Influx of Thyroid Hormones into Rat Liver In Vivo

DIFFERENTIAL AVAILABILITY OF THYROXINE AND TRIIODOTHYRONINE BOUND BY PLASMA PROTEINS

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ABSTRACT The transport of [^{125}I]thyroxine (T_4) and [^{125}I]triiodothyronine (T_3) into liver was investigated with a tissue sampling-portal vein injection technique in the anesthetized rat. The method allows the investigation of the effects of plasma proteins in human serum on the unidirectional influx of T_4 or T_3 into liver cells. The percent extraction of unidirectional clearance of T_3 and T_4 was 77±2% and 43±2%, respectively, after portal injection of a bolus of Ringer's solution. Cell membrane transport of T_4 or T_3 was nonsaturable because 50-μM concentrations of unlabeled hormone had no effect on transport. The addition of bovine albumin in concentrations of 1, 5, or 10 g/100 ml bound >98% of T_4 or T_3 in vitro, but had no significant effect on T_3 or T_4 transport in vivo. Conversely, 10% rabbit antisera specific for T_3 or T_4, completely abolished the intracellular distribution of thyroid hormone into liver. In the presence of rat serum, which contains albumin and thyroid hormone binding prealbumin (TBPA), 18 and 81% of total plasma T_4 and T_3, respectively, were available for transport in vivo. The fraction of hormone available for transport in the presence of normal human serum, which contains albumin, TBPA, and thyroid hormone binding globulin (TBG) was 11% for T_4 and 72% for T_3. The fraction of hormone transported into liver after injection of serum obtained from pregnant or birth control pill-treated volunteers was 4% for T_4 (but this was not significantly different from zero) and 54% for T_3.

These data suggest: (*a*) The mechanism by which T_4 and T_3 traverse the liver cell membrane is probably free diffusion. (*b*) Albumin-bound T_4 or T_3 is freely cleared by liver, ~50% of TBG-bound T_3 is transported, but little, if any, of TBPA-bound T_4 or TBG-bound T_4 is cleared by liver cells. (*c*) Although the albumin-bound fraction of T_4 greatly exceeds the free (dialyzable) moiety, the two fractions are both inversely related to the existing TBA or TBG level; therefore, in vitro measurements of free T_4 would be expected to accurately reflect what is available for transport in vivo. Conversely, TBG-bound T_3 is readily transported in vivo; therefore, it is proposed that in vitro measurements of free T_3 do not reliably predict the fraction of T_3 available for transport into liver in vivo.

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INTRODUCTION

The thyroid and steroid hormones are tightly bound by plasma proteins (1), and it is generally regarded that only the small fraction that is free at equilibrium in vitro is available for transport in vivo (2). However, in vitro equilibrium measurements of ligand protein interactions do not bear on three important determinants of ligand transport in vivo: (*a*) the rate of unidirectional ligand dissociation from the plasma protein, which is generally on the order of milliseconds to seconds, (*b*) the capillary transit time (1–10 s), i.e., the duration of exposure of the plasma protein to the tissue cell membranes, and (*c*) membrane permeability, which determines the rate at which the ligand is transported through the biological membrane. Given favorable realtionships between these latter three determinants, the transport of protein-bound ligands may be extensive. Our previous studies with the steroid hormones have shown that albumin-bound, but not globulin-bound hormone is readily transported through the brain capillary wall, i.e., the blood-brain barrier (3); these results correlate with the fact that the rates of unidirectional dissociation of steroid from albumin and from globulin binding sites are fast and slow, respectively, relative to the capillary transit time in brain, ~1 s (3). Conversely, the rate of estradiol or cortisol dissociation from sex hormone binding globulin or

corticosteroid binding globulin, respectively, is relatively fast (4, 5) compared with the long transit time in liver, 5–10 s (6). Moreover, both albumin-bound and globulin-bound estradiol or cortisol are available for transport into liver (7).

