

# Stereospecificity of Triiodothyronine Transport into Brain, Liver, and Salivary Gland: Role of Carrier- and Plasma Protein-Mediated Transport\*

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**ABSTRACT.** The stereospecificity of  $T_3$  transport through the walls of the brain capillary, *i.e.* the blood-brain barrier (BBB), and the salivary gland capillary and through the hepatocyte plasma membrane was studied with a tissue-sampling single injection technique in rats. In the absence of plasma proteins, the  $ED_{50}$  of inhibition of the saturable transport of [ $^{125}$ I]L- $T_3$  through the BBB was 1  $\mu$ M for unlabeled L- $T_3$  and 9  $\mu$ M for unlabeled D- $T_3$ . The brain extraction of [ $^{125}$ I]D- $T_3$ ,  $5.9 \pm 0.1\%$  ( $\pm$ SE), was about one third that of [ $^{125}$ I]L- $T_3$ . Conversely, no saturable and no stereospecific  $T_3$  transport was observed for the salivary gland capillary, which, unlike the brain capillary, is porous. The hepatic extraction of  $T_3$  was minimally stereospecific in the absence of plasma proteins. In the presence of 5 g/dl bovine albumin, the fraction of circulating D- or L- $T_3$  that was available for transport into liver (50–100%) was many-fold greater than the fraction that was free *in vitro* ( $\sim 2\%$ ); therefore, both D- $T_3$  and L- $T_3$  were available for uptake by liver from the circulating albumin-bound pool. This plasma protein-mediated transport of  $T_3$  is believed to represent a process of enhanced dissociation of  $T_3$  from the albumin-binding site, since the plasma protein *per se* is not significantly taken up by liver on a

single pass. However, in the presence of 5 g/dl bovine albumin, the extravascular hepatic extraction of [ $^{125}$ I]D- $T_3$  ( $50 \pm 2\%$ ) was nearly half that for [ $^{125}$ I]L- $T_3$  ( $93 \pm 12\%$ ), although no significant difference in the *in vitro* binding of [ $^{125}$ I]D- $T_3$  and [ $^{125}$ I]L- $T_3$  to 5 g/dl bovine albumin was found with equilibrium dialysis. In addition, the isoelectric point of bovine albumin bound to [ $^{125}$ I]L- $T_3$  (5.1) was higher than that of bovine albumin bound to [ $^{125}$ I]D- $T_3$  (5.0), as determined by isoelectric focusing, which indicates that the surface of the bovine albumin molecule is slightly more positive when the protein binds L- $T_3$  as opposed to D- $T_3$ . The isoelectric focusing and *in vivo* transport data together suggest that the interaction between the surfaces of the plasma protein and the hepatic microcirculation that is presumed to cause enhanced hormone dissociation from the protein-binding site is electrostatic in nature. These studies (1) show that the BBB thyroid hormone transport system is sharply stereospecific, and this property is probably a major factor underlying the low biological potency of D- $T_3$  in brain; and (2) provide the first evidence for stereospecificity of plasma protein-mediated transport of hormones into tissues *in vivo*. (*Endocrinology* 121: 1185–1191, 1987)

THE biological potency of D- $T_3$  is 3–10 times less than that of L- $T_3$  (1–4). However, the affinity of D- $T_3$  for the nuclear receptor that mediates  $T_3$  action (5, 6) is equal to or slightly less than that of L- $T_3$  (7–9). Since  $T_3$  nuclear receptor occupancy is also a function of the relative rates of cellular uptake of circulating isomers of  $T_3$ , stereospecific transport mechanisms for  $T_3$  may provide the major locus of hormone stereospecificity. Since  $T_3$  is avidly bound by plasma proteins (10), there are two major sites where stereospecific transport mechanisms may occur. The first site is the plasma membrane. Previous studies have shown that  $T_3$  enters the brain via a carrier-mediated transport mechanism

localized within the brain capillary wall, *i.e.* the blood-brain barrier (BBB) (11). The operation of a similar transport system in liver seems less likely, since previous studies have shown that  $T_3$  transport into liver cells either *in vivo* (12) or *in vitro* (13) is consistent with free diffusion of this relatively lipid-soluble hormone. A second potential site of transport stereospecificity is the  $T_3$ -binding site on plasma proteins, such as albumin. Previous studies have shown that  $T_3$  is readily available for transport into liver from the circulating albumin-bound pool (12). Since the albumin molecule *per se* is not significantly taken up by liver on a single circulatory passage (14), the transport of  $T_3$  into liver from the albumin-bound pool is believed to arise from a mechanism of enhanced dissociation of  $T_3$  from the albumin-binding site (15–17). That is, owing to transient interactions between amino acid residues on the surface of the albumin molecule and components of the microcirculation surface, conformational changes in the binding

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site ensue, which result in markedly increased rates of hormone dissociation from the plasma protein *in vivo* compared to what occurs *in vitro*. The overall surface charge of the albumin molecule, which is a function of its conformational state, may be a stereospecific function of the ligand bound to albumin. Within the context of this model, plasma protein-mediated T<sub>3</sub> transport into tissues such as liver may show stereospecificity. Therefore, the purpose of the present study was to examine the stereospecificity of carrier-mediated T<sub>3</sub> transport into brain, liver, and salivary gland and the potential stereospecificity of albumin-mediated transport of T<sub>3</sub> into liver *in vivo*. Salivary gland was examined as a control peripheral tissue, since the capillaries in this organ (18), in contrast to those in the brain (19), are porous and allow for free diffusion of T<sub>3</sub> into the tissue.

