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HORMONE-INDUCED MODIFICATIONS OF FREE TYROSINE IN THE RAT THYROID GLAND

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SUMMARY

The concentrations of free tyrosine in liver, muscle, kidney and thyroid gland were determined in separate groups of rats maintained on a low iodine diet and treated with intraperitoneal injections of thyroxine (T 4), thyrotrophin (TSH) and TSH plus propylthiouracil (PTU). Groups of hypophysectomized rats also were given T 4. Significant changes in tissue tyrosine were generally confined to the thyroid gland. Animals treated with T 4 showed a decrease of mean thyroid tyrosine from $113{\cdot}3 \pm 17{\cdot}9$ (S.D.) μg/g wet weight of gland to $76{\cdot}2 \pm 5{\cdot}7$ μg/g ($P < 0{\cdot}01$). Although there was little change in content when TSH was given alone, a significant increase in tyrosine levels was observed when TSH plus PTU were administered ($P < 0{\cdot}01$). In hypophysectomized rats thyroid tyrosine decreased and was further lowered (to $46{\cdot}0 \pm 3{\cdot}1$ μg/g; $P < 0{\cdot}05$) by T 4 treatment. When tyrosine concentrations were expressed in relation to ribonucleic acid (RNA) or protein content of the assayed gland, all differences in tyrosine content became greater.

INTRODUCTION

Recent work has shown a specific elevation of plasma tyrosine concentration in patients with hyperthyroidism and in normal subjects given L-triiodothyronine (T 3) (Levine, Oates, Vendsalu & Sjoerdsma, 1962; Melmon, Rivlin, Oates & Sjoerdsma, 1964). Furthermore, after oral administration of tyrosine, rises in the plasma concentration of this amino acid were greater in hyperthyroid patients than in normal subjects

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(Melmon *et al.* 1964). The tyrosine changes are apparently not due to increased absorption of the amino acid (Melmon *et al.* 1964), or alteration in metabolism due to enzyme changes induced by thyroid hormone (Levine *et al.* 1962; Rivlin & Levine, 1963; Davis, 1963). Melmon *et al.* (1964) suggested that thyroid hormones may produce an alteration in partition of tyrosine between plasma and various tissues. To investigate this possibility further, free tyrosine concentrations were determined in body tissues of the rat under conditions of normal and abnormal thyroid function.

METHODS

Male Holtzman or Sprague Dawley rats, 1–3 months of age and weighing 150–250 g, were fed throughout on a low-iodine diet (Nutritional Biochemicals). Litter-mates were used as controls in all studies. All agents used in the study were administered by I.P. injection.

Groups of rats were made hyperthyroid by injection of L-thyroxine (200 μg/ml. dissolved in saline brought to pH 10·5 by addition of 1 N-NaOH) for periods of 9, 15 and 30 days. A single dose of 200 μg daily was used regardless of body weight; by the ninth day this dose produced manifestations of severe hyperthyroidism, including apparent irritability, slowed weight gain despite polyphagia, and increase in the ratios of heart and liver to body weight. Thyroid glands were smaller and less vascular than those of control animals and the uptake of ^{131}I (see below) by the glands was much lower. Control rats received injections of saline adjusted to the same pH as that of the thyroxine solution.

Three further groups of rats were given thyrotrophic hormone (TSH, Armour and Co.) in a single dose of 2 u. daily in normal saline solution for 6 days. One of these groups received additionally propylthiouracil (10 mg three times daily per animal) to block iodination of tyrosine.

Holtzman rats were obtained from Hormone Assay Laboratories, Chicago, Illinois, within 2 days after hypophysectomy; the adequacy of operation was confirmed by thyroid uptake of ^{131}I (see below) and at autopsy by gross examination of the pituitary fossae and histological examination of scrapings from those with any remaining tissue. Studies were started on these animals 5 days after hypophysectomy. Some of these rats were given injections of T4 as above, and results were compared with those of hypophysectomized controls and normal rats with and without T4 treatment.

Radiothyroidectomy was carried out in one group of normal rats by a single injection of ^{131}I (250 μc/100 g body weight) in rats fed a low iodine diet for 10 days. The animals were killed 30 days after ^{131}I administration.

The weights of all animals were recorded at weekly intervals. Twenty-four hours before being killed most groups of rats were given a tracer dose (1 μc per rat) of carrier-free ^{131}I (Oak Ridge National Laboratories), and thyroid uptake was measured by direct counting of the excised glands.

In all studies food was withheld for 18 hr before sacrifice but access to water was continued. Animals were anaesthetized by I.P. injection of sodium pentobarbitone (30 mg/kg) and tissues for tyrosine determination were removed, weighed and frozen. Samples of some or all of the following tissues were obtained: kidney, liver, thyroid, brain, heart and skeletal muscle. The tissues were homogenized and the proteins precipitated with trichloroacetic acid. After suitable dilution of supernatant the nitrosonaphthol derivative of tyrosine was measured fluorometrically (Waalkes & Udenfriend, 1957). Each tissue sample was run separately through the assay procedure; only in the case of the thyroid glands from the 15-day thyroxine-treated group were samples from different animals pooled. Recovery of

tyrosine added to tissue homogenates was 85–100%. Values for tyrosine concentrations given in the Tables represent the means (± S.D.) of all individual values.