Based on studies reported by Hillier (8, 9) there is reason to believe that transport of protein-bound thyroid hormones into tissues is as extensive as observed for the steroid hormones. Hillier has shown that the $t_{1/2}$ at 37°C of triiodothyronine (T_3)[1] dissociation from thyroid hormone binding globulin (TBG) or albumin is 4 s and <1 s, respectively (8). The $t_{1/2}$ of unidirectional dissociation of thyroxine (T_4) from TBG or thyroid hormone binding prealbumin (TBPA) at 37°C is 39 and 8 s, respectively (9); the $t_{1/2}$ of T_4 dissociation from albumin has apparently not been measured, but is probably on the same order of magnitude as T_3. It is assumed the data of Hillier (8, 9) provide at least estimates of the upper limit of thyronine debinding rates. Given these data and knowledge of the capillary transit times in tissues such as brain (~1 s) or liver (~5 s), it might be expected that T_3 bound to albumin, but not to TBG, is transported into brain. Indeed, recent studies have shown that the albumin-bound fraction of T_3 transported into brain is 10 times the free (dialyzable) fraction (10). Moreover, it might be anticipated that T_3 bound to both albumin and to TBG is transported into a tissue such as liver with a long transit time of ~5 s. Similarly, a rapid unidirectional dissociation of T_4 from albumin might lead to transport of albumin-bound T_4 into liver, whereas T_4 bound to TBG and perhaps to TBPA may not dissociate within the hepatic capillary transit time. Therefore, the present studies were designed to investigate the hypothesis that the rates of unidirectional dissociation of thyroid hormones from plasma proteins reported by Hillier (8, 9) are predictive of which fraction (albumin, TBPA, TBG) of protein-bound hormone is transported into liver.

METHODS

All isotopes were purchased from New England Nuclear, Boston, Mass. The manufacturer specific activity was L-[^{125}I]-thyroxine, 1,300 μCi/μg; L-[^{125}I]3,5,3′-triiodothyronine, 1,100 μCi/μg; [^{125}I]sodium iodide, 17,000 μCi/μg; and water-^{3}H, 1 mCi/g. Bovine albumin (nondefatted), 1-octanol and unlabeled T_3 and T_4 were purchased from Sigma Chemical Co., St. Louis, Mo. Serum was obtained from the rat, and the following human sources: normal male or female on no medications (ages 20–45 yr), females on birth control pills (Norinyl 1 + 50 [Syntex Laboratories, Inc., Palo Alto, Calif.]; 1 mg norethindrone and 50 μg mestranol), third trimester pregnancy, and both male and female cord blood. Rabbit antisera specific for either T_3 or T_4 were obtained from Dr. Inder J. Chopra and Mr. Darrel Mayes (Endocrine Sciences, Inc., Tarzana, Calif.).

All ^{125}I-labeled compounds were at least 98% radiochemically pure based on cellulose thin-layer chromatography and radioscanning (Packard model 7230 radiochromatogram scanner, Packard Instrument Co., Inc., Downers Grove, Ill.). The solvent system used was chloroform 60, tertiary butyl alcohol 376, 2 N ammonia 70; the following retardation factor (R_f) values were obtained: thyroxine 0.15, iodide 0.30, triiodothyronine 0.41. Silica gel H (Analtech, Inc., Newark, Del.) was initially used as the stationary phase, but this material appeared to cause degradation of the thyronines.

The permeability of the hepatocyte cell membrane to the labeled compounds was measured by the tissue sampling-single injection technique of Oldendorf (11) as adapted to liver (12, 13). A 200–300-g male Sprague-Dawley rat fed ad lib. was anesthetized with intraperitoneal pentobarbital (Diabutal, 45 mg/kg, Diamond Laboratories, Des Moines, Iowa). The animal was placed in a supine position, laparotomized, and the hepatic artery was ligated. The portal vein was immediately cannulated with a 25-gauge needle and ~200 μl of buffered Ringer's solution (pH = 7.4, 5 mM Hepes) was rapidly injected. This solution contained 2.5 μCi/ml of [^{125}I]thyronine, 25 μCi/ml of ^{3}H-water, a highly diffusible internal reference, and 0.1 g/100 ml bovine albumin. The albumin was added to prevent thyronine binding to the injection syringe. At 18 s after injection, a time sufficient for a single circulatory pass of the bolus through liver (12), the portal vein was transected and the right major lobe of liver was immediately removed. The liver tissue and the injection solution were prepared for double isotope ^{3}H, ^{125}I liquid scintillation counting as described previously (7, 10). The liver uptake index (LUI) was calculated as LUI(%) = (^{125}I/^{3}H) disintegrations per minute (liver)/(^{125}I/^{3}H) disintegrations per minute (injection solution) × 100. The LUI = E_T/E_R where E_T and E_R are the percent extraction of the test and reference isotopes, respectively, at 18 s after rapid portal injection. Because E_R is known under these experimental conditions (E_R = 0.65±0.04, mean±SE, n = 8 rats, [7]), the LUI may be converted to E_T. The rate of liver blood flow under the present experimental conditions is 1.4 ml/min per g (7); assuming a hematocrit of 40%, the rate of plasma flow is ~2.3 ml/min per g. Given the rate of plasma flow (F), the E_T may be converted to unidirectional clearance (Cl), i.e., Cl = E_TF. Since an E_T of 13–15% reflects distribution only into the interstitial space of liver (see Results), the Cl for T_3 or T_4 must be corrected for extracellular distribution, so that a measure of unidirectional clearance into liver cells is obtained, e.g., Cl = (E_T − 14%)F.