## Materials and Methods

### Materials

Unlabeled L-T<sub>3</sub>, L-T<sub>4</sub>, D-T<sub>3</sub>, Sephadex G-25 (fine), *N,N,N'*, *N'*-tetramethylethylenediamine, and glycerol were purchased from Sigma Chemical Co. (St. Louis, MO). Acrylamide, *N,N'*-methylene-bis-acrylamide, and ammonium persulfate were purchased from Bio-Rad Laboratories (Richmond, CA). Pharmalyte (pH 3–10 and pH 4–6.5) was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Ultrofilm <sup>3</sup>H was purchased from LKB (Gaithersburg, MD). Kodak GBX developer and fixer were purchased from Eastman Kodak Co. (Rochester, NY). BSA (Pentex, fraction V) was purchased from Miles Laboratories (Elkhart, IN). Serum was obtained from ketamine/xylazine-anesthetized rats. All other reagents were commercially available.

All isotopes except [<sup>125</sup>I]D-T<sub>3</sub> were obtained from New England Nuclear (Boston, MA). The specific activities of the isotopes are as follows: [<sup>125</sup>I]L-T<sub>3</sub>, 150 μCi/μg; [<sup>125</sup>I]L-T<sub>4</sub>, 150 μCi/μg; [<sup>125</sup>I]iodine, 2.17 mCi/nmol; and <sup>3</sup>H<sub>2</sub>O, 1 mCi/g.

### Iodination of D-T<sub>3</sub>

D-T<sub>3</sub> was iodinated with [<sup>125</sup>I]iodine to a specific activity of 100 μCi/μg with the use of chloramine-T. The iodination was carried out by adding sequentially, to a glass tube, 7 μl 1 mg/ml D-T<sub>3</sub>, 10 μl 0.3 M sodium phosphate buffer (pH 7.4), 10 μl 500 mCi/ml [<sup>125</sup>I]iodine, and 10 μl 100 μM chloramine-T. The mixture was incubated for 1 min at room temperature, and 200 μl 0.1 M sodium acetate (pH 5.6) were added. The solution was then transferred to a 0.7 × 18-cm Sephadex G-25 (fine) column. The column was eluted with 16 ml 0.1 M sodium acetate (pH 5.6) and then with 22 ml 0.1 N NaOH. Initially, the column was eluted with 12 ml 0.1 M sodium acetate (pH 5.6), but this incompletely removed free iodine (Fig. 1). Elution with 16 ml acetate buffer removed any detectable iodine contamination in the [<sup>125</sup>I]D-T<sub>3</sub> preparation, as detected by TLC (see below).

The radiochemical purities of [<sup>125</sup>I]L-T<sub>3</sub>, [<sup>125</sup>I]L-T<sub>4</sub>, and [<sup>125</sup>I]D-T<sub>3</sub> were at least 99%, as judged by cellulose (250 μm) TLC and radiochromatogram scanning (model 7230 radiochromato-

gram scanner, Packard, Downers Grove, IL) using the solvent system of chloroform-*t*-butanol-2 N NH<sub>4</sub>OH (60:376:70). The radiochemical purity of [<sup>125</sup>I]D-T<sub>3</sub> was also checked by HPLC (model 110A, Altex Scientific, Inc., Berkeley, CA) (20). The analytical column was a reverse phase C<sub>18</sub> Ultrasphere column (id, 15 cm × 4.6 mm; Altex). The mobile phase was 30% acetonitrile in 0.1 M sodium acetate with 4 mM cupric sulfate and 8 mM L-proline. The solvent flow rate was 1.5 ml/min. The eluate was collected every minute, and each 1.5-ml fraction was counted for <sup>125</sup>I.

