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Uptake, metabolism and subcellular distribution of [^{125}I]T_4 in calf thyroid slices

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Abstract. Previous work from our laboratory has shown that iodothyronines have a direct inhibitory action on the thyroid. In the present studies the uptake, metabolism and subcellular distribution of labelled thyroxine were analyzed. The entry of this hormone reaches a plateau after 15–20 min incubation and is temperature-dependent. The T/M values for T_4 were much lower than those of labelled T_3 and the curve was different from that obtained with [^{125}I]. The influence of a series of compounds on the T/M values for thyroxine was studied. KI, T_3, IOP, PTU and MMI were without effect. Absence of Ca^{++} in the buffer, or addition of $KClO_4$ or ouabain caused a slight decrease, while TSH produced a stimulation in the T/M ratio. Calf thyroid slices dehalogenated thyroxine, producing both T_3 and rT_3. TSH increased the generation of these two compounds. Neither PTU nor IOP altered the production of T_3 and rT_3 significantly. The lack of effect of IOP on T_3 and rT_3 generation was confirmed in calf thyroid homogenates. However, and in agreement with previous reports, IOP significantly decreased production of both triiodothyronines in rat liver slices and in rat liver homogenates.
When the subcellular distribution of labelled thyroxine was examined most of the radioactivity appeared in the soluble fraction and less than 1% was present in purified nuclei. The addition of unlabelled thyroxine to the slices only caused a significant displacement in the radioactivity of purified nuclei. The presence of labelled thyroxine in the nuclei was confirmed by paper chromatography.

Iodine has been used for many years in the treatment of goitre and to relieve symptoms of Graves' disease. Many studies have been performed in order to clarify its mechanism of action (see reviews Wolff 1976; Pisarev & Kleiman de Pisarev 1980). Results from different laboratories have shown that iodine inhibits a number of thyroid parameters, such as adenylate cyclase and cyclic AMP formation (Van Sande & Dumont 1973; Rapoport et al. 1975;), protein synthesis (Sherwin & Tong 1975; Pisarev & Aiello 1976), thyroid growth (Pisarev & Itoiz 1972) and RNA labelling (Pisarev et al. 1976).

These actions of KI are inhibited by perchlorate, propylthiouracil and methylmercaptoimidazole*, indicating that they are mediated by an organified iodine intracellular compound (see Wolff 1976). Previous data from our laboratory as well as from others (Pisarev & Aiello 1976; Pisarev et al. 1976; Friedman et al. 1978) showed that iodothyronines have a direct in vitro inhibitory effect on thyroid RNA labelling and on ODC activity, and these

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* Abbreviations: MMI: methylmercaptoimidazole; PTU: propylthiouracil; T/M: thyroid/medium ratio; IOP: iopanoic acid; DTT: dithiothreitol; β-ME: beta-mercaptoethanol; TCA: trichloroacetic acid.

results led to the hypothesis that they may be the intermediates of iodine action.

Since the inhibitory effect of iodothyronines on the thyroid was not altered by addition of PTU or MMI, and labelled T_3 was not significantly dehalogenated under the same experimental conditions (Juvenal et al. 1981), it is suggested that T_3 has a direct inhibitory action on the thyroid, which is probably related to the autoregulatory mechanism. Labelled T_3 is concentrated in the thyroid cell by a temperature-dependent process and is specifically localized in the nuclei (Pisarev et al. 1981). We have analyzed the uptake, metabolism and subcellular localization of labelled thyroxine under the same experimental conditions as those in which it inhibits protein and RNA synthesis. The action of different compounds on these parameters was also examined.

Materials and Methods

Calf thyroid slices obtained from the same gland were prepared with a Stadie-Riggs hand microtome from glands recently obtained at a local slaughterhouse. The slices were kept in cold isotonic saline.

Uptake of [^{125}I]T_4 *by thyroid slices*

Calf slices were pre-incubated for around 15 min in saline, at 37°C. Afterwards they were incubated in quadruplicate in 4 ml of Krebs-Ringer-Bicarbonate buffer, pH 7.4 under 95% O_2 and 5% CO_2 at 37°C. After 30 min a tracer dose of [^{125}I]T_4 (around 1.9 μCi; specific activity 135 μCi/μg, New England Nuclear, USA) was added, and the incubations were continued for different periods of time. The slices were extensively washed with cold saline, blotted on filter paper and weighed. The radioactivity of each slice and of an aliquot of the incubation medium was measured. The T/M ratio was calculated as follows:

