

## **In vivo stimulation of sugar uptake in rat thymocytes. An extranuclear action of 3,5,3'-triiodothyronine.**

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### **Research Article**

In previous studies we have demonstrated that 3,5,3'-triiodothyronine (T3) in vitro produces a prompt increase in the uptake of the sugar analogue 2-deoxyglucose (2-DG) by freshly isolated rat thymocytes. This effect is prompt, being evident at 20 min after addition of T3, is independent of new protein synthesis, and can be elicited by physiologic concentrations of the hormone. In the present studies, we have sought to determine whether physiologic doses of T3 are capable of inducing an increase in 2-DG uptake in the thymocytes of the living animal. Therefore, 26-28-d-old female rats were injected with increasing doses of i.v. T3, followed 60 min later by 3H-labeled 2-DG. 30 min later, animals were killed, thymocytes were isolated, and their 3H content determined. Uptake of [3H]2-DG was increased by T3 in a dose-dependent manner. The lowest effective dose was 10 ng/100 g of body weight (30% above control) and the maximally effective dose 1 microgram/100 g of body weight (116% above control). The effect of T3 was independent of new protein synthesis in that it was not blocked by a dose of cycloheximide that inhibited the incorporation of [3H]leucine into thymocyte protein by 92-95%. Comparable studies with various thyronine analogues revealed the following rank order of potency: L-T3 greater than L-3,5,3'5'-tetraiodothyronine (L-T4) greater than D-T3 greater than or equal to D-T4 greater [...]

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# In Vivo Stimulation of Sugar Uptake in Rat Thymocytes

## An Extranuclear Action of 3,5,3'-Triiodothyronine

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### Abstract

In previous studies we have demonstrated that 3,5,3'-triiodothyronine ( $T_3$ ) in vitro produces a prompt increase in the uptake of the sugar analogue 2-deoxyglucose (2-DG) by freshly isolated rat thymocytes. This effect is prompt, being evident at 20 min after addition of  $T_3$ , is independent of new protein synthesis, and can be elicited by physiologic concentrations of the hormone. In the present studies, we have sought to determine whether physiologic doses of  $T_3$  are capable of inducing an increase in 2-DG uptake in the thymocytes of the living animal. Therefore, 26–28-d-old female rats were injected with increasing doses of i.v.  $T_3$ , followed 60 min later by  $^3\text{H}$ -labeled 2-DG. 30 min later, animals were killed, thymocytes were isolated, and their  $^3\text{H}$  content determined. Uptake of [ $^3\text{H}$ ]2-DG was increased by  $T_3$  in a dose-dependent manner. The lowest effective dose was 10 ng/100 g of body weight (30% above control) and the maximally effective dose 1  $\mu\text{g}/100$  g of body weight (116% above control). The effect of  $T_3$  was independent of new protein synthesis in that it was not blocked by a dose of cycloheximide that inhibited the incorporation of [ $^3\text{H}$ ]leucine into thymocyte protein by 92–95%. Comparable studies with various thyronine analogues revealed the following rank order of potency:  $L\text{-}T_3 > L\text{-}3,5,3'5'\text{-tetraiodothyronine (L-}T_4) > D\text{-}T_3 \geq D\text{-}T_4 > L\text{-}3,3'5'\text{-triiodothyronine} > 3'\text{-isopropyl-}3,5\text{-L-diiiodothyronine (}T_2) = 3,5\text{-L-}T_2$ . DL-thyronine was without effect.

These studies indicate that  $T_3$  in physiologic doses acts in vivo to increase the uptake of sugar by rat thymocytes by a mechanism that is extranuclear in origin, in that it is independent of new protein synthesis. The findings support the conclusion that the previously demonstrated effects of  $T_3$  on thymocyte sugar uptake in vitro, which seem clearly to be mediated at the level of the plasma membrane, have physiologic relevance.

### Introduction

We have previously demonstrated that 3,5,3'-triiodothyronine ( $T_3$ )<sup>1</sup> increases the uptake of the glucose analogue 2-deoxyglucose (2-DG) in isolated rat thymocytes in vitro (1–5). This effect of  $T_3$  is evident within 20 min after the addition of the hormones;

it is independent of new protein synthesis, is calcium-dependent, and is mediated by an increase in cellular cyclic AMP (cAMP) concentration secondary to an increase in adenylate cyclase activity. On the basis of these and other findings, we have suggested that this action of  $T_3$  is mediated at the level of the thymocyte plasma membrane, and consonant with this is our demonstration of the presence of specific, saturable binding sites for  $T_3$  in highly purified thymocyte plasma membrane preparations (6).