### Tissue-sampling single injection technique

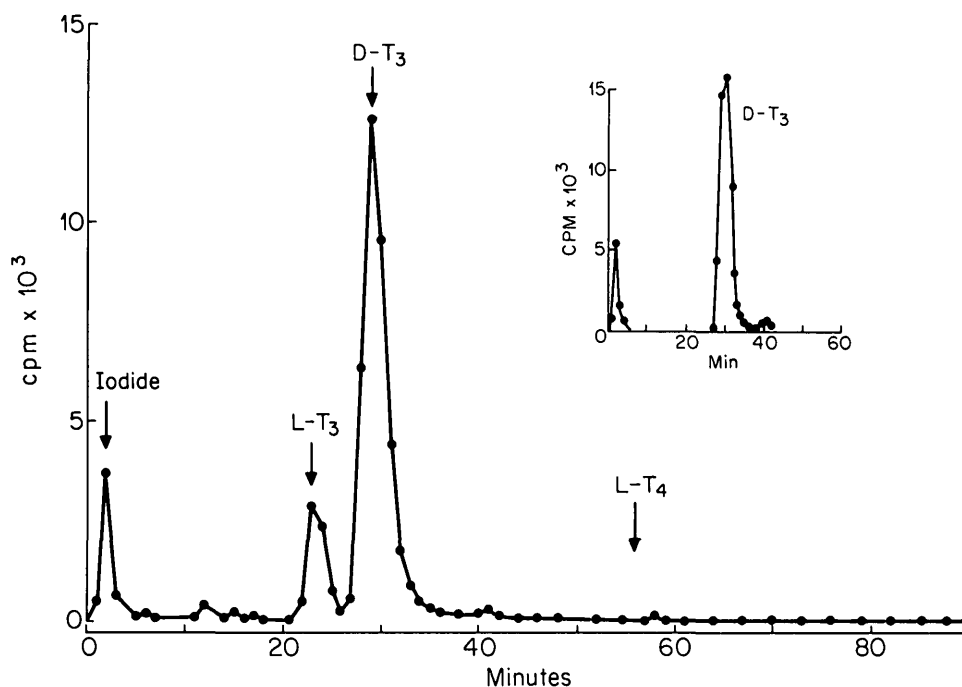
The extraction of unidirectional influx for [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> relative to a <sup>3</sup>H<sub>2</sub>O reference was measured by the tissue-sampling single injection technique in adult male Sprague-Dawley rats (240–290 g) under ketamine/xylazine anesthesia (ketamine, 235 mg/kg, im; xylazine, 2.3 mg/kg, im). An approximately 200-μl bolus of HEPES-buffered Ringer's solution (pH 7.4, RHB) was rapidly injected (<1 sec) via a 27-gauge needle into the common carotid artery for the BBB transport (11) and salivary gland transport studies (18) or into the portal vein after ligation of the hepatic artery for the hepatic transport study (12). The injection vehicle contained [<sup>125</sup>I]D-T<sub>3</sub> or [<sup>125</sup>I]L-T<sub>3</sub> (2 μCi/ml), <sup>3</sup>H<sub>2</sub>O (20 μCi/ml) and various concentrations of albumin or serum in RHB. Trace concentrations of BSA (0.05–0.1%) were added to the RHB solution to prevent T<sub>3</sub> adsorption to the injection syringe. This concentration of BSA causes no inhibition of T<sub>3</sub> transport into brain or liver (11, 12). Mixing of the injectate with the circulating blood has been found to be less than 5% (21). In the case of BBB and salivary gland transport studies, the rat was decapitated 15 sec after carotid injection, and then the hemisphere and the mandibular gland ipsilateral to the injection site were removed. Eighteen seconds after the injection, the portal vein was transected, and the right major lobe of liver was rapidly removed for the hepatic transport study. Portions of brain, salivary gland, and liver were solubilized in 2.0 ml Soluene-350 (Packard) at 50 C for 1 h before double isotope liquid scintillation counting.

Counts per min were converted to disintegrations per min by standard quench corrections, and the percent brain uptake index (BUI), salivary gland uptake index (SUI), and liver uptake index (LUI) were calculated as follows:

$$\text{BUI, SUI, or LUI} = \frac{(^{125}\text{I dpm}/^3\text{H dpm}) \text{ tissue}}{(^{125}\text{I dpm}/^3\text{H dpm}) \text{ injectate}} \times 100$$

The percent extractions of T<sub>3</sub> in brain (E<sub>b</sub>), salivary gland (E<sub>s</sub>), and liver (E<sub>l</sub>) were calculated: E<sub>b</sub> = BUI × E<sub>rb</sub>, E<sub>s</sub> = SUI × E<sub>rs</sub>, and E<sub>l</sub> = LUI × E<sub>rl</sub>, where E<sub>rb</sub>, E<sub>rs</sub>, and E<sub>rl</sub> represent the fractional brain, salivary gland, and liver extractions of <sup>3</sup>H<sub>2</sub>O, respectively. E<sub>rb</sub>, E<sub>rs</sub>, and E<sub>rl</sub> are 0.64, 0.70, and 0.84, as previously reported (16, 18, 22, 23). Although the vascular volume relative to whole tissue volume of brain is so small that it is negligible for the calculation of extravascular brain extraction (19), the vascular volume of salivary gland and liver is not negligible (12, 18). Therefore, the percent extraction by salivary gland (E<sub>x,s</sub>) and liver (E<sub>x,l</sub>) due to extravascular uptake of T<sub>3</sub>

FIG. 1. Chromatogram of the enantiomers of [<sup>125</sup>I]T<sub>3</sub>. The mixture of 0.1 μCi [<sup>125</sup>I]L-T<sub>3</sub> and 0.5 μCi [<sup>125</sup>I]D-T<sub>3</sub> was injected into the reverse phase C<sub>18</sub> column. The eluate was collected at 1-min intervals. The separation conditions were as follows: mobile phase, 30% acetonitrile in 0.1 M sodium acetate with 4 mM cupric sulfate and 8 mM L-proline; flow rate, 1.5 ml/min. A chromatogram of the [<sup>125</sup>I]D-T<sub>3</sub> is illustrated in *inset*.



were calculated:

$$E_{x,s} = \frac{E_s - E_{al,s}}{100 - E_{al,s}} \times 100$$

$$E_{x,l} = \frac{E_l - E_{al,l}}{100 - E_{al,l}} \times 100$$

where  $E_{al,s}$  and  $E_{al,l}$  represent the percent extraction of albumin in salivary gland and liver, respectively. The  $E_{al,s}$  and  $E_{al,l}$  values are  $17 \pm 2\%$  and  $13 \pm 2\%$ , which were determined previously (12, 18).