$$T/M = \frac{\text{CPM/g tissue}}{\text{CPM/ml medium}}$$

Metabolism of [^{125}I]T_4

a) *Studies with slices.* Thyroid slices were pre-incubated in quadruplicate in 4 ml KRB containing the different compounds. A tracer dose (usually 1.9 μCi) of labelled T_4 was added and the incubation was continued for another 60 min. At the end of this period the slices were extensively washed with cold saline and each slice was homogenized in 1 ml of 1 mM PTU with an all-glass Potter-Elvehjem tissue homogenator. Aliquots of each homogenate were analysed by paper chromatography utilizing two different solvent systems: I: n-butanol: ethanol: 1 N ammonium hydroxyde (5:1:2; v v), ascending for 20–22 h; II: hexane: tertiary amyl alcohol: 2 N ammonium hydroxyde (1:5:6; v v), descending for 20–22 h. Standards of iodine and of iodoaminoacids were run simultaneously to help in identification of the spots. Each strip was cut and, after counting in an automatic gamma spectrometer, the distribution of radioactivity was calculated. Very good agreement was found between both chromatographic systems.

Similar studies were performed with rat liver slices. The animals were sacrificed by a blow on the neck and the liver was thoroughly washed with cold saline. Slices were obtained as described, and incubations were performed under the same conditions as those applied to thyroid slices.

b) *Studies in homogenates.* Labelled T_4 metabolism in tissue homogenates was studied following the method described by Kaplan et al. (1979). Two g of calf thyroid or rat liver were homogenized at 4°C in 6 ml 50 mM Tris-HCl pH 7.6, with a Potter-Elvehjem type tissue homogenator teflon pestle. The homogenates was filtered through several layers of cheesecloth and centrifuged for 15 min at 2000 × *g* at 4°C. Incubations were performed in glass tubes containing 0.9 ml of the 2000 × *g* supernatant, with 10 mM β-ME. After pre-incubating for 30 min, at 37°C a tracer dose (around 2.5×10^6 CPM) of labelled T_4 was added. Sixty min later, aliquots of the incubation mixture were rapidly spotted on Whatman No. 1 paper and analysed by ascending and descending chromatography as already described.

The influence of iopanoic acid on [^{125}I]T_4 metabolism was also analysed. This compound was dissolved in ethyleneglycol to prepare a stock solution and final dilutions were made in the appropriate buffers.

Subcellular distribution of [^{125}I]T_4

Around 1 g of thyroid slices was pre-incubated in quadruplicate, in 10 ml of KRB-buffer as described above, with and without T_3 or T_4 at 10^{-5} M. After 30 min, a tracer dose of 2.2 μCi of labelled T_4 (1064 μCi μg, New England Nuclear, USA) was added and the incubation continued for another 60 min. The slices were extensively washed with cold saline and homogenized in 5 ml 0.32 M sucrose, 20 mM Tris-HCl pH 7.7, 1 mM $MgCl_2$ and 1 mM DTT with a Potter-Elvehjem tissue homogenator with a teflon pestle, at 4°C. After filtrating through cheesecloth the homogenate was centrifuged at 800 × *g* for 15 min. The supernatant was submitted to 20 000 × *g* for 30 min. Each 800 × *g* pellet was resuspended in 2.1 M sucrose, 20 mM Tris-HCl pH 7.7, 1 mM $MgCl_2$, 1 mM DTT and centrifuged at 50 000 × *g* for 1 h. The resulting pellet was washed once with 0.25 M sucrose, 20 mM Tris-HCl pH 7.7, 1 mM $MgCl_2$, 2 mM DTT, 0.5% Triton X-100 and once with the same buffer without Triton. The radioactivity present in each fraction was detemined and the per cent distribution calculated.

Nuclear localization of [^{125}I]T_4

Around 1–2 g of thyroid slices was pre-incubated in quadruplicate in KRB-buffer, as described above, containing 1 mM $KClO_4$ and 1 mM PTU. After 30 min a tracer dose (30 μCi) of [^{125}I]T_4 (135 μCi/μg) was added and the incubation continued for another 60 min. Nuclei were purified as described above. Between 85–90% of the nuclear radioactivity was extracted with absolute ethanol and the samples were examined by paper chromatography as already described.

Iodine organification

Triplicate calf thyroid slices were incubated at 37°C, in KRB-buffer in the presence or in the absence of the different test substances. After 30 min a tracer dose (around 2×10^6 CPM) of [^{125}I] was added and the incubations were continued for another 60 min. After extensive washing with cold saline each slice was homogenized in 0.5 ml 1 mM PTU. 0.5 ml of 20% TCA was added and the precipitate obtained after 2500 r.p.m. during 15 min was washed twice with 10% TCA. Total and TCA precipitable radioactivity were measured and the percentage present in the latter was calculated.

All chemicals utilized were reagent grade. Labelled compounds were purchased from New England Nuclear, USA. Bovine TSH (B9) was a gift from the National Pituitary Agency (USA). Iopanoic acid was kindly donated by FARMASA, SA (Argentina). Protein was determined according to Lowry et al. (1951). Statistical analysis was by Student's *t*-test.