After determining the LUI and E_T of T_3 or T_4 following injection of Ringer's solution, the effects of unlabeled T_3 and T_4 or the effects of plasma proteins, e.g., albumin or sera from various sources, may be investigated. Assuming plasma proteins inhibit T_3 or T_4 transport by binding the hormone and making the molecule unable to traverse the hepatocyte cell membrane, then the percent inhibition of hormone transport is a measure of the in vivo binding of the hormone by the plasma protein. The percentage of plasma hormone that is transported in vivo may be calculated (7), Percent transported = $LUI_{serum} - LUI_{antiserum}/LUI_{control} - LUI_{antiserum} \times 100$; where LUI_{serum} = the LUI for T_3 or T_4 after injection of serum, $LUI_{control}$ = LUI after injection of Ringer's solution, and $LUI_{antiserum}$ = the LUI after injection of a 10% solution of rabbit antiserum specific for T_3 or T_4. The $LUI_{antiserum}$ was found to represent nonspecific distribution into the extracellular space (see Results).

The percentage of free (dialyzable) [^{125}I]T_3 or [^{125}I]T_4 in

[1] *Abbreviations used in this paper*: E_T, extraction of test compound; LUI, liver uptake index; TBG, thyroid hormone binding globulin; TBPA, thyroid hormone binding prealbumin; T_4, thyroxine; T_3, triiodothyronine.

the presence of either sera or a 2-g/100 ml concentration of bovine albumin was determined as previously described (10).

Subsequent to the unidirectional influx of T_4 or T_3 into liver, the labeled hormone may either return to blood via efflux or be sequestered in liver cells by either a cell membrane active transport system or by binding to cytoplasmic protein. The sequestration of T_4 or T_3 was studied by measuring the rate of change in LUI during a 90-s period after portal injection. Because LUI = E_T/E_R, the change in LUI with time is a function of dE_T/dt and dE_R/dt. Water radioactivity is not sequestered by liver, so $dE_R/dt = -K_R E_R$ or $E_R(t) = E_{R_0}e^{-K_R t}$, where E_{R_0} = the extraction of the references at t = 0 and K_R = the efflux rate constant of the ^{3}H-water reference. Previous studies have shown $K_R = 1.4$ min^{-1} (7). The test compound may either be sequestered by liver or return to blood. If it is assumed that any [^{125}I]T_3 or [^{125}I]T_4 that enters liver cells is completely sequestered by liver during the 90-s circulation period such that no efflux of T_3 or T_4 back to blood occurs, then $dE_T/dt = 0$ and $E_T(t) = E_{T_0}$. Under these conditions $LUI(t) = E_T(t)/E_R(t) = (E_{T_0}/E_{R_0})e^{K_R t}$ and $\ln LUI(t) = \ln LUI_0 + K_R t$ (14). Therefore, a plot of ln LUI vs. time should be linear with a slope $K_R = 1.4$ min^{-1} (7) and intercept = LUI at zero time, if T_3 or T_4 were completely sequestered by liver.

Since sequestration of T_3 or T_4 radioactivity could be due to metabolism of the hormone to a nondiffusible labeled metabolite, the following experiment was done. A 5-μCi bolus of [^{125}I]T_3 was rapidly injected into the portal vein and 90 s later the right major lobe was freeze-clamped by aluminum blocks precooled in liquid nitrogen. The frozen tissue was pulverized with a mortar and pestle and was homogenized in a glass tissue grinder in cold 95% methanol:ammonia (99:1). The homogenate was centrifuged at 4°C for 20 min at 3,000 *g* and the supernate was concentrated in a vacuum centrifuge evaporator (Savant Instruments Inc., Hicksville, N. Y.). The concentrated homogenate was spotted on a 250-μm thin layer cellulose plate (Analtech Inc.) and developed in butanol 50: acetone 25:ammonia 18. The addition of 1 μCi of [^{125}I]T_3 to unlabeled liver homogenate served as a control and this was treated exactly as the experimental sample.