Although supraphysiologic concentrations of  $T_3$  are required to produce these effects when  $T_3$  is tested alone, in the presence of adequate concentrations of insulin and epinephrine, the in vitro effect of  $T_3$  on sugar uptake in the intact thymocyte is elicited by physiologic concentrations of  $T_3$  as low as 5 pM (2). Nonetheless, in respect to these actions of  $T_3$ , as with other in vitro metabolic responses to a hormonal agent, ultimate proof of their physiologic relevance depends on the demonstration that they can be reproduced by physiologic doses in the living animal. In the present studies, we have shown that very small doses of  $T_3$  (10 ng/100 g of body weight [bw]) increase the uptake of 2-DG by thymocytes in the living rat, a response that, like its in vitro counterpart, is dose-dependent and independent of new protein synthesis.

### Methods

**Animals and reagents.** Animals used were 26–28-d-old female rats of the Sprague-Dawley (CD) strain purchased from Charles River Laboratories, Wilmington, MA, and weighing ~65 g at the time of study.<sup>2</sup> Rats were maintained for up to 5 d on tap water and a standard diet (OVAL, Charles River RMH 3000) supplied by Agways, Inc., Syracuse, NY.

Other materials used in these studies were purchased from the following sources:  $^{125}\text{I-}T_3$  (sp act 1,200  $\mu\text{Ci}/\mu\text{g}$ ), [ $^3\text{H}$ ]2-DG (sp act 5–8 Ci/mmol), [ $^3\text{H}$ ]mannitol (sp act 17 Ci/mmol), and [ $^3\text{H}$ ]leucine (sp act 141 Ci/mmol) from New England Nuclear, Boston, MA; cycloheximide and DL-thyronine from Sigma Chemical Co., St. Louis, MO;  $L\text{-}T_3$ ,  $L\text{-}3,5,3'5'\text{-tetraiodothyronine (L-}T_4)$ ,  $D\text{-}T_3$ ,  $D\text{-}T_4$ , and  $L\text{-}3,5\text{-diiiodothyronine (}T_2)$  from Henning GmbH, Berlin, Federal Republic of Germany;  $L\text{-}3'5'\text{-triiodothyronine (r}T_3)$  from Calbiochem-Behring, San Diego, CA.  $3'\text{-isopropyl-}T_2$  was a gift from Henning GmbH.

Unless otherwise stated, the various materials administered were dissolved in saline or mildly alkaline saline solution and injected into the tail vein in a volume of 0.1 ml/100 g of bw.

**Measurement of tissue  $^{125}\text{I-}T_3$  accumulation.** Initial experiments were conducted to determine the time course of accumulation of administered  $T_3$  in several organs, including the thymus gland. Animals were injected with 250 nCi of  $^{125}\text{I-}T_3/100$  g of bw (0.21 ng of  $T_3/100$  g of bw)<sup>3</sup> and

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1. Abbreviations used in this paper: bw, body weight; 2-DG, 2-deoxy-D-glucose;  $T_2$ , diiodothyronine;  $T_3$ , triiodothyronine;  $T_4$ , tetraiodothyronine.

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2. Animals used in this study were maintained in accord with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication No. (NIH) 78-32, revised 1978).

3. Purity of  $^{125}\text{I-}T_3$ , measured by descending paper chromatography (7), was >98%.

were killed by means of a guillotine at various times. Blood was collected and the thymus gland, liver, and kidneys were quickly removed. The  $^{125}\text{I-T}_3$  concentration in serum and in these organs was then measured in a gamma-scintillation counter.

**Measurement of [ $^3\text{H}$ ]2-DG uptake by thymocytes.** Additional experiments examined the time course of the uptake of [ $^3\text{H}$ ]2-DG in thymocytes. Here, animals were injected with 30  $\mu\text{Ci}$  of [ $^3\text{H}$ ]2-DG/100 g of bw (0.8  $\mu\text{g}$  of 2-DG/100 g of bw) and were killed by means of a guillotine at various times thereafter. Blood was collected, from which serum was obtained, and thymus glands were quickly removed and placed into ice-cold Tris-Krebs-Ringer buffer, pH 7.4. Thymocytes were then isolated by methods previously described (1). The isolated cells were resuspended in ice-cold buffer, and 200- $\mu\text{l}$  aliquots ( $\sim 30 \times 10^6$  cells/ml) were transferred to microtubes and centrifuged in a Beckman Microfuge (Beckman Instruments, Inc., Palo Alto, CA) for 30 s. The supernatant was aspirated, and the pelleted cells were transferred to vials containing liquid scintillation cocktail, as were aliquots of serum.  $^3\text{H}$  content of the samples was measured in a liquid scintillation counter.<sup>4</sup>

