{125}\text{I}_2$ compared to animals receiving $^{125}\text{I}^-$, at all dose levels.

TLC is utilized to separate serum lipids in animals receiving $^{125}\text{I}_2$ or $^{125}\text{I}^-$ into individual classes. A radioactive band co-migrating with the standard cholesteryl

iodide ($R_f = 0.85$), and a second unidentified band ($R_f = 0.19$) were detected in animals treated with $^{125}\text{I}_2$ (data not shown). Together, the radioactivity in these two bands contain 1.8 u Eq I/ml whole blood, at the 2 mg/Kg dose level. This corresponds to 20% of the radioactivity which distributes to whole blood, and 57% of the radioactivity in lipids. No specific localization of radioactivity is detected in animals treated with $^{125}\text{I}^-$ with the majority of the radioactivity migrating with the solvent front.

The radioactivity that associates with serum proteins is 3 to 5 times higher in animals treated with $^{125}\text{I}_2$ compared to animals receiving $^{125}\text{I}^-$; at all dose levels (Figure 5). In animals treated with I_2 this association reaches a maxima at doses of 2 mg/Kg and does not increase further with higher doses.

The association of radioactivity in serum protein is further investigated by fractionating the proteins into the two major forms, albumin (Figure 6) and globulin (Figure 7). In both fractions at all dose levels, the amount of radioactivity that associates is approximately 3 times higher in animals receiving $^{125}\text{I}_2$ compared to those receiving $^{125}\text{I}^-$. Radioactivity associates with albumin in an apparently saturable fashion. On the other hand, association with globulin appears linear with dose. At the 2 mg/Kg dose level, distribution into albumin accounts for approximately 75% of the radioactivity in serum protein and

10% of the radioactivity in whole blood. At the same dose, distribution into globulin accounts for approximately 22 and 10% of the radioactivity in proteins, and 3 and 1% of the radioactivity in whole blood, with I_2 and I^- treatment, respectively.

The highest fraction, approximately 40% of the radioactivity distributing to whole blood, with either treatment, is accounted for in serum water, which consists of deproteinated serum and pooled water from washing the cellular component. In animals treated with I_2 this distribution is not linear with dose, but reaches a maxima at doses of 2 mg/Kg and does not significantly increase further with increasing dose (Data not shown). The distribution in animals treated with I^- continues to increase with increasing dose.

A summary of the amounts of radioactivity associated with each blood fraction at the 2 mg/Kg dose level is provided in Table 2. Higher amounts of radioactivity associates in all blood fractions in animals treated with I_2 compared to animals receiving I^- . At least 93% of the radioactivity in whole blood was accounted for in the individual fractions. Therefore, the differences cannot be attributed to incomplete recovery.

Discussion

The experiments presented indicate clear differences in the distribution of I^- and I_2 in the blood. These data support our previous conclusion that the form of iodine in the blood is different in animals receiving I_2 than animals receiving I^- (Thrall and Bull, 1990). The observations key to this are discussed below.

As shown by HPLC analysis, some iodine in $^{125}I_2$ -treated animals does evidently get converted to I^- and transported into the plasma, although the levels of I^- appear to be substantially lower than in animals receiving equivalent doses of $^{125}I^-$. These data also show that little, if any, iodine reaches the blood in the form of I_2 . Since no unreacted I_2 was detected in plasma, it is reasonable to conclude that much of the radiolabel has become associated with iodinated products in the gastrointestinal tract. These products appear to be absorbed and distributed to specific blood compartments.

The amount of radioactivity in animals treated with I_2 that distributes to the serum water fraction is not linear with dose, but reaches a maxima at doses of 2 mg/Kg. If the non-linear association of radioactivity with other blood components in animals receiving I_2 was a result of a limited number of binding sites within the blood, this component should continue to increase once binding sites were occupied..

The non-linear association of these products with certain blood components suggests that these reactions are largely of a specific nature. Therefore, either (1), the substrates for these reactions are present in limited quantities in the gastrointestinal tract. (2), Reactions are confined to a very limited site, or (3), A transport system carrying iodinated compounds into the blood is of limited capacity.

A large fraction of radioactivity in whole blood is accounted for in serum lipids. In animals treated with $^{125}\text{I}_2$, 57% of this radioactivity is detected in cholesteryl iodide. It is difficult to imagine that the production of these by-products is limited by body stores of cholesterol. Therefore, it is more reasonable to conclude that iodine had depleted cholesterol in a more limited compartment, and the resulting cholesteryl iodide migrates to the serum lipid pool.

The association of radioactivity derived from I_2 -treated animals with serum albumin is interesting in view of recent work by Sherer et al. (1991), showing that in the rat, oral I_2 treatment results in a significant increase in plasma thyroxine (T_4) levels. In the rat, T_4 is carried primarily by albumin, and secondly by globulin. Although the rat is frequently used as a model for thyroid effects in man, the primary carrier for T_4 and T_3 in man is thyroxine-binding globulin (TBG) (Berson and Yalow, 1954). The data

presented here suggest that I_2 may be reacting within the gastrointestinal tract to form T_4 , which is subsequently transported into the blood.

It was noted that despite the large differences in ^{125}I distribution to blood when derived from I_2 versus I^- , the relative distribution to each blood compartment was similar. Some of this radioactivity from $^{125}I^-$ could exist in similar forms as seen with I_2 . Peroxidase activity in the small intestine is comparable to that of the stomach and at least 3.5 fold as high as that of the thyroid (Banerjee and Datta, 1982). This enzyme could oxidize I^- to I_2 , and increase its reactivity. However, TLC failed to detect radioactivity with a particular lipid in $^{125}I^-$ -treated animals.

In summary, we find that the distribution of radioactivity derived from ^{125}I treated animals into blood components is significantly higher in all fractions when administered as I_2 compared to I^- . Little, if any, iodine in the form of I_2 is present in the plasma. The difference in recovery of total radioactivity in blood components and HPLC suggests that some radioactivity may have been retained on the HPLC column. Some radioactivity (20% of whole blood) derived from $^{125}I_2$ -treated animals is identified in serum lipids in the form of cholesteryl iodide. This provides direct evidence that I_2 reacts with organic materials to produce iodinated products. A substantial fraction of radioactivity in whole blood was found to associate with

albumin. In other experiments we have demonstrated that iodine reacts with thyroxine (T_4) metabolites in the gastrointestinal tract to resynthesize T_4 . Therefore, some of the radioactivity bound to albumin could be attributed to thyroxine binding.

Figure 1a: Radiochromatogram of HPLC fractions from plasma derived from $^{125}\text{I}_2$ treated animals compared to I_2 and I^- standards in water. Animals were administered a single oral dose of ^{125}I in the form of I_2 or I^- and blood was collected two hours later. Plasma proteins were precipitated by twice the volume of acetonitrile and the supernatant was concentrated under a stream of N_2 gas and injected (100 μl) on an isocratic HPLC system capable of simultaneous UV and radioisotope detection (CN 4.6 X 250 mm column, 10:90:0.1% $\text{MeOH}:\text{H}_2\text{O}:\text{H}_3\text{PO}_4$). Fractions were collected every 12 seconds and counted for radioactivity using a LKB gamma counter.

Figure 1b: Radiochromatogram of HPLC fractions from $^{125}\text{I}_2$ and $^{125}\text{I}^-$ treated animals as described in the 1a.

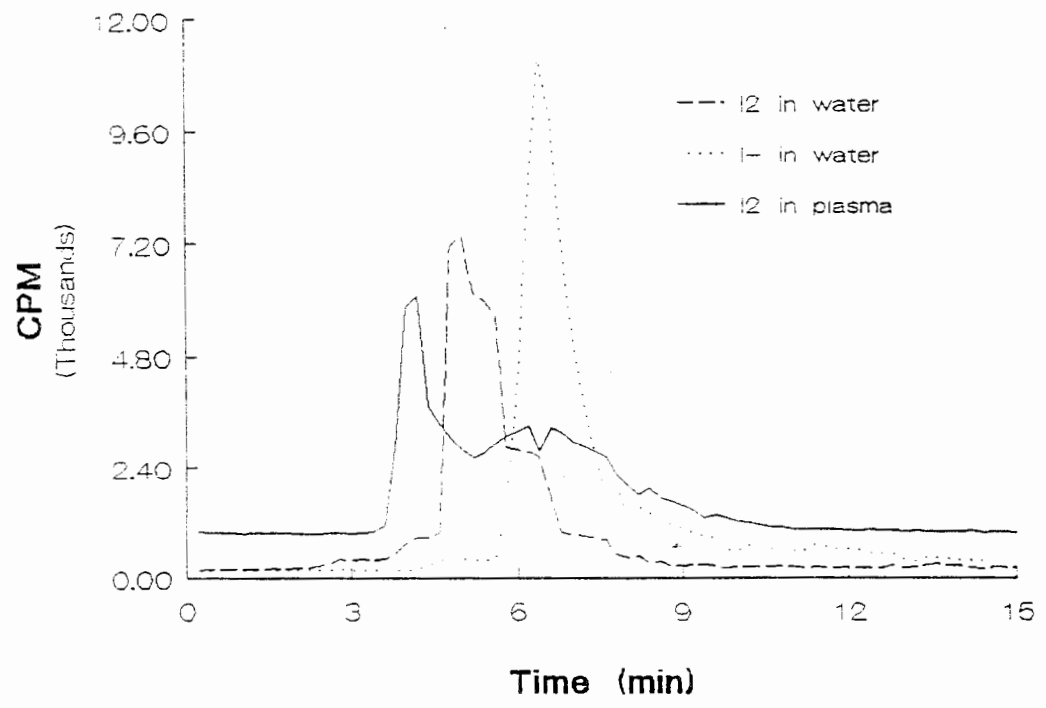


Figure 1a.

