Triiodothyronine Binding in Rat Anterior Pituitary, Posterior Pituitary, Median Eminence and Brain

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ABSTRACT. *In vivo* studies of the exchange of tracer [^{125}I]L-triiodothyronine (T_3) between plasma (P), and the anterior pituitary (AP), posterior pituitary (PP), median eminence (ME) and the frontal lobes of the brain (B), in the rat show that from 2.5 h onwards the concentration of net [^{125}I]T_3 in AP, PP and B were parallel to that of the plasma, with a $t_{\frac{1}{2}}$ of 7.4 h; the $t_{\frac{1}{2}}$ for ME was 10.3 h.

The extrapolation of these curves to zero time was assumed to indicate the relative concentration of T_3 per unit weight in terms of total body T_3. T_3 content of these tissues was determined by radioimmunoassay. The values obtained validated the steady state parameters derived from the radio-isotopic measurements.

As an indicator of the concentration gradient between tissue and plasma the organ/plasma (O/P) ratio was calculated; these data indicate that under steady state conditions, the order of T_3 concentration is AP > PP > ME > B.

Binding studies have shown that AP and PP contain "specific," saturable binders while ME and B do not. Evaluation of the binding parameters of the high affinity binders in both AP and PP gave similar association constants. These association constants, when corrected for the binding strength of T_3 to plasma proteins, resulted in values similar to those of nuclear T_3 binders. (*Endocrinology* 96: 1357, 1975)

THE anterior and posterior pituitary were shown by Ford *et al.* (1) to concentrate triiodothyronine. Recently, Schadlow *et al.* (2) have indicated the presence of "specific" T_3 binding sites in the anterior pituitary. It was further shown by Bar-Sela *et al.* (3,4) that T_3 is localized mainly in the somatotrophs and thyrotrophs of the anterior pituitary (AP), in the pituicytes of the posterior pituitary (PP) and in the ependyma, glia and some nerve endings of the median eminence (ME). Since T_3 concentrates within cells of certain hypophyseal structures, it was of interest to know whether specific binding sites for T_3 exist within these structures. In this study we examined the kinetics of interchange of labeled T_3 between the plasma and the AP, PP, ME, and the brain (B), the latter serving as a control tissue. Furthermore, binding capacity studies were performed in each tissue in order to determine the existence of specific binding sites for T_3. And finally, the T_3 content of each tissue was measured and these values served to confirm the data obtained from radioisotopic studies.

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Materials and Methods

[^{125}I]T_3 distribution between plasma and organs

Male Hebrew University rats weighing 90–120 g were injected through the tail vein with a combined dose of 20 μCi of [^{125}I]T_3 (SA 1.5 mCi/μg prepared in this laboratory by chloramine-T iodination) and 5 μCi of [^{131}I]human serum albumin (SA 1 μCi/mg, purchased from the Israel Atomic Energy Commission, Nuclear Research Center, Negev). The purity of [^{125}I]T_3 was tested chromatographically (7) and always exceeded 96%.

Groups of animals were sacrificed at intervals of 0.5 to 24 h after the injection, by exsanguination from the abdominal aorta and inferior vena cava. Blood was collected and the anterior pituitary (AP), posterior pituitary (PP), the median eminence (ME) and parts of the brain's frontal lobes (B) were rapidly removed, weighed and homogenized in 2.5 ml of human plasma. The tissue homogenates as well as 0.1 ml of rat plasma in carrier human plasma were precipitated by the addition of 10% TCA vol/vol and the precipitates were counted to a statistical counting error less than 3.4% in a double

channel scintillation counter (Packard Auto Gamma Spectrometer).

In order to determine the net tissue or plasma exchangeable $[^{125}I]T_3$ concentrations, the $PB^{125}I$ values were corrected as follows:

(a) A correction was made for the $[^{125}I]T_3$ bound to trapped plasma proteins within the tissues, by the formula suggested by Hasen *et al*, (5) in which the $[^{131}I]$albumin was used as a marker of plasma proteins:

$$C = O_{^{125}I} - \frac{P_{^{125}I}}{P_{^{131}I}} \times O_{^{131}I}$$

where:

C = corrected organ concentration of $[^{125}I]T_3$ (% dose/g)
$O^{125}I$ = total organ concentration of $[^{125}I]T_3$ (% dose/g)
$P^{125}I$ = plasma concentration of $[^{125}I]T_3$ (% dose/ml)
$P^{131}I$ = plasma concentration of $[^{131}I]$albumin (% dose/ml)
$O^{131}I$ = organ concentration of $[^{131}I]$albumin (% dose/g)

Trapped plasma was found to be (mean ± SE):

AP:	0.161 ± 0.006 ml/g tissue
PP:	0.135 ± 0.012 ml/g tissue
ME:	0.023 ± 0.002 ml/g tissue
B:	0.013 ± 0.006 ml/g tissue

The $PB^{125}I$ values were reduced, therefore, by a factor of 2–4% representing the $[^{125}I]T_3$ trapped with plasma proteins.

