JCI The Journal of Clinical Investigation

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J Clin Invest. 1984;74(3):1035-1049. https://doi.org/10.1172/JCI111471.

Research Article

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Production Rates and Turnover of Triiodothyronine in Rat-developing Cerebral Cortex and Cerebellum

Responses to Hypothyroidism

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Abstract. Local 5'-deiodination of serum thyroxine (T4) is the main source of triiodothyronine (T3) for the brain. Since we noted in previous studies that the cerebral cortex of neonatal rats tolerated marked reductions in serum T4 without biochemical hypothyroidism, we examined the in vivo T4 and T3 metabolism in that tissue and in the cerebellum of euthyroid and hypothyroid 2-wk-old rats. We also assessed the contribution of enhanced tissue T4 to T3 conversion and decreased T3 removal from the tissues to the T3 homeostasis in hypothyroid brain. Congenital and neonatal hypothyroidism was induced by adding methimazole to the drinking water. Serum, cerebral cortex (Cx), cerebellum (Cm), liver (L) and kidney (R) concentrations of ^{125}I-T4, ^{125}I-T3(T4), and ^{131}I-T3 were measured at various times after injecting ^{125}I-T4 and ^{131}I-T3. The rate of T3 removal from the tissues was measured after injecting an excess of anti-T3-antibody to rats previously injected with tracer T3. In euthyroid rats, fractional turnover rates of T3 per hour were: Cx, 0.26±0.02 (SE); Cm, 0.20±0.02; L, 0.98±0.07; R, 0.97±0.12; and the calculated unidirectional plasma T3 clearance by these tissues were, in milliliters per gram per hour: Cx = 0.38, Cm = 0.32, L = 5.0, and R = 5.6. In hypothyroidism, the fractional removal rates and clearances were reduced in all tissues, in cortex and cerebellum by 70%, and in liver and kidney ranging from 30 to 50%. While >80% of the ^{125}I-T3(T4) in the brain tissues of euthyroid rats was locally produced, in hypothyroid cerebral cortex and cerebellum the integrated concentrations of ^{125}I-T3(T4) were 2.7- and 1.5-fold greater than in euthyroid rats. In the Cx, this response resulted from an approximately sixfold increase in fractional conversion and an approximately fourfold decrease in T3 removal rate hampered by a decreased uptake of T4 from plasma, whereas in Cm the response resulted only from the reduced T3 removal rate. In euthyroid rats, the calculated production rate of T3 in nanograms per gram per hour by the Cx was 0.96 and 0.89 by the Cm, which on a per organ basis equals 15 and 2%, respectively, of the extrathyroidal production rate as assessed in the body pool exchanging with plasma. Several conclusions can be drawn: (*a*) Production of T3 by developing brain is a very active process in agreement with the need of thyroid hormones during this period. (*b*) The brain-plasma exchange of T3 is slow compared with that of L or R. (*c*) This, along with the active local production, explains the predominant role of the latter as a source of T3 for the brain. (*d*) In hypothyroidism, the Cx is protected by an increase in the efficiency of T4 to T3 conversion and a prolonged residence time of T3 in the tissue, whereas the Cm is protected only by the latter. Because of the large fraction of the tissue T3 produced locally and the active turnover rate of T3 in the brain, reductions in T3 removal rate are of utmost importance for T3 homeostasis in these tissues.

Introduction

Even though thyroxine (T4)[1] is about 10-fold more abundant than triiodothyronine (T3) in the thyroidal secretion, the latter

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Received for publication February 21, 1984 and in revised form May 24, 1984.

J. Clin. Invest.

0021-9738/84/09/1035/15 $1.00
Volume 74, September 1984, 1035–1049

1. *Abbreviations used in this paper:* AOV, analysis of variance; PTU, propylthiouracil; T3, triiodothyronine; T4, thyroxine; TWAOV, two-way analysis of variance.

ultimately accounts for most of the biological potency of the secretion. The enzymatically catalyzed deiodination of T4 in the 5' position, by giving rise to about two-thirds of the T3 produced daily, is of utmost importance in thyroid physiology (1, 2). In vivo and in vitro obtained evidences indicate that there are two separate enzymatic pathways of extrathyroidal T3 generation (3–5). The only iodothyronine 5'-deiodinase found in liver and kidney, 5'D-I, is uncompetitively inhibited by propylthiouracil (PTU), is decreased in hypothyroidism, and increased in hyperthyroidism (6–9). Another 5'-deiodinase, 5'D-II, has been found in significant quantities in the brain, in the anterior pituitary (4, 5), and more recently, in the brown adipose tissue (10). This enzyme is insensitive to PTU, increases markedly in hypothyroidism, and is highly sensitive to inhibition by thyroid hormones (3–5, 9, 11–13). This pathway accounts for all the T3 produced in the brain and the pituitary, whereas 5'D-I seems to catalyze the production of most of the extrathyroidally generated T3 in the adult rat (3).

While the variations in 5'D-I activity would affect the tissue T3 concentration through changes in the plasma concentration of this hormone, the fluctuations in 5'D-II activity are bound to affect the concentration of T3 in those tissues where most of their T3 content is produced locally. Recently, we have reported that the increment of 5'D-II observed in the cerebral cortex of neonatal rats with congenital hypothyroidism may protect this tissue from becoming biochemically hypothyroid in spite of a 80–90% reduction in serum T4 concentration (14). At age 2 wk, when the increment of 5'D-II in response to hypothyroidism was maximal, only the animals with <5% of normal serum T4 showed evidence of cerebrocortical hypothyroidism. This high efficiency of the cerebral cortex to adapt to hypothyroxinemia suggests that there could be additional mechanisms other than the increase in the efficiency of T4 to T3 conversion (14). For example, the inhibition of conversion with iopanoic acid showed that in the cortex of hypothyroid animals there was more T3, after giving T4, than could be accounted for by the increased rate of conversion, suggesting that the rate of disappearance of T3 from the brain was decreased in hypothyroid rats. The relative importance of these two factors leading to increased concentration of T3 derived from T4 in the hypothyroid cerebral cortex, namely the more efficient conversion and the prolonged residence of T3 in the brain, was not examined in those studies. On the other hand, a prolonged transit time of T3 through the central nervous system is consistent with findings of decreased T3 5-deiodinating activity in homogenates of brain from hypothyroid rats (12). Given the levels of T3-deiodinating activity in euthyroid central nervous system, it is reasonable to assume that a substantial fraction of the T3 disappearing from the brain is degraded via this pathway, and further, that a reduction in the activity of this enzyme may have a significant impact in the concentration of T3 in the brain. Consequently, we have undertaken the study of T3 turnover and the kinetics of exchange with plasma in cortex and cerebellum in 2-wk-old euthyroid and hypothyroid rats. As before (14), we have chosen this model because it is the age when most rapid differentiation of the cerebral cortex takes place and because rat brain development at this age probably corresponds to that occurring in late stages of fetal life and early postnatally in humans (15).

Methods

Studies were performed in 2-wk-old Sprague-Dawley rats. Pregnant rats were obtained (Zivic-Miller Laboratories, Allison Park, PA) at gestational age 15–16 d. They were housed in individual cages upon reception, and to induce congenital hypothyroidism, approximately half of them were given 0.02% (wt/vol) methimazole in the drinking water. At birth, litters were culled to ~10 pups each and the same regimen of methimazole was maintained until age 2 wk when the experiments were performed. In making experimental groups, rats were taken from various litters in order to make the groups as homogeneous as possible. Details of this model of congenital neonatal hypothyroidism, as well as the nutritional conditions of the litters, have been given elsewhere (14).

Tracers

^{125}I-T4 (sp act, ~4,200 μCi/μg) and ^{131}I-T3 (sp act, ~2,800 μCi/μg) were prepared from T3 and 3,5-L-diiodothyronine, respectively, as previously described (16, 17). Tracers were prepared within 48 h of the experiments and contained <5% iodide as major contaminant; ^{125}I-T4 had <1% ^{125}I-T3, and ^{131}I-T3 <0.5% ^{131}I-T4 as determined by a combination of affinity and paper chromatography (18, 19) and just paper chromatography, respectively (20). The tracers were stored in 70% ethanol until the time of injection when they were dried under a stream of nitrogen and redissolved in 10% rat serum in 0.9% sodium chloride containing 0.1% of sodium iodide. The tracers were injected mixed into one of the external jugular veins in 0.1 ml bolus containing ~40 μCi of ^{125}I-T4 and 15 μCi of ^{131}I-T3 or otherwise as indicated.

Collection of sera and tissues samples

At the indicated times after the tracers injection, the animals were anesthetized with ether and exsanguinated through the abdominal aorta at the level of the bifurcation. 0.5–1.5 ml of blood was obtained. Subsequently, 0.5–1.0 g of liver and both kidneys were removed, the skull opened, and the cerebral cortex and cerebellum dissected out as described previously (21). All four tissues were immediately frozen in dry ice acetone. The blood was allowed to clot, and the sera were collected and frozen. Tissue and serum samples were analyzed in the next following days. (Even though all circulating iodothyronines were measured in serum, the term plasma will be used in the text for extrapolations to in vivo situations.)