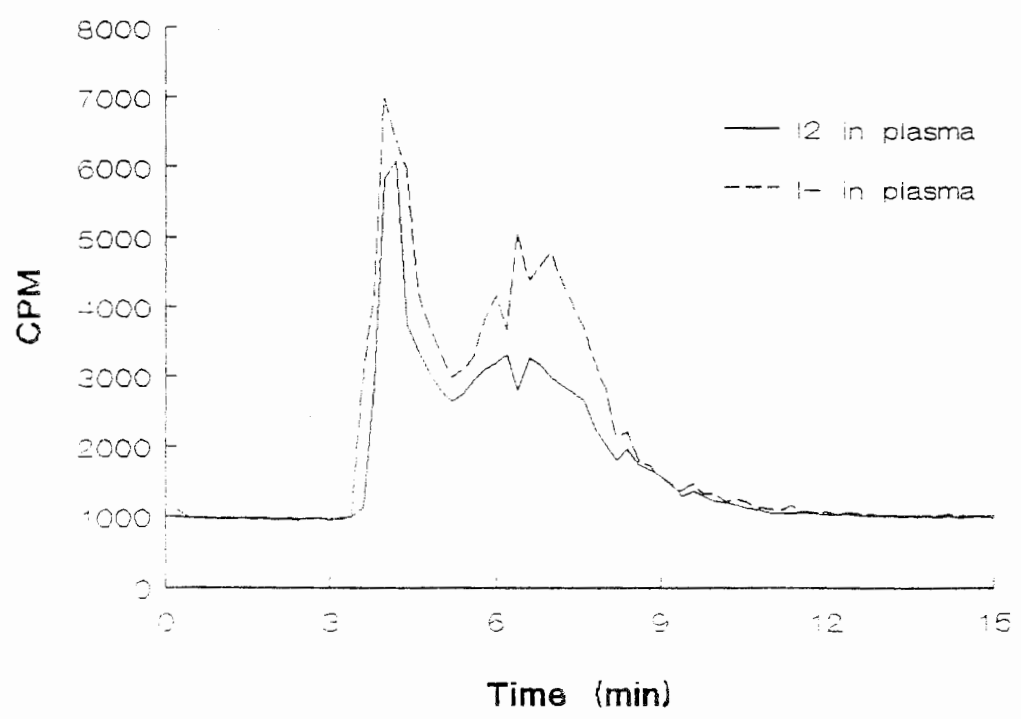


Figure 1b.

Table 1
CPM and Ratio of radioactive peaks following I₂ or I⁻
treatment

	Peak 1	Peak 2	Ratio
I ₂	33600 ± 4828 ^a	64235 ± 5333	1.73 ± 0.10
I ⁻	46973 ± 4699	114911 ± 12814 [*]	2.46 ± 0.16 [*]

^a Average CPM ± S.E.M. of n=8 animals

^{*} Value differs significantly from animals treated with I₂,
P < 0.05 by two sample t-test.

Figure 2: Distribution of iodine into whole blood depending on chemical form. Animals were administered a single oral dose of $^{125}\text{I}^-$ or $^{125}\text{I}_2$ at various dose levels. Blood was collected 2 hrs following treatment and counted for radioactivity. Each experimental point represents the mean of 6 animals \pm S.E.M. In some cases the S.E.M. is smaller than the symbols that identifies the mean value.

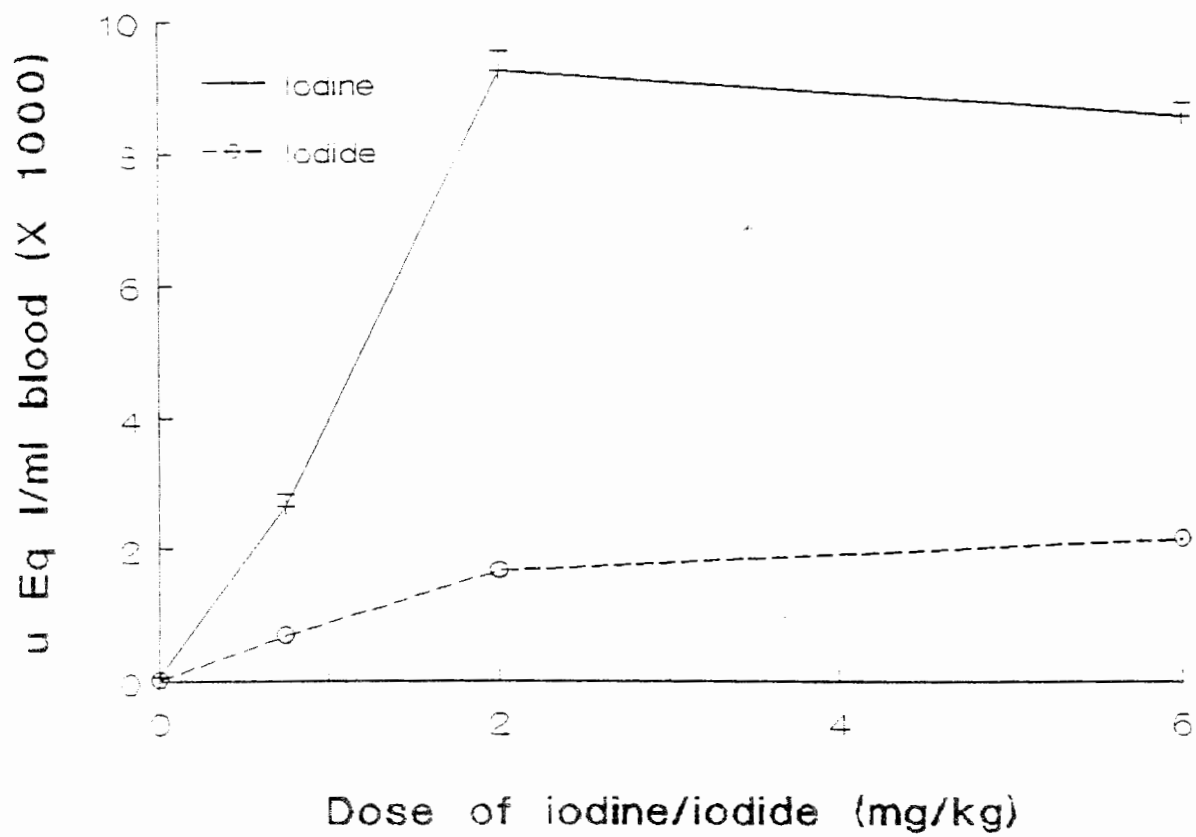


Figure 3: The dose-dependence of iodine distribution into red and white blood cells depending on the chemical form administered. Animals were administered a single oral dose of $^{125}\text{I}_2$ or $^{125}\text{I}^-$ at the dose levels indicated. Blood was collected 2 hrs following treatment, blood cells were separated as described in the methods, and counted for radioactivity. Each experimental point represents the mean of 6 animals \pm S.E.M. The S.E.M. is smaller than the symbol that identifies the mean value in some cases.