In each experimental group, the extracellular [ $^3\text{H}$ ]2-DG included in the pellet of isolated thymocytes was determined. Prior to centrifugation, [ $^3\text{H}$ ]mannitol was added to cell suspensions obtained from animals that had not been injected with [ $^3\text{H}$ ]2-DG in each of the experimental groups. Cells were pelleted, and the [ $^3\text{H}$ ]mannitol contents of the pellets determined. These values were used to correct values of the [ $^3\text{H}$ ]2-DG uptake for the labeled sugar occluded in the cell pellet. Values for occluded  $^3\text{H}$  did not exceed 0.1% of the total added and did not differ among various experimental groups.

In each animal injected with [ $^3\text{H}$ ]2-DG, blood was obtained at the time of sacrifice and serum derived therefrom was analyzed for  $^3\text{H}$  concentration. To determine the chemical nature of the  $^3\text{H}$ -labeled compounds within the thymocyte, thin-layer chromatographic analysis for sugars was performed in cellulose, using ethyl acetate, pyridine, water, and glacial acetic acid (36:36:21:7) as the running solvent (8).

**Measurement of [ $^3\text{H}$ ]leucine incorporation in thymocytes.** Animals were injected with 40  $\mu\text{Ci}$  of [ $^3\text{H}$ ]leucine/100 g of bw into the tail vein. 15 min later, animals were killed by means of a guillotine and thymocytes were immediately isolated. 150- $\mu\text{l}$  aliquots of thymocyte suspension were transferred into small plastic tubes containing 150  $\mu\text{l}$  of ice-cold 12% trichloroacetic acid (TCA), mixed well and put on ice for 20 min. Tube contents were transferred onto 0.45- $\mu\text{m}$  Millipore filters (Millipore Corp., Medford, MA) under vacuum. Tubes and filters were washed three times with 2 ml of ice-cold 6% TCA, filters were dried, transferred to vials containing liquid scintillation cocktail, and their content of  $^3\text{H}$  was measured with a beta-scintillation counter.

**Cell viability.** Cell viability was assessed by means of the trypan blue technique (1), in which a viable cell is one that excludes the dye.

**Statistical analysis.** Where appropriate, statistical analyses of the differences between experimental groups were performed using the analysis of variance followed by the Newman-Keuls multiple range test for comparison of multiple groups with one another (9).

## Results

**Tissue accumulation of  $^{125}\text{I-T}_3$  in vivo.** Serum  $^{125}\text{I-T}_3$  concentration declined rapidly, in an apparent exponential fashion, following i.v. injection of the labeled hormone (Fig. 1). Concentrations of  $^{125}\text{I}$  in liver and kidney rose rapidly, reaching a peak at  $\sim 20$  min, decreasing rapidly thereafter, and ultimately

4. The thymocyte content of [ $^3\text{H}$ ]2-DG was also measured by a second method in which 200- $\mu\text{l}$  aliquots of the suspended cells were transferred onto GF/A glass-fiber filters (Whatman Chemical Separation Inc., Clifton, NJ), and washed three times with 2 ml of ice-cold buffer. Filters were then dried and transferred to vials containing liquid scintillation cocktail, and their content of  $^3\text{H}$  was measured. Similar results were obtained with both procedures. Because the procedure utilizing the microtubes was more convenient, it was employed in ensuing experiments.