Measurement of tracers in the serum

Serum ^{125}I-T4 and ^{131}I-T3 were separated and quantitated by paper chromatography (20). When there were >1,000 cpm of the least abundant isotope in 20 μl of serum, this volume was directly applied to chromatographic paper (Whatman 3 MM, Whatman Laboratory Products, Inc., Clifton, NJ) with 50 μl of marker (~2 mg/ml T4, T3, and sodium iodide in ethanol made alkaline by adding ~5% of concentrated NH_4OH). Since larger volumes of serum induced tailing of the T4 peak and hence poor resolution from iodide, when there were <1,000 cpm/20 μl, the appropriate volume of serum (usually 30–50 μl) to accumulate ≥1,000 cpm of the least abundant iodothyronine was extracted with 50 μl of the marker described above and 100 μl of butanol saturated with 2 N NH_4OH. The 2,000 *g* supernatant was transferred to paper in two or three applications and the pellet washed once with 100 μl of ethanol:2 N NH_4OH (9:1); this ensured >90% extraction. ^{125}I-T3 derived from ^{125}I-T4, ^{125}I-T3(T4), was measured by

a combination of affinity and paper chromatography as described (18, 19); the quantity of ^{131}I-T3 determined by paper chromatography in the same serum sample as above was used as internal recovery standard. The concentration of all three iodothyronines was expressed as percent of a dose per milliliter of serum. All values were normalized to 30 g body weight to avoid the variation derived from differences in size; the body weights are given in Results. Serum ^{125}I-T3 was corrected for the ^{125}I-T3 contaminating the ^{125}I-T4 (usually negligible 4 h after the injection) and for the halving of the specific activity inherent to the ^{125}I 5′-deiodination of the tracer T4.

Measurement of labeled iodothyronines in the tissues

The tissues were weighed and rapidly counted before complete thawing. They were subsequently extracted with ~2 vol/wt butanol:2 N HCl (9:1); extractions were repeated (two or three times) until >90% of the counts had been extracted. All or part of the extract was concentrated under a stream of nitrogen and chromatographed as described for the serum (20). From the chromatographic distribution of radioactivity and the total number of counts in the intact tissue, the contents of ^{125}I-T4, ^{125}I-T3, and ^{131}I-T3 in the tissues were calculated. In pilot experiments, extractions were performed adding known amounts of tracer iodothyronines to the tissues to monitor the losses during the extraction and chromatography. There was no significant degradation of radiolabeled T3, but there was 10–15% degradation of ^{125}I-T4, which was recovered as iodide and as unidentified radioactivity in the front of the chromatogram; yet, no significant T4 to T3 conversion was detected during the extraction and chromatography procedure. These results were not affected by the thyroid status.

In estimating the concentration of radioiodinated iodothyronines in the tissues, the volume of plasma trapped in the tissue at the time of initiating the extraction had to be taken into account. To do this, we obtained a pool of the 60,000–100,000 D rat serum proteins by standard gel filtration techniques and labeled them with radioactive iodine. Approximately 40 μg of this mixture of proteins, largely albumin, was injected intravenously to 2-wk-old euthyroid and hypothyroid rats, and the animals were sacrificed at 2 and 5 min after the injections. The volume of plasma trapped was calculated from the TCA-precipitable radioactivity of the serum and tissue homogenates. There were no significant differences between the figures obtained 2 and 5 min after the injections.

The tissue ^{125}I-T3(T4) was also corrected by the ^{125}I-T3 contaminating the ^{125}I-T4 dose, a correction that was insignificant 4 h after the injection. The corrected value was multiplied by two for the loss of one atom of radioactive iodine in the process of conversion. After all these corrections had been made, the tissue concentrations of all three radiolabeled iodothyronines were expressed as percentage of dose per gram of tissue and normalized to a 30 g rat as for serum concentrations. Body weights are given in Results (Table II).

Time course of plasma and tissue concentrations of labeled iodothyronines

Groups of four rats, both euthyroid and hypothyroid, injected with ^{125}I-T4 and ^{131}I-T3 were killed at 1, 2, 3, 6, 8, 12, and 16 h after the injections by exsanguination under light ether anesthesia. Serum and tissues were handled as described above, and the corrected radiolabeled iodothyronines in both tissues and serum were plotted against time in semilog paper.

Studies injecting anti-T3 antibodies

The gamma globulin fraction of highly specific rabbit anti-T3 serum was obtained by standard ammonium sulfate precipitation and DEAE cellulose chromatography techniques. The affinity and maximal binding capacity of this partially purified antibody were checked, and sufficient antibody to bind at least 10 ng of T3 per milliliter of serum was injected intravenously after preincubating it with ^{131}I-T3. At −2 h, groups of four euthyroid or hypothyroid rats were injected with ~10 μCi of ^{125}I-T3 in 50 μl of the vehicle described above into one external jugular vein. At time 0, ~4 μCi of ^{131}I-T3 antibody complex, in a volume of 100 μl, was injected in the other jugular vein. The animals were killed at 2, 4, 6, and 8 h later as described above. Groups of animals injected with ^{125}I-T3 but not with the ^{131}I-T3 antibody complex were killed at the time of injecting this complex (time 0) and at various times thereafter to serve as controls.

The goal of this experiment was to measure the rate of disappearance of T3 from the tissues after stopping the inflow of plasma ^{125}I-T3 into the tissues. ^{131}I-T3 bound to the antibody was to control for both the slow dissociation of the T3 bound to the antibody reentering the tissues and for the plasma trapped in the vascular bed of the tissues. Thus, at each time point after the injection of the ^{131}I-T3 antibody complex, the product of the ^{131}I-T3 tissue to serum ratio times the serum ^{125}I-T3 concentration was subtracted from the total observed tissue ^{125}I-T3 concentrations. The corrected tissue ^{125}I-T3 concentration was plotted in semilog paper against time after the injection of the antibody.

Other procedures

Serum T4 and T3 concentrations were measured by previously described methods (22, 23). Iodothyronine stripped rat serum instead of human serum was used for the standard curves in the radioimmunoassays.

Data were statistically analyzed by Student *t* test and by one or two-way analysis of variance, AOV and TWAOV, respectively (24).

Calculation of kinetic parameters

The disappearance of ^{125}I-T4 and ^{131}I-T3 from the plasma, and the appearance of ^{125}I-T3(T4) in circulation were analyzed by noncompartmental analysis (25, 26). Detailed methodology and discussion on the calculation of the various kinetic parameters as well as the fractional conversion of ^{125}I-T4 to ^{125}I-T3 have been reported elsewhere (19, 27).

Modelling of intracellular T3 kinetics. Our view of intracellular T3 economy is schematically represented in Fig. 1. In general, there are two inputs of T3 into the cells, the T3 entering from plasma, T3(T3), and that produced locally, T3(T4). The absolute and relative magnitude of each source varies from one tissue to another (2). In Fig. 1, A and B represent hypothetical compartments, the site of entry and the site of T4-to-T3 conversion, respectively; they are the "lobbies" of both T3's before mixing and distributing into the rest of the cell compartment, which is represented by C in Fig. 1. This compartment constitutes the bulk of cellular T3 and includes the cell nucleus. This assumption is supported by the evidence that follows. Since 5′D-I and 5′D-II are membrane-bound enzymes (28, 29), it is likely that compartments A and B physically overlap within the cell, at least to some extent. Secondly, in liver and brain, tracer T3 seems to distribute rapidly with the cell since the nuclear-to-tissue ratio was maximal by a half hour after the injection of the tracer (30, 31), although equilibration of brain with plasma T3 takes 2–3 h (30, 31). Similarly, in unpublished pilot experiments, we have found that the ratio of ^{125}I-T3(T4) to ^{131}I-T3 3 h after injecting ^{125}I-T4 and ^{131}I-T3 was the same in the nuclei as in the extranuclear fraction of cerebral cortex and cerebellum. Lastly, the injection of cold T3 to rats (20 μg/100 g body weight) injected with ^{125}I-T4 reduced by >90% the nuclear content of ^{125}I-T3(T4) in all tissues examined including the brain (21, 32). In the

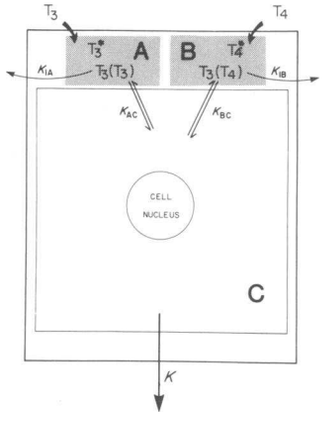

Figure 1. Schematic representation of tissue T3 economy. A and B are hypothetical compartments, the areas adjacent to the site of entry from plasma, T3(T3), or the site of production from T4, T3(T4), respectively. C represents the rest of the cell where T3 from both sources are mixed. K_{IA} and K_{IB} are the fractional removal rates of T3 from A and B, respectively, either by degradation or exit to plasma before mixing. K_{AC} and K_{BC} are the fractional transfer rates from A or B into C. K represents the actual fractional turnover rate of T3 from the cellular pool. A and B probably overlap physically within the cells since 5'D-I and 5'D-II are membrane-bound enzymes. If they exist, these compartments are small and rapidly turned-over since T3 from either source reaches rapidly the innermost compartment, the nucleus, already mixed with the T3 from the other source. In these studies, the cellular pool of T3 has been labeled with ^{131}I-T3 or ^{125}I-T4. See text for details.

present experiments, we have labeled tissue T3 by injecting either tracer T3 or tracer T4. From Fig. 1, one would anticipate that the specific activity of tissue T3 relative to plasma T3 will depend on the rate of exchange of T3 with plasma, the rate of intracellular conversion of T4 to T3 and degradation of T3, and the turnover of T3 and T4 in plasma. When tracer T4 is injected, the isotopic dilution outside the tissue is slower than when tracer T3 is used, and if conversion in a tissue is active and the exchange of T3 with plasma slow, the specific activity of tissue T3, relative to plasma T3, attainable with tracer T4 will be higher than with tracer T3. This is reflected in higher tissue to plasma ratios of T3(T4) than T3(T3) and is demonstrable by pulse injections (21) as well as by constant infusion (33). The higher ratios for T3(T4) than for T3(T3) do not imply that T3 from both sources does not mix within the tissue.