(b) A correction was made for non-exchangeable (NEI) iodine formed within the tissues and plasma (6). Its amount was determined by extracting the TCA precipitates 6 times with 4 volumes of 96% ethanol (6). The percent of ^{125}I remained in the pellet was subtracted from the corrected $PB^{125}I$ values. The % NEI was found to be maximal in plasma (ranging between 7–13%). Tissues % NEI ranged between 0.04–3.8%

(c) Chromatographic analysis (7) of the ethanolic extracts indicated that between 90–96% of the ^{125}I of AP, PP, ME and plasma consisted of $[^{125}I]T_3$. In the brain 64–78% of the ^{125}I was recovered as T_3 while 15–20% of the ^{125}I was found in a spot migrating slower than T_3; therefore the $[^{125}I]T_3$ concentration of the brain was corrected accordingly.

The net $[^{125}I]T_3$ content of the organs and plasma were expressed in terms of percent injected dose/g tissue (or ml plasma), normalized for 100 g body weight. The net organ/plasma ratio (O/P) for the various organs were calculated for each time interval.

The effect of T_3 loading on $[^{125}I]T_3$ distribution

In these experiments normal as well as thyroidectomized rats were used. Surgical thyroidectomy was performed either 6 days or 1 month before sacrifice. The thyroidectomized animals were kept on a low iodine diet and distilled water containing 1% $CaCl_2$.

In order to test the efficiency of the thyroidectomy the T_4 and T_3 content of the sera were determined (Tetralute® for T_4 and Seralute® for T_3, Ames, Elkhart USA). The T_3-radioimmunoassay coefficients of variance for quadruplicates were 6% for intra-assays and 7% for interassay. T_3 content fell from the normal control values of 0.81 ± 0.09 (mean ± SD) ng/ml to 0.08 ± 0.010 ng/ml in the 7 days post-thyroidectomy rats and to undetectable levels at 1 month after thyroidectomy. Likewise, T_4 values fell from normal 28 ± 2.2 ng/ml to 1 ng/ml ± 0.6, 7 days after thyroidectomy, and to undetectable levels at 1 month after thyroidectomy.

The effect of T_3 on $[^{125}I]T_3$ distribution in both normal and thyroidectomized rats was tested as follows: groups of animals were injected with either tracer $[^{125}I]T_3$ or $[^{125}I]T_3$ together with various loads of T_3 (ranging from 6 ng to 11 μg T_3/100 g BW). Three or four hours later the animals were sacrificed and their tissues and plasma were treated as previously described. The O/P ratio was determined for each of the organs studied.

In order to test the net strength of the plasma binding of T_3, the T_3 dialyzable fraction (DF_3) was determined in the plasma of normal and thyroidectomized rats (8).

T_3 content of tissues

100 males rats were sacrificed by exsanguination. Their AP, PP, ME, as well as parts of the frontal lobes of their brain were removed, weighed, pooled and homogenized in H_2O.

The appropriate tissues from 2 rats, labeled *in vivo* 3 h previously with $[^{125}I]T_3$, were homogenized together with the pooled organs and their label served for recovery calculations. The homogenates were extracted with 4 ml of

96% ethanol with a recovery of about 80% of [^{125}I]T_3. The extracts were dried with N_2 at room temperature and the residue was dissolved in T_3-free serum. T_3 was determined by radioimmunoassay (Seralute® Ames) and the tissue content was corrected for [^{125}I]T_3 recovery, as well as for T_3 bound to trapped plasma proteins, by using the average values of trapped plasma obtained previously. DNA content of each tissue was determined by a fluorometric technique adapted for the central nervous system (9). T_3 content of tissue was also calculated as ng T_3/mg DNA.