The relative lipid solubility of T_3 and T_4 were assessed by measuring the 1-octanol/Ringer's (pH 7.4) partition coefficient as described previously (3). The solution of [^{125}I]T_3 or [^{125}I]T_4 was made 25 μM by addition of unlabeled T_3 or T_4, respectively, to minimize binding of the thyronines to glass. As noted previously, sequential 1-octanol/Ringer's solution partitions were run (3); the first partition removed any small, but highly polar impurity, e.g., [^{125}I]iodide, which would tend to markedly lower the measured partition coefficient of the highly lipid soluble thyronines.

Statistical significance was assessed by Students' *t* test.

RESULTS

The LUI of labeled T_3 and T_4 was 118±2% and 66±2% (Table I), respectively, after a bolus injection of hormone in Ringer's solution containing 0.1 g/100 ml bovine albumin. The corresponding extractions, 77±2% for T_3 and 43±2% for T_4, are given in Fig. 1. A small fraction of the E_T for T_3 or T_4 represents distribution into the interstitial space of liver. A measure of extracellular distribution was obtained by injecting labeled T_3 or T_4 mixed with a solution of 10% rabbit antiserum that was specific for T_3 or T_4. Because of the slow debinding of T_3 or T_4 from an antibody (15), no transport of protein-bound hormone into liver occurs. Since only the trivial fraction of hormone that is free (dialyzable) will be available for transport after injection of antibody, the use of antisera provide a reliable measure of nonspecific distribution into the extracellular space. For example, the LUI of antibody-bound T_3 or T_4 (Table I) approximated the LUI for such extra-

TABLE I
*Transport of T_3 and T_4 into Liver**

Portal vein injection vehicle	T_3‡,§		T_4‡	
	LUI	Transported	LUI	Transported
	%	%	%	%
Ringer's solution	118±2¶	100	66±2¶	100
Rat	100±5¶	81	31±2¶	18
Normal human	91±6¶	72	28±2¶	11
Oral contraceptives	83±5¶	64	25±2	4
Pregnancy	81±4¶	62	25±2	4
Cord blood	77±6¶	58	25±1	4
Antiserum (10%)	20±3	0	22±1	0

* Data are mean±SEM (n = 6–8 rats per group; serum was obtained from 6–8 different volunteers or patients in each group).

‡ LUI = liver uptake index; % transported = the fraction of total hormone available for transport into liver cells (see text for calculations).

§ Comparison of the LUI for T_3 after injection of rat serum to the LUI after injection of human serum resulted in the following P values; normal human ($P < 0.15$), oral contraceptives ($P < 0.025$), pregnancy ($P < 0.01$), cord ($P < 0.01$).

¶ Significantly different from antiserum ($P < 0.05$).

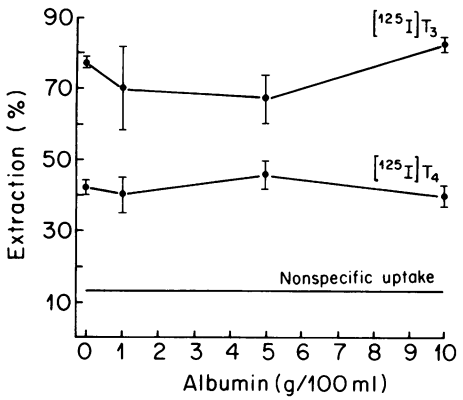

FIGURE 1 The extraction of unidirectional influx of T_4 and T_3 into liver vs. the concentration of bovine albumin in the portal vein injection solution. The nonspecific uptake reflects the distribution into the interstitial space and is equal to T_4 or T_3 clearance after injection of antiserum (Table I).

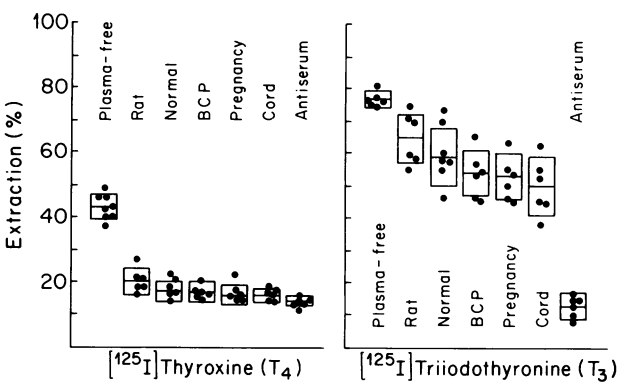

FIGURE 2 The extraction of T_4 or T_3 by rat liver vs. the type of serum added to the injection solution. Plasma-free solutions contained 0.1 g/100 ml bovine albumin; T_4 or T_3 antiserum solutions were diluted to 10%; all other samples were injected as 67% serum solutions. Each point represents a different patient, volunteer, or animal. The boxes represent the mean±SD. Normal human and cord samples were obtained from both males and females; BCP, birth control pills.