Therefore, we have assumed that regardless of whether we label the cellular pools with ^{131}I-T3 or ^{125}I-T4, most of the intracellular T3 will be rapidly labeled, and if the input of the tracer is stopped, the fractional disappearance of the labeled T3 will represent the fractional turnover rate of the bulk of the cellular pool of T3. For convenience we have chosen tracer T3, since to stop the generation of intracellular T3 or to chase the T3(T4) would markedly perturb the system. We allowed the pool to be labeled for 2 h, which is close to the time when maximal specific activity is reached by this route, and then followed the disappearance of the tracer T3 preventing further uptake from plasma as described.

Further support for this approach and its implications and limitations are further analyzed in the Discussion Section.

Tissue-plasma exchange of T3. After bolus injection of radioactive T3, there is a time when the concentration of radioactivity in the tissue is maximum; at this time, the rate of change of tissue radiolabeled T3 concentration is zero which means that the velocity of entry from plasma and disappearance from the tissue is equal. This is the equilibrium time point, t_m (30, 31).

The exchange of labeled T3 between plasma and tissues can be described by:

$$\frac{d[T3]t}{dt} = TCl \times [T3]_s - [T3]t \times K \quad (1)$$

Where $[T3]t$ and $[T3]_s$ are the concentrations of labeled T3 in the tissue and the serum, respectively; TCl is the unidirectional clearance of plasma T3 into the tissue; and K is the fractional removal rate of T3 from the tissue. At the time of transient equilibrium, t_m:

$$TCl \times [T3]_s = [T3]t \times K \quad (2)$$

$$\frac{TCl}{K} = \frac{[T3]t}{[T3]_s} = \left(\frac{T}{S}\right)_e \quad (3)$$

in milliliters of plasma per gram of tissue. In these studies, K was obtained experimentally from the disappearance curves of T3 from the tissues after injecting antibodies, as described above. The equilibrium tissue to serum ratio of T3, $(T/S)_e$, was obtained for liver and kidney by averaging the T/S ratios obtained at the various times after the injections, since the numbers were not significantly different by AOV. For cerebral cortex and cerebellum, the $(T/S)_e$'s were obtained by plotting the T/S's at the various times and interpolating the value for the time at which the radioactivity in the tissue was maximal. The clearance of plasma T3 by the corresponding tissues was then calculated from Eq. 3.

Knowing TCl and K for each tissue and serum concentrations of T3 at various times after the injections, the temporal profile of the tissue concentration of T3 derived from the serum can be easily obtained with the use of programmable desk top calculator. Thus, from the serum ^{125}I-T3(T4) concentration curve, we calculated the contribution of plasma ^{125}I-T3(T4) to the total observed tissue ^{125}I-T3(T4) at any given time, and by subtraction, we obtained the temporal profile of the ^{125}I-T3(T4) generated locally in the tissue.

Local production of T3 from T4 in cortex and cerebellum. At variance with the disappearance of T3 from the tissues, the disappearance of T4 in all four tissues examined was rapid and paralleled the disappearance of T4 from the serum after the level in the tissues had peaked. The fraction of ^{125}I-T4 converted to ^{125}I-T3 by these tissues can be calculated from the area under the locally produced tissue concentrations of ^{125}I-T3(T4) as discussed below. These integrated tissue concentrations from 0–16 h were measured by planimetry but after 16 h had to be calculated. Since in euthyroid rats the fractional rates of removal of T3 in cortex and cerebellum were faster than the rates of removal of T4 from the tissues, and at 16 h, >70% of the ^{125}I-T4 had been consumed, the tissue level of ^{125}I-T3(T4) after 16 h is expected to approach the fractional removal rate of ^{125}I-T4. Therefore, in euthyroid rats, the 16 h-to-infinity integrated tissue concentrations were calculated by dividing the 16-h concentration by $\lambda 4$, the fractional removal rate of T4. In the case of hypothyroid rats, the rates of T3 disappearance from the cerebral cortex and the cerebellum were of smaller magnitude than the fractional removal rate of T4 from plasma (see Results), and therefore, the tissue level of ^{125}I-T3(T4) is expected to decrease at a rate approaching that of T3 from the respective tissues rather than that of T4 from the serum. Consequently, in these hypothyroid animals, the 16 h-to-infinity integrated concentrations of ^{125}I-T3(T4) were calculated by dividing the 16-h values by the fractional rate of disappearance of T3 from the corresponding tissues, as determined by the studies with T3 antibodies.

With the fractional removal rate of T3 from the tissues independently determined and with the integrated concentration of locally produced ^{125}I-T3(T4) from time zero to infinity, it was possible to calculate the production rates of ^{125}I-T3 from the ^{125}I-T4 dose by the cerebral cortex and cerebellum. The calculation is based on the general principles

ruling the volume:flow and mass:flux ratios in an open system (34). If in such a system we have a solute in dynamic steady state, and we inject a tracer amount of an indicator as a "slow bolus," the fractional turnover rate of the system can be obtained by dividing the dose injected by the area under the residual amount of the indicator from time zero to infinity (34). By extension then:

$$K = \frac{F}{\int_0^\infty [^{125}\text{I-T3(T4)}]_t \cdot dt} \quad (4)$$

where F is percentage of the ^{125}I-T4 dose converted to T3 by the tissue and $[^{125}\text{I-T3(T4)}]_t$ is the tissue concentration of locally produced ^{125}I-T3(T4) integrated from 0 to infinity. Since K, the fraction turnover rate of T3 in the tissue, has been determined, F can be calculated and as the system has not been perturbed by the injection of the tracer, the value obtained is equal to the percentage of the total T4 metabolized in the body in any given interval of time, in steady state conditions, that is converted to T3 by the tissue. As the total T4 metabolized in the body can be calculated from the plasma clearance and the steady state serum T4 by radioimmunoassay, the quantity of T3 produced can be calculated as follows:

$$(\text{T3PR})_t = [\text{T4}]_s \times \text{PCl} \times \frac{F}{100} \times \frac{651}{777} \quad (5)$$

where $(\text{T3PR})_t$ means the T3 production rate by any given tissue, $[\text{T4}]_s$ is the serum concentration of T4 by radioimmunoassay, PCl is the clearance of T4 from the plasma, F is the percentage of T4 converted to T3 as defined for Eq. 4, and 651/777 is the correction for the change in molecular weight inherent to the conversion; if serum T4 is expressed in nanograms per milliliter, the clearance in milliliters per hour, and F fraction per gram of tissue, the production rate results in nanograms of T3 per hour per gram of tissue.

Results

The volume of plasma trapped in the tissues expressed in microliters of plasma per gram of wet weight are shown in Table I. The thyroid status did not affect these volumes in any of the tissues but the values obtained for liver and kidney were much higher than those obtained for both nervous tissues. The only instance in which the volume of plasma present in the tissue was a significant contribution to the observed iodothyronine content was for ^{125}I-T4 in the brain tissues of hypothyroid animals. Although the correction fell within the intragroup experimental variation for all three iodothyronines in other tissues, the individual values were corrected by the numbers presented in Table I.

Table I. Volume of Plasma Trapped in Various Tissues Collected from 2-wk-old Rats after Aortic Exsanguination (μl of plasma/g of wet weight)

	Euthyroid	Hypothyroid
Cerebral cortex	9.5±0.5	8.4±0.5
Cerebellum	14.0±0.6	12.2±1.3
Liver	81±4	74±3
Kidney	62±1	57±5

Each entry is the mean±SEM of tissues from five rats. None of the differences between euthyroid and hypothyroid rats are statistically significant.

The serum and tissue concentrations of ^{131}I-T3 at various times after the injection are presented in Fig. 2. Details on plasma kinetics have been reported elsewhere (27). The main plasma kinetic parameters calculated by noncompartmental analysis are shown in Table II. In both liver and kidney, the tissue T3 concentration decreased at all times of observation paralleling the serum concentrations, whereas in both cerebral cortex and cerebellum, the concentration of radioactive T3 did not reach a maximum before 2 to 4 h. After maximal concentrations of ^{131}I-T3 in the cerebral cortex and cerebellum of euthyroid animals, the tissue levels of ^{131}I-T3 fell approaching the slope of the serum concentration of the hormone. In the hypothyroid pups, the maximal tissue concentration was reached later and subsequently decreased at a slower rate than the levels of serum ^{131}I-T3. In addition, in the hypothyroid animals, the concentrations of ^{131}I-T3 in the cortex and the cerebellum were higher than in the euthyroid animals by TWAOV ($P < 0.001$).

The tissue-to-serum ratios of ^{131}I-T3 are shown in Fig. 3. As predicted from Fig. 2, the ratios of liver and kidney did not change significantly over the period of observation. In contrast, the ratios for cerebral cortex and cerebellum were ascending throughout the 16 h of observation. During the first 3–4 h, the slopes were steeper than thereafter, although they remained significantly greater than zero and significantly steeper in the hypothyroid than in euthyroid pups ($P < 0.005$). Altogether these data indicate that the rate of removal of T3 from the brain tissues is relatively slow compared with the plasma removal rate and that this phenomenon is exaggerated in hypothyroidism.