Results

[^{125}I]T_4 *uptake*

Fig. 1 shows the uptake of [^{125}I]T_4 by calf thyroid slices. The T/M ratio increased to a value of around 3 at 15 min, and stood at a plateau for up to 60 min. The curve of [^{125}I]T_3 uptake under the same conditions is also shown. For the latter, the T/M ratio reached a value of around 12–15 after 60 min. On the other hand, the T/M ratio for ^{125}I (performed without blockade by PTU or MMI) showed a linear rise up to 60 min. As shown in Fig. 2, the entry of labelled T_4 into the thyroid is a temperature dependent process, being higher at 37°C than at 4°C.

The influence of different compounds on the entry of labelled T_4 is shown in Table 1. In these studies a short period of incubation (5 min) was chosen in order to avoid the contribution of 'spurious' counts due to [^{125}I]T_4 dehalogenation. However it should be mentioned that essentially the same results were obtained with longer periods of pulse labelling. A series of compounds were devoid of action on the T/M values, namely, KI, T_3, IOP, PTU and MMI. Addition of $KClO_4$ caused a 25% decrease, while ouabaine inhibited only by 21%. Omission of Ca^{++} in the KRB-buffer reduced the entry of T_4 by 32%. Similar studies were

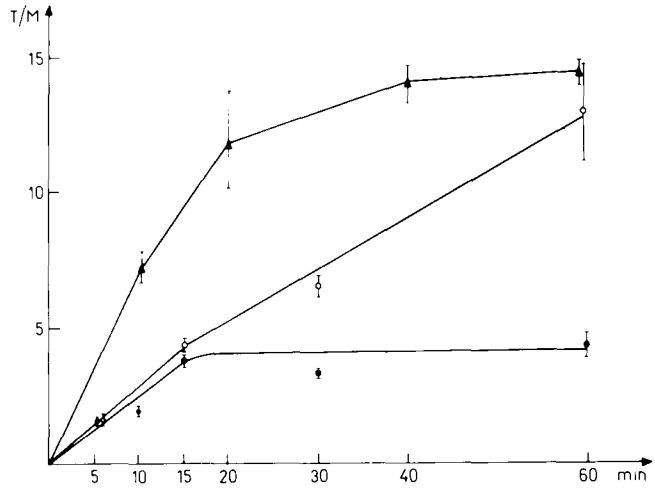

Fig. 1.

Time-course of entrance of [^{125}I]T_3: ▲——▲; ^{125}I: o——o and [^{125}I]T_4: ●——●. Each value is the average of four slices ± SD. The T/M ratio was determined as described in Materials and Methods.

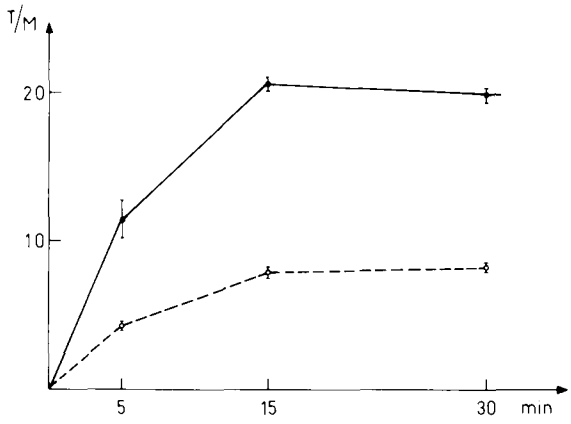

Fig. 2.
Effect of the temperature of incubation on the entrance of [125I]T_4. Calf thyroid slices were incubated at 37°C: ●——● or at 0–4°C: o——o. Each value is the average of four slices ± SD.

Table 1.
Influence of various compounds on [125I]T_4 and [125I]T_3 uptake by calf thyroid slices.

Label	Treatment	Concentration	% Hormone uptake
[125I]T_4	Control		100
	KI	10^{-5} M	90
	T_3	10^{-5} M	86
	T_4	10^{-5} M	374
		10^{-6} M	206
		10^{-7} M	116
	IOP	10^{-4} M	110
	PTU	10^{-3} M	93
	MMI	10^{-3} M	99
	$KClO_4$	10^{-3} M	75
	Ouabain	10^{-4} M	79
	TSH	20 mU ml	130
	α Ca^{++}		68
[125I]T_3	*Control*		100
	Oubain	10^{-4} M	92
	α Ca^{++}		76

Quadruplicate calf thyroid slices were incubated in KRB containing different compounds for 30 min. The uptake of the labelled hormone was measured after 5 min of incubation. The T/M ratio was calculated as described under Materials and Methods. Results are expressed as percentage of controls (100%). The T/M values after 5 min of incubation were 1.71 ± 0.43 for T_4 and 3.44 ± 0.42 for T_3. Similar results were obtained in 4–5 additional experiments.

conducted with labelled T_3. The absence of Ca^{++} decreased the T/M value of T_3 about 25%, while ouabain did not alter this value significantly. Pre-incubation of the slices with increasing concentrations of unlabelled T_4 (10^{-8} to 10^{-5}) caused a progressive rise in the T/M values of labelled T_4.