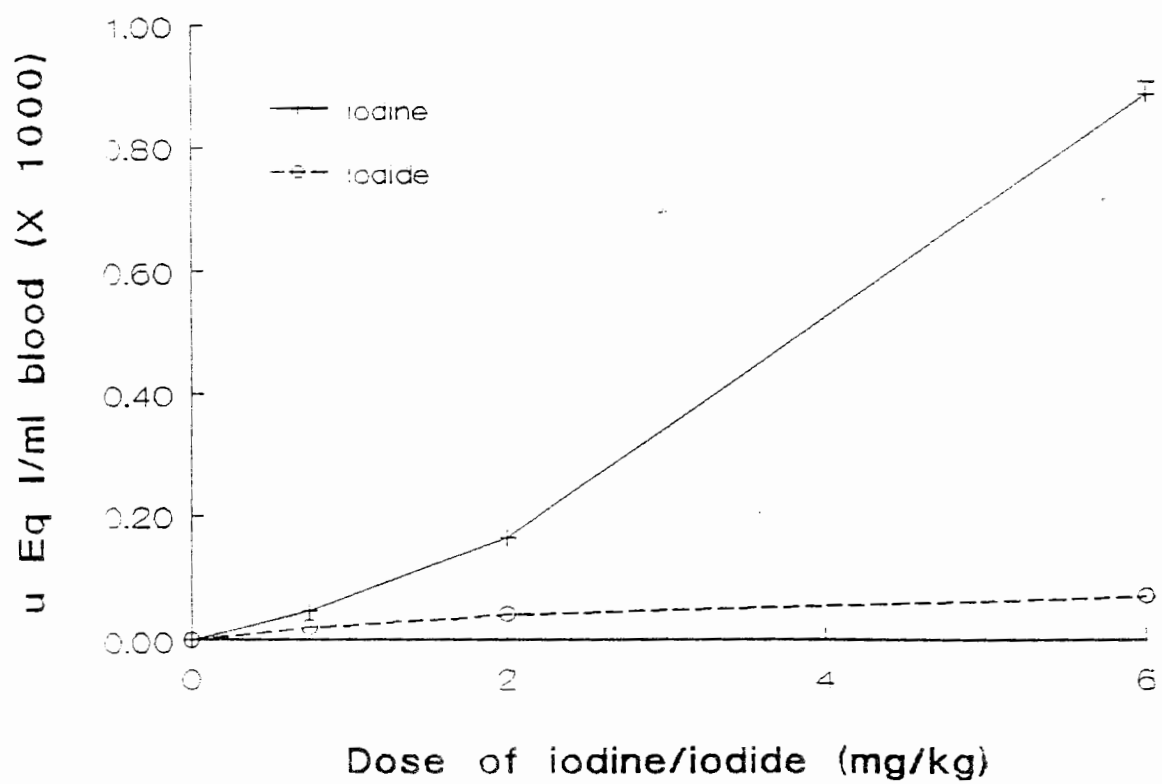


Figure 4: Distribution of iodine into serum lipids depending on chemical form administered. Animals were administered a single oral dose of $^{125}\text{I}_2$ or $^{125}\text{I}^-$ at various dose levels. Blood was collected 2 hrs following treatment, lipids were extracted as described in the methods, and counted for radioactivity. Each experimental point represents the mean of 6 animals \pm S.E.M. In some cases the S.E.M. is smaller than the symbol which identifies the mean value.

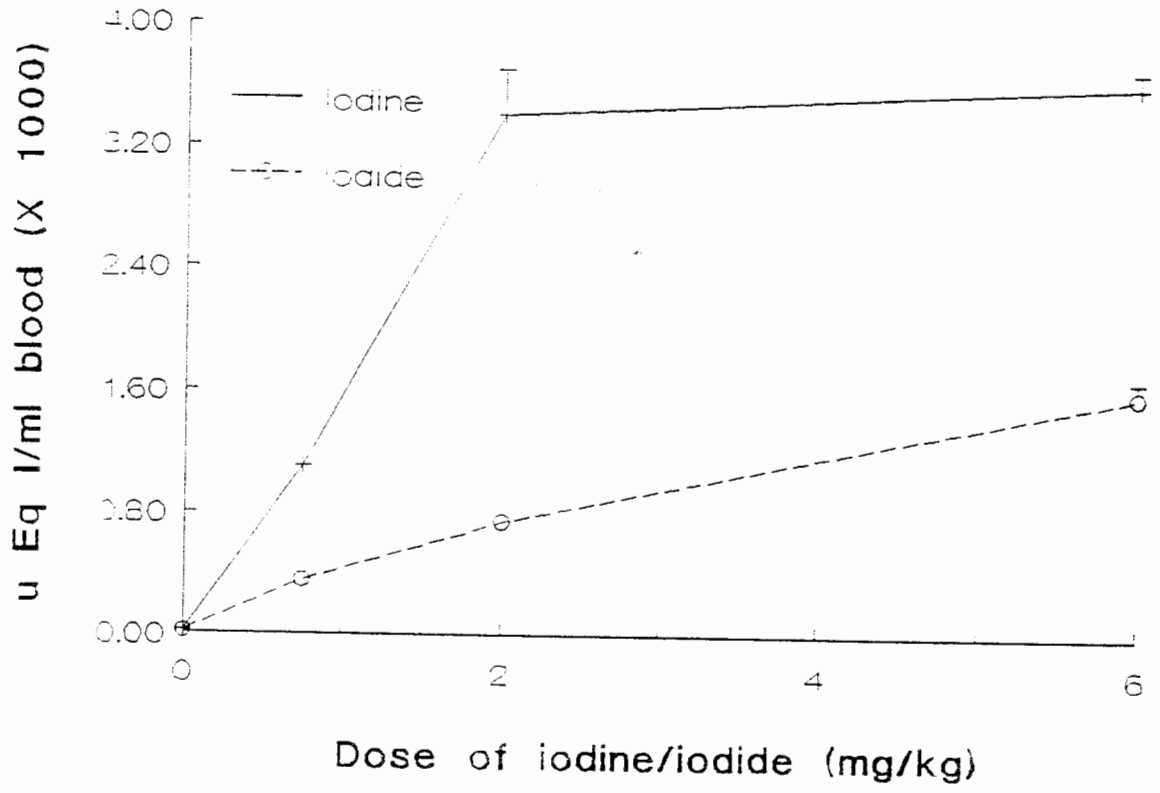


Figure 5: Distribution of iodine into serum proteins depending on chemical form administered. Animals were administered a single oral dose of $^{125}\text{I}_2$ or $^{125}\text{I}^-$ at various dose levels. Blood was collected 2 hrs following treatment, proteins were precipitated as described in the methods, and radioactivity determined. Each experimental point represents the mean of 6 animals \pm S.E.M. In some cases the S.E.M. is smaller than the symbol which identifies the mean value.

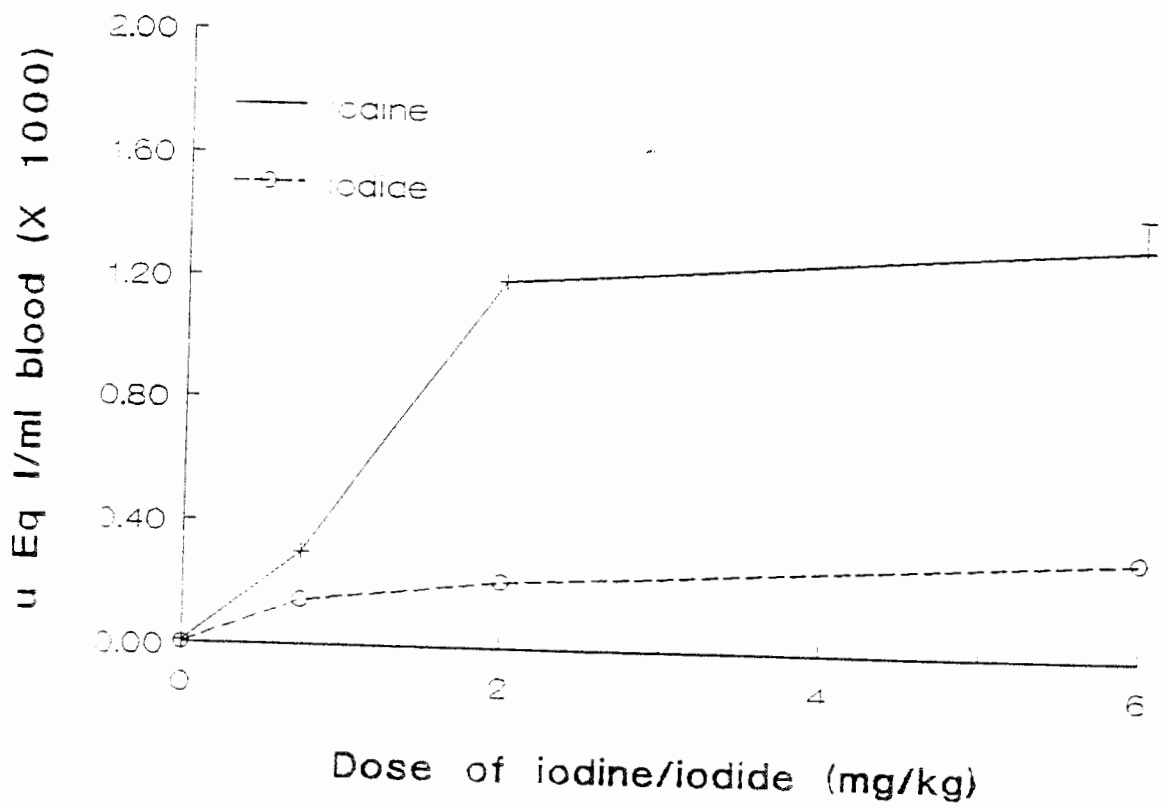


Figure 6: Distribution of iodine into serum albumin depending on chemical form administered. Animals were administered a single oral dose of $^{125}\text{I}_2$ or $^{125}\text{I}^-$ at various dose levels. Blood was collected 2 hrs following treatment, albumin was separated as described in the methods, and radioactivity determined. Each experimental point represents the mean of 6 animals \pm S.E.M. In some cases the S.E.M. is smaller than the symbol which identifies the mean value.