The results of injecting an excess of anti-T3 antibody to measure the disappearance rate of T3 from all these tissues are shown in Tables III, IV, and in Fig. 4. Table III shows the results obtained in serum, cerebral cortex, and cerebellum of euthyroid and hypothyroid pups. At 2 h after the injection of the antibody, there was an approximately sixfold increase in the serum concentration of ^{125}I-T3. The ^{131}I-T3 tissue-to-serum ratios were very low regardless of the thyroid status when compared with those shown in Fig. 3. These ratios times the corresponding ^{125}I-T3 serum concentrations were subtracted from the total observed ^{125}I-T3 at the various times. These corrected ^{125}I-T3 tissue concentrations are shown in Table III. The plots of the log of these values vs. time after the injection of the antibodies are shown in Fig. 4. The coefficients of correlation were >0.92, indicating that the disappearance of T3 from these tissues, that is the combination of degradation and return to the blood stream, followed first order kinetics. For simplicity, the data for kidney and liver have been omitted from Table III and Fig. 4, but the coefficients of correlation for similar plots were >0.92. Table IV shows the fractional disappearance rates, the equilibrium tissue-to-serum ratios, and the calculated unidirectional clearance rates of T3 from

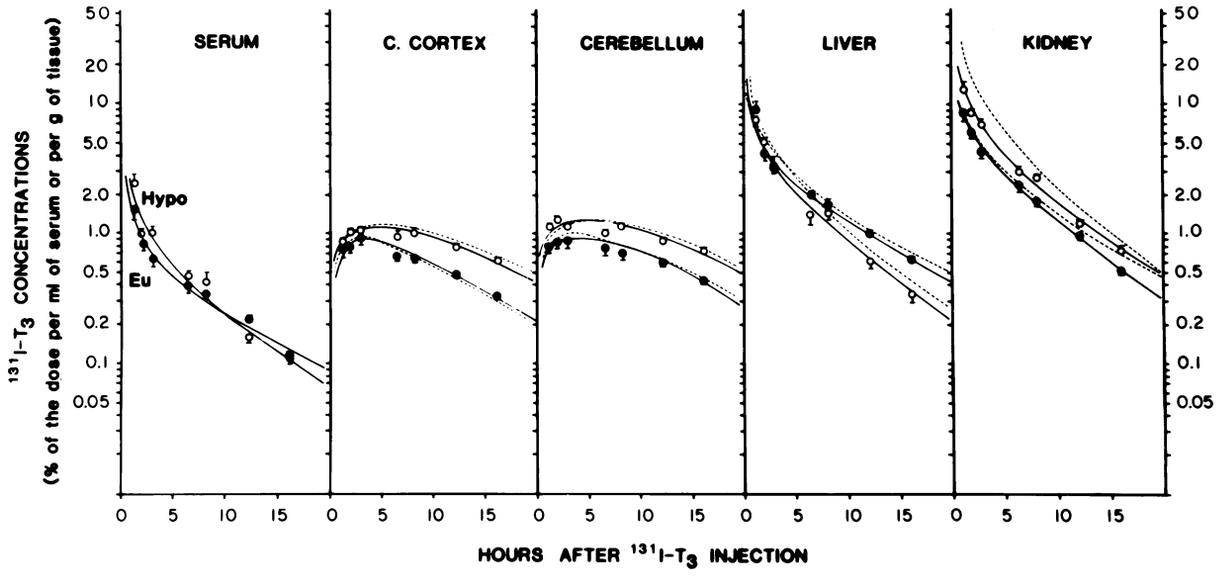

Figure 2. Serum and tissue concentrations of ^{131}I-T3 in 2-wk-old euthyroid (Eu) and hypothyroid (Hypo) rats at various times after injecting ^{131}I-T3. Curves have been fitted visually. Dotted lines represent calculated tissue values (see text). C. cortex, cerebral cortex.

plasma by all four tissues. The unidirectional clearances of T3 from plasma by liver and kidney are 15-fold greater than those by central cortex and cerebellum, whereas the rates of fractional disappearance were four- to fivefold greater in liver and kidney than in the nervous tissues. These differences explain the faster equilibration of plasma T3 with liver kidney when compared with cortex and cerebellum. Hypothyroidism reduced the fractional removal rates and unidirectional clearances in all four tissues, but in the cortex and cerebellum the reductions were of greater magnitude than in liver and kidney. Whereas in the nervous tissues, the fractional removal rates and clearance rates were decreased by approximately the same factor, in liver and kidney they were affected differently by hypothyroidism as reflected by lower and higher tissues to plasma ratios, respectively (Table IV), in hypothyroid than in euthyroid pups.

The dotted lines in Fig. 2 represent the calculated tissue ^{131}I-T3 concentrations using the serum ^{131}I-T3 levels, and the

Table II. Main Serum T4 and T3 Kinetic Parameters Obtained by Noncompartmental Analysis after ^{125}I-T4 and ^{131}I-T3 Injections in 2-wk-old Euthyroid and Hypothyroid Rats

	n	VD	PCl	λ	Fractional T4 to T3 conversion	RIA
		ml	*ml/h*	h^{-1}	%	*ng/ml*
T4: Eu	28	6.44	0.50	0.078	35.5	36±0.8
Hypo	28	5.99	0.71	0.118	39.0	—
T3: Eu	28	77.1	10.9	0.145	—	0.61±0.03
Hypo	28	57.1	8.7	0.172	—	—

Abbreviations: VD, volume of distribution; PCl, plasma clearance; λ, fractional removal rate. Both VD and Cl have been normalized to 30 g. Euthyroid rats weighed 26±3 g (SD) and hypothyroid 21.4±3.4 g. Fractional conversion is the percent of T4 metabolized that is converted to T3. Details of the calculations have been presented elsewhere (27).

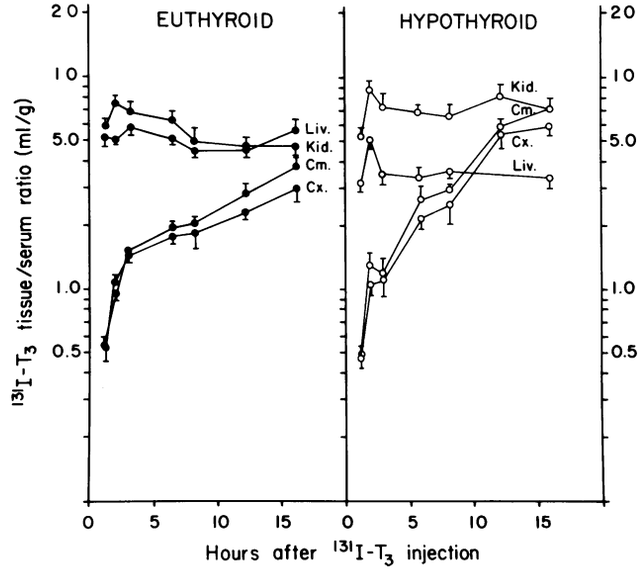

Figure 3. ^{131}I-T3 tissue-to-serum ratios at various times after injecting ^{131}I-T3. The data base are those presented in Fig. 1. From 3 h on, the rate of increase in the ratio is greater in hypothyroid than in euthyroid tissues ($P < 0.005$). Liv, liver; Kid, kidney; Cm, cerebellum; Cx, cerebral cortex.

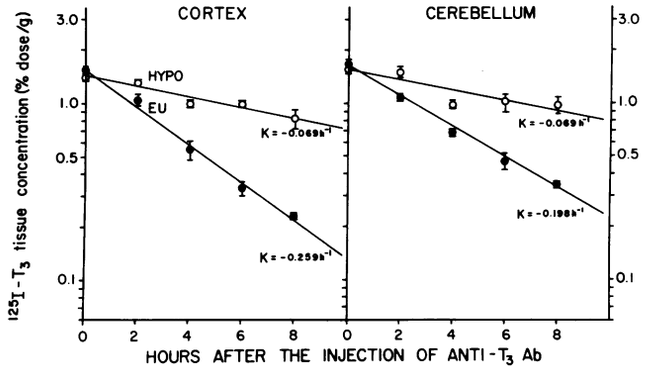

Figure 4. Disappearance of ^{125}I-T3 from cerebral cortex and cerebellum of euthyroid (Eu) and hypothyroid (Hypo) rats. Rats were injected with ^{125}I-T3 and 2 h later, with an excess of anti-T3-antibody to prevent further uptake of serum ^{125}I-T3 by the tissues. Values have been corrected as described in Methods.

corresponding unidirectional clearances and fractional removal rates are shown in Table IV. The agreement of these curves with the experimental ones is excellent, except for liver and kidney in hypothyroid rats. Notwithstanding, the agreement in all other instances suggests that the fractional turnover rates obtained as explained above at least describe well the turnover of the tissue T3 entering from plasma. Since the fractional removal rates were obtained in different rats, major errors in the determination due to artefacts derived from the methodology used, would have generated curves markedly discrepant.