[125I]T_4 *metabolism*

The results of the studies on [125I]T_4 metabolism are shown in Table 2. Thyroid slices dehalogenated labelled thyroxine, generating around 2% of T_3, 3% reverse T_3 and 10% iodide. Incubation with 20 mU/ml of bovine TSH caused an increase in T_4 dehalogenation, with a significant increase in both T_3 ($P < 0.0005$) and reverse T_3 ($P < 0.0005$). TSH also caused a significant increase in the radioactivity of the origin in the chromatogram. When calf slices were incubated with 125I the radioactivity precipitable by TCA was 81.36 ± 2.09% (n = 3), while PTU treated slices had 6.75 ± 0.15% radioactivity in the TCA precipitate ($P < 0.0005$; 93% of inhibition). However PTU at 1 mM failed to alter the generation of T_3 and rT_3 in thyroid slices, as shown in Table 3.

In the same experiments thyroid slices incubated with 0.1 mM IOP produced 2.56 ± 0.60% of rT_3 and 2.79 ± 0.61% of T_3. The action of IOP on T_4 metabolism in liver slices was also examined. IOP-treated slices showed 48% inhibition in T_3 production and 51% inhibition in rT_3 production. The difference between liver and thyroid vis a vis their sensitivity to IOP was further assessed in tissue homogenates.

When the 2000 × *g* supernatant from liver was incubated with 0.1 mM IOP a 48% decrease in T_3 generation from labelled thyroxine was again observed, while no change occurred in calf thyroid homogenate (Table 4).

When the subcellular distribution of [125I]T_4 was examined, most of the radioactivity was found in the 20 000 × *g* supernatant. Less than 1% was localized in purified nuclei (Fig. 3). When the pattern of subcellular distribution was compared in control and 10^{-5} M T_4 – treated slices, a significant decrease in nuclear radioactivity was found in the latter, while no significant changes occurred in the

Table 2.
[^{125}I]T_4 metabolism in calf thyroid slices.

Treatment	Origin	MIT	DIT	Iodide	rT_3	T_4	T_3
Controls	1.97 ± 0.52	0.68 ± 0.38	0.68 ± 0.34	10.43 ± 1.94	2.55 ± 0.28	76.62 ± 3.89	1.86 ± 0.76
TSH 20 mU/ml	3.65 ± 0.71[3]	0.60 ± 0.16	1.45 ± 0.69	14.53 ± 2.12[4]	3.47 ± 0.54[1]	58.85 ± 7.05[2]	4.20 ± 0.30[1]
Standard [^{125}I]T_4	0.13	0.22	0.19	1.35	1.61	94.49	0.93

Thyroid slices were pre-incubated with different compounds. A tracer dose of labelled T_4 was added and the incubations continued for 16 min. After homogenization with 1 mM PTU, the percent distribution of radioactivity was examined by paper chromatography (the results with the BEA system are shown here, similar results were obtained with hexane: tertiary amyl alcohol: ammonium). Each value is the average of four slices ± 1 SD.
[1] $P < 0.0005$; [2] $P < 0.002$; [3] $P < 0.005$; [4] $P < 0.005$.

other fractions. The effect of unlabelled thyroxine and triiodothyronine on the nuclear localization of labelled thyroxine was examined further in three additional experiments. As shown in Table 5, T_4 at 10^{-5} M caused a 25% displacement in nuclear radioactivity ($P < 0.002$), while T_3 at the same concentration consistently increased this parameter by 34% ($P < 0.0005$). The same results were obtained in three different experiments.

The possibility that the counts found in the purified nuclei after incubating the slices with [^{125}I]T_4 could be due to contamination from the supernatant was explored in a series of experiments. The 800 × *g* pellet obtained from non-radioactive slices was mixed with the 800 × *g* supernatant from slices incubated with labelled hormone. It was determined that 93% of the radioactivity present in the nuclei purified by centrifugation in 2.1 M sucrose was due to true localization of the labelled hormone during the incubation with thyroid slices (results not shown).

The nature of the nuclear radioactivity observed after 1 h incubation of thyroid slices with [^{125}I]T_4 was analysed. Around 50% of total nuclear radioactivity is due to labelled T_4, while T_3 accounted for around 7.5% (Table 6).

Table 3.
Action of IOP on [^{125}I]T_4 metabolism in thyroid and liver slices.