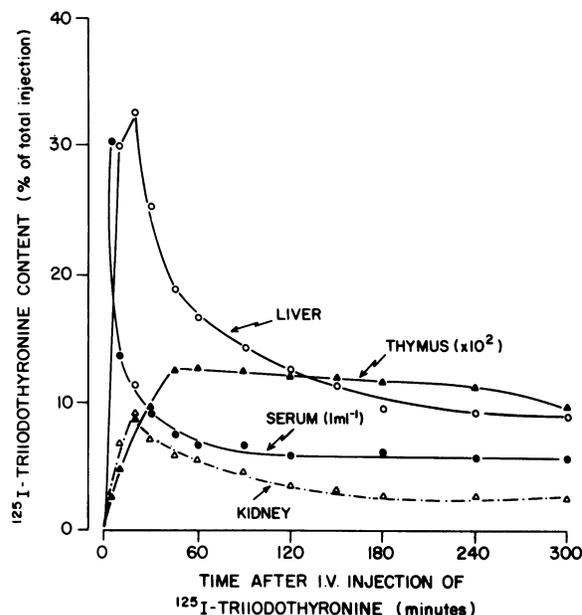


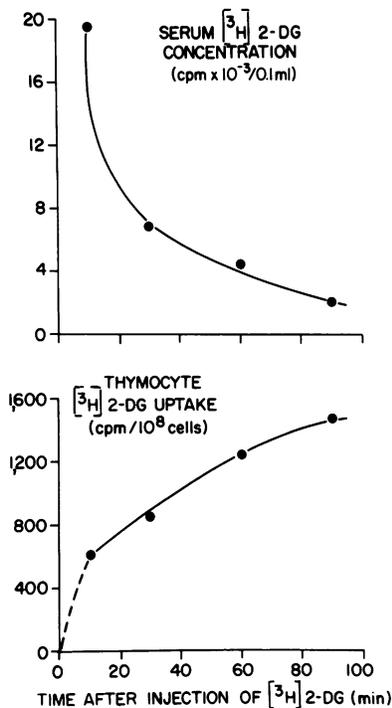
Figure 1. Time course of  $^{125}\text{I}$ -triiodothyronine accumulation in several tissues in the rat. Animals were injected with 250 nCi of  $^{125}\text{I-T}_3$ /100 g of bw. Animals were killed at the time periods indicated, and serum, thymus, liver, and kidneys were collected and their content of  $^{125}\text{I}$  was measured. Values shown are the results obtained in a single experiment involving one animal per time point, but are typical of those obtained in two additional experiments.

declining in parallel with the decrease in serum  $^{125}\text{I}$  concentration. In contrast, the concentration of  $^{125}\text{I}$  in the whole thymus gland reached a peak at  $\sim 40$  min, but remained relatively constant thereafter.

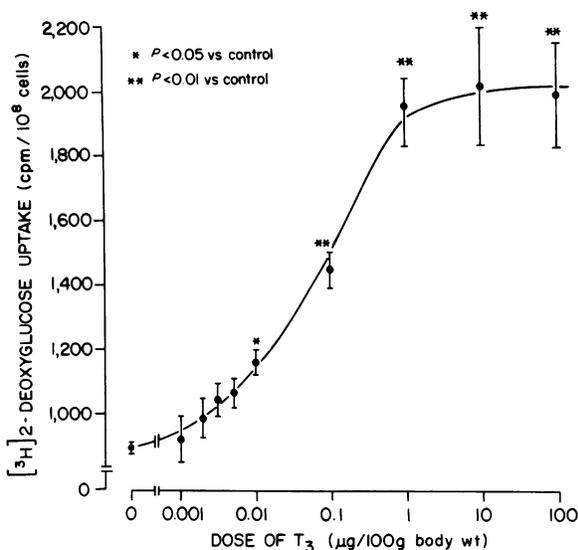
**[ $^3\text{H}$ ]2-deoxyglucose uptake.** After the i.v. injection of [ $^3\text{H}$ ]2-DG, the serum concentration of [ $^3\text{H}$ ]2-DG displayed a rapid, apparently exponential, rate of decline. This was accompanied by a progressive increase in the thymocyte uptake of the labeled sugar, which appeared to represent an exponential approach to a maximum, reflecting in a mirror-image fashion the decline in serum [ $^3\text{H}$ ]2-DG concentration (Fig. 2). This behavior is consonant with the known metabolism of 2-DG, i.e., uptake into the cell, phosphorylation by hexokinase, and the retention of 2-DG-6-phosphate within the cell without further metabolism (10, 11). In subsequent experiments, animals were killed for measurement of thymocyte [ $^3\text{H}$ ]2-DG uptake 30 min after administration of [ $^3\text{H}$ ]2-DG. This was an interval at which serum [ $^3\text{H}$ ]2-DG concentration was declining more slowly than it would have been at earlier time periods, making the precision of the time of killing animals less critical.

**Effect of  $T_3$  on thymocyte 2-DG uptake.** Animals were given i.v.  $T_3$  in doses ranging from 2 ng to 100  $\mu\text{g}$ /100 g of bw, followed in 60 min by [ $^3\text{H}$ ]2-DG (30  $\mu\text{Ci}$ /100 g of bw). 30 min later, animals were killed, thymocytes were isolated, and their content of [ $^3\text{H}$ ]2-DG was measured.