cellular space markers as [^{125}I]iodide, 21±1% (mean ±SEM, $n = 3$ rats), or [^{14}C]sucrose, 20±5% (7).[2] Since the average E_T for extracellular distribution is 14% (Fig. 1), the corrected E_T representing intracellular distribution of T_3 and T_4 is 63 and 29%, respectively, in the absence of plasma protein binding. After injection of labeled hormone mixed in rat serum, the E_T (corrected for extracellular distribution) for T_3 and T_4 is 51 and 6%, respectively (Fig. 2). Therefore, the unidirectional clearance for T_3 or T_4 by liver in the rat is 1.2 and 0.14 ml $min^{-1}g^{-1}$, respectively, given $Cl = E_TF$ and $F = 2.3$ ml $min^{-1}g^{-1}$ (Methods). The latter estimate for unidirectional T_4 clearance by rat liver approximates previous measurements by Hasen et al. (16).

Albumin is known to actively bind both T_3 and T_4. For example, a 2-g/100 ml concentration of bovine albumin bound 98.4±0.2 and 99.3±0.2% of a tracer concentration (10 nM) of [^{125}I]T_3 and T_4, respectively, in vitro as determined by equilibrium dialysis at 37°C. Conversely, when increasing concentrations of albumin were added to the injection solution, there was no significant change in the extraction of T_3 or T_4 by liver (Fig. 1). These results indicate T_3 or T_4 bound by albumin is freely available for transport into liver cells.

The availability for transport of hormone bound to TBPA was investigated by making the final injection solution 67% rat serum. The only thyroid hormone binding proteins in rat serum are albumin and TBPA; the latter protein actively binds T_4, but binds T_3 to a much lesser extent (1). Aš shown in Fig. 2, rat serum had a marked inhibitory effect on T_4 transport and a much lesser effect on T_3 flux into liver, i.e., 18% of total T_4 was available for transport vs. 81% for T_3 (Table I). In addition to albumin and TBPA, human serum contains TBG (1), and this latter protein is elevated in either pregnancy or oral estrogen use (1). The effects of 67% serum solution from four different human sources (normal, oral contraceptive, pregnancy, and cord blood) on T_3 or T_4 transport into liver are shown in Fig. 2. Human serum had a marked inhibitory effect on T_4 transport (Fig. 2) and only the LUI of T_4 bound to normal serum was statistically significantly different from the LUI of antiserum-bound T_4. Conversely, T_3 was readily transported into liver after injection of T_3 bound to human serum (Fig. 2). The amount of total T_3 available for transport into liver ranged from 58 to 72% for the human serum samples (Table I). However, the fraction of plasma T_3 cleared by liver after injection of serum containing elevated TBG (oral contraceptives or pregnancy) was not statistically different from normal human serum (Table I). Yet in the case of the sera samples obtained from pregnant volunteers, the free (dialyzable) fraction in vitro was decreased 50% compared with normal human serum (Table II). Cord serum, which contains high normal levels of TBG (17) and which binds high normal fractions of T_3 in vitro (Table II) inhibited [^{125}I]T_3 transport to the greatest degree (Table I); these results suggest the T_3 debinding rate from cord TBG may be somewhat slower than normal.

The saturability of labeled T_3 or T_4 transport was assessed by making the final injection solution 50 μM in either T_3 or T_4. Under these conditions, the LUI for T_3, 117±9%, or for T_4, 60±1%, was not significantly different from the control (Table I).

In order for the LUI to accurately reflect the extrac-

[2] In addition, the LUI for antisera-bound T_3 or T_4 approximates the value for a plasma protein, e.g., the LUI of ^{125}I-fetuin is 22±3%. Pardridge, W. M., and A. J. Van Herle, unpublished results.

TABLE II
*Free (Dialyzable) T_3 In Vitro**

Serum source	Dialyzable [^{125}I]T_3
	%
Rat	0.93±0.08
Normal human	0.30±0.03
Cord blood	0.25±0.02
Pregnancy	0.15±0.01
T_3 antiserum (10%)	0.053±0.003

* Data are mean±SEM (n = 6 per group). Dialysis was performed on serum diluted to 10% and on antiserum diluted to 1%, and the measured percentages were divided by 10 to arrive at the reported values.

tion or unidirectional influx into liver, it must be shown that the test compound cleared by liver cells does not efflux back to blood during the 18-s circulation period (13). Hormone efflux was studied by prolonging the circulation time after injection up to 90 s (Fig. 3). The rate of increase in the LUI for T_3 or T_4, 1.4 min^{-1}, is exactly equal to the rate of washout of the ^{3}H-water reference (7). As indicated in Methods, these observations indicate that T_3 or T_4 radioactivity is completely sequestered by liver for at least 90 s after injection. Therefore, no efflux of T_3 or T_4 during the 18-s circulation period occurs, and the LUI can be considered a reliable index of E_T.