Tissue	Treatment	% T_3	% rT_3
Thyroid	Control	2.66 ± 0.33	3.23 ± 0.58
	PTU 1 mM	2.43 ± 0.12	3.19 ± 0.86
	IOP 0.1 mM	2.79 ± 0.61	2.56 ± 0.60
Liver	Control	5.83 ± 0.58	8.33 ± 0.95
	IOP 0.1 mM	3.05 ± 0.12[1]	4.14 ± 0.12[2]

Slices were incubated for 60 min with [^{125}I]T_4. Each value is the average of quadruplicates ± SD.
[1] $P < 0.0005$; [2] $P < 0.005$ when compared to the controls.

Table 4.
Effect of IOP on [^{125}I]T_3 generation from [^{125}I]T_4 in liver and thyroid homogenates.

Tissue	Addition	% T_3	$P <$
Liver	None	4.67 ± 0.55	–
	IOP 0.1 mM	2.44 ± 0.54	0.0005
Thyroid	None	1.71 ± 0.33	–
	IOP 0.1 mM	2.08 ± 0.56	N.S.

The 2000 × *g* supernatant obtained from a 2 g homogenate from rat liver or from calf thyroid were incubated with labelled T_4. The per cent conversion into T_3 was obtained by paper chromatography. Each value is the average of quadruplicates ± SD. Protein content was 7.06 and 8.66 mg/ml for liver and thyroid respectively.

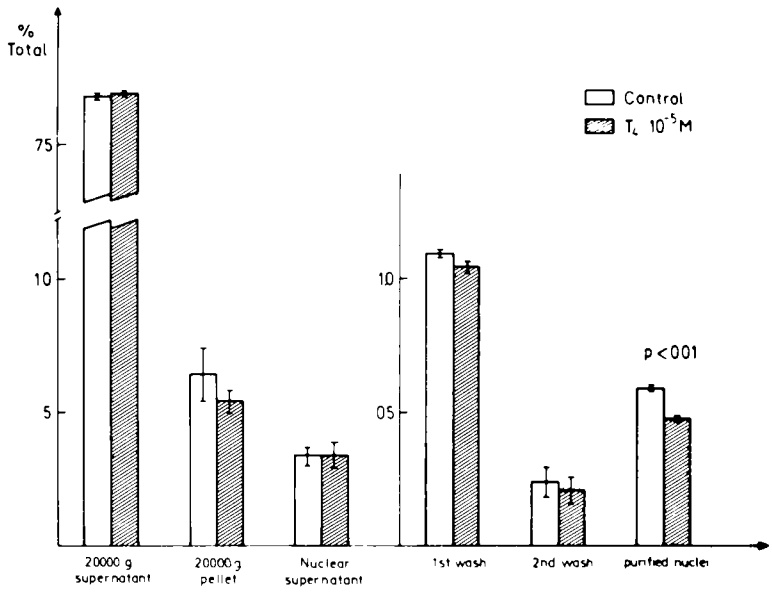

Fig. 3.

Subcellular distribution of [^{125}I]T_4. Calf thyroid slices were incubated and homogenized as described in Materials and Methods. Each column represents the % radioactivity of the fraction referred to total radioactivity and is the average of four different groups ± SD.

Discussion

Previous studies from our laboratory, and others, have shown that iodine and iodothyronines exert an inhibitory action on different thyroid parameters (Pisarev & Itoiz 1972; Van Sande & Dumont 1973; Sherwin & Tong 1975; Rapoport et al. 1975; Yu et al. 1976; Takasu et al. 1974; Shishiba et al. 1975; Pisarev et al. 1976; Kleiman de Pisarev et al. 1978; Friedman et al. 1978; Kielczynski & Nauman 1978). As far as T_3 is concerned we have recently presented evidence indicating that this hormone has a direct effect on the gland, which may probably be related to the autoregulatory mechanism (Juvenal et al. 1981).

Table 5.

Effect of T_4 and T_3 on the nuclear localization of [^{125}I]T_4 in thyroid slices.

Treatment	Nuclear radioactivity % from control	$P <$
Controls	100 ± 8.0	–
T_4 10^{-5} M	76.6 ± 5.4	0.002
T_3 10^{-5} M	134.0 ± 5.0	0.0005

Quadruplicate calf thyroid slices were incubated with cold T_3 or T_4, and after 30 min, a tracer dose of [^{125}I]T_4 was added. Nuclear localization of the radioactivity was determined as described in Materials and Methods. The values from control slices were taken as 100% and the average corresponding to three different experiments (12 flasks per group) ± SD was calculated.

Our present results show that T_4 enters calf thyroid slices and is progressively concentrated, with values greater than 1 after 5 min of incubation, reaching a plateau of around 3–4 at 20–30 min. This process, similar to the entry of T_3 (Juvenal et al. 1981), is temperature-dependent. The T/M values for thyroxine are lower than those found with labelled triiodothyronine, which attained levels of about 12–15 under similar conditions (Juvenal et al. 1981 and present results). Previous work by Haibach (1971) and Green (1978) has also demonstrated that exogenous labelled thyroxine enters the rat thyroid. The latter also reported that the T/M value of T_3 is greater than that of T_4 in the rat. Additonal differences apart from those of the T/M values were observed in the

Table 6.
Distribution of radioactivity in purified thyroid nuclei obtained from calf slices incubated with [^{125}I]T_4.