#### *In vitro* equilibrium dialysis

The *in vitro* percentage of free (dialyzable) [<sup>125</sup>I]D-T<sub>3</sub> or [<sup>125</sup>I]L-T<sub>3</sub> in the presence of either sera or albumin was determined by equilibrium dialysis (12). One and one half milliliters of the injectate containing 0.5 μCi/ml [<sup>125</sup>I]T<sub>3</sub> were applied to a dialysis bag made of a 1.5 × 15-cm strip of dialysis tubing (mol wt cut-off, 12,000–14,000; Spectrum Medical Industries, Inc., Los Angeles, CA) and dialyzed against 15 ml RHB containing 0.05% sodium azide at 37 C for 20 h. The *in vitro* percent free was calculated from the ratio of disintegrations per min/ml in the dialysis buffer to that in the dialysis bag.

#### *Isoelectric focusing (IEF) and autoradiography*

IEF was carried out on prefocused horizontal polyacrylamide slab gels over a pH range of 3.8–6.5 at 4 C, with the use of a flatbed electrophoresis apparatus (C.B.S. Scientific Co., Del Mar, CA), an electrophoresis constant power supply (ECPS 3000/150, Pharmacia Fine Chemicals), and a volt-hour integrator (VH-1, Pharmacia Fine Chemicals) (24). Twenty-five milliliters of the gel solution containing 4.875% acrylamide, 0.125% N,N'-methylene-bis-acrylamide, 8% glycerol, 1.06 ml Phar-

malyte (pH 4–6.5), and 0.6 ml Pharmalyte (pH 3–10) were prepared. After degassing, polymerization was carried out in a casting mold (thickness, 1 mm; width, 140 mm; length, 160 mm) in the presence of 250 μl 3% ammonium persulfate and 28 μl N,N,N',N'-tetramethylethylenediamine. Prefocusing was done with an anode solution (25 mM DL-aspartic acid and 25 mM L-glutamic acid) and a cathode solution (250 mM glycine) in a setting of constant power at 9 watts for 60 min, reaching a maximum of 2000 V. Then, 7 μl 5 g/dl BSA containing 10 μCi/ml [<sup>125</sup>I]D-T<sub>3</sub> or [<sup>125</sup>I]L-T<sub>3</sub> were applied to the center of the gel. IEF was carried out for 5 h at a constant power of 13 watts, reaching a maximum of 2300 V. Immediately after the current was turned off, the pH gradient in the gel was determined with a pH meter (pH meter 140, Corning Science Products, Medfield, MA). Half-centimeter slices of the gel were eluted in 2 ml 50 mM KCl. The gel was then transferred to the filter paper, covered with plastic film, and dried for 1 h at 60 C on a gel slab dryer (model 224, Bio-Rad Laboratories). The dried gel was exposed to LKB Ultrafilm for 2 days at room temperature and then developed by Kodak GBX developer for 4 min. The development was stopped by 2% acetic acid for 30 sec and washed in water for 30 sec. Then, the film was fixed with Kodak GBX fixer for 5 min, followed by a wash with water for 20 min and complete drying.

Statistical differences were assessed by Student's *t* test.

## Results

The chromatogram of a mixture of [<sup>125</sup>I]L-T<sub>3</sub> and [<sup>125</sup>I]D-T<sub>3</sub> is shown in Fig. 1. The retention times of [<sup>125</sup>I]L-T<sub>3</sub>, [<sup>125</sup>I]D-T<sub>3</sub>, and [<sup>125</sup>I]L-T<sub>4</sub> were 23, 29, and 56 min, respectively. The typical chromatogram of the [<sup>125</sup>I]D-T<sub>3</sub>, which was prepared by the chloramine-T iodination method, is also shown in the *inset* of Fig. 1. The small

amount of free iodine found in the HPLC run after elution of the G-25 column with 12 ml acetate buffer (Fig. 1) was not found on subsequent HPLC runs after elution of the G-25 column with 16 ml acetate buffer (see *Materials and Methods*). The HPLC system separated the D- and L-isomers of T<sub>3</sub> (Fig. 1), and no [<sup>125</sup>I]L-T<sub>3</sub> was found in the [<sup>125</sup>I]D-T<sub>3</sub> preparation (Fig. 1, *inset*).