Thyroid-tissue plasma exchange. Serum-disappearance and tissue-accumulation curves for ^{125}I-T4 injected simultaneously with the ^{131}I-T3 are depicted in Fig. 5. The main serum kinetic parameters for T4 obtained by noncompartmental analyses are shown in Table II. As happened with ^{131}I-T3, liver and kidney ^{125}I-T4 content decreased throughout the period of observation at a rate not different from that of the serum ^{125}I-T4. In contrast, maximal cerebrocortical and cerebellar ^{125}I-T4 concentrations in euthyroid rats were reached around 3 h after the tracer injections, and subsequently decreased paralleling serum T4. Although in the cortex of hypothyroid rats the time for maximal concentration was clearly delayed, the tracer T4 in both cortex and cerebellum of hypothyroid pups decreased paralleling the serum levels. Accordingly, the tissue-to-serum ratios of ^{125}I-T4 plateaued at ~3 h in the euthyroid

Table III. Cerebral Cortex, Cerebellum and Serum Concentrations of ^{125}I-T3 at Different Times after Injecting an Excess of Anti-T3 Antibody With ^{131}I-T3

	Hours after antibody				
	0	2	4	6	8
Euthyroid					
Serum ^{125}I-T3	1.68±0.18	9.20±0.71	9.96±0.38	4.97±0.48	3.9±0.43
Cortex					
Total ^{125}I-T3	1.56±0.05	1.44±0.10	1.04±0.06	0.89±0.02	0.77±0.04
T/S of ^{131}I-T3 (×10^3)	—	40±4	73±7	116±10	136±6
Corrected ^{125}I-T3	1.56±0.05	1.06±0.07	0.55±0.07	0.33±0.03	0.23±0.01
Cerebellum					
Total ^{125}I-T3	1.68±0.07	1.48±0.03	1.17±0.07	1.08±0.03	0.97±0.03
T/S of ^{131}I-T3 (×10^3)	—	44±5	80±7	127±12	159±6
Corrected ^{125}I-T3	1.68±0.07	1.08±0.01	0.68±0.03	0.47±0.05	0.35±0.01
Hypothyroid					
Serum ^{125}I-T3	1.78±0.16	10.8±0.88	9.18±0.22	8.69±0.44	9.39±1.37
Cortex					
Total ^{125}I-T3	1.42±0.07	1.54±0.04	1.34±0.05	1.64±0.19	1.20±0.15
T/S of ^{131}I-T3 (×10^3)	—	22±2	38±4	46±5	51±10
Corrected ^{125}I-T3	1.42±0.06	1.31±0.04	0.99±0.05	0.99±0.03	0.82±0.11
Cerebellum					
Total ^{125}I-T3	1.59±0.07	1.82±0.08	1.38±0.06	1.77±0.67	1.52±0.27
T/S of ^{131}I-T3 (×10^3)	—	29±2	44±3	85±30	57±10
Corrected ^{125}I-T3	1.59±0.07	1.51±0.10	0.98±0.03	1.03±0.11	1.00±0.13

Groups of four rats were injected intravenously (jugular) with ^{125}I-T3 and 2 h later injected with anti-T3 antibody containing ^{131}I-T3. Rats were killed at the indicated number of hours after the antibody. Each entry is the mean±SEM of four rats. For each time point serum, cortex and cerebellum are from the same rats. Serum concentrations are expressed as percent of the dose per milliliter and tissue concentrations in percent of the dose per gram. The tissue-to-serum ratio (T/S) resulted from dividing (percent dose per gram) ÷ (percent dose per milliliter), multiplied by 10^3 as indicated. The corrected ^{125}I-T3 was obtained by subtracting [(serum ^{125}I-T3) × (^{131}I-T3 T/S) × 10^{-3}] to the total tissue ^{125}I-T3, and was used to calculate the rate of disappearance of T3 from the tissues.

Table IV. Parameters of Tissue-Plasma Exchange of T3 for Various Tissues of 2-wk-old Rats; Effects of Thyroid Status

		Cerebral cortex	Cerebellum	Liver	Kidney
Equilibrium T/S (*ml/g*)	Eu	1.5	1.6	5.1	5.8
		(1.3–1.7)	(1.4–1.8)	(4.8–5.4)	(5.1–6.5)
	Hypo	1.6	2.0	3.8	7.2†
		(1.3–2.0)	(1.6–2.4)	(3.6–4.0)	(5.7–8.7)
		NS	NS	<0.001	NS
Fractional Removal Rates (h^{-1})	Eu	0.26	0.20*	0.98	0.97
		(0.23–0.29)	(0.17–0.23)	(0.81–1.15)	(0.70–1.23)
	Hypo	0.07	0.07	0.67	0.46§
		(0.04–0.10)	(0.03–0.10)	(0.53–0.81)	(0.31–0.01)
		<0.001	<0.001	<0.01	<0.005
Clearance from Plasma ([*ml/g*] × h^{-1})	Eu	0.38	0.32	5.0	5.6
	Hypo	0.10	0.13	2.5	3.3

* $P < 0.01$ when compared with euthyroid cortex. † $P < 0.001$ and § $P < 0.05$ when compared with hypothyroid liver. The tissue-to-serum ratios at equilibrium $(T/S)_e$, the fractional removal rate from these tissues, K, and the clearance of T3 from plasma by these tissues, Cl_t have been defined and their derivation described in Methods. Cerebral cortex and cerebellum K values are those shown in Fig. 3. Liver and kidney values were obtained in the same way although they are not shown in Fig. 4 (see text). The numbers in parenthesis are the 95% confidence limits. Since the clearances have been calculated from the $(T/S)_e$'s and the K's, it is not possible to obtain a figure for statistical significance.

rats and about 6 h in the hypothyroid ones, i.e., there was no discernible slope after these times by AOV. This situation was different from that observed with the ^{131}I-T3 tissue-to-serum ratios (Fig. 3). The ^{125}I-T4 concentrations in the cortex of euthyroid rats were higher than in hypothyroid rats ($P < 0.001$), so that the area circumscribed by the curve in euthyroid rats was about eightfold greater than in hypothyroid rats (16.1[%/g]h vs. 1.93[%/g]h). The difference was only in part accounted for by the reduced serum levels of T4 in the hypothyroid rats, since the cortex to serum ratios of ^{125}I-T4 are still significantly higher in euthyroid than in hypothyroid animals ($P < 0.001$, Table V). In the cerebellum of hypothyroid

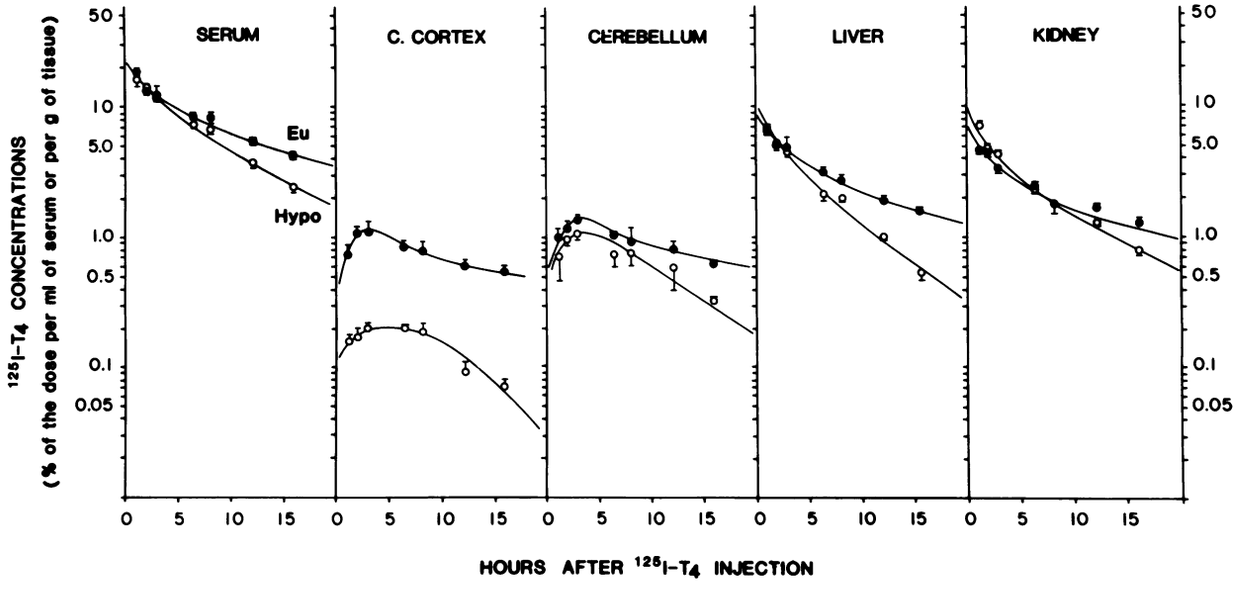

Figure 5. Serum and tissue concentrations of ^{125}I-T4 at various times after injecting ^{125}I-T4 to 2-wk-old euthyroid (Eu) and hypothyroid (Hypo) rats. Curves have been fitted visually. All tissue values have been corrected by losses in the extraction/chromatography and for plasma trapped in the tissues. C. cortex, cerebral cortex.

Table V. Tissue-to-Serum Ratios of ^{125}I-T4 in Various Tissues after Injecting ^{125}I-T4 into 2-wk-old Euthyroid and Hypothyroid Rats

		Hours after ^{125}I-T4 injection*						
		1.3	2	3	6.5	8.3	12.3	16.1
	Eu	4.0±0.9‡	7.6±1.6	9.5±2.1	9.7±1.5	11±2	11±1	13±1
Cortex ($\times 10^2$)	Hypo§	1.2±0.3‡	1.3±0.2	1.6±0.2‡	2.7±0.2	2.7±0.3	2.4±0.04	2.9±0.03
	Eu	5.2±0.9‡	9.0±1.5‡	11±1.5‡	12±0.4	14±1.1	15±1.6	15±1.2
Cerebellum ($\times 10^2$)	Hypo	4.3±1.3‡	6.9±0.2‡	8.4±0.6	10±1.6	11±1.9	10±0.8	11±2.9
	Eu	0.36±0.05	0.38±0.09	0.41±0.05	0.37±0.04	0.34±0.07	0.36±0.02	0.38±0.03
Liver	Hypo	0.42±0.04	0.38±0.01	0.39±0.01	0.29±0.02	0.31±0.02	0.26±0.04	0.24±0.02
	Eu	0.25±0.02	0.34±0.07	0.29±0.03	0.29±0.03	0.24±0.03	0.30±0.02	0.31±0.02
Kidney	Hypo	0.46±0.11	0.35±0.03	0.35±0.03	0.32±0.01	0.35±0.02	0.36±0.02	0.34±0.01

Each entry is the mean±SEM of four rats. Since tissue and serum concentrations were expressed as percentage of the dose per gram or milliliter respectively, the ratios are in milliliters per gram. * These are the actual bleeding times after the tracer injection. For simplicity, approximate times were given in Methods. ‡ Indicate values that are significantly different from the rest by AOV. § Hypothyroid cerebral cortex ratios were significantly lower than those of euthyroid rats by TWAOV ($P < 0.001$).

rats, the smaller area under the ^{125}I-T4 levels was fully accounted for by the difference in serum levels since, as shown in Table V, the tissue-to-serum ratios of ^{125}I-T4 were not different between euthyroid and hypothyroid pups.