$T_3$  produced a dose-related increase in the uptake of [ $^3\text{H}$ ]2-DG by thymocytes (Fig. 3). The lowest effective dose was 10 ng/100 g of bw (130% of control values,  $P < 0.05$ ), and the maximal effect was reached at a dose of 1  $\mu\text{g}$ /100 g of bw (216% of control values,  $P < 0.01$ ). From the dose-response curve, the calculated half-maximal effect was obtained with a dose of 50-100 ng/100 g of bw.



**Figure 2.** Time course of the uptake of  $[^3\text{H}]2\text{-DG}$  by rat thymocytes. Animals were injected with  $30\ \mu\text{Ci}$  of  $[^3\text{H}]2\text{-DG}/100\ \text{g}$  of bw into the tail vein. Thereafter, at the periods of time indicated, animals were killed, and the content of  $^3\text{H}$  in serum (upper panel) or thymocytes (lower panel) was measured. Values shown are the results obtained in a single experiment involving one animal per time point, but are typical of those obtained in two additional experiments.



**Figure 3.** Dose-related effect of 3,5,3'- $\text{T}_3$  on  $[^3\text{H}]2\text{-DG}$  uptake by rat thymocytes in vivo. Animals were given the indicated doses of  $\text{T}_3$  by a single injection into the tail vein. 60 min later,  $[^3\text{H}]2\text{-DG}$  ( $30\ \mu\text{Ci}/100\ \text{g}$  of bw) was injected into the tail vein and its 30-min uptake by thymocytes was then measured. Values shown are the mean  $\pm$  standard deviation of pooled data obtained in two (0.001 and  $0.002\ \mu\text{g}$  of  $\text{T}_3$ ), three (0.005, 0.01, 2, and  $100\ \mu\text{g}$  of  $\text{T}_3$ ), four (0.003, 0.1, and  $10\ \mu\text{g}$  of  $\text{T}_3$ ), or seven (control and  $1\ \mu\text{g}$   $\text{T}_3$ ) separate experiments, each involving a single animal.

Effects of  $\text{T}_3$  on thymocyte 2-DG uptake could not be explained by a reduction in uptake of the sugar by other tissues leading to a higher serum  $[^3\text{H}]2\text{-DG}$  concentration, since the concentration of  $^3\text{H}$  in the serum of animals given the maximally effective dose of  $\text{T}_3$  ( $1\ \mu\text{g}/100\ \text{g}$  of bw) was  $93.0 \pm 16.2\%$  (mean  $\pm$  SD) of that found in control animals. Additional experiments related to this point are described below.

As judged from thin-layer chromatographic analysis,  $>90\%$  of the  $^3\text{H}$  in thymocytes from control and  $\text{T}_3$ -treated animals was  $[^3\text{H}]2\text{-DG-6-phosphate}$ .

#### *Inhibition of thymocyte protein synthesis by cycloheximide.*

Rats were given a single i.p. injection of cycloheximide in various doses, and 30 min later  $[^3\text{H}]$ leucine ( $40\ \mu\text{Ci}/100\ \text{g}$  of bw) was injected into the tail vein. Rats were killed 15 min later, their thymocytes were harvested, and  $[^3\text{H}]$ leucine incorporation into thymocyte protein was measured. Cycloheximide produced a dose-related decrease in  $[^3\text{H}]$ leucine incorporation;  $0.1\ \text{mg}$  and  $1.0\ \text{mg}/100\ \text{g}$  of bw inhibited  $[^3\text{H}]$ leucine incorporation by 65% and 95%, respectively. In additional experiments, cycloheximide ( $1\ \text{mg}/100\ \text{g}$  of bw) was injected i.p., and 30, 60, 120, and 180 min later animals were injected with  $[^3\text{H}]$ leucine. After 15 min, animals were killed and incorporation of  $[^3\text{H}]$ leucine into thymocyte protein was measured. In all the four time periods studied, cycloheximide inhibited  $[^3\text{H}]$ leucine incorporation by not  $<92\%$ . Hence, in subsequent experiments, animals were injected with i.p. cycloheximide ( $1\ \text{mg}/100\ \text{g}$  of bw) 30 min prior to the injection of  $\text{T}_3$ .