The concentration dependency for the brain extraction of [<sup>125</sup>I]L-T<sub>3</sub> is illustrated in Fig. 2. The nonsaturable brain extraction ( $5.86 \pm 0.02\%$ ) was obtained from the extraction of [<sup>125</sup>I]L-T<sub>3</sub> in the presence of 100  $\mu\text{M}$  L-T<sub>3</sub>. The ED<sub>50</sub> for the saturable brain extraction of L-T<sub>3</sub> was 1  $\mu\text{M}$ . The inhibition of the brain extraction of [<sup>125</sup>I]L-T<sub>3</sub> by D-T<sub>3</sub> is also illustrated in Fig. 2. The ED<sub>50</sub> of D-T<sub>3</sub> inhibition of [<sup>125</sup>I]L-T<sub>3</sub> extraction was 9  $\mu\text{M}$ . The brain extraction of [<sup>125</sup>I]D-T<sub>3</sub> in RHB with 0.05 g/dl BSA is compared to that of [<sup>125</sup>I]L-T<sub>3</sub> in the *inset* of Fig. 2. The brain extraction of [<sup>125</sup>I]D-T<sub>3</sub> was significantly less than that of [<sup>125</sup>I]L-T<sub>3</sub>, and no difference was observed between the brain extraction of [<sup>125</sup>I]D-T<sub>3</sub> ( $5.78 \pm 0.35\%$ ) and the nonsaturable extraction of [<sup>125</sup>I]L-T<sub>3</sub> ( $5.86 \pm 0.07\%$ ).

The extractions of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> by rat salivary gland were also examined (Table 1). No significant difference was observed between extravascular salivary gland extraction of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> in the presence of 0.05 g/dl BSA. Additionally, there was no significant difference in the extraction of [<sup>125</sup>I]L-T<sub>3</sub> in the absence or presence of 25  $\mu\text{M}$  L-T<sub>3</sub> and 25  $\mu\text{M}$  D-T<sub>3</sub> in the injectate. Furthermore, no significant difference was found for the extraction of [<sup>125</sup>I]D-T<sub>3</sub> in the absence or presence of 25  $\mu\text{M}$  D-T<sub>3</sub> in the injectate (Table 1).

When plasma proteins were essentially absent from the portal venous injection vehicle (e.g. 0.1 g/dl BSA;

Table 2), the extravascular hepatic extraction of L-T<sub>3</sub> was only 12% greater than that for D-T<sub>3</sub>. The effect of serum protein binding on hepatic extraction of L-T<sub>3</sub> and D-T<sub>3</sub> was investigated by adding 5 g/dl BSA or 92% rat serum to the injection solutions (Table 2). The extravascular hepatic extraction of [<sup>125</sup>I]D-T<sub>3</sub> in the presence of 5 g/dl BSA was significantly less than that of [<sup>125</sup>I]L-T<sub>3</sub> ( $P < 0.05$ ). On the contrary, there was no difference between the extravascular extraction of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> in the presence of 92% rat serum (Table 2).

The *in vitro* binding of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> to 5 g/dl BSA and 100% rat serum was determined by the equilibrium dialysis technique. The *in vitro* unbound percentages (dialyzable fraction) of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> in the presence of 5 g/dl BSA were  $1.95 \pm 0.16\%$  and  $1.79 \pm 0.28\%$ , respectively, and these values were not significantly different. The *in vitro* unbound percentages of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> in the presence of 100% rat serum were  $4.30 \pm 0.31\%$  and  $2.42 \pm 0.28\%$ , respectively ( $P < 0.01$ ).

The isoelectric point (pI) of BSA was determined by sequential IEF and autoradiography in the presence of 5 g/dl BSA bound by either [<sup>125</sup>I]L-T<sub>3</sub> or [<sup>125</sup>I]D-T<sub>3</sub>. As shown in Fig. 3, the pI value of BSA bound to [<sup>125</sup>I]L-T<sub>3</sub> was 5.1, which was greater than that of BSA bound to [<sup>125</sup>I]D-T<sub>3</sub> (pI 5.0).

## Discussion

The present studies provide evidence for 1) stereospecific carrier-mediated transport of T<sub>3</sub> through the brain capillary wall, i.e., the BBB; 2) nonstereospecific transport of T<sub>3</sub> through the salivary gland capillary wall and essentially nonstereospecific transport of T<sub>3</sub> through the

FIG. 2. The brain extraction of [<sup>125</sup>I]L-T<sub>3</sub> is decreased by the presence of L-T<sub>3</sub> or D-T<sub>3</sub> in the carotid injection solution. O, The brain extraction of [<sup>125</sup>I]L-T<sub>3</sub> inhibited by unlabeled L-T<sub>3</sub>. ●, The brain extraction of [<sup>125</sup>I]L-T<sub>3</sub> inhibited by unlabeled D-T<sub>3</sub>. The nonsaturable extraction ( $5.86 \pm 0.02\%$ ) represents the extraction of [<sup>125</sup>I]L-T<sub>3</sub> after injection of labeled hormone in 100  $\mu\text{M}$  L-T<sub>3</sub>. The brain extractions of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> after injection of isotope mixed in RHB containing 0.05 g/dl BSA are compared in the *inset*. Data are the mean  $\pm$  SE for three experiments.