	Origin	DIT	MIT	Iodide	T_4	T_3	SF
Nuclei	4.50 ± 2.28	3.95 ± 0.93	3.34 ± 1.94	9.03 ± 1.80	50.56 ± 3.90	7.54 ± 1.49	5.55 ± 0.97
Incubating medium	0.63 ± 0.17	0.13 ± 0.03	0.25 ± 0.07	3.61 ± 1.22	92.91 ± 0.86	0.96 ± 0.18	0.67 ± 0.22

Quadruplicate calf thyroid slices were incubated as described in Materials and Methods. Nuclei were purified, extracted with ethanol and submitted to paper chromatography. Aliquots of the incubating medium were equally studied. Each value is the average of four samples ± SD. SF: solvent front. Results are expressed as % of total radioactivity.

present studies, between T_3 and T_4. In a previous paper it was shown that incubation with unlabelled T_4 failed to alter the entrance of labelled T_3 into the thyroid (Juvenal et al. 1981), while our present data indicate that cold T_3 does not alter the uptake of labelled T_4. Therefore it is reasonable to assume that there are independent mechanisms for the entry of both thyroxine and triiodothyronine.

The influence of different compounds on the entry of labelled thyroxine into the thyroid was also analysed. KI, IOP, PTU and MMI were without effect. This lack of effect of PTU on T_4 uptake was also observed by Green (1978) in the rat. Both $KClO_4$ and ouabain caused some decrease in the T/M ratio, which can partially be explained by a selective impairment in the uptake of the contaminating iodide. The entrance of labelled triiodothyronine into calf thyroid slices was also unaffected by these agents (Juvenal et al. 1981). A similarity between the entrance of T_4 and T_3 was observed when the influence of Ca^{++} was studied. The absence of Ca^{++} in the KRB-buffer caused a small decrease in the entry of both labelled iodothyronines. Since this decrease was due to a diminution in tissue radioactivity, with no change in medium radioactivity, we may conclude that these results are due to a net decrease in entry, and not to an increase in the efflux, as described by Maayan et al. (1981) in the mouse thyroid under different experimental conditions. Pre-incubation of the calf slices with unlabelled T_4 caused a dose-dependent increase in the entry of labelled thyroxine. Similar results were obtained when labelled and unlabelled T_4 were added simultaneously and when the slices were incubated in siliconized flasks. These results are in sharp contrast with those obtained with labelled triiodothyronine, in which pre-incubation with increasing concentrations of T_3 caused a progressive decrease in rate of entry (Juvenal et al. 1981). Studies are being performed in our laboratory in order to clarify this point.

Calf thyroid slices dehalogenate labelled thyroxine after 1 h of incubation, in contrast with our previous results with labelled triiodothyronine, whose dehalogenation was almost negligible under the same experimental conditions (Juvenal et al. 1981). Both triiodothyronine and reverse T_3 originated from thyroxine, in a fashion similar to that in rat (Haibach 1971; Green 1978; Erickson et al. 1981), dog (Laurberg 1980) and human thyroid (Bidey et al. 1976; Ishii et al. 1980), as well as in many peripheral tissues (Chopra 1981). However, some differences between the thyroid and the other organs became evident when the influence of several agents on labelled thyroxine metabolism was examined. The addition of TSH to the slices significantly increased the generation of T_3 and rT_3. The generation of T_3 and rT_3 was not significantly altered by PTU and IOP. PTU is active under our experimental conditions, since it completely blocked ^{125}I organification. Moreover IOP also failed to modify the generation of T_3 and rT_3 in thyroid slices, a finding in striking contrast with the metabolism of T_4 in peripheral tissues (Bürgi et al. 1976; Kaplan & Utiger 1978; Kaplan et al. 1979; Obregon et al. 1980; Chopra 1981). Under the same experimental conditions, IOP caused a significant decrease in T_3 generation in rat liver slices, as reported by others (Kaplan & Utiger 1978; Chopra 1981). One possible explanation would be that the entrance of IOP is different in liver and in thyroid slices. However, when tissue homogenates were used as a source of the dehalogenating system the same difference in the re-

sponse to IOP between liver and thyroid was found. Two possible explanations may be offered concerning this difference in sensitivity to IOP: a) IOP may be handled in a dissimilar fashion; b) the dehalogenating system may be different in rat liver and in calf thyroid (isoenzymes?).