Results

Kinetics of interchange of T_3 between organs and plasma

The change in the exchangeable [^{125}I]T_3 concentrations in organs and plasma with time is demonstrated in Fig. 1. From 2.5 h onwards the disappearance curves of [^{125}I]T_3 from AP, PP, and B are parallel to that of the plasma, with a half life of 7–7.8 h (Table 1). The $t_{\frac{1}{2}}$ for the ME was found to be significantly longer (10.3 h, $P < 0.001$). The data presented in Fig. 1 show therefore that the O/P ratios remain constant over the 24 h of observation (Table 1, column 2). The kinetics of the exchangeable [^{125}I]T_3 could be approximated by a single compartment distribution. Therefore, the zero time intercepts of the regression lines could be taken as a measure of the relative T_3 pool size of the organs in terms of percent of total body T_3. These extrapolated values are summarized in the 3rd column of Table 1, and were used to calculate the extrapolated organ/plasma ratios (O/P) appearing in column 4 of Table 1. As seen, the extrapolated O/P ratios representing the pool sizes approximate closely the O/P ratios derived from direct observations.

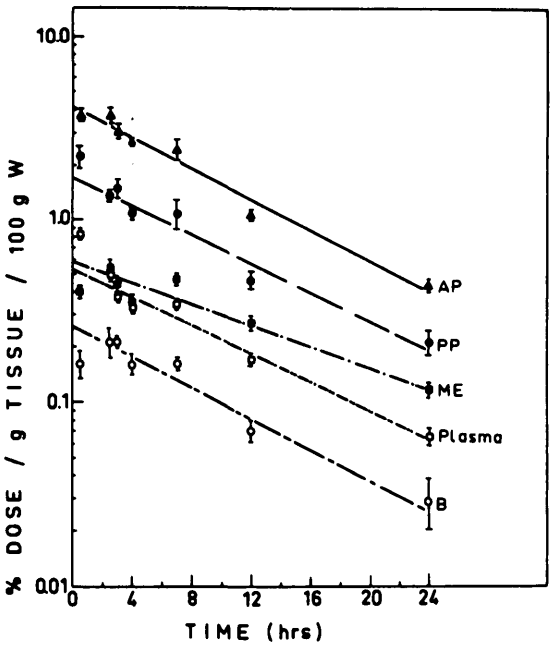

FIG. 1. Net exchangeable [^{125}I]T_3 concentration in the various tissues at different time intervals after an iv injection of tracer T_3. The increase in plasma T_3 concentration due to the injected isotope did not exceed 4% of the endogenous plasma T_3. Each point represents at least 4 animals. The bars around each point mark the mean ± SEM. The points of the regression curve were obtained in 5 different experiments. The net exchangeable [^{125}I]T_3 was determined by the modifications detailed in Materials and Methods.

TABLE 1. Relative pool size and half-life of exchangeable [^{125}I]T_3

Organ	$t_{\frac{1}{2}}$ in h*	Measured O/P mean ± SEM (N)	Extrapolated % ID/g**	Calculated O/P***
Plasma	7.8	—	0.53	—
Anterior pituitary	7.1	7.16 ± 0.25 (23)	4.16	7.85
Posterior pituitary	7.6	3.19 ± 0.15 (25)	1.70	3.20
Median Eminence	10.3	1.38 ± 0.67 (26)	0.58	1.09
Frontal lobe of brain	7.0	0.46 ± 0.02 (23)	0.28	0.49

* $t_{\frac{1}{2}}$ was calculated from the regression presented in Fig. 1.
** This value is the extrapolated zero time [^{125}I]T_3 content as % of injected dose (ID)/g tissue or ml plasma.
*** Organ [^{125}I]T_3/plasma [^{125}I]T_3 concentration ratios, are obtained using the values appearing in column 3. The number of animals are shown in parenthesis (N).

TABLE 2. T_3 content of the plasma, anterior pituitary, posterior pituitary, median eminence, and frontal lobes of the rat's brain

Organ	Calculated* T_3 ng/g	T_3 Measured by** R.I.A. ng/g mean ± SEM	DNA mg/g mean ± SEM	T_3 ng/mg DNA
Plasma	—	0.81 ± 0.05	—	
Anterior pituitary	6.38	6.78 ± 0.06	15.6 ± 0.5	0.43 (0.72)***
Posterior pituitary	2.60	2.63 ± 0.69	4.2 ± 0.3	0.63
Median eminence	0.89	1.47 ± 0.36	0.4 ± 0.3	3.68
Frontal lobe of brain	0.40	0.55****	3.7 ± 0.3	0.15

* Sample of calculation: extrapolated O/P value for AP from column 3 of Table 1 = 7.85; [T_3]plasma = 0.81 ng/ml. [T_3]AP = [T_3]plasma × O/P = 0.81 ng/ml × 7.85 = 6.38 ng/g.