Tissue concentrations of ^{125}I-T3(T4). The concentrations of ^{125}I-T3 at various times after the injection of ^{125}I-T4 in the serum, cerebral cortex, cerebellum, liver, and kidney are shown in Fig. 6. All values have been corrected as described in Methods.

The detailed analysis of the serum concentration curves has been reported elsewhere (27). By noncompartmental analysis, the serum ^{125}I-T3(T4) curves shown in Fig. 6 represent a fractional conversion of T4 to T3 of 35.5 and 39% in euthyroid and hypothyroid rats, respectively (Table II). The early peak in serum T3 observed in the euthyroid rats and the shoulder at the corresponding times observed in the hypothyroid animals probably represent a "burst" of T3 from compartments taking up a large fraction of T4 during the first passages after the

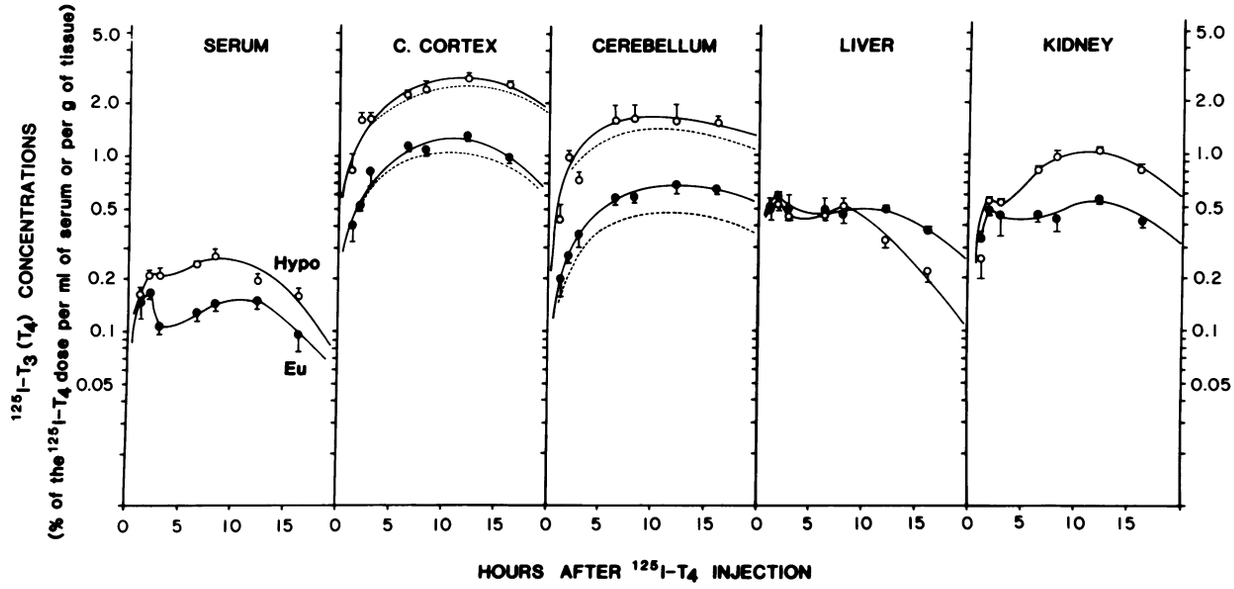

Figure 6. Serum and tissue concentrations of ^{125}I-T3 at various times after injecting ^{125}I-T4 to 2-wk-old euthyroid (Eu) and hypothyroid (Hypo) rats. All values have been corrected for the ^{125}I-T3 present in the dose of ^{125}I-T4 (0.6%) and multiplied by two. Curves were fitted visually. The dotted lines in cerebral cortex (C. cortex) and cerebellum represent the locally produced ^{125}I-T3. For serum, cerebral cortex, cerebellum, and kidney differences between euthyroid and hypothyroid rats were significant by TWAOV ($P < 0.001$).

injections, that is, before complete mixing with endogenous plasma T4; these rapid pools probably are liver and kidney. This early burst of T3 is largely blunted by PTU (27), further suggesting that it comes from 5'D-I containing tissues, and is highly reproducible in both the neonatal (27) and in adult rats (19).

The temporal profile of ^{125}I-T3(T4) in liver and kidney mimics that in the serum, whereas in cerebral cortex and cerebellum it is completely different. In these two tissues, the tissue-to-serum ^{125}I-T3(T4) ratios were markedly higher than that observed with ^{131}I-T3 ($P < 0.001$ by TWAOV, Table VI) in striking contrast with the liver- and kidney-to-serum ratios of ^{125}I-T3, which were significantly lower than those of ^{131}I-T3 ($P < 0.001$ by TWAOV, Table VI). Altogether, these data reflect the large fraction of T3 produced locally in the brain, and the rapid exchange of T3 between liver or kidney and plasma. These data suggest further that the T3 produced in liver and kidney has no time to distribute thoroughly in the cell.

The contribution of plasma ^{125}I-T3(T4) to the total observed cerebrocortical and cerebellar ^{125}I-T3(T4) was calculated from the unidirectional clearances by these tissues, the fractional removal rates shown in Table IV, and the serum concentrations of ^{125}I-T3(T4) at various times. The differences between each of these values and the observed total tissue ^{125}I-T3(T4) generated the dotted lines shown in Fig. 6. The areas under these curves represent the residual ^{125}I-T3(T4) from that produced by these tissues during the 16-h period of observation. Since by 16 h, 72 and 85% of the ^{125}I-T4 has been consumed in euthyroid and hypothyroid rats, the rest of the area, i.e., from 16 h-to-infinity, can be calculated as described in Methods. Table VII shows the integrated ^{125}I-T3(T4) concentrations from time zero-to-infinity in cortex and cerebellum. The total production of ^{125}I-T3(T4) by these tissues was estimated using these values and the fractional removal rates of T3 (Table IV). The estimates represent the percentage of the ^{125}I-T4 dose converted to ^{125}I-T3 per gram of tissue with no time frame. Knowing the integrated ^{125}I-T4 concentrations, which are also shown in Table VIII, one can estimate the fraction of ^{125}I-T4 metabolized in these tissues converted to ^{125}I-T3 per unit of time dividing the production of ^{125}I-T3(T4) (in percent of ^{125}I-T4/g) by the integrated concentration of ^{125}I-T4 (in [percent

Table VI. Tissue-to-Serum Ratios of ^{131}I-T3 and ^{125}I-T3(T4) at Various Times after Injecting ^{131}I-T3 and ^{125}I-T4 into 2-wk-old Euthyroid and Hypothyroid Rats

		Hours after tracer injections*						
		1.3	2	3	6.5	8.3	12.3	16.1
Cortex								
Euthyroid	T3(T3)	0.53±0.08	0.96±0.05	1.51±0.16	1.74±0.09	1.82±0.27	2.30±0.15	2.98±0.38
	T3(T4)	3.7±0.9	3.2±0.3	8.2±1.8	9.2±0.9	7.6±0.4	7.7±0.1	8.2±0.6†
Hypothyroid	T3(T3)	0.47±0.03	1.06±0.10	1.11±0.17	2.15±0.21	2.50±0.45	5.44±0.76	5.90±0.60‖
	T3(T4)	5.4±0.8	8.0±1.0	8.1±1.2	9.3±1.0	10.8±1.0	15.6±1.3	16.4±2.0†
Cerebellum								
Euthyroid	T3(T3)	0.54±0.03	1.08±0.03	1.44±0.23	1.98±0.05	2.00±0.18	2.85±0.23	3.85±0.04
	T3(T4)	1.5±0.3	1.70±0.2	3.5±0.5	4.6±0.4	4.1±0.3	4.8±0.6	7.5±1.3†
Hypothyroid	T3(T3)	0.49±0.08	1.29±0.18	1.18±0.20	2.64±0.38	2.95±0.13	5.85±0.43	7.06±0.60‖
	T3(T4)	2.8±0.6	4.9±0.7	3.7±0.6	6.8±1.7	6.5±1.8	8.6±1.7	10.1±1.9†
Liver								
Euthyroid	T3(T3)	5.2±0.3	5.1±0.3	5.8±0.3	5.1±0.3	4.4±0.4	4.6±0.3	5.7±0.4
	T3(T4)	4.2±0.4	3.6±0.7	4.3±0.8	3.9±0.7	2.7±0.6	3.6±0.6	2.0±0.5§
Hypothyroid	T3(T3)	3.2±0.3	5.1±0.3	3.5±0.4	3.3±0.2	3.6±0.2	4.7±0.2	3.3±0.9¶
	T3(T4)	3.4±0.1	2.6±0.3	2.3±0.3	1.7±0.2	1.9±0.3	1.8±0.1	1.3±0.2§
Kidney								
Euthyroid	T3(T3)	5.9±0.3	7.5±0.7	6.9±0.7	6.3±0.7	4.9±0.8	4.4±0.1	4.7±0.6
	T3(T4)	2.5±0.7	2.9±0.3	4.6±1.0	3.7±0.3	3.2±0.7	4.0±0.4	4.6±1.2§
Hypothyroid	T3(T3)	5.3±0.4	8.8±0.6	7.3±1.1	6.9±0.3	6.6±0.7	8.3±0.9	7.2±1.4‖
	T3(T4)	1.7±0.3	2.7±0.3	2.8±0.3	3.5±0.2	3.8±0.3	5.9±0.1	5.1±0.4§

Tissue concentrations are expressed as percentage of the dose per gram of wet weight, and serum concentrations as percentage of the dose per milliliter. T3(T3) = ^{131}I-T3, T3(T4) = ^{125}I-T3(T4). * Actual times after tracers. See footnote in Table V. TWAOV: † T3(T4) ratios higher than T3(T3), $P < 0.001$; § T3(T4) ratios lower than T3(T3), $P < 0.001$; ‖ T3(T3) ratios higher than euthyroid, $P < 0.001$; ¶ T3(T3) ratios lower than in euthyroid, $P < 0.001$.