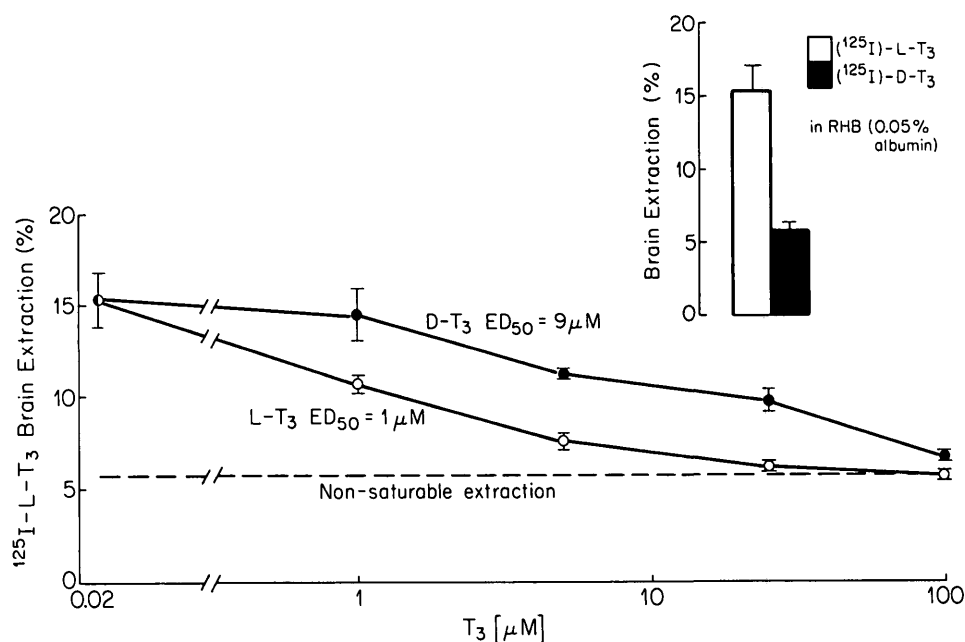


TABLE 1. Extravascular salivary gland extraction of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub>

Injection vehicle (RHB)	Extravascular salivary gland extraction (%)	
	D-T <sub>3</sub>	L-T <sub>3</sub>
0.05% albumin	30.3 ± 4.1	34.2 ± 2.4
25 μM L-T <sub>3</sub>		33.7 ± 2.2
25 μM D-T <sub>3</sub>	30.3 ± 2.0	29.0 ± 1.8

Values given are the mean ± SE (n = 3).

TABLE 2. Comparison of rat extravascular hepatic extraction of [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> in the presence of various serum proteins

Injection vehicle <sup>a</sup>	Extravascular hepatic extraction (%)	
	D-T <sub>3</sub>	L-T <sub>3</sub>
0.1% BSA	73.4 ± 1.1	84.3 ± 3.7 <sup>b</sup>
5.0% BSA	49.9 ± 2.2	93.1 ± 11.7 <sup>b</sup>
92% Rat serum	55.2 ± 3.8	52.0 ± 1.7

Values given are the mean ± SE (n = 3).

<sup>a</sup> All injectate solutions were diluted with RHB (pH 7.4).

<sup>b</sup> Significantly different (*P* < 0.05, by Student's *t* test) from D-T<sub>3</sub> hepatic extraction at the same injectate concentration.

hepatocyte cell membrane; 3) biochemical evidence for different albumin conformational states, depending on whether the plasma protein binds L-T<sub>3</sub> or D-T<sub>3</sub>; and 4) the first evidence of stereospecificity of plasma protein-mediated transport of albumin-bound ligands into tissues *in vivo*. These results are discussed within the context of 1) plasma membrane T<sub>3</sub> transport in brain, salivary gland, or liver; 2) plasma protein-mediated transport of T<sub>3</sub> in liver; and 3) the implications of these findings to the differential biological potencies of the L- and D-isomers of T<sub>3</sub>.

Previous *in vivo* studies have shown that the uptake of T<sub>3</sub> by brain is saturable, whereas T<sub>3</sub> uptake by other organs, such as liver, is nonsaturable (25, 26). Earlier studies from this laboratory have shown that a specific thyroid hormone carrier-mediated transport system is present in the brain capillary wall or BBB (11). The

BBB thyroid hormone transport system was found to have a higher affinity for T<sub>3</sub> (*K<sub>m</sub>* = 1 μM) than for T<sub>4</sub> or T<sub>3</sub>, and was found to be a distinct entity from the known neutral amino acid transport system that is also active within the BBB (11). The availability of methods for iodinating D-T<sub>3</sub> and for determining the isomeric purity of the iodinated D-T<sub>3</sub> preparation (20) provided the opportunity for assessing the stereospecificity of the BBB thyroid hormone transport system. The present studies show that this carrier has a 9 times higher affinity for L-T<sub>3</sub> than for D-T<sub>3</sub>. Owing to the very low affinity of D-T<sub>3</sub> for this transport system, virtually the only transport pathway for D-T<sub>3</sub> movement through the BBB is free diffusion. For example, the first pass extraction of [<sup>125</sup>I]T<sub>3</sub> (5.8%) is no greater than the nonsaturable, *i.e.* not carrier mediated, extraction of [<sup>125</sup>I]L-T<sub>3</sub> (5.9%). The flux of D-T<sub>3</sub> through the nonsaturable mechanism, *i.e.* free diffusion, is about one third of the total influx of L-T<sub>3</sub> through both the nonsaturable and carrier-mediated mechanisms.