** T_3 RIA measured as described in Materials and Methods. Values are means of 3 experiments.

*** T_3 ng/mg DNA for the AP in brackets, indicates the value obtained if only 60% of the cells of the AP are involved in concentrating T_3.

**** The corresponding value for the whole brain was found to be 2.23 ± 0.28 corresponding to an O/P of 2.7, probably reflecting concentration of T_3 in the choroid plexus (1).

The T_3 content in 4 pools of normal rat plasma was found to be 0.81 ± 0.09 ng/ml (mean ± SD). When this value was used, the expected organ concentration could be easily calculated by multiplying the extrapolated O/P ratio of each organ by the average plasma concentration (Table 2, column 1, and footnote for an example of calculation). Since these figures were based on tracer measurements their validity was tested by measuring directly the T_3 content of the various tissues. Table 2 indicates that the two sets of data, the calculated and the measured (col. 1 and col. 2), correspond well with each other. Therefore the O/P values obtained by tracer technique closely approximate the steady state situation.

It was shown by Bar-Sela *et al.* (3,4) that T_3 concentrated within the pituicytes of the PP. In the AP T_3 concentrated primarily within the somatotrophs and thyrotrophs and to a much lesser degree in the gonadotrophs; while in the median eminence T_3 concentrated within the ependyma, glia, as well as some of the nerve endings. Therefore, the T_3 content per unit DNA was calculated for each tissue (Table 2, column 3). Since approximately 60% of the cells of AP accumulate T_3, the value obtained for the AP should be corrected. Therefore, the corrected value of the AP, 0.72 ng T_3/mg DNA, is similar to that of the PP, 0.63 ng T_3/mg DNA, and both are about five times higher than the T_3 concentration per mg DNA of the frontal lobes of the brain.

The high value of T_3/mg DNA for the ME cannot be compared with the other tissues, since T_3 in this organ concentrates also in the nerve endings which originate in hypothalmic nuclei not sampled, while the DNA measured is primarily that of the glia cells.

Saturation characteristics of T_3 binders

The effect of T_3 loading on the isotopic O/P values of the various organs at 3 h is depicted in Fig. 2. A significant decrease of the O/P values in both AP and PP is seen, while in both ME and B the O/P ratios were not affected by T_3 loading. The drop in O/P values was due to changes in tissue content, since plasma [^{125}I]T_3 concentration did not vary with the increasing loads of T_3. In the AP the change of O/P was noted in normal animals loaded with T_3 as high as 1.5 μg/100 g BW while the changes in the PP were noted only in the hypothyroid animals given small loads of T_3.

The O/P values of the AP in the normal and 6 days post-thyroidectomy animals were always higher than the values obtained in the 1 month post-thyroidectomy animals given the same load of T_3 (Fig. 2). However, the change in O/P per unit load