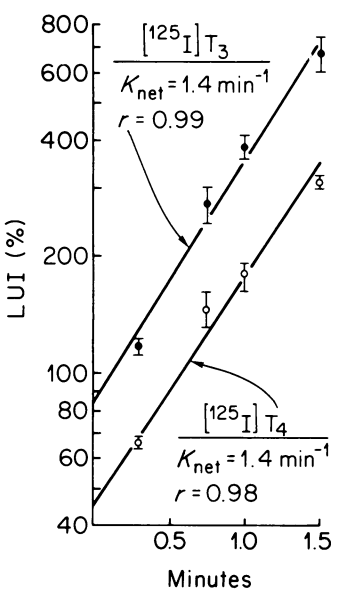

FIGURE 3 The LUI for T_3 or T_4 is plotted vs. circulation time after rapid portal injection. The K_{net} = the slope determined by linear regression analysis. The rate of increase in LUI is equal to the rate of washout of the ^{3}H-water reference, K = 1.4 min^{-1} (7). The observation that the change in LUI with time is only a function of ^{3}H-water washout indicates both T_3 and T_4, after unidirectional clearance by liver cells, are essentially completely sequestered by liver for at least 90 s after injection.

The process mediating sequestration of T_3 radioactivity was not saturated by making the injection solution 50 μM T_3; e.g., the 90-s LUI, 523±49 (mean ±SEM, n = 3 rats), was not significantly different from the control (Fig. 3). Conversion of T_3 to a polar metabolite could explain the sequestration of the hormone; therefore, the liver was freeze-clamped 90 s after injection of labeled T_3 (Methods). Thin-layer chromatography and radioscanning of the liver homogenate revealed only one peak which comigrated exactly with the [^{125}I]T_3 standard; these results suggest T_3 is not significantly metabolized during the 90-s period after injection.

The 1-octanol/Ringer's (pH 7.4) partition coefficients for [^{125}I]T_3 and [^{125}I]T_4 were 180±10 and 91±14 (mean ±SEM, n = 3), respectively.

DISCUSSION

These studies bear on two aspects of hepatocyte plasma membrane transport of T_4 and T_3; (*a*) the mechanism by which T_4 or T_3 traverses the hepatocyte cell membrane, and (*b*) the role of the thyroid hormone binding plasma proteins in limiting transmembrane transport.

In general, compounds such as T_4 or T_3 may traverse the liver cell membrane by one of two mechanisms: (*a*) lipid-mediation (free diffusion), which does not involve ligand binding at the cell membrane, or (*b*) carrier-mediation (facilitated diffusion, active transport), which involves T_4 or T_3 binding to a cell membrane transport system. The evidence presented here and by others (18) suggest T_3 and T_4 enter liver cells via lipid mediation. Hillier (18) has previously shown that T_4 or T_3 transport into the perfused rat liver is nonsaturable up to a thyronine concentration of 1 μM. Similarly, [^{125}I]T_3 or [^{125}I]T_4 transport in vivo is not affected by a 50-μM concentration of hormone (Results). Further evidence in favor of lipid mediation is the high lipid solutility of T_3 or T_4, e.g., the octanol/Ringer's partition coefficients for T_3 and T_4 is 180 and 90, respectively (Results).

The high partition coefficients for T_4 and T_3 measured here are in contrast to the heptane/saline ratios reported for T_4, 0.0004, and T_3, 0.3 (19). However, Dietschy (20) has emphasized the importance of using polar organic solvents, not nonpolar solvents such as heptane, to mimic the lipid solubility of biological membranes. For example, if a highly polar lipid such as lecithin is used, the lipid partition coefficient for T_4 (12,000) or T_3 (22,000) is very high (21).