**$\text{T}_3$  effect and cycloheximide.** To examine whether the stimulatory effect of  $\text{T}_3$  on thymocyte 2-DG uptake requires new protein synthesis, animals were first injected with i.p. cycloheximide ( $1\ \text{mg}/100\ \text{g}$  of bw). 30 min later,  $\text{T}_3$  in doses ranging from  $0.1$  to  $1\ \mu\text{g}/100\ \text{g}$  of bw was injected into the tail vein, followed in 60 min by  $[^3\text{H}]2\text{-DG}$ . Animals were killed 30 min later and both the  $[^3\text{H}]2\text{-DG}$  concentration in serum and uptake by thymocytes were measured.

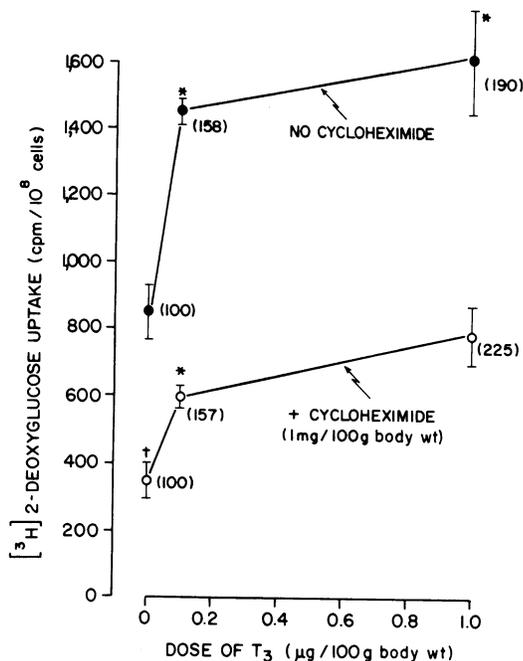
Cycloheximide reduced basal (control) thymocyte 2-DG uptake by 60%, an effect similar to that produced by both puromycin and cycloheximide in thymocytes in vitro (1). However, the proportionate increase in thymocyte 2-DG uptake that  $\text{T}_3$  produced was generally the same in animals that were treated with cycloheximide as in those that were not (Fig. 4).

#### *Effect of $\text{T}_3$ and cycloheximide on serum $^3\text{H}$ concentration.*

Because the uptake of  $[^3\text{H}]2\text{-DG}$  by tissues over time is in part a function of the integrated plasma  $[^3\text{H}]2\text{-DG}$  concentration to which they are exposed during this period, additional experiments were undertaken to verify that the changes in thymocyte 2-DG uptake seen in response to  $\text{T}_3$ , cycloheximide, or both were not merely the result of a change in overall  $[^3\text{H}]2\text{-DG}$  disposal. In these experiments, animals were injected with  $\text{T}_3$  ( $1\ \mu\text{g}/100\ \text{g}$  of bw), cycloheximide ( $1\ \text{mg}/100\ \text{g}$  of bw), or both, followed by  $[^3\text{H}]2\text{-DG}$  according to the protocols described above. Animals in each of the groups were killed at 5, 10, 20, and 30 min after injection of  $[^3\text{H}]2\text{-DG}$  and the  $^3\text{H}$  concentration in specimens of serum obtained at those times was measured.

In animals given  $\text{T}_3$  alone, serum  $^3\text{H}$  concentration was consistently  $\sim 10\%$  lower than in controls at all time points. The converse was true in animals treated with cycloheximide, the average increase being  $\sim 50\%$ . Serum  $^3\text{H}$  concentrations in animals given both cycloheximide and  $\text{T}_3$  were  $\sim 10\text{--}20\%$  lower than those in animals given cycloheximide alone (Table I).

**Thyronine analogues.** The effect of various thyronine analogues on the in vivo uptake of  $[^3\text{H}]2\text{-DG}$  by rat thymocytes