In contrast to brain capillaries, which have no pores, salivary gland capillaries are fenestrated (18), and the presence of pores in salivary gland capillaries obviates the need for carrier-mediated transport mechanisms. The absence of a specific T<sub>3</sub> transport system from salivary gland capillaries is supported by the observation that T<sub>3</sub> transport in this organ is neither saturable nor stereospecific.

Hepatic microvessels are sinusoids, which have very large pores and no basement membrane (27). Thus, the sinusoidal wall provides no diffusion barrier to either small molecules, such as free T<sub>3</sub>, or plasma proteins, such as albumin. In liver the limiting membrane preventing free diffusion of circulating substances into the hepatocyte cell water is the hepatocyte plasma membrane. Previous studies of T<sub>3</sub> transport *in vivo* have shown that these relatively lipid-soluble hormones undergo rapid nonsaturable transport across the hepatocyte cell membrane (12). Similarly, hepatocyte cell membrane transport of T<sub>3</sub> is weakly, if at all, stereospecific. As shown in

D-T<sub>3</sub> with 5% BSA

L-T<sub>3</sub> with 5% BSA

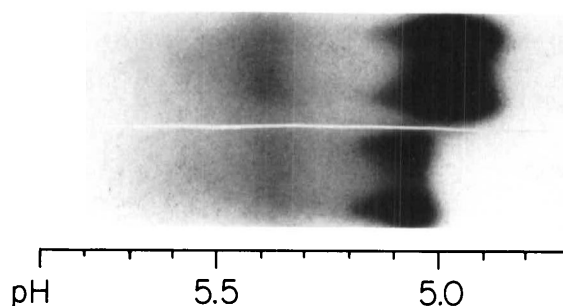


FIG. 3. Autoradiograph of the IEF gel for [<sup>125</sup>I]L-T<sub>3</sub> and [<sup>125</sup>I]D-T<sub>3</sub> bound to BSA. The pH of the gel is shown on the ordinate of the figure. The content of the sample is shown on the abscissa. The concentration of both [<sup>125</sup>I]D-T<sub>3</sub> and [<sup>125</sup>I]L-T<sub>3</sub> were 2 μCi/ml, and the albumin concentration was 5 g/dl. The minor band at pH 5.4 represents unbound [<sup>125</sup>I]L-T<sub>3</sub> or [<sup>125</sup>I]D-T<sub>3</sub>, as this peak comigrated with free [<sup>125</sup>I]T<sub>3</sub>.

Table 2, the extravascular hepatic extraction of D-T<sub>3</sub> (in the presence of 0.1 g/dl BSA) is only 12% lower than the extravascular hepatic extraction of L-T<sub>3</sub>. The physiological significance of this difference is questionable, and it is likely that D-T<sub>3</sub> traverses the hepatocyte cell membrane by a nonsaturable nonstereospecific mechanism involving free diffusion.

Although the transport of T<sub>3</sub> across the hepatocyte cell membrane is essentially nonstereospecific, the present studies provide evidence for stereospecificity of albumin-mediated transport of T<sub>3</sub> into liver *in vivo*. In these experiments, liver was used to assess the stereospecificity of plasma protein-mediated transport, since the uptake of T<sub>3</sub> by liver is several times larger than the uptake of T<sub>3</sub> by the brain or the salivary gland. The high hepatic extraction facilitates the measurement of plasma protein-binding effects *in vivo*. In the presence of 5 g/dl BSA, the extravascular hepatic extraction of D-T<sub>3</sub> was about half that of L-T<sub>3</sub>, whereas the *in vitro* albumin binding of D-T<sub>3</sub> was comparable to that of L-T<sub>3</sub> (*cf. Results*). A similar pattern was also found for rat albumin. *In vitro*, binding of L-T<sub>3</sub> to rat serum was nearly twice as great as that of D-T<sub>3</sub> (*cf. Results*). However, the *in vivo* extravascular hepatic extractions of D-T<sub>3</sub> and L-T<sub>3</sub> in the presence of rat serum were not significantly different. Therefore, there are species differences in T<sub>3</sub> binding to albumin *in vivo* and *in vitro*. T<sub>3</sub> binding to BSA is stereospecific *in vivo* but not *in vitro*, whereas T<sub>3</sub> binding to rat albumin is stereospecific *in vitro* but not

*in vivo*. Since the stereospecific binding of T<sub>3</sub> to albumin is a function of the conformation about the protein binding site, these studies suggest that the T<sub>3</sub>-binding site on albumin is different *in vivo* in the liver microcirculation compared to *in vitro*.