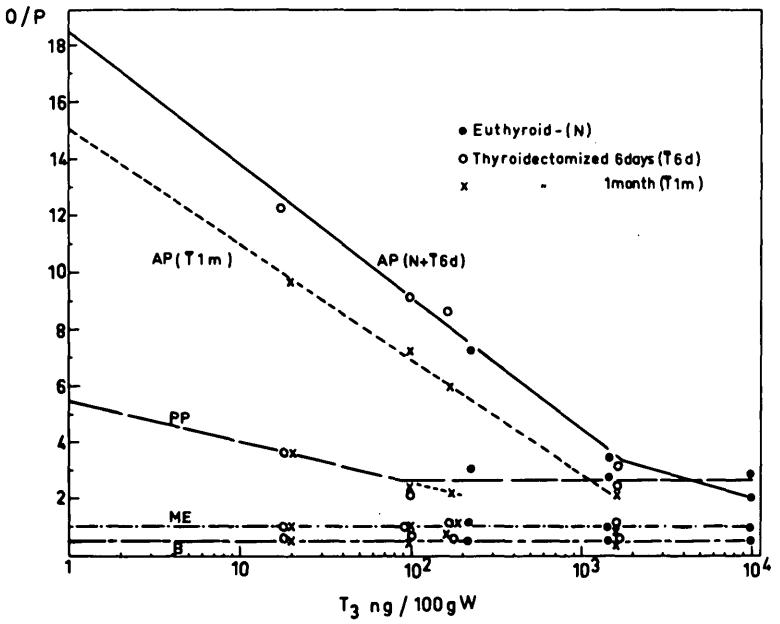

FIG. 2. Effect of T_3 loading on the organ/plasma ratio. Normal as well as thyroidectomized rats were injected through the tail vein with [^{125}I]T_3 and T_3 mixture. The animals were killed at 3 h after the injection; organ and plasma concentrations of exchangeable [^{125}I]T_3 were determined as described in the methods. The T_3 load (ng/100 g BW) was taken as the sum of the endogenous T_3 and the injected dose. The lowest load in each experiment represents endogenous T_3 plus the amount of T_3 present in the tracer dose (ranging between 6–30 ng/100 g BW). The endogenous T_3 is the product of blood T_3 level and the space of distribution (determined for euthyroid rats from Fig. 1, and for 6 days post-thyroidectomy rats in separate experiment not shown here). The endogenous T_3 in the 1 month post-thyroidectomy rats was disregarded since their blood T_3 levels were undetectable (see Materials and Methods). Each point represents at least 4 animals. Lines were calculated by the method of least squares. The SD around each mean did not exceed 25% of the mean.

of T_3 was the same in both types of animals.

Figures 3 and 4 describe the relationship between the T_3 concentration in plasma (P) and organ (O), where O/P is plotted against O. These curves are essentially Scatchard type curves where O/P is used instead of B/F, according to the following formulation:

Let

P = plasma T_3 concentration,
O = organ T_3 concentration,
N = organ "empty" binding sites,
M = organ maximal binding capacity.

If P is in equilibrium with O then:

$$P + N \rightleftharpoons O$$

$$Ka = \frac{[O]}{[P][N]} = \frac{[O]}{[P][M - O]}$$

$$O/P = KaM - KaO$$

The slope of these curves equals the "relative" association constant (Ka) and the Y axis intercept equals KaM where M is the maximal binding capacity. The validity of using this relationship for the determination of both Ka and M was thoroughly discussed by Oppenheimer *et al.* (10), and by Coutsoftides and Gordon (11).

As is evident from Fig. 2 the O/P values of AP from normal and 6 days post-thyroidectomy animals fall essentially on the same regression curve while those of 1 month post-thyroidectomy animals belong to another curve with a similar slope but a somewhat lower intercept. Therefore in Fig. 3 two "Scatchard" plots are presented: one for T_3 in the AP of euthyroid and 6 day post-thyroidectomy rats, and the other for 1 month post-thyroidectomy rats. Both plots indicate the presence of "specific" T_3 binder with a similar high affinity and a low capacity. A second T_3 binder with a lower affinity and a much higher capacity is demonstrated also in the Scatchard plot of normal and 6 days post-thyroidectomy rats. Figure 4 shows the corresponding situation for the PP from the thyroidectomized rats. As in the AP two T_3

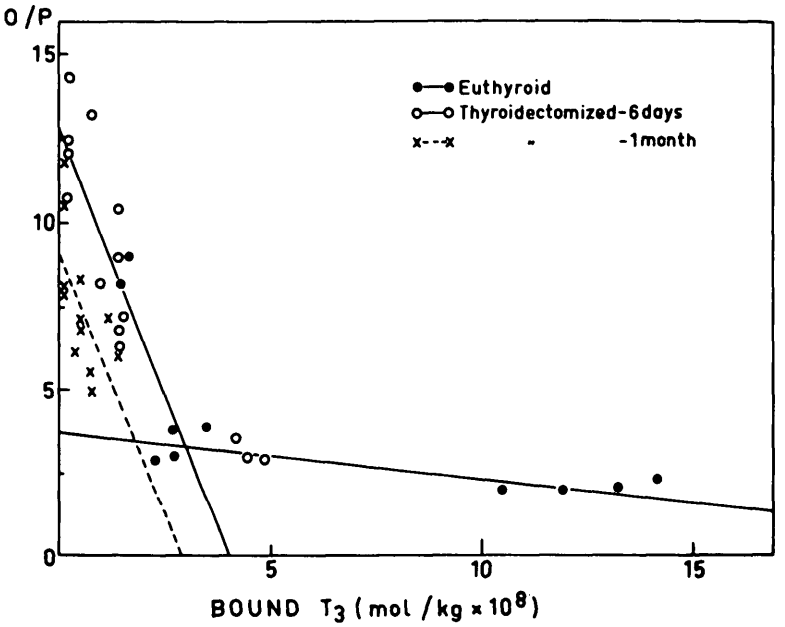

FIG. 3. "Scatchard"-type plots for the anterior pituitary binding of T_3. Solid curve represents normal and 6 days post-thyroidectomy rats and the dotted curve, 1 month post-thyroidectomy rats. Organ/Plasma ratio is plotted against the organ concentration of T_3 in rats which were injected iv with various loads of T_3 and their tissue analyzed 3 h later. For rationale of using this approach see section of Results. Each point represents 1 rat. Lines were calculated by the method of least squares.