The pattern of subcellular distribution of [^{125}I]T_4 is very similar to that of labelled T_3 (Juvenal et al. 1981). Although most of the radioactivity was found in the soluble fraction, the addition of unlabelled thyroxine to the slices only caused a significant displacement in the purified nuclei fraction. Similar observations were made when the influence of unlabelled T_3 on labelled T_3 distribution was studied (Juvenal et al. 1981). We have previously found that 'cold' T_4 displaced labelled T_3 from its binding to purified nuclei, although less potently than cold T_3 (Pisarev et al. 1981). In the present studies, the addition of unlabelled T_3 to the incubating slices caused a significant increase in the localization of labelled thyroxine in the purified nuclei, an observation that deserves further study.

When the nuclear radioactivity was analysed by paper chromatography after incubation of slices with labelled thyroxine, radioactive thyroxine comprised 50–55% and triiodothyronine was around 8% of the total. These data again support the view that both iodothyronines can localize in thyroid nuclei. The labelled T_3 could originate from the dehalogenation of T_4 in the cytoplasm since under our conditions labelled T_3 comprised around 2–3% in the total homogenate (Table 2) and around 7–9% in purified nuclei (Table 6), it is likely that a specific mechanism for the selective concentration of T_3 exists in the thyroid nucleus.

In a previous report we showed that when calf thyroid slices are incubated with ^{125}I, both labelled T_4 and T_3 are found in purified nuclei (Pisarev et al. 1981 and submitted for publication). Therefore, and taking into account our present results, we may conclude that nuclear iodothyronines may arise from two different sources: 1) hormone which remained in the gland; 2) hormone that enters the thyroid after having been circulating in the blood. Since the T/M ratio was rather low, we might conclude that the former is a more important pathway. On the other hand T_3 may come from extrathyroidal sources (including the hormone originated from T_4 at the peripheral tissue) and from intrathyroidal formation, both from iodine organification and from T_4 dehalogenation (Fig. 4). We have previously shown that T_3 has a direct inhibitory effect on the thyroid (Juvenal et al. 1981). The present demonstration that T_4 is converted into T_3 in the thyroid suggests that, as in other tissues T_3 would be the active hormone.

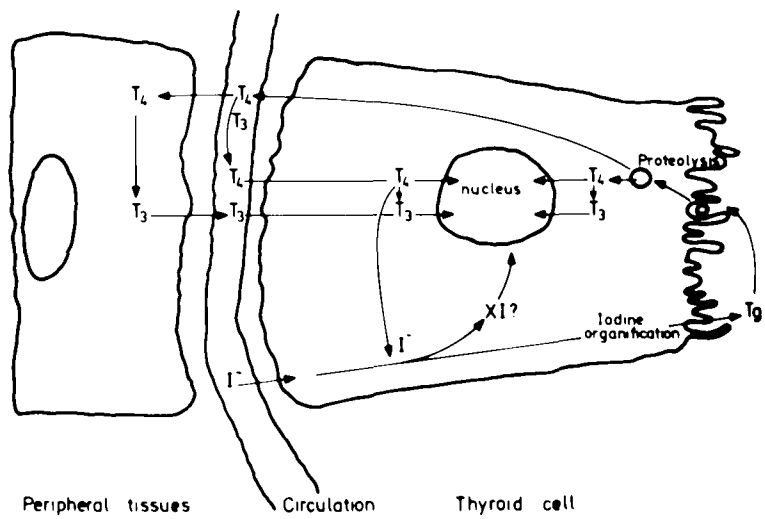

Fig. 4.
Possible origin of the iodothyronines for the control of thyroid cell function in the autoregulatory mechanism.

References

Bidey S P, Anderson J, Marsden P & McKerron C G (1976): Post secretory deiodination of iodothyronines released from normal human thyroid cells in vitro. Biochem Biophys Res Commun 72: 67–73.

Bürgi H, Wimpfheimer C, Burger A, Zaunbauer W, Rosler H & Lamarchand-Béraud T (1976): Changes of circulating thyroxine, triiodothyronine and reverse triiodothyronine after radiographic contrast agents. J Clin Endocrinol Metab 43: 1203–1210.

Chopra I J (1981): Triiodothyronines in health and disease. Springer Verlag, Berlin, New York.

Erickson V J, Cavalieri R R, Rosenberg L L (1981): Phenolic and non-phenolic ring iodothyronine deiodination from rat thyroid gland. Endocrinology 108: 1257–1264.

Friedman Y, Lang M & Burke G (1978): Inhibition of thyroidal cyclic AMP-dependent protein kinase by thyroid hormone. Endocr Res Commun 5: 109–124.

Green W L (1978): Metabolism of thyroid hormones by rat thyroid tissue in vitro. Endocrinology 103: 826–837.

Haibach H (1971): Evidence for a thyroxine deiodinating mechanism in the rat thyroid different from iodothyrosine deiodinase. Metabolism 20: 819–829.