Table VII. Integrated Cerebro-cortical and Cerebellar Concentrations of ^{125}I-T3(T4) and ^{125}I-T4, and Percent of the ^{125}I-T4 Converted to ^{125}I-T3 by these Tissues after a Bolus Injection of ^{125}I-T4 into 2-wk-old Euthyroid and Hypothyroid Rats

	Tissue weight (g)	Integrated ^{125}I-T3(T4)‡ (% of ^{125}I-T4/g)·h	Total tissue ^{125}I-T3 production§ (% of ^{125}I-T4 dose/g)	Integrated ^{125}I-T4‖ (% of dose/g)·h	Fractional conversion rate (h^{-1})¶
Cerebral Cortex					
Euthyroid	0.72±0.03*	25.5	6.34	16.1	0.39
Hypothyroid	0.65±0.05	69.0	4.82	2.0	2.41
Hypo/Eu	0.90	2.74	0.76	0.12	6.12
Cerebellum					
Euthyroid	0.14±0.01	29.8	5.91	20.6	0.29
Hypothyroid	0.11±0.01	44.7	3.08	12.4	0.25
Hypo/Eu	0.79	1.50	0.52	0.60	0.87

* Mean±SEM of 28 euthyroid or hypothyroid rats. ‡ From data shown in Fig. 5, after subtracting the contribution of plasma ^{125}I-T3(T4). 88 and 73% of the ^{125}I-T3(T4) was locally produced in euthyroid cerebral cortex and cerebellum, whereas in hypothyroid rats this figure was >90% for both tissues. § Obtained by multiplying the integrated local ^{125}I-T3(T4) concentration shown in the column on the left by the fractional removal rate of T3 from these tissues, as shown in Table IV. ‖ From data shown in Fig. 5. ¶ Obtained from dividing the production of ^{125}I-T3(T4) by the integrated tissue concentration of ^{125}I-T4.

of ^{125}I-T4/g] h). This figure represents the efficiency of conversion in the tissue. In euthyroid rats, the rate was 0.39/h in the cortex and 0.29/h in the cerebellum, but in hypothyroid rats was increased only in the cortex. Thus, the 2.7-fold increase in the integrated ^{125}I-T3(T4) concentration in the cortex of hypothyroid rats was the combined result of enhanced fractional conversion and reduced rate of T3 disappearance, whereas the 1.5-fold increase in the integrated ^{125}I-T3(T4) in the cerebellum seems totally accounted for by the reduced rate of T3 removal.

The approach used to calculate ^{125}I-T3(T4) production rate in cerebral cortex and cerebellum could not be used for liver and kidney. In these two tissues, the ^{125}I-T3(T4) concentration relative to plasma was lower (Table VI) than the corresponding tissue to plasma ratios of ^{131}I-T3, probably due to the very rapid exchange of T3 with plasma as it will be discussed later.

Source and turnover of T3 in cerebral cortex and cerebellum of 2-wk-old euthyroid rats. Table VIII shows the wet weight of cerebral cortex and cerebellum, and the estimates of the concentrations of T4, T3, and the turnover of T3. The concentration of T4 was obtained from the equilibrium tissue-to-serum ratio (Table V) and the serum T4 by radioimmunoassay (RIA). The locally produced T3 was calculated as described in Methods, and the T3 coming from plasma was obtained from the unidirectional clearance of T3 by these two

Table VIII. Cerebral Cortex and Cerebellum T4 and T3 Concentrations, and T3 Turnover Rate in 2-wk-old Euthyroid Rats

	Weight		T4		T3		T3 Turnover		
	g	%BW	ng/g	%BP	ng/g	%BP	ng/g/h		% Extrathyroidal T3 production
Cortex	0.72	2.7	3.6	1.3	4.6	8.1	Local	0.96	15
							Plasma	0.21	—
							Total	1.17	—
Cerebellum	0.14	0.5	4.3	0.3	5.5	1.9	Local	0.89	2.3
							Plasma	0.21	—
							Total	1.10	—

BW, body weight; BP, body pool of the respective iodothyronine. T4 concentration was obtained from the equilibrium tissue-to-serum ratio (Table V) and serum T4 (Table II). T3 concentration was calculated from the total turnover shown in the next column divided by the fractional removal from the tissue (Table IV). Local T3 production was calculated as described in the text and the plasma contribution, "plasma," was obtained from multiplying the plasma T3 clearance by the corresponding tissue by the serum T3 (Table II). When T4 and T3 content are expressed as percent of the BP, or T3 production as percent of the extrathyroidal production, the values have been previously adjusted to the actual weight of the cortex and cerebellum, i.e., 0.72 and 0.14 g, respectively.

tissues (Table IV) and the radioimmunoassayable serum T3 concentration shown in Table II. The steady state concentrations of T3, in the last column of this table, were obtained from the total T3 turnover and the fractional removal rates shown in Table IV. Whereas the cerebral cortex constitutes ~2.7% of the body weight and the cerebrocortical pool of T4 is ~1.3% of the total body pool, the local production rate of T3 equals 15% of the total extrathyroidal T3 production rate. Similar calculations for the cerebellum revealed that even though the production rate of T3 in this tissue is also very rapid on a weight basis, because of the smaller size of this organ, is not as impressive as that of the cerebral cortex when expressed as a fraction of the total extrathyroidal T3 production.

Discussion

The present results suggest that T3 production and turnover are very active in the developing central nervous system and that marked changes take place in neonatal, congenitally hypothyroid rats. In the cerebral cortex of these rats, the efficiency of T4 to T3 conversion is increased and in both cortex and cerebellum, the mean transit time of T3 is prolonged. The data further suggest that the uptake of T3 and T4 by these tissues from the plasma is decreased in hypothyroidism, so that the increased in vitro (12, 14) and in vivo efficiency of T4 to T3 conversion observed in this condition is hampered by a decreased availability of T4 at any given plasma concentration. Under these circumstances, the prolonged residence time of T3 in the brain becomes also fundamental in the adaptation to hypothyroidism observed previously in neonatal rats (14).

The analyses of the time course of tissue and serum concentrations of labeled iodothyronines after injecting the appropriate tracers provides only limited information on the kinetics of tissue-to-plasma exchange and tissue T3 kinetics. Our goals were to estimate the turnover rate of T3 in the brain and define the changes in intracerebral T3 metabolism occurring in hypothyroidism. To achieve this goal it was necessary to obtain experimentally at least one more kinetic parameter, and the one found feasible was the fractional rate of T3 removal from the tissues. The approach used assumes that once the tracer T3 enters the cell it mixes rapidly with the intracellular T3, regardless of the source, and that the perturbation induced by the antibody does not alter the intrinsic rate of T3 disappearance during the time frame of the experiment. Evidence supporting the first assumption has been provided in the Methods. The second assumption is supported by our own results, in that the calculated tissue concentrations of T3 from the plasma concentrations and the fractional turnover rate obtained in different rats, generated curves in excellent agreement with the experimental ones. Had the antibody perturbed markedly the intrinsic turnover of T3 or given a grossly erroneous value, there would have been a proportional error in the calculation of clearance (see Eq. 3), and the application of these parameters to the plasma concentration of intact rats would have generated curves overtly deviating from the experimental ones.

The results are also consistent with the assumption that tracer T3(T3) is well mixed with the intracellular pool. If during the 6-h interval of observation after the injection of the antibody, the mixing process had been taking place, that is T3 being transferred from A into C in Fig. 1, the observed rate of disappearance from the tissue would have reflected the residual tracer T3 in A and C at various times, and K_{1A} and K (Fig. 1). Unless these two constants were very similar and the amounts of tracer in A and C at 2 h were approximately the same, the disappearance of the tracer during the 6 h of observations would have shown two components. The single component observed is compatible with most of the T3 in one compartment, either A or C. The evidence provided above suggests that already by 0.5 h at least a large fraction of tracer T3 is in compartment C. It is then reasonable to assume that the measured fractional removal rate reflects well the disappearance from the tracer from compartment C, that is, reflects the fractional removal rate of the bulk of cell T3.