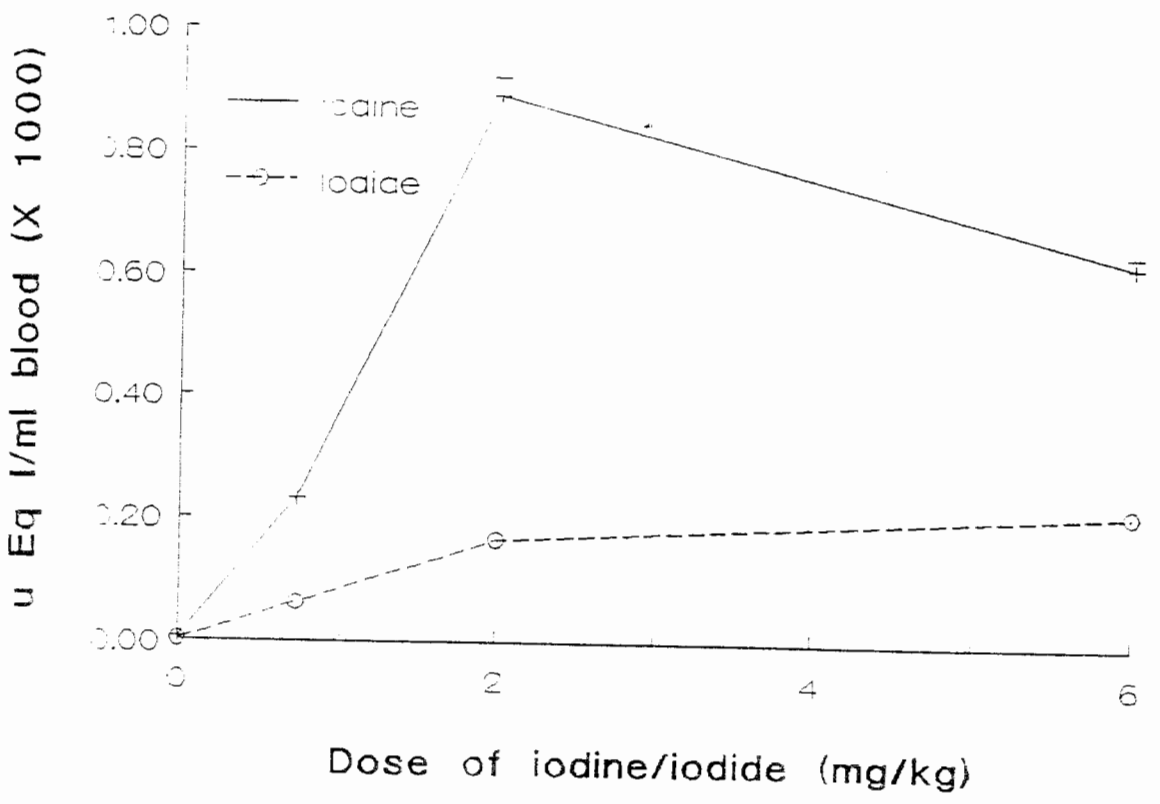


Figure 7: Distribution of iodine into serum globulin depending on chemical form administered. Animals were administered a single oral dose of $^{125}\text{I}_2$ or $^{125}\text{I}^-$ at various dose levels. Blood was collected 2 hrs following treatment, globulin was separated as described in the methods, and radioactivity determined. Each experimental point represents the mean of 6 animals \pm S.E.M. In some cases the S.E.M. is smaller than the symbol used to identify the mean value.

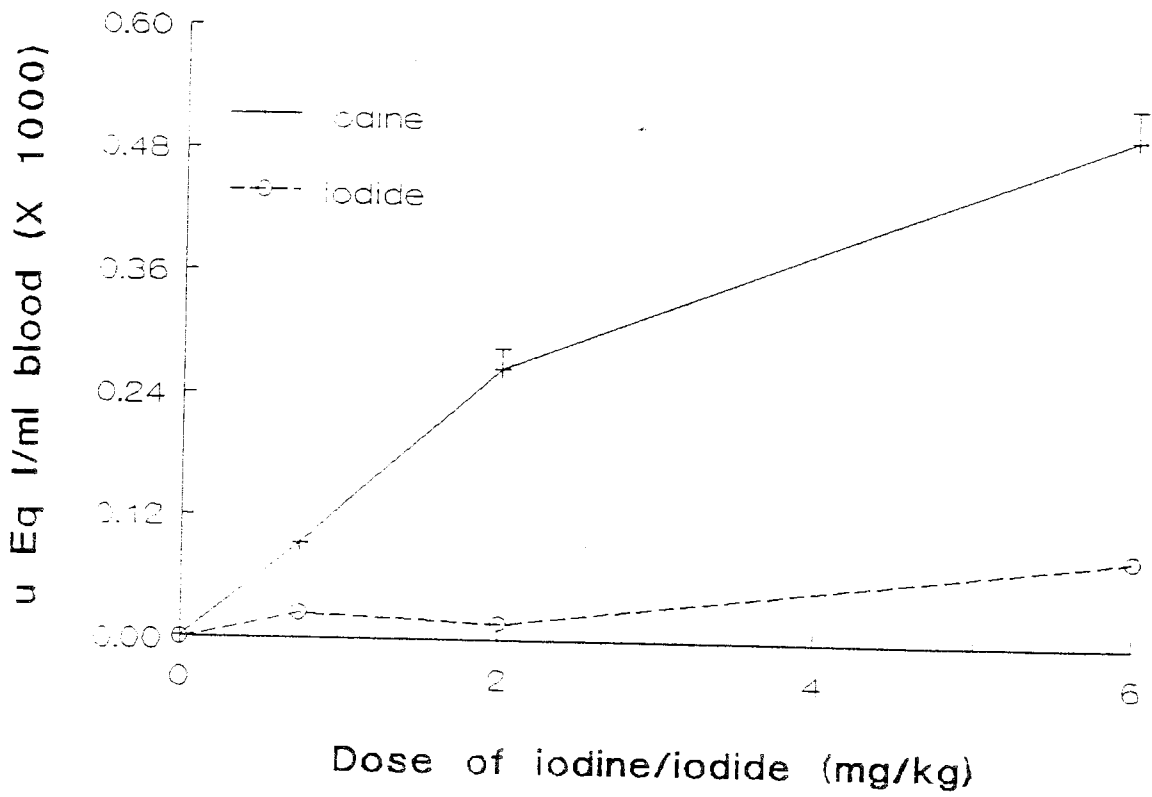


Table 2
 Association of radioactivity in blood components in animals
 treated with 2 mg/Kg $^{125}\text{I}_2$ or $^{125}\text{I}^-$

Compartment	Treatment (2 mg/Kg)	
	$^{125}\text{I}_2$	$^{125}\text{I}^-$
Whole blood	9.27 ± 0.3^a	1.68 ± 0.07
Blood cells	0.16 ± 0.004 (1.8%) ^b	0.04 ± 0.003 (2.5%)
Lipids	3.40 ± 0.3 (36.7%)	0.75 ± 0.04 (44%)
Cholesteryl I	1.84 ± 0.5 (20%)	
Proteins	1.20 ± 0.03 (12.9%)	0.22 ± 0.02 (13%)
Albumin	0.89 ± 0.03 (9.6%)	0.17 ± 0.01 (9.9%)
Globulin	0.27 ± 0.02 (2.9%)	0.02 ± 0.002 (1%)
Serum Water ^c	3.86 ± 0.2 (41.6)	0.62 ± 0.03 (37%)
Total Recovery	8.62 ± 0.4 (93%)	1.64 ± 0.07 (97.6%)

^a Data is expressed as $\mu\text{Eq I/ml}$ blood (X 1000) based on the specific activity of administered iodine. Each value represents the mean of 6 animals \pm S.E.M.

^b Numbers in parentheses indicate the percentage of radioactivity in whole blood accounted for in each compartment.

^c Serum water consists of deproteinated serum and water collected from the washing of the cellular component.

CHAPTER IV

**Evidence of Thyroxine (T₄) Formation Following Iodine (I₂)
Administration in Sprague-Dawley Rats**Abstract

Iodine (I₂) has been proposed as a disinfectant for recycled water on the manned space station. Previous work by Sherer et al. (1991), has shown that subchronic administration of I₂ to Sprague-Dawley rats in drinking water significantly increases plasma thyroid hormone thyroxine/triiodothyronine (T₄/T₃) levels. This is not observed with I⁻ treatment at equivalent doses. The present study addresses two hypotheses that could account for the increase in T₄/T₃ ratios: (1) an inhibition of 5'-deiodinase, (2) production of T₄ by reaction of I₂ with metabolites of thyroid hormones in the gastrointestinal tract. Incubation of diiodothyronine (T₂), T₃ or reverse T₃ (rT₃) with I₂ in phosphate buffered saline (PBS) resulted formation of T₄ as measured by radioimmunoassay (RIA). Washes from a small segment of the small intestine show that substrates are present in the small intestine that react with I₂ to produce T₄. Administration of single doses of I₂ to rats by gavage produces significant dose-related increases in serum T₄ and decreases in T₃ concentrations 2

hours post dosing. This effect reaches a maximum at a dose of 2 mg/Kg I_2 . Administration of an equivalent dose of I^- produces a small effect in the opposite direction; a decrease in T_4 and an increase in T_3 concentrations. Higher concentrations of a radioactive substance that bound an antibody specific for T_4 is found in the plasma of animals treated with $^{125}I_2$ compared to those treated with $^{125}I^-$. Therefore, the increase in T_4 concentrations in rats treated with I_2 results from direct reaction of I_2 with T_4 metabolites in the gastrointestinal tract to resynthesize T_4 and elevate its levels in blood.