Stereospecificity differences in L-T<sub>3</sub> vs. D-T<sub>3</sub> binding to albumin *in vivo* may provide insight into the mechanism of plasma protein-mediated T<sub>3</sub> delivery to liver. The amount of circulating L-T<sub>3</sub> or D-T<sub>3</sub> available for uptake by liver *in vivo* was many times greater than the fraction of T<sub>3</sub> that was unbound *in vitro*. Therefore, circulating T<sub>3</sub> is readily available for uptake by liver from either the circulating bovine or rat albumin-bound pools. Since the plasma protein *per se* is not significantly taken up by liver on a single circulatory passage (14), the transport of hormone from the circulating albumin-bound pool represents a process of enhanced hormone dissociation from the albumin-binding site (15–17). It is hypothesized that transient interactions between the surface of the albumin molecule and the surface of the hepatocyte cause conformational changes in the T<sub>3</sub>-binding site on the albumin molecule that result in a binding site uncoiling and markedly increased rates of T<sub>3</sub> dissociation from albumin *in vivo* in the liver microcirculation, but this does not occur *in vitro*. Biochemical evidence in support of the tissue-mediated conformational change in hormone-binding proteins has been recently reported (28). The present studies provide additional biochemical evidence for this model and suggest that the basis for the interaction between albumin and the hepatic microcirculatory surface is an electrostatic one. The IEF data in Fig. 3 show that the surface charge of albumin is a stereospecific function of the nature of the bound ligand. The albumin surface charge is slightly but significantly less negative when the plasma protein is carrying L-T<sub>3</sub> compared to D-T<sub>3</sub>. Thus, the binding of L-T<sub>3</sub> to albumin may allow for the exposure of an important lysine or arginine residue on the surface of the plasma protein that is not completely exposed when D-T<sub>3</sub> is bound to albumin (see model in Fig. 4). The data in Table 2, showing the restricted transport of albumin-bound D-T<sub>3</sub> compared to albumin-bound L-T<sub>3</sub>, indicate that there is a greater uncoiling around the T<sub>3</sub>-binding site on albumin when the plasma protein assumes the conformation that yields the less negative surface charge. The charge selectivity model shown in Fig. 4 is also consistent with the finding by other workers that plasma proteins such as albumin normally undergo rapid electrostatic interactions with the cellular surface of the microcirculation (29, 30).

Finally, in regard to the difference in biological potency between D- and L-T<sub>3</sub>, the present studies indicate that membrane transport mechanisms provide marked stereospecificity for the T<sub>3</sub> stereoisomers in brain, but not in

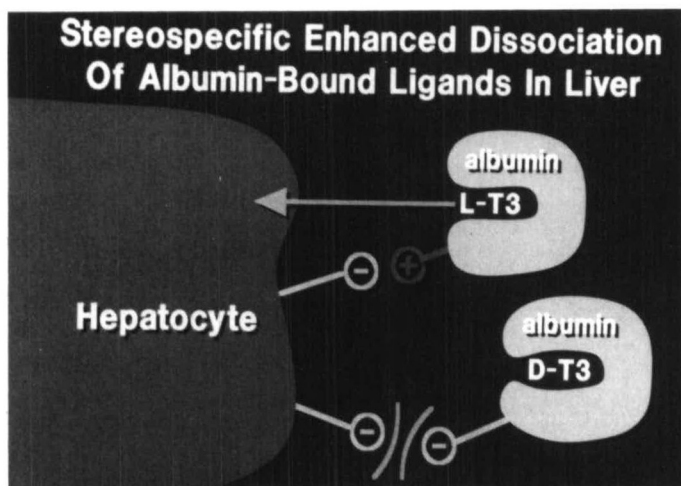


FIG. 4. Hypothetical charge selectivity mechanism for stereospecific enhanced dissociation of albumin-bound T<sub>3</sub> within the liver microcirculation. Albumin carrying L-T<sub>3</sub> is slightly more basic than albumin carrying D-T<sub>3</sub> (see Fig. 3), and L-T<sub>3</sub> is more readily available for uptake by hepatocytes from the circulating albumin-bound pool than is albumin-bound D-T<sub>3</sub> (see Table 2). Therefore, it is proposed that an electrostatic interaction between a positively charged residue on the surface of the albumin molecule carrying D-T<sub>3</sub> and a negatively charged residue on the hepatocyte surface causes a conformational change in the T<sub>3</sub>-binding site that results in enhanced dissociation of T<sub>3</sub> from albumin that is preferential for the L-T<sub>3</sub> isomer.

salivary gland or liver. The marked stereospecific differences between D- and L-T<sub>3</sub> transport through the BBB, however, may explain a large component of the overall differences in biological potency of the two stereoisomers of T<sub>3</sub>, if T<sub>3</sub> action in the brain regulates organ function in organs other than the brain. For example, Goldman and co-workers (31) have recently shown that intracerebral administration of T<sub>3</sub> to hypothyroid rats results in a greater increase in heart rate than when an equal dose of T<sub>3</sub> is administered iv. Thus, the acceleration of bradycardia in hypothyroidism after T<sub>3</sub> administration may actually represent T<sub>3</sub> action in brain, rather than direct T<sub>3</sub> action on the myocardium. Not all of T<sub>3</sub> actions are mediated via the central nervous system. However, those functions in peripheral organs that are regulated by brain would be expected to show stereospecific differences in the biological potencies of D- and L-T<sub>3</sub>, since these two stereoisomers are transported through the BBB at markedly different rates.

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