binders are indicated, except that the second binder of the PP is not saturable within the range of T_3 loads used in this experiment. The characteristics of the first binders of both PP and AP are summarized in Table 3. The association constant in both organs do not differ significantly from each other. On the other hand the maximal binding capacity for the PP is about one sixth of the maximal binding capacity of the AP (0.46×10^{-8}mol/kg vs 2.84×10^{-8}mol/kg). The maximal binding capacity of the 1 month post-thyroidectomy AP is slightly, though significantly, lower (2.0×10^{-8}mol/kg) than that of the normal and 6 days post-thyroidectomy rats. The weight of the AP in the 1 month hypothyroid rats is significantly increased over that of the normal rats. If then, the maximal binding capacity is expressed in terms

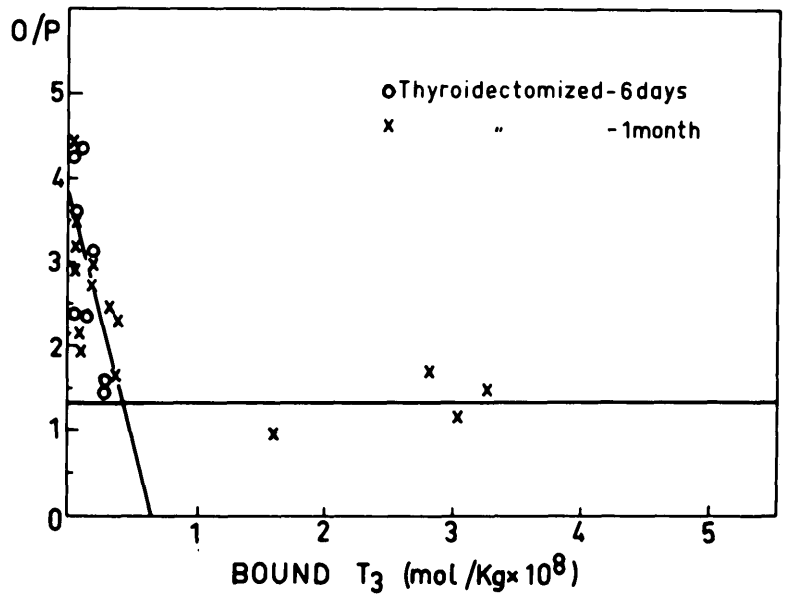

FIG. 4. "Scatchard"-type plot for the posterior pituitary binding of T_3 of 6 days and 1 month post-thyroidectomy rats. O/P ratio is plotted against the organ concentration of T_3 in rats which were injected with various loads of T_3 and their tissue analyzed 3 h later. Each point represents 1 rat. Lines were calculated by the method of least squares.

TABLE 3. Association constant (Ka) and maximal binding capacity (M) of T_3 first binder in the anterior and posterior pituitary

Organ	Ka kg/mol*	M mol/kg	Weight of gland in mg mean ± SEM	M mol/gland
Anterior pituitary				
Euthyroid + $\bar{T}$ 6 days	3.4×10^8	2.84×10^{-8}	3.79 ± 0.12	1.08×10^{-13}
$\bar{T}$ 1 month	3.3×10^8	2.00×10^{-8}	4.93 ± 0.19	0.98×10^{-13}
Posterior pituitary				
$\bar{T}$ 6 days + $\bar{T}$ 1 month	5.6×10^8	0.46×10^{-8}	0.79 ± 0.02	3.63×10^{-15}

* Ka = association constant relative to plasma; when corrected for plasma binding strength (10), the association constant increases to an order of magnitude of 10^{11} (see text).
$\bar{T}$ = thyroidectomized animals.

of moles per gland rather than mol/kg gland, the M of the AP in both groups becomes indistinguishable (Table 3), as if hypothyroidism resulted in a "dilution" of binding sites within the enlarged gland.