Ishii H, Tanaka K, Naito K, Nishikawa M & Inada M (1980): Conversion of thyroxine to 3, 5, 3' L-triiodothyronine in human thyroid. 62nd Annual Meeting of the Endocrine Society, Abstr 512. Washington DC.

Juvenal G J, Pisarev M A, Kleiman de Pisarev D L & Altschuler N (1981): Uptake, metabolism and action of triiodothyronine in calf thyroid slices. Mol Cell Endocrinol 22: 31–40.

Kaplan M M & Utiger R D (1978): Iodothyronine metabolism in rat liver homogenates. J Clin Invest 61: 459–471.

Kaplan M M, Tatro J B, Breitbart R & Larsen P R (1979): Comparison of thyroxine and 3, 5, 3'-triiodothyronine metabolism in rat kidney and liver homogenates. Metabolism 28: 1139–1146.

Kielczynski W & Nauman J (1978): Inhibition of thyrotropin binding to human thyroid plasma membranes by thyroid hormones and 'reverse triiodothyronine'. Biochem Biophys Res Commun 82: 811–819.

Kleiman de Pisarev D L, Pisarev M A & Juvenal G J (1978): Action of KI and several iodocompounds on [^{3}H]uridine incorporation into thyroid RNA. Acta Endocrinol (Copenh) 89: 316–322.

Laurberg P (1980): Iodothyronine release from the perfused canine thyroid. Acta Endocrinol (Copenh), Suppl 236: 1–37.

Lowry O H, Rosebrough N J, Farr A L & Randall R S (1951): Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275.

Maayan M L, Volpert E M & From A (1981): Calcium depletion stimulates thyroxine release from the thyroid. Endocrinology 108: 1987–1988.

Obregon M J, Pascual A, Mallol J, Morreale de Escobar G & Escobar del Rey F (1980): Evidence against a major role of L-thyroxine at the pituitary level: studies in rats treated with iopanoic acid (telepaque). Endocrinology 106: 1827–1836.

Pisarev M A & Itoiz M E (1972): Action of KI on stimulated thyroid protein biosynthesis. Endocrinology 90: 1409–1412.

Pisarev M A & Aiello L O (1976): Studies on the mechanism of action of KI on thyroid protein biosyntheses. Acta Endocrinol (Copenh) 82: 298–305.

Pisarev M A, Aiello L O & Kleiman de Pisarev D L (1976): Action of KI, thyroxine and cyclic AMP on [^{3}H]uridine incorporation into the RNA of thyroid slices. Acta Endocrinol (Copenh) 83: 313–320.

Pisarev M A & Kleiman de Pisarev D L (1980): Biochemistry of thyroid regulation under normal and abnormal conditions. J Endocrinol Invest 3: 317–329.

Pisarev M A, Juvenal G J & Kleiman de Pisarev D L (1981): Inhibitory action of iodothyronines on the thyroid. Possible role in the autoregulatory mechanism. In: Soto R J, DeNicola A & Blaquier J (eds). Physiopathol Endocr Dis and Mechanism of Hormone Action: Alan R Liss Inc, p 103–114. New York.

Rapoport B, West M N & Ingbar S H (1975): Inhibitory effect of dietary iodine on the thyroid adenylate cyclase response to thyrotropin in the hypophysectomized rat. J Clin Invest 56: 516–519.

Sherwin J R & Tong W (1975): Thyroid autoregulation – iodide induced suppression of thyrotropin-stimulated cyclic AMP production and iodinating activity in thyroid cells. Biochim Biophys Acta 404: 20–39.

Shishiba Y, Takaishi M, Miyachi Y & Ozawa Y (1975): Alteration of thyroidal responsiveness to TSH under the influence of circulating thyroid hormone; short feed-back regulatory effect. Endocrinol Jpn 22: 367–371.

Takasu N, Sato S, Tsuki T, Yamada T, Furihata R & Mariuchi M (1974): Inhibitory action of thyroid hormone on the activation of adenyl cyclase-cyclic AMP system by thyroid stimulating hormone in human thyroid tissue from euthyroid and thyrotoxic patients. J Clin Endocrinol Metab 39: 772–778.

Van Sande J & Dumont J E (1973): Effects of thyrotropin, prostaglandin E_1 and iodide on cyclic 3'5'AMP concentration in dog thyroid slices. Biochim Biophys Acta 313: 320–328.

Wolff J (1976): Iodine homeostasis. In: Klein E & Reinwein D (eds). Regulation of Thyroid Function. Int Thyr Symp, p 65–82. Düsseldorf FRG, Shattauer Verlag, Stuttgart.

Yu S, Friedman Y, Richman R & Burke G (1976): Altered thyroidal responsivity to thyrotropin induced by circulating thyroid hormones. A »short-loop« regulatory mechanism? J Clin Invest 57: 745–755.

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