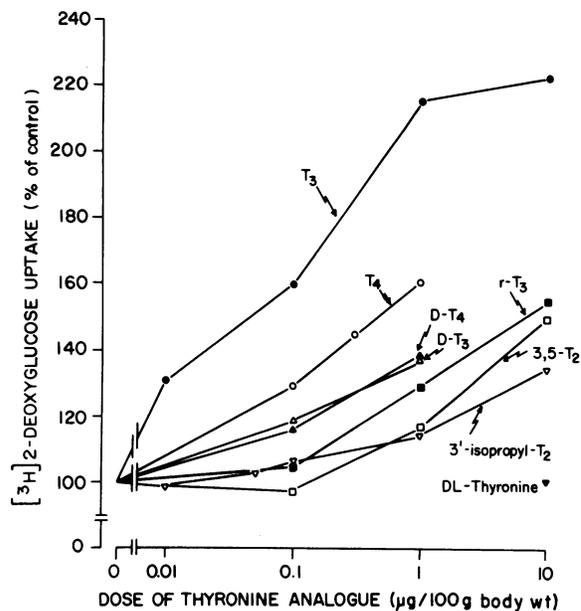
**Figure 4.** The effect of cycloheximide on the T<sub>3</sub>-induced increase in 2-DG uptake by rat thymocytes in vivo. Animals were first injected i.p. with 1 mg of cycloheximide/100 g of bw. 30 min later, T<sub>3</sub> in various doses was injected into the tail vein, and 60 min later the thymocyte uptake of [<sup>3</sup>H]2-DG was measured. Values shown are the mean ± standard deviation of those obtained in four separate experiments, each involving a single animal. \*, significantly greater than the corresponding value in the absence of T<sub>3</sub> administration ( $P < 0.01$ ). +, significantly lower than the corresponding value in the absence of cycloheximide and T<sub>3</sub> ( $P < 0.01$ ). The numbers in parentheses are the values in T<sub>3</sub>-treated animals expressed as a percent of those in corresponding animals given no T<sub>3</sub>.

was studied, using the same experimental procedure as that used for T<sub>3</sub>. Relative potencies were estimated from dose-response curves (Fig. 5), and the following rank-order of potency was

**Table I.** Effects of T<sub>3</sub> and Cycloheximide, Alone and Together, on the Disappearance of <sup>3</sup>H from the Serum of Animals Injected with [<sup>3</sup>H]2-DG

Time after [ <sup>3</sup> H]2-DG injection min	Serum <sup>3</sup> H concentration			
	Control	T <sub>3</sub>	CH	CH + T <sub>3</sub>
	cpm × 10 <sup>-3</sup> /0.1 ml			
5	33.3 ± 11.4	28.2 ± 6.6	58.7 ± 21.1	47.6 ± 11.9
10	26.4 ± 11.8	18.5 ± 5.7	35.1 ± 15.4	32.0 ± 13.1
20	10.7 ± 3.1	10.1 ± 7.4	19.2 ± 4.7	15.9 ± 6.0
30	8.0 ± 2.4	7.2 ± 2.2	13.3 ± 4.7	9.5 ± 1.6

Animals were injected with T<sub>3</sub> (1 µg/100 g of bw) and/or cycloheximide (CH) (1 mg/100 g of bw) as described in Methods. [<sup>3</sup>H]2-DG was injected i.v. and animals were killed at the indicated intervals thereafter. Values shown are mean ± standard deviation of those obtained in three separate experiments involving one animal for each data point. As judged from Dunnett's test (9), results in the three experimental groups are not significantly different from those in the corresponding control group at any time point.



**Figure 5.** The effect of several thyronine analogues on 2-DG uptake by rat thymocytes in vivo. The experimental procedure was the same as that described in the legend to Fig. 3. Values shown are the mean of those obtained in eight (L-T<sub>3</sub>) or three (other analogues) separate experiments, each involving a single animal per point. Values of standard deviations were 5–15% of their respective mean values.

observed: L-T<sub>3</sub> > L-T<sub>4</sub> > D-T<sub>3</sub> = D-T<sub>4</sub> > r-T<sub>3</sub> > 3'-isopropyl-T<sub>2</sub> = L-3,5-T<sub>2</sub>. DL-thyronine was without effect.

## Discussion

In the present studies, we have shown that administration of T<sub>3</sub> leads to an increase in the uptake of the glucose analogue 2-DG by thymocytes in the living female weanling rat. The effect of T<sub>3</sub> was relatively prompt in onset, being evident 90 min after T<sub>3</sub> administration, the earliest time point studied. This response to T<sub>3</sub> was independent of new protein synthesis, because it was not blocked by a dose of cycloheximide that profoundly inhibited thymocyte protein synthesis throughout the entire 90 min period of T<sub>3</sub> action. These effects of T<sub>3</sub> and cycloheximide, alone and together, on thymocyte 2-DG uptake could not be explained by unrelated effects of these agents on the clearance of [<sup>3</sup>H]2-DG from the circulation. Thus over the entire 30 min period during which [<sup>3</sup>H]2-DG uptake was measured, <sup>3</sup>H concentrations in serum tended to be lower than in controls in animals that received T<sub>3</sub>, higher than in controls in animals that received cycloheximide, and lowered by T<sub>3</sub> in animals of the latter group. Indeed, the apparent lowering of serum <sup>3</sup>H concentration would be consistent with an increased uptake of [<sup>3</sup>H]2-DG by tissues other than thymocytes, in that the increase in [<sup>3</sup>H]2-DG uptake by thymocytes alone could not have accounted for the lowering of serum <sup>3</sup>H concentrations that was observed.