Discussion

Exchange of tracer $[^{125}I]T_3$ between plasma and the anterior pituitary, posterior pituitary, and the frontal lobes of the brain in the rat shows that isotopic equilibrium is already attained at 2.5 h. The rate of disappearance of exchangeable $[^{125}I]T_3$ in these tissues is similar, having a $t_{\frac{1}{2}}$ of 7 to 7.8 h. The organ/plasma ratio of exchangeable $[^{125}I]T_3$ remained therefore, relatively constant over the 24 h of observation. Similar results were found for the AP by Schadlow *et al.* (2). The O/P ratios obtained were considered to reflect the steady state O/P ratio, and by substituting the plasma T_3 concentration, the expected concentration of T_3 in the tissue was calculated. The fact that T_3 determination of tissue content was in close agreement with those expected from the isotopic studies validates the following implicit assumptions: 1) That isotopic steady state values reflect the true tissue parameters; 2) That T_3, as determined by radioimmunoassay, measures primarily exhangeable T_3.

The median eminence exhibited a $[^{125}I]T_3$ disappearance curve with a $t_{\frac{1}{2}}$ of 10.3 h, significantly longer than that of plasma. This phenomena may indicate that the ME equilibrates with an additional source of hormone, possibly the CSF of the third ventricle (12). Therefore in the ME the T_3 content calculated from the extrapolated O/P values was only 60% of T_3 value actually found by radioimmunoassay measurement.

Our experiments show that in the normal animal the order of T_3 concentrations is AP > PP > ME > P > B. However when T_3 concentration is calculated per cell (in terms of DNA), the AP and the PP show similar values.

Furthermore the data presented on T_3 binding characteristics in the various tissues indicate that both AP and PP show "specific" T_3 binding with two types of binders. The first binder of the PP and both binders of the AP are saturable "specific" binders. The second binder of the PP and the binders of the ME and brain are practically nonsaturable with T_3 loads as high as 11 μg T_3/100 g BW, and therefore considered non-"specific".

The association constants obtained were "relative" association constant since they were determined from O/P ratios where P was not corrected for its free T_3 concentration. The "true" association contant could be estimated from a formula suggested by Oppenheimer *et al.* (10); where $K = \frac{[Ka][O/P]}{[DF_3]}$; K = "true" association constant, Ka = "relative" association constant, [O/P] = the steady state O/P ratio and

$[DF_3]$ = the T_3 dialyzable fraction. The "true" association constants for the first binder of the AP and PP were calculated to be 9.0×10^{11} and 6.0×10^{11} kg/mol respectively.

Samuels and Tsai (13) studied the equilibrium of T_3 with the nuclei of GH_1 cells grown in tissue culture and observed that equilibrium of T_3 with the nuclear receptors occurred at 3 h, and that these binders had an association constant of 0.34×10^{11} kg/mol. Likewise Oppenheimer *et al.* (10), studying rat liver nuclear binding *in vivo*, noted that equilibration with the nuclear receptors occurred between 1–2 h and that the apparent or "true" association constant for these binders was 4.7×10^{11} kg/mol.

In our experiment *in vivo* the equilibration of both AP and PP occurred at about 2.5 h and the apparent or "true" association constant calculated for the tissue as a whole was in the order of 10^{11}–10^{12} kg/mol. Thus, it appears that the equilibration of these tissues with plasma is occurring with tissue receptors having binding parameters similar to those described for nuclear receptors in other tissues of the rat (10,13).

The maximal binding capacity of the first binder in the AP was found to be 2.84×10^{-8} mol/kg. This value agrees with 1.9–2.4×10^{-8} mol/kg values obtained by Schadlow *et al.* (2), who concluded from their experiments that the anterior pituitary binding of T_3 is close to saturation in the normal rat. This has not been validated by our data; we have found both from isotopic estimation and actual measurements of T_3 that the AP contains about 1.0×10^{-8} mol/kg T_3 in the normal animal, which is about 40–50% of the binding capacity of the first binder.

The comparison of the binding parameters of anterior pituitaries from normal rats to those of rats 30 days after thyroidectomy may shed some understanding on the type of cells responsible for T_3 binding in the AP of the normal rat.

The cell population of the normal AP in the rat consists of 45% acidophilic cells and 10% basophilic cells (14). Hypothyroidism (1 month) results in an increase of the number of cells and their size (15) and in a shift in the cellular proportions, so that 55% of the cells became basophils while the acidophils (somatotrophs) became degranulated and appeared microscopically as chromophobes (14). The fate of these degranulated somatotrophs is not as yet clearly known.