Although the high lipid solubility of T_4 and T_3 and the nonsaturability of thyronine transport into liver is evidence in favor of lipid-mediation, other studies re-

port data in favor of carrier-mediation (22, 23). The transport of T_3 into isolated liver cells at 21–23°C has been shown to be saturable (22, 23). A similar discrepancy has been observed for liver cell membrane transport of the steroid hormones, wherein steroid transport in vivo is nonsaturable (7), but hormone transport in vitro is saturable (24). However, it is likely that extracellular steroid or thyroid hormone equilibrates instantaneously in vitro where the volume of extracellular fluid is log orders greater than the intracellular space; under these conditions, the major factor determining hormone uptake might be intracellular binding systems. Indeed, the cell/medium ratio of labeled T_3 in isolated liver cells is 20–30 by only 20 s of incubation (23); these data suggest that the process of transmembrane movement of T_3 reaches equilibrium nearly instantaneously in vitro, and that the net uptake of T_3 by the liver cell is determined primarily by an intracellular sequestration system. The data in Fig. 3 indicate that once radiolabeled T_3 or T_4 enter liver cells, these molecules are immediately and completely sequestered by intracellular binding systems. In regard to the present in vivo approach, it is unlikely that T_3 or T_4 equilibrate instantaneously across the hepatocyte cell membrane because the extraction of T_3 (E_T = 77±2%) or T_4 (E_T = 43±2%) is not in excess of 95%, which would be expected if instantaneous equilibration occurred. Therefore, results obtained with a portal vein injection method can be considered to accurately reflect the kinetics of transmembrane transport of T_3 or T_4 and to be independent of intracellular binding systems.

The need for an experimental approach that accurately measures unidirectional influx of T_3 or T_4 through the liver cell membrane is also of importance in assessing the effect of the plasma proteins on the transport of circulating T_4 or T_3 into liver cells. As shown by Oppenheimer and associates (25), the net uptake of thyroid hormones is strongly influenced by intracellular binding systems; therefore, if net clearance of thyroid hormone is measured, the separation of plasma protein effects from cytoplasmic protein effects may be difficult.

In addition to permitting the measurement of unidirectional influx, the portal vein injection technique used in the present studies has the novel advantage of allowing the study of human sera in an in vivo rat liver paradigm (7). Because the rate of portal injection exceeds the rate of portal blood flow, the injection solution enters the liver circulation as a bolus, which makes a single pass of liver without appreciable mixing with circulating rat plasma. Therefore, the concentration of plasma proteins added to the injection solution approximates the composition of the microvasculature at the time of [^{125}I]T_3 or [^{125}I]T_4 transport. The maintenance of bolus flow has been documented in numerous studies of brain capillary transport using a carotid injection technique (3, 10, 26). A similar maintenance of bolus flow occurs in liver transport studies, as shown by the marked difference in T_4 transport, depending on whether Ringer's solution (E_T = 43±2%) or rat serum (E_T = 20±2%) is injected (Fig. 2). If appreciable mixing of the injection solution with rat plasma occurred during bolus transit through the liver, then the relatively high E_T for T_4 after injection of Ringer's solution would not be observed.[3]

Rat plasma contains two T_4 binding proteins, albumin and TBPA (27, 28). Although albumin binds considerable quantities of T_4, albumin-bound T_4 is readily dissociated and transported into liver (Fig. 1). Therefore, the 82% inhibition (Table I) of intracellular distribution of T_4 by rat serum may be attributed to TBPA. Based on the data of Sutherland and associates (27), it may be calculated that 86% of rat plasma T_4 is TBPA-bound and 14% is albumin bound.[4] Therefore, the percent inhibition of T_4 transport by rat plasma correlates with the percent distribution of T_4 to TBPA; and the albumin-bound moiety approximates the fraction of T_4 transported in vivo (Fig. 1). Human serum results in an even greater inhibition of T_4 transport (Fig. 2); the presence of TBG results in a lowering to 5–10% of the percent distribution of T_4 to albumin (29). Since the percent of T_4 bound to albumin in human serum (5–10%) approximates the experimental variability (5–10%) of the technique (Table I), a LUI for T_4 that is statistically different from the antiserum background is obtained only for normal human serum (Table I). In addition to the data in Figs. 1 and 2, there is experimental evidence in man, consistent with the proposal that albumin-bound T_4 is readily available for transport into liver. Unidirectional extraction of T_4 clearance by human liver, as determined by a venous sampling-single injection technique, is 7% (30). The value is 200 times the free (dialyzable) T_4 fraction, ~0.03% (1), and approximates the albumin-bound T_4 fraction in human serum (29). Because the hepatic capillary transit times are likely to be of similar magnitude in the rat and in man, it is probable that the

[3] It might be argued that the real E_T for T_4 is much higher than 43±2% and mixing of the bolus with circulating rat TBPA did occur and resulted in a lowering of E_T to 43%. However, if this were the case, the injection of 50 μM unlabeled T_4 (Results) would have saturated all TBPA binding sites and caused an elevation in E_T for T_4. Since no increase was observed, it is concluded that no significant mixing of the bolus with rat plasma occurred.