Tissue-plasma exchange of T3 and T4 to T3 conversion in cerebral cortex and cerebellum of neonatal rats

Euthyroid rats. Previous studies, which are confirmed here, have shown that whereas serum T3 equilibrates very rapidly with the liver and kidney (in less than 0.5 h), it takes about 3 h to reach maximum concentration of T3 in brain after a bolus of tracer T3 (30, 31); these are observations that we had also made previously (21). The slow equilibration and the low tissue-to-serum ratio at equilibrium (compared with that of the liver and kidney) is in agreement with the low transfer rate of tracer T3 from plasma to brain found by Pardrige (35), and is not due to slow mixing within the cell. Furthermore, the relatively prolonged time it takes the tracer T3 to reach maximum suggests that the fraction rate of removal from these tissues is also slow, as was demonstrated by the studies described. The low volume of plasma cleared of T3 by the nervous tissue is in agreement with the minor contribution of plasma T3 to the T3 pool of these tissues. On the other hand, the slow fractional removal rate of T3 tends to retain T3 in the central nervous system, and since the production rate of T3 from T4 is a very active process, the relative contribution of this source is large, particularly in the cerebral cortex that produced an amount of ^{125}I-T3(T4) equal to ~15% of ^{125}I-T3(T4) produced in the pool in rapid exchange with plasma.

Responses to hypothyroidism. Of all four tissues examined, hypothyroidism induced the most marked reduction in fractional removal rate in the cerebral cortex followed by the cerebellum. The plasma clearance of T3 by these two tissues were proportionally reduced by hypothyroidism, and consequently, these tissues did not accumulate much more ^{131}I-T3 from plasma than that observed in the corresponding tissues of euthyroid rats. However, the slower fractional removal rate of T3 from the brain in hypothyroidism had a marked effect on the concentration of T3 derived from T4 in the central nervous system, particularly in the cerebral cortex.

At first glance, the reduced areas under the ^{125}I-T4 concentration in the cortex of hypothyroid animals and the greater areas under the ^{125}I-T3(T4) curves suggest that it is the enhanced formation ^{125}I-T3 which accounts for the difference. However, when the decreased fractional removal rate of T3 is taken into consideration, the fraction of the total ^{125}I-T4 converted to T3 in the cortex and the cerebellum turns out to be decreased not increased, indicating that the larger areas under the ^{125}I-T3(T4) curves observed in hypothyroid rats are largely due to the decreased fractional removal rate of T3. The reduced content of ^{125}I-T4 in the hypothyroid rats has then to be explained either by increased metabolism of T4 by other pathways or decreased uptake of T4 from plasma. The delay in reaching the maximum ^{125}I-T4 concentration in the cortex of the hypothyroid rats suggest that is not the accelerated disappearance of T4 via other pathways, but a decreased cerebrocortical uptake of ^{125}I-T4 from plasma which accounts for most of the reduction in cortex ^{125}I-T4 in hypothyroidism. As shown in Table VII, the fraction of ^{125}I-T4 available to this tissue converted to T3 per unit of time is increased, indicating a reduction of the T4 disposed by other pathways. This increased efficiency in conversion is in agreement with the elevated 5'D-II activity in cortex homogenates from hypothyroid rats (12, 14).

In the cerebellum, no evidence of enhanced T4-to-T3 conversion was found in these in vivo studies in contrast with the rather marked increments in 5'D-II activity in homogenates of hypothyroid cerebella from rats of this age (36). This discrepancy may indicate either an underestimation of the fractional removal rate of T3 (used to calculate ^{125}I-T3(T4) production) in these studies or that the in vitro assay conditions do not reproduce the conditions prevailing in vivo, e.g., the excess of dithiothreitol used in the assay may not have a counterpart in the reducing cofactors present in vivo.

The cerebral cortex is thus protected by a double mechanism in hypothyroidism, an increased efficiency of T4-to-T3 conversion, and reduced removal of T3, whereas the cerebellum is only protected by the latter. However, since the unidirectional clearance of T4 by the cortex is reduced, it is the reduced fractional removal of T3 which ultimately accounts for the greater T3 concentrations seen after T4 injections in these and in previous studies (14). In these two tissues with a large proportion of T3 fluxes derived from local production, reductions in the rate of T3 disposal may have a major impact in the tissue concentration of T3. At present we do not know the mechanisms involved, but given the relatively high levels of T3 inner ring deiodinase activity in homogenates of central nervous system tissues and the reduction in the activity of this enzyme in hypothyroidism (12), it is likely that a reduced rate of 5-deiodination of T3 plays an important role.

T3 economy in liver and kidney

These two tissues clear about 15 times more plasma of T3 than the cerebral cortex and cerebellum, but the fractional disappearance rates of T3 from liver and kidney are only four- to fivefold greater than those in the brain. This difference is reflected in higher tissue-to-serum ratios of ^{131}I-T3 than those observed in the nervous tissues.

A marked difference of liver and kidney with the brain is that the tissue-to-serum ratios of ^{125}I-T3(T4) are significantly lower than those of ^{131}I-T3 in the hepatic and renal tissue. Several factors may contribute to this discrepancy. One is the fact that the maximal tissue specific activity after injecting tracer T3 is reached shortly after the injection (30), at a time when the concentration of T3 in the plasma is decreasing very rapidly (20–30 min after the injection). Thus, during the short time when the tissue level turns from ascending to descending, the plasma concentration has decreased markedly, which elevates the tissue-to-serum ratio. Other factors are the location of the enzyme, 5'D-I, on the plasma membrane (28) and the rapid exchange with plasma. In liver and kidney, the T3(T4) does not distribute fully into the cell, for the nuclear to tissue ratio of ^{125}I-T3(T4) is less than that of ^{131}I-T3 ($P < 0.005$) 3 h after injecting ^{125}I-T4 and ^{131}I-T3 (J. E. Silva, unpublished observation). In the scheme shown in Fig. 1, although the turnover through compartment B is very active because of the large amounts of 5'D-I in liver and kidney, it is likely that either because of the location of the enzyme or the rapid transfer of T3 from plasma, or both, a large fraction of ^{125}I-T3(T4) is transferred into circulation before distributing throughout the cell compartment, i.e., K_{1B} is large compared with K_{BC}. Therefore, the approach used here to estimate T3 turnover and production in the central nervous system is not appropriate for liver and kidney.

Physiological and clinical implications of these studies

The present in vivo studies in 2-wk-old rats show that although the cerebral cortex is only ~2.5% of the body weight, with not more than 2% of the total T4 pool, it contains ~8% of the total T3 pool, and what is more important, that the total production rate of T3 by this tissue equals 15% of the extrathyroidal T3 production in the body pool exchangeable with plasma. These figures illustrate the high in vivo efficiency of 5'D-II, and an important question emerging from this is whether the central nervous system could contribute significantly to the plasma pool of T3. We have found that in rats this age, 80% of the T3 is formed extrathyroidally (27) and on a weight basis, the production rate of T3 is approximately twofold that observed in adult animals. In 2-wk-old rats, liver and kidney 5'-deiodinase activity is reduced compared with adult levels and the extrathyroidal production of T3 is minimally inhibited by propylthiouracil, observations that suggest that serum T3 comes largely from a 5'D-II type of enzyme (27). The data only show that the brain has the potential to produce a significant fraction of total body T3 production, but does not provide evidence as to how much. For one thing, the brain contains significant amounts of T3 5-deiodinase (12); it is conceivable that a significant fraction of the T3 produced is degraded in situ without reaching the circulation. On the other hand, our estimates of brain T3 production are based on the ^{125}I-T3(T4) seen in the tissues, so that it is also conceivable that a significant fraction of the T3 produced by the brain is

transferred directly into the circulation, without being seen in the tissue, as it may occur in liver and kidney (see above). Further studies are needed to define the anatomical sources of plasma T3 and their relative contributions.

Whereas the increased thyroid-stimulating hormone observed in hypothyroxinemia is fundamental for the maintenance of the serum T3 and thereby, the concentration of T3 in those tissues for which the serum is the main source of the hormone, the mechanisms described in the present studies illustrate the adaptation of tissues where most of the T3 derives from local conversion of T4 to T3. In the absence of such mechanisms, these tissues would be markedly affected in all those conditions of partial thyroid hypofunction where a reduced serum T4 is a constant finding. These studies may explain why in areas where there is moderate iodine deficiency, the modestly reduced serum levels of T4 in the cord blood (37) are not reflected in higher incidence of endemic cretinism as seen in areas of severe iodine deficiency (38). It is likely that the degree of hypothyroxinemia observed in areas of moderate deficiency is compensated by the mechanisms described here. Although we do not have figures of T3 production by the brain at other ages, the ontogeny of 5'D-II in the central nervous system (39) suggests that it is at this time when the production of T3 is maximal, a remarkable coincidence with the essential role of thyroid hormones during this period for brain development (15). Likewise, the responses of cerebrocortical tissue to hypothyroidism seem to be more efficient for T3 homeostasis at 2 wk than at later ages (14). Since the age of 2 wk is roughly equivalent to the perinatal period in the human in terms of brain maturation, we might be less concerned about modest reductions of serum T4 at birth, but certainly the treatment should not be delayed because the compensation mechanisms become less efficient.

The present studies also show that, even though the residence time of T3 in the central nervous system is prolonged in hypothyroidism, the unidirectional clearance of T3 from plasma by the central nervous system is proportionally reduced. It follows that if one of the mains goals of treating congenital hypothyroidism is to normalize the intracerebral T3 concentration, the best way of achieving the goal is with T4 replacement. In this form of hypothyroidism, as probably in any other, the brain is prepared to efficiently use modest amounts of T4 (14).

Acknowledgments

The secretarial work of Kathleen Danaher, Anne Keller, and Jeanne Gaz is well appreciated. We also appreciate Dr. P. R. Larsen's valuable suggestions in the preparation of this manuscript.

This work was supported in part by National Institutes of Health grant AM-18616.

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