Introduction

On short-term space missions, NASA has used a nominal iodine (I_2) residual of about 2 mg/L to disinfect water for drinking purposes (Janik and Thorstenson, 1986). With NASA contemplating more extended space missions, a water disinfectant will be used for longer periods. However, the safety of long-term consumption of elevated iodine (I_2) has received little study. Most studies of elevated iodine intake have focussed on iodide (I^-), iodate (IO_3^-) and other dietary forms of iodine (Mahmoud, et al., 1986; Savoie, et al., 1975), ignoring the fact that I_2 is the chemical species used in water disinfection.

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

There are at least two hypotheses that could explain the increase in T_4/T_3 ratios following I_2 treatment: (1) I_2 , or a reaction product of I_2 , is capable of inhibiting the 5'-deiodinase enzyme responsible for the conversion of T_4 to T_3 ; or (2) I_2 reacts with T_4 metabolites in the gastrointestinal tract to resynthesize T_4 and elevate its levels in blood. The work presented here was designed to test these two hypotheses.

Methods and Materials

Chemicals. ^{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, MA). Neutral pH was achieved by the addition of HCl. Oxidation of $^{125}I^-$ to $^{125}I_2$ was achieved by reacting $Na^{125}I$ with H_2O_2 and HCl according to the method described by McAlpine (1945). The purity of $^{125}I_2$ (specific activity 17.4 Ci/mg), was found to be 100% as measured using an isocratic HPLC system capable of

simultaneous UV and radioisotope detection (C₁₈, 4.6 X 250-mm column, 70:30:0.1% MeOH:H₂O:H₃PO₄, Beckman Instruments, Inc., San Ramon, CA). L-[¹²⁵I]-Thyroxine (¹²⁵I-T₄) in a solution containing 1-propanol:water, 1:1, (specific activity 4400 Ci/mmol, concentration 329 uCi/ml), was purchased from Dupont-NEN. The radiochemical purity of ¹²⁵I-T₄ was found to be greater than 93% as determined by HPLC.

Animals. Male, Sprague-Dawley rats were purchased from Laboratory Animal Resource Center (Washington State University, Pullman WA), unless otherwise specified. Occasionally it was necessary to purchase animals from Simonsen Laboratories, Inc., (Gilroy, CA) because they were unavailable at the Washington State University Laboratory Animal Resource Center.

Comparison of the short-term administration of iodine and iodide. Animals weighed approximately 80-100 g prior to treatment, and were provided Purina Rodent Chow *ad libitum*. Thirty rats were randomly assigned to 5 treatment groups (6 animals/dose). Rats were maintained on distilled water containing I₂ at dose levels of 0, 3, 10, 30 and 100 mg/L of drinking water for 14 days. Blood (500 ul) was collected from the tail vein on days 0, 7 and 14 of treatment. Total

plasma T_3 and T_4 levels were determined using radioimmunoassay (RIA) Amerlex kits obtained from Amersham Corp. (Arlington Heights, IL). The human standards supplied with these kits were used to construct the standard curve as previous studies conducted in our laboratory have detected no differences between these standards and rat standards prepared in our laboratory.

Immediate effects of iodine and iodide treatment on thyroxine and triiodothyronine levels. Animals weighed approximately 100-120 g and had food removed from their cage 18 hours prior to use. 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 oral gavage. The total doses of iodine were 0, 0.75, 2.0 and 6.0 mg/kg body weight. Blood (500 ul) was collected from the tail vein immediately prior to treatment and at 2 hours following treatment.

Demonstration that iodination of thyroxine metabolites is chemically feasible. T_4 (12 ng/ml) and T_4 precursors (T_3 (2.6 ng/ml), rT_3 (1 ng/ml), T_2 (1 ng/ml) and MIT (5.5 ng/ml)) were added to phosphate buffered saline (PBS) (50ul) was added to I_2 or I^- (200 ng/ml) and allowed to react for 60 minutes. T_4 levels were then measured in the solution. A

standard curve was constructed using known concentrations of T_4 dissolved in 0.8% saline.

Detection of substrates for thyroxine precursors in the small intestine. Animals weighed approximately 180-220 g and had food removed from the cage 18 hours prior to use. Animals were sacrificed by a lethal IP injection of Ketaset (Ketamine HCl, Fort Dodge, IA) followed by destruction 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 a test tube. The intestinal wash (50 ul) was added to I_2 or I^- (2, 20 or 200 ng/ml) and allowed to react for 0, 5, 10, 15, 30, 60, and 120 minutes. T_4 levels were then measured in the wash. A standard curve was constructed using known concentrations of T_4 dissolved in 0.8% saline. To demonstrate the conditions were proper for reaction with T_3 , rT_3 and T_2 , these metabolites were added to 50 ul of isolated wash with H_2O , I_2 (200 ng/ml) or I^- (200 ng/ml) and allowed to react for 60 min and T_4 production assayed.

Demonstration that thyroxine increase in vivo results from direct iodination reactions. Rats were purchased from Simonsen Laboratories, Inc., and weighed approximately 120-150 g prior to use. Animals had food removed from their

cage 18 hours prior to administration of equivalent doses of I_2 or I^- containing tracer amounts (50 uCi) of ^{125}I by gavage. The total dose of iodine was 2.0 mg/kg body weight. Two hours following administration of the radiolabeled iodine, blood (5 ml) was drawn from the caudal vena cava and plasma separated. Radioactive plasma (50 ul) was incubated with T_3 - or T_4 -specific antibody available in the Amerlex RIA kit for 1 hour at $37^{\circ}C$. Radioactivity associated with the antibody was measured in an LKB gamma counter. To test for non-specific binding, increasing amounts of non-radioactive T_4 or T_3 was 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.

Effect of iodine treatment on deiodinase activity. Animals weighed approximately 150-180 g and were provided Purina Rodent Chow *ad libitum*. Twelve rats were randomly assigned to 2 treatment groups (6 animals/dose). Rats were maintained on either distilled water or distilled water containing I_2 at a dose level of 100 mg/l of drinking water for 7 days. Blood (500 ul) was collected from the tail vein on day 7 of treatment and total plasma T_3 and T_4 levels were determined using an RIA kit as described previously. Immediately following the collection of blood, animals

received a single IV dose of $^{125}\text{I}-\text{T}_4$ at a concentration of 6 uCi (329 uCi/ml). Blood was collected via the caudal vena cava at 0, 60 and 120 min following dosing and plasma separated. Radioactive plasma (50 ul) was incubated with T_3 or T_4 specific antibody available in the Amerlex RIA kit for 1 hour at 37°C . Radioactivity associated with the antibody was measured in an LKB gamma counter.

Results

Table 1 shows the effect on plasma T_4/T_3 levels in rats following administration of I_2 in drinking water for 7 days. Iodine treatment results in a significant increase in the plasma T_4/T_3 ratio at the 100 mg/L dose level. This change results from a significant decrease in T_3 levels as well as an increase in T_4 levels at both 7 and 14 days post treatment, relative to concurrent controls. These effects are more pronounced at day 7 of treatment than at day 14. During the course of the experiment an increase in T_4 levels and decrease in T_3 levels occurs in the control animals. This is because young rats are utilized (38 days of age at the beginning of the experiment) and this pattern of change is consistent with previously reported developmental patterns of plasma thyroid hormone levels in young rats (Dohler et al., 1979).

The feasibility of iodination of T_4 metabolites is explored in an *in vitro* solution of phosphate buffered saline (PBS) (Table 2). On a molar basis, the addition of I_2 to T_3 , rT_3 or T_2 results in a 73, 69 and 51% conversion of the metabolite to T_4 , respectively. The addition of I^- to T_4 metabolites does not produce T_4 . These data show that the iodination of T_3 , rT_3 or T_2 with I_2 is chemically possible at a respectable yield. MIT does not produce T_4 in this system, ruling out interference of monocyclic compounds with the T_4 determinations.

T_4 production in isolated intestinal washes following the addition of various concentrations of I_2 or I^- is presented in Figure 1a. The rate of T_4 production increases with I_2 concentration. Maximal levels result from a concentration of 20 ng/ml I_2 . Higher levels of I_2 produce the maximum T_4 yield at an earlier time. These data show that I_2 reacts to produce a total of approximately 10 ng of T_4 from precursors available in this short section of the small intestine. Equivalent concentrations of I^- did not increase T_4 levels.

Figure 1b shows that the total amount of T_3 decreases in isolated intestinal wash following the addition of I_2 or I^- at various concentrations. The rate and extent of T_3 loss was dependent on increasing concentrations of I_2 . However, the decrease in T_3 is too small to account for the

increase in T_4 (0.6 ng loss of T_3 and a 11 ng increase in T_4).

Addition of I_2 to T_4 , T_3 , rT_3 or T_2 to the intestinal wash shows that conditions are appropriate for the iodination of these T_4 metabolites to form T_4 (Table 3). The net production of T_4 is calculated as the difference between solutions containing T_4 or metabolites with I_2 (column 3), and control I_2 solutions (17.6 ng). The addition of 12 ng T_4 to I_2 produces a 16.6 ng increase in T_4 levels, indicating complete recovery of added T_4 . With the addition of I_2 to T_3 , rT_3 or T_2 , an increase in T_4 results, ranging from 52-67%, on a molar basis. The addition of I^- does not change T_4 levels. These data indicate that T_4 metabolites are present in the gastrointestinal tract and that I_2 will react with them to resynthesize T_4 .