[4] The percent distribution of T_4 or T_3 may be approximated by (BIa)/ΣBI, where BIa is the binding index (binder concentration ÷ dissociation constant) of the protein in question and ΣBI is the sum of binding indices of all thyronine binding plasma proteins (1).

7% unidirectional extraction of T_4 in human liver represents distribution into liver cells of T_4 bound to albumin.

Since both the free (dialyzable) and albumin-bound fractions are inversely related to the existing TBG level, unidirectional T_4 clearance by liver is regulated by changes in TBG. In contrast to T_4, the data in Fig. 2 and Table I indicate that the transport of T_3 bound to TBG into liver cells is extensive. About 70% of total T_3 is bound to TBG and about 30% is bound to albumin (31). Because the T_3 that is albumin-bound is freely cleared by liver, the observation that 60–70% of total T_3 is available for transport (Table I) indicates that half of the T_3 bound to TBG is available for transport into liver. Moreover, the observation that conditions in which TBG is elevated, e.g., pregnancy or oral contraceptive use (1), are characterized by only a negligible inhibition of T_3 transport (Fig. 2) is consistent with the proposal that TBG-bound T_3 is readily available for transport into liver. The conclusion that TBG exerts relatively little influence over the unidirectional hepatic clearance of T_3 has been suggested previously by Zaninovich et al. (32). Although the experimental approach used by these workers (32) has since been criticized as an unreliable measurement of unidirectional clearance (33), it is significant that the studies reported herein lead to the same conclusions originally suggested by Zaninovich and co-workers (32).

The fractions of plasma T_4 or T_3 that are available for transport into liver are different from the fraction of hormone available for transport into brain. Only a small fraction (~10%) of albumin-bound T_3, and probably no TBG-bound T_3, is transported into the brain (10). The basis for the different rate of transport of protein-bound thyroid hormone into brain and liver would appear to be twofold. Firstly, the permeability of the liver cell membrane to T_3 and T_4 is much greater in liver (Table I, [10]). Secondly, the capillary transit time is considerably longer in hepatic sinusoids relative to brain capillaries (3, 6). As mentioned in the introduction, the capillary transit time, membrane permeability, and ligand unidirectional debinding rates, appear to be the major factors that determine the extent to which protein-bound hormones are transported into tissues (3, 7, 10, 34–36).

Finally, the relationship between the unidirectional clearance estimates for T_4 and T_3 reported in the present studies and the net or bidirectional hepatic clearance of hormone should be discussed. As emphasized by Oppenheimer and associates (25), the net retention of thyroid hormone by liver is a function of the tissue binding index[3] relative to the plasma binding index. Assuming no changes occur in tissue binding of thyroid hormone, then alterations in plasma binding parameters should reliably predict the net transport of the hormone into the tissue. However, what is less certain is whether in vitro measurements, e.g., Table II, accurately estimate the in vivo binding index of thyroid binding plasma proteins. Despite the extensive unidirectional transport of protein-bound thyroid hormones into tissues, it might be argued that the net flux of hormone is inversely related to the total binding index of the plasma, which is reliably estimated by the in vitro free (dialyzable) fraction (25). However, it is our view (3, 7, 10, 34–36) that, under many circumstances, the transport of protein-bound hormones into tissues in vivo cannot be accurately predicted by the in vitro binding index. The in vivo measurement of plasma protein binding in the model we have used, i.e., Table I, may provide a more accurate index of what is actually transported than does an in vitro assay. However, if only albumin-bound hormone is available for transport into the tissue, e.g., T_4 or T_3 influx into brain (10) or T_4 influx into liver (Fig. 1), then the in vitro free hormone fraction should reliably predict the amount of plasma hormone available for transport. This is because both the free (dialyzable) and albumin-bound fractions will, under most circumstances, be inversely related to the existing TBG or TBPA levels. Conversely, both albumin-bound and TBG-bound T_3 are readily available for transport into liver (Fig. 1). Under these conditions, increases in TBG concentrations, e.g., pregnancy or estrogen treatment, would be expected to substantially reduce the free (dialyzable) fraction in vitro (Table II), yet have relatively little influence on the plasma binding of T_3 in liver sinusoids in vivo (Table I, Fig. 2). Therefore, it is probable that in vitro measurements of free T_3 do not reliably predict T_3 clearance by liver in vivo.

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