If we assume that in the euthyroid AP the binding parameters of the first binder represented binding exclusively within the thyrotrophs, then 30 days after thyroidectomy a marked increase in the binding capacity would be expected, parallel to the increase in the thyrotroph population (14,15). The finding that hypothyroidism did not result in a rise in the M of the first binder of the AP lends itself to the following hypothetical interpretations:

(a) The first binder of T_3 in the AP does not belong to the thyrotrophs but to the somatotrophs, which continue to bind T_3 in an unchanged manner despite their degranulation.

(b) The first binder of the AP belongs to both somatotrophs and thyrotrophs. Therefore in the euthyroid AP, T_3 binds primarily to somatotrophs which constitute the majority of the binding cells. In the hypothyroid AP the T_3 binding by thyrotrophs is increased and the constancy of the binding capacity suggests a decrease in binding by the degranulated somatotrophs.

Both hypotheses are compatible with the assumption that in the euthyroid animal the first T_3 binder in the AP is found primarily within the somatotrophs.

Based on the data of this work and on the interpretation presented in the discussion, the following tentative conclusions can be drawn:

1) The association constants of the first T_3 binders in both AP and PP suggest that *in vivo* the T_3 gradients established between organ and plasma are due to equilibration

of the plasma, indirectly, with tissue nuclear binders.

2) The first binder of the AP in the euthyroid animal is only 50% saturated while the first binder of the PP is about 85% saturated.

3) In the AP of the normal rat the binding parameters for T_3 are primarily those of the somatotrophs. The binding of T_3 by the thyrotrophs cannot be evaluated from the data.

4) In the ME and brain the binding curves of T_3 suggest non-"specific" binding.

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Addendum

Since the completion of this manuscript the work of Oppenheimer *et al.* entitled: "Tissue Differences in the Concentration of Triiodothyronine Nuclear Binding Sites in the Rat: Liver, Kidney, Pituitary, Heart, Brain, Spleen and Testis" appeared in Endocrinology **95:** 897, 1974. The results of this group deal with nuclear binding and therefore are not directly comparable with our results. However, the observation that in the anterior pituitary nuclear binding sites for T_3 at steady state are 50% saturated agrees with our results for the specific first binder of anterior pituitary, determined in whole tissue.

References

1. Ford, D. H., and J. Gross, *Endocrinology* **62:** 416, 1958.
2. Schadlow, A. R., M. I. Surks, H. L. Schwartz, and J. H. Oppenheimer, *Science* **176:** 1252, 1972.
3. Bar-Sela, P., O. Stein, and J. Gross, *Endocrinology* **93:** 1410, 1973.
4. ———, Ph.D. Thesis, The Hebrew University, Jerusalem, 1973.
5. Hasen, J., G. Bernstein, E. Volpert, and J. H. Oppenheimer, *Endocrinology* **82:** 37, 1968.
6. Surks, M. I., H. L. Schwartz, and J. H. Oppenheimer, *J Clin Invest* **48:** 2168, 1969.
7. Shapiro, O., and A. Gordon, *Proc Soc Exp Biol Med* **121:** 577, 1966.
8. Oppenheimer, J. H., R. Squef, M. I. Surks, and H. Hauer, *J Clin Invest* **42:** 1769, 1963.
9. Kissane, J. M., and E. Robbins, *J Biol Chem* **233:** 184, 1958.
10. Oppenheimer, J. H., H. L. Schwartz, D. Koerner, and M. I. Surks, *J Clin Invest* **53:** 768, 1974.
11. Coutsoftides, T., and A. Gordon, *Acta Endocrinol (Kbh)* **65:** 409, 1970.
12. Knigge, K. M., and A. J. Silverman, *In* Knigge, K. M., D. E. Scott, and A. Weindel (eds.), Brain Interaction, Median Eminence Structure and Function, Karger, Basel, 1972, p. 530.
13. Samuels, H. H., and J. S. Tsai, *Proc Natl Acad Sci (USA)* **70:** 3488, 1973.
14. Ching, M., A. B. Evans, E. S. Evans, S. Joseph, and S. Sorrentino, Jr., *Acta Endocrinol (kbh)* **75:** 221, 1974.
15. Kraicer, J., and S. C. Cheng, *Am J Physiol* **214:** 158, 1968.