$HOCl$ is added to the isolated intestinal wash in an attempt to block vacant sites on thyronines (Table 4). Treatment of intestinal washes with 100 ng $HOCl/ml$ completely blocks the formation of T_4 . This inhibition of iodination occurred with an apparent K_i of 16.8 ng $HOCl/ml$. Added T_4 along with 100 ng $HOCl/ml$ was accounted for completely in a parallel assay, demonstrating that the T_4 antibody is not altered by high levels of chlorine.

Single doses of non-radioactive iodine administered by gavage produce a dose-related increase in plasma T_4 levels

(Figure 2a) to a maxima at 2 mg/Kg. Plasma T_3 levels (Figure 2b) reach a minima at the same dose level. No further increase in T_4 nor decrease in T_3 is observed at higher dose levels. In contrast, a slight decrease (maximum of 13%) in plasma T_4 levels and an increase in plasma T_3 levels (maximum of 16%) is observed in animals receiving equivalent doses of I^- .

The amount of radioactivity in plasma that binds to antibodies specific for T_4 and T_3 following 2 mg/Kg oral doses of $^{125}I_2$ or $^{125}I^-$ is shown in Table 5. Twice as much radioactivity associates with both the T_4 and the T_3 antibodies in animals treated with $^{125}I_2$ compared to animals treated with $^{125}I^-$. In addition, three times the radioactivity derived from $^{125}I_2$ -treated animals is displaceable from either the T_4 or the T_3 antibody by excess non-radioactive T_4 or T_3 , respectively. This provides strong evidence that I_2 is acting by direct reaction with T_4 precursors. Incubating the antibody complex with increasing concentrations of cold T_4 to displace T_4 indicated that an average of 25 ng/ml of T_4 was in the blood of the rat, if it is assumed that only one molecule of ^{125}I bound to each precursor molecule. This assumption may be incorrect since more than one ^{125}I may be bound to some T_4 precursors (ie. T_2), and thus this measure probably overestimates the amount of T_4 formed.

In an experiment designed to test the hypothesis that inhibition of the deiodinase enzyme is responsible for increasing the T_4/T_3 ratio, animals are treated with 0 or 100 mg/L I_2 for 7 days in their drinking water prior to a single IV injection of ^{125}I - T_4 . The elimination of radioactive T_4 from plasma with time is shown in Figure 3. The half-life of elimination of radioactivity from the plasma is 79.8 min for treated animals and 86.6 min for concurrent controls. These differences in half-lives of elimination were not significant by a two sample t-test ($P = 0.3$).

Discussion

Our results confirm earlier results that I_2 in drinking water increases plasma T_4/T_3 ratios (Sherer et al., 1990). Moreover, data obtained with single doses of I_2 makes it clear that this effect occurs in a matter of hours.

The experimental results of this study strongly support the hypothesis that iodine reacts with deiodinated 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 specifically iodinates T_3 , T_2 and rT_3 *in vitro* in PBS. (2) Precursors that react with I_2 to form T_4 are present in the small intestine of the rat as demonstrated by *in vitro* production of T_4 in washes

collected from the intestine. (3) A single oral dose of I_2 significantly increases plasma T_4 levels *in vivo*. (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.

The hypothesis that I_2 , or a by-product of I_2 , inhibits the deiodinase enzyme is ruled out by the fact that the half-life of elimination of radioactive T_4 does not differ significantly between animals treated for one week with I_2 versus controls. If the enzyme is inhibited, we would expect a significantly longer half-life of T_4 in I_2 treated animals to occur.

Reactions with precursors present in the gastrointestinal tract appear sufficiently large to account for most, if not all, of the increase in plasma T_4 levels. In a separate experiment we determined that a 10 ng oral dose of T_4 produced a 7 ng/ml (± 1.4) increase in plasma T_4 (Data not shown). *In vivo*, a single oral dose of I_2 produced a 11 ng/ml increase in plasma T_4 levels. Based on the previous experiment, we calculate that 16 ng of T_4 were likely formed in the gastrointestinal tract. *In vitro* experiments using intestinal wash indicates that enough T_4 precursors are available to produce 11 ng of T_4 . Therefore,

73% of the increase in plasma T_4 levels following I_2 administration can be accounted for by the iodination of T_4 metabolites in the small segment of the intestine that was utilized for these experiments.

Although the rat is a useful model for thyroid function in man, some species differences do exist. Namely, in man T_4 is carried primarily by thyroxine-binding globulin (TBG) (Berson and Yalow, 1954), rather than by albumin like the rat. The binding affinity of TBG is approximately 1000 times higher than the binding affinity of albumin (Rall, 1976). Since hormone binding is generally accompanied by slower metabolic degradation of the hormone, the turnover of T_4 in the rat will likely occur at a faster rate than in man. The biological half-life of T_4 in human plasma (5-9 days) (Sterling and Lazarus, 1977) is longer than in rat plasma (12-24 hr) (Harland and Orr, 1969; Larsen and Frumess, 1977). A decreased turnover of T_4 in the human would suggest that an iodine induced increase in T_4 levels will require repeated doses of I_2 . Therefore, less iodination of T_4 metabolites in the gastrointestinal tract may be expected in man than demonstrated in rats.

Astronauts returning from Apollo and Skylab space missions were found to have elevated T_4 levels (Johnston et al., 1975; Nicogossian, 1977; Leach and Rambaut, 1977). It has been previously assumed that this is a result of the

physiological adaptation to a weightlessness environment. However, poor control over iodine concentrations in drinking water occurred on some space flights, with levels reaching an estimated 10-15 mg/L (Compton and Benson, 1983). The data presented here suggests that the elevated T₄ levels in these astronauts may have resulted from direct iodination of T₄ metabolites. If this is the case, the lower turnover rate of T₄ is influencing the result seen in humans.

Table 1
Effect of I₂ treatment on serum thyroid hormone levels

Treatment	T ₄	T ₃	T ₄ /T ₃
<u>Day 0</u>			
Control	17.4 ± 1.7 ^a	0.98 ± 0.14 ^a	20.0 ± 4.0
3 mg/L	18.8 ± 3.2	1.05 ± 0.13	21.5 ± 4.8
10	18.3 ± 1.3	0.85 ± 0.09	17.5 ± 2.0
30	17.1 ± 1.5	1.06 ± 0.10	23.0 ± 3.0
100	17.7 ± 1.7	0.78 ± 0.06	17.2 ± 2.5
<u>Day 7</u>			
Control	18.1 ± 1.4	0.46 ± 0.05	40.4 ± 3.3
3 mg/L	15.9 ± 1.8	0.50 ± 0.03	31.7 ± 3.2
10	20.3 ± 1.7	0.46 ± 0.04	40.0 ± 7.4
30	17.7 ± 3.5	0.35 ± 0.05	70.4 ± 31.8
100	30.4 ± 3.3 [*]	0.28 ± 0.03 [*]	116.4 ± 17.4 ^{**}
<u>Day 14</u>			
Control	26.1 ± 0.9	0.69 ± 0.06	40.3 ± 4.6
3 mg/L	24.3 ± 2.7	0.64 ± 0.02	41.0 ± 8.0
10	20.2 ± 3.6	0.69 ± 0.06	29.6 ± 2.9
30	30.1 ± 4.4	0.57 ± 0.08	53.7 ± 8.4
100	30.3 ± 1.9	0.48 ± 0.07 [*]	71.0 ± 12.4 [*]

^a Values reported as ng/ml ± S.E.M. of n = 6 animals in duplicate

^{*} Indicates that the value differs significantly from the control at P < 0.05 or ^{**} P < 0.005 by two sample t-test.

Table 3

T₄ levels in solution following treatment with I⁻ or I₂ and metabolite supplementation

Addition	Treatment		
	None	I ₂	I ⁻
T ₄ (12 ng/ml)	13.14 ± 0.90	12.38 ± 1.10	11.14 ± 0.84
T ₃ (2.6 ng/ml)	----	1.49 ± 0.40	0
rT ₃ (1 ng/ml)	----	0.68 ± 0.15	0.09 ± 0.03
T ₂ (1 ng/ml)	----	0.70 ± 0.10	0
MIT (5.5 ng/ml)	----	0	0

Values expressed as average total concentration in phosphate buffered saline (PBS) solution in ng/ml ± S.E.M. of n = 4 samples in duplicate.

Figure 1a: *In vitro* production of T_4 by iodine in isolated intestinal wash with time. Animals were sacrificed and the upper intestine was dissected out and flushed with 0.8% saline. Wash (50 ul) 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 methods section.

Figure 1b: Decrease in T_3 levels in isolated intestinal wash with time following treatment with iodine. The procedure is described in the previous figure.