Studies with various thyronine analogues revealed that, except in the case of 3'-isopropyl-T<sub>2</sub>, which was much less potent than T<sub>3</sub>, their rank order of potency in stimulating 2-DG uptake in vivo generally accorded well with that of their classical thyromimetic effects (12–15). Thus, L-T<sub>3</sub> was more potent than L-T<sub>4</sub>, the L-isomers of the two hormones were more potent than their D-isomers, and r-T<sub>3</sub>, 3,5-T<sub>2</sub>, and DL-thyronine were much less effective or not effective at all.

Over a range of  $T_3$  doses from 10 ng/100 g of bw to 1  $\mu\text{g}/100$  g of bw, the response to  $T_3$  was dose-related. Doses within this range can clearly be considered physiologic, and the response thereto to be physiologically relevant, in that the daily replacement dose of  $T_3$  in the rat is  $\sim 0.5$   $\mu\text{g}/100$  g of bw (16).

The major features of the in vivo increase in thymocyte sugar uptake induced by  $T_3$  are similar to those of the increase in thymocyte sugar uptake produced by  $T_3$  in vitro (1). This is true of the promptness of the effect, its independence of new protein synthesis, and the rank order of potency of various thyronine analogues, including the relative lack of potency of 3'-isopropyl- $T_2$ . Further, as with the very small doses of  $T_3$  required in vivo, responses to  $T_3$  in vitro were elicited by physiologic concentrations of  $T_3$ , in the presence of insulin and epinephrine (2). These similarities suggest that the mechanism by which  $T_3$  increases thymocyte 2-DG uptake in vivo is the same as that by which it does so in the in vitro system, a mechanism that seems clearly to be initiated at the level of the plasma membrane.

If this is so, then from the findings obtained in vitro, we would suggest that  $T_3$  initiates its action in vivo by binding to specific receptors on the thymocyte plasma membrane (6). This is followed by an increase in cytoplasmic free calcium concentration resulting from an influx of extracellular  $\text{Ca}^{2+}$  (5, 17). This, in turn, leads to a calmodulin-mediated increase in adenylate cyclase activity (18), and the resulting increase in cellular cAMP concentration in some manner leads to an increase in the activity of sugar transport carriers within the thymocyte plasma membrane (4).

Iqbal and co-workers (19) have demonstrated that  $T_3$  in vitro in near physiologic concentrations promptly increases the uptake of 2-DG, as well as amino acids, into slices of cerebral cortex from the hypothyroid mouse, findings that seem to accord with those in the thymocyte. It remains to be determined in what other tissues  $T_3$  may influence sugar uptake in this way, and studies on that point are in progress, but the present findings lend credence to the concept that thyroid hormones exert their diverse metabolic effects in various tissues by more than a single mechanism and at more than a single primary site. In some cases, these actions may be complementary to one another, as in the case of the prompt, plasma membrane-mediated effect of  $T_3$  to increase sugar transport into the chick embryo myocardial cell, an action that is followed by a delayed, nucleus-mediated increase in hexokinase activity (20–22).

An unusual aspect of the current findings is the low potency of 3'-isopropyl- $T_2$  in stimulating thymocyte 2-DG uptake in vivo, in that this analogue is more potent than  $T_3$  and indeed is the most potent analogue known with respect to classic thyromimetic effects, such as oxygen consumption and goiter prevention (12, 13, 15). On the other hand, the present in vivo findings correlate very well with results obtained in vitro, since 3'-isopropyl- $T_2$  was far less potent than  $T_3$  in stimulating the in vitro thymocyte uptake of 2-DG and in enhancing adenylate cyclase activity in thymocyte membrane preparations (23). Seemingly analogous are the observations of Will-Shahab and co-workers (24), who found 3'-isopropyl- $T_2$  much less potent than  $T_3$  in stimulating adenylate cyclase activity in cat and rat myocardium. These and numerous other findings suggest that responses to thyroid hormones, and the relative potencies of thyroid hormone analogues in eliciting them, are much more heterogeneous than has generally been recognized, varying with the nature of the response and perhaps its locus of initiation, and differing in some cases from tissue to tissue (14, 24–39).

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