Table 3
 Production of T₄ from T₄ metabolites in intestinal wash^a
 following treatment with iodine

Addition	Treatment			Increase above control with I ₂ ^b
	None	I ⁻	I ₂	
Control	8.6 ± 2.2 ^c	9.4 ± 3.1	17.6 ± 2.0	---
T ₄ (12 ng)	19.8 ± 5.2	25.7 ± 3.3	34.2 ± 5.6*	16.6
T ₃ (10 ng)	8.9 ± 2.1	10.8 ± 3.2	25.3 ± 4.0*	7.7
rT ₃ (10 ng)	9.7 ± 2.5	9.8 ± 3.1	29.0 ± 3.2*	11.4
T ₂ (10 ng)	9.3 ± 2.0	8.3 ± 3.2	24.8 ± 3.2*	7.2

^a 50 ul of wash collected from the duodenum was added to the indicated substrate along with H₂O, I₂ or I⁻ and allowed to react for 60 min prior to determination of T₄ levels by a commercially available RIA kit.

^b Difference between solutions containing I₂ (column 3) and control with I₂ (17.6 ng).

^c Values expressed as average amount of T₄ in isolated intestinal wash (*in vitro* system) in ng ± S.E.M. of n = 6 animals in duplicate.

* ng of T₄ in intestinal wash treated with I₂ differs significantly from control, P < 0.05 by paired t-test.

Table 5

Production of T₄ in intestinal wash following treatment with iodine and HOCl

Addition	Treatment		
	None	I ₂	I ⁻
None	8.6 ± 2.2 ^a	17.6 ± 2.0*	9.4 ± 3.1
HOCl (2 ng/ml)	7.9 ± 3.0	14.8 ± 2.3*	8.2 ± 1.9
HOCl (10 ng/ml)	8.6 ± 2.1	14.0 ± 2.4*	9.0 ± 2.2
HOCl (100 ng/ml)	6.3 ± 3.3	9.4 ± 2.2	8.0 ± 2.2
HOCl + T ₄ ^b	18.6 ± 4.3	17.9 ± 5.2	18.8 ± 3.1

^a Values expressed as average amount of T₄ in isolated intestinal wash (*in vitro* system) in ng ± S.E.M. of n = 6 animals in duplicate.

^b 100 ng HOCl/ml and 10 ng T₄ added together.

* ng of T₄ in intestinal wash treated with I₂ differs significantly from control, P < 0.05 by two sample t-test.

Figure 2a: Change in T_4 levels following I_2 or I^- treatment. Animals were treated with various doses of $^{125}I_2$ or $^{125}I^-$ as a single oral dose. Blood (500 ul) was collected from the tail vein immediately prior to treatment and at 2 hours following treatment. Total plasma T_4 levels were determined using RIA kits as described in the methods section.

Figure 2b: Change in T_3 levels following I_2 or I^- treatment. Animals were treated as described in the previous figure.

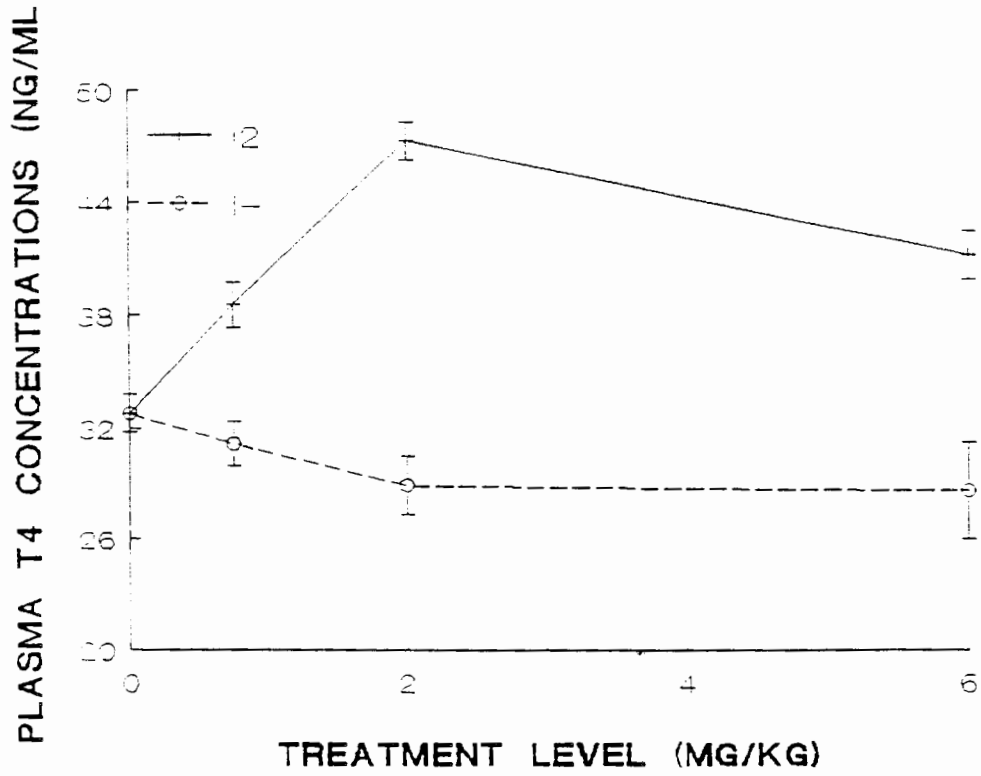


Figure 2a.

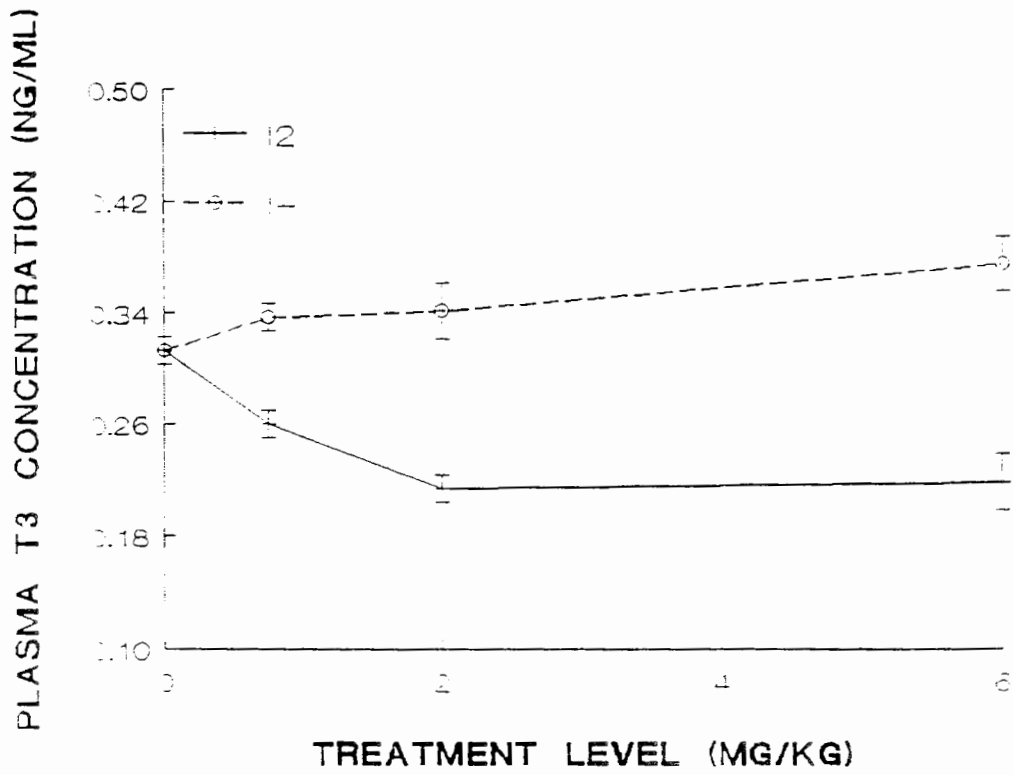


Figure 2b.

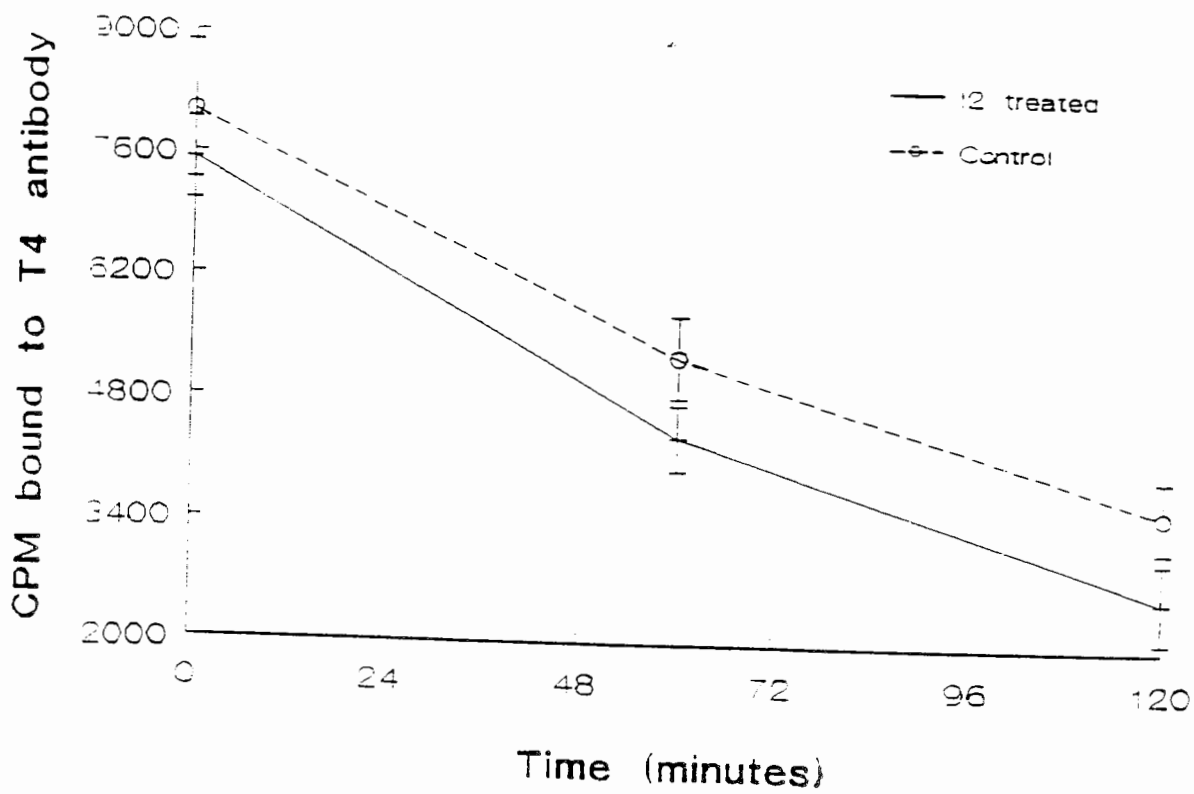
Table 5

Detection of ^{125}I in plasma T_4 and T_3 antibodies following treatment of rats with 2 mg/kg $^{125}\text{I}_2$ or $^{125}\text{I}^-$

	Associated with antibody	CPM	Displaced from antibody
		T_4	
$^{125}\text{I}_2$	1563 ± 42^a		1209 ± 31
$^{125}\text{I}^-$	703 ± 51		397 ± 47
		T_3	
$^{125}\text{I}_2$	783 ± 39		491 ± 21
$^{125}\text{I}^-$	433 ± 17		153 ± 5

^a Average and S.E.M. of 4 animals in duplicate.

Figure 3: Decrease in radioactivity binding to a T₄ antibody in plasma of treated and control animals with time. Animals received either 6 mg I₂/L of drinking water or distilled water for 7 days. At the end of 7 days blood was collected (500 ul) and T₄ levels determined by RIA as described in the methods section. Animals then received a single IV dose of ¹²⁵I-T₄. Blood, (500 ul) was collected immediately following injection, and at 1 and 2 hours following dosing. Radioactivity in plasma bound to a T₄-specific antibody was determined. Each experimental point represents the mean of 6 animals ± S.E.M.



Chapter V

Summary and Conclusions

It is clear from the present studies that iodide (I^-) and iodine (I_2) cannot be treated as if they are physiologically or toxicologically equivalent. Not only is the distribution of radioiodine in the Sprague-Dawley rat different depending on chemical form administered, but iodinated by-products are formed in the gastrointestinal tract following oral administration of I_2 . Thyroxine (T_4), the precursor to the active thyroid hormone, triiodothyronine (T_3), is among these by-products.

Iodine behaves differently than I^- pharmacokinetically. Experiments in fasted animals involving acute exposure to radiolabeled I^- indicate rapid distribution of radioactivity into the thyroid gland. In contrast, thyroid uptake of radioactivity from fasted animals treated orally with $^{125}I_2$ is depressed. Repeated exposure of I_2 in drinking water is less effective in suppressing thyroid uptake of a challenge dose of $^{125}I^-$ than daily I^- exposure. These findings indicate that I_2 is not quantitatively converted to I^- following ingestion, as suggested in classical texts (Haynes and Murad, 1985).

There is evidence of by-product formation in the gastrointestinal tract with I_2 treatment. In fed animals treated orally with $^{125}I_2$ a large portion of the radioactivity is retained in the stomach contents relative to animals treated with $^{125}I^-$, indicating that reactions are occurring with I_2 which hinder the uptake of radioiodine into the blood. More specifically, I_2 was shown to produce a by-product which co-migrated with cholesteryl iodide on thin layer chromatography.

The reaction of I_2 with lipids is not linear with dose but reaches a maximum at a dose of 2 mg/Kg. This suggests that reactions with lipids occur in a local site, perhaps the gastrointestinal tract, since it is otherwise difficult to believe that lipids could be limiting in this reaction.

Experiments conducted both *in vitro* and *in vivo* indicate that I_2 treatment results in the iodination of T_4 metabolites in the gastrointestinal tract to resynthesize T_4 . Evidence for this is as follows: (1) The iodination of T_3 , rT_3 , and T_2 with I_2 could be demonstrated in a cell-free system. (2) T_4 was also produced in wash collected from the rat duodenum in solutions containing I_2 . (3) A single oral dose of I_2 produced an approximate 34% increase in plasma T_4 levels 2 hours after dosing, providing evidence that the iodination of T_4 metabolites can occur *in vivo* as well. (4) Binding of radioactivity in the plasma to an antibody specific for T_4 following oral administration of $^{125}I_2$

demonstrated that the increase in T_4 arises directly from reactions with I_2 . (5) All of the above effects were either much smaller or absent when equivalent levels of I^- were utilized.

In subchronic studies, (Sherer et al., 1991) significant increases in thyroid size were seen in male rats treated with I^- . This was not observed in animals receiving I_2 , where the trend was toward decreased thyroid size. Classically, decreased levels of T_4 and T_3 are typical of excessive iodide intakes in humans and can give rise to a thyroid goiter (Ingbar, 1985). Increase in thyroid size is in response to inadequate release of hormone which leads to hypersecretion of TSH (Haynes and Murad, 1985). In contrast, excessive levels of thyroid hormones produce the opposite effect, or inhibition of TSH secretion, resulting in a diminished thyroid size. Thus, the differential effects of I_2 and I^- on thyroid size in the subchronic study (Sherer et al., 1991) is consistent with the present data, where I_2 treatment produced an increased T_4/T_3 ratio and I^- treatment produced a slight decrease.

It is possible for I_2 to react with material other than those identified. Following oral administration of radioactive I_2 , approximately 14% of the radioactivity in whole blood is identified as free I^- by HPLC analysis. Twenty percent of the radioactivity in whole blood associates with lipids primarily in the form of a product

co-migrating with cholesteryl iodide. Of the remaining 66%, a large portion (an estimated 48% of the radioactivity in whole blood) is incorporated into T_4 metabolites to produce T_4 as determined by radioactivity in the plasma binding to the T_4 antibody following oral administration of $^{125}I_2$. This would indicate that if I_2 reacts with other materials, these reaction products are in low concentrations.

Although the rat has been used as a model for thyroid function in man, T_4 is metabolized at a faster rate in the rat than in man (Sterling and Lazarus, 1977). A slower rate of thyroid hormone turn-over in man, indicates that fewer T_4 metabolites will circulate enterohepatically and thus less substrate may be available for iodination with I_2 administration. Based on this, the effect of increased T_4/T_3 ratios following I_2 administration may occur in man, but the sensitivity of plasma T_4 concentrations may be significantly less than in the rat.

These data have some bearing on the use of I_2 as a drinking water disinfectant. On previous space missions, NASA has used an iodine residual of 2 mg/L to disinfect water for consumption. In our animal studies, the steady state levels of thyroid hormones are not increased until animals are exposed to repeated doses of I_2 at a concentration of approximately 10 mg/Kg/day. 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 2 mg/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.

It is interesting to note that Skylab and Apollo astronauts were found to have increased T_4 levels upon return, relative to preflight levels (Johnston et al., 1975; Nicogossian, 1977; Leach and Rambaut, 1977). This increase (approximately 12%) has been previously attributed to a physiological adaptation to a weightless environment. The data presented here suggest that this may have actually resulted from poor control over iodine concentrations in drinking water, which reached an estimated 10-15 mg/L on some space flights (Compton and Benson, 1983). However, elevated T_4 appeared to occur at a lower mg/kg dose in man than in the rat, suggesting that the slower rate of turnover in man may be responsible for the build up of T_4 .

Skylab and Apollo astronauts were also reported to have elevated TSH levels postflight (Leach and Rambaut, 1977). Although T_4 rapidly returned to preflight levels, TSH remained elevated in astronauts at 14 days postflight. This could indicate a more severe alteration in the thyroid pituitary axis that is not easily explainable by available data. Consequently, the interaction between I_2 intake and microgravity conditions requires further investigation.

The major criticism of iodinated drinking water is the potential for development of iodide-induced goiter in sensitive individuals. The data in the present study, as well as studies by Sherer et al. (1991), indicate that I_2 actually produces the reverse effect expected of I^- . Therefore, prior arguments that I_2 may not be a safe drinking water disinfectant appear to be unfounded. If this is true, there is a clear need to be certain that I_2 is not significantly reduced to I^- in finished water. The elevated TSH observed in Skylab and Apollo astronauts should remain as a warning that other neuroendocrine effects could arise, perhaps in the presence of microgravity.

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