Reason for RNA determinations. Since the thyroid gland has varying cellular content, the usefulness of expressing tyrosine levels only in terms of total gland weight is open to question. For this reason measurements of total thyroid gland protein and RNA concentration (μg/g) were made in parallel with some of the tyrosine determinations and the tyrosine levels expressed in terms of total protein and RNA rather than wet weight. Total protein determinations were made by a modification of the phenol reagent assay (Rabinowitz & Pricer, 1962) and RNA determinations by the Schmidt–Tannhauser–Schneider procedure (Schneider, 1946).

Specificity of tyrosine assays on thyroid gland. Whereas tyrosine has been presumed to be the only major nitrosonaphthol-reacting material present in tissues (Waalkes & Udenfriend, 1957), it was considered important to check the specificity of the method when applied to thyroid glands because of the presence of other tyrosine derivatives in this tissue. Monoiodotyrosine (MIT) and diiodotyrosine (DIT) were found to yield only 1/800 the fluorescence of tyrosine on reaction with nitrosonaphthol. Examination of the glands for tyramine and

TABLE 1. Specific activity measurements confirming the validity of the tyrosine assay in thyroid gland

Fraction	Tyrosine (cpm/μg)
Supernatant of homogenate	127,139
Eluate from Dowex-50	101,850
Eluate from high-voltage electrophoresis	122,000

See text for details.

synephrine (Spector, Melmon, Lovenberg & Sjoerdsma, 1963) showed that these amines were undetectable. In addition, when salt-saturated acidified thyroid homogenate was shaken with peroxide-free ether, which was in turn shaken with a neutral aqueous buffer (0·5 M phosphate), there were no fluorescent compounds (acids) detected when the aqueous phase was allowed to react with nitrosonaphthol.

Finally, the reliability of the tyrosine assay was verified by an isotope dilution experiment in which purified tritiated tyrosine (398 mc/m-mole) was added to the supernatant fraction of the homogenate of thyroid tissue. The ratios of counts per minute (cpm) to apparent tyrosine (fluorometric assay) were determined first in the crude supernatant and also after two purification steps. In these studies, 90 mg thyroid tissue was homogenized and [^{3}H]-tyrosine (500,000 cpm) was added to the supernatant (10 ml.). An aliquot was assayed fluorometrically and another aliquot was evaporated and counted in Bray's solution (Table 1). The remainder of the supernatant was desalted by the addition of 10 volumes acetone–alcohol (7:3) solution. Insoluble residues were centrifuged and the supernatant evaporated to 0·5 ml. This fraction was dissolved in 5 ml. water and 0·5 ml. concentrated HCl, and was chromatographed on a Dowex 50 (H^+ form) column by a modification of the method of Tompsett (1962). Aliquots of the eluate were assayed as above (Table 1). The rest of the eluate was evaporated, resuspended in 0·1 ml. formic acid (4 %, w/v) and placed on Whatman 3 MM paper for paper electrophoresis (5 kV for 3 hr in 4 % formic acid medium). The tyrosine area on the paper was eluted and aliquots assayed as before (Table 1). Since specific activities after these two purifications were essentially the same as that of the original supernatant, it is reasonable to conclude that only free tyrosine was being measured by the assay procedure.

RESULTS

Effects of L-*thyroxine and radiothyroidectomy.* Mean plasma concentrations of free tyrosine (controls 15·7 μg/ml.; after T 4 treatment 17·6 μg/ml.) were similar to those in the study by Rivlin & Levine (1963) but the scatter of individual observations was too wide for this difference to be statistically significant. Free tyrosine concentrations in various tissues showed considerable variation and were not significantly influenced by T 4 treatment for 9, 15 or 30 days (Table 2). The thyroids from such animals, on the other hand, exhibited remarkable decreases in free tyrosine concentrations in all periods of treatment with T 4 (Table 3). When expressed on the basis of tissue protein concentration this amounted to about a 50% decrease. Although tyrosine concentrations were lower in 30-day control animals than for animals on the low iodine for shorter periods (Table 3), again T 4 treatment resulted in a significant reduction of thyroid tyrosine.

Eight animals rendered hypothyroid by radiothyroidectomy were more sluggish than control rats and gained weight more slowly; some died towards the end of the 30-day observation period. There were no significant differences in tyrosine concentration of kidney, liver, brain, skeletal muscle or heart between hypothyroid and control groups.

Effects of TSH alone and in combination with propylthiouracil. Administration of TSH for 6 days failed to influence free tyrosine concentration of the thyroid despite an adequate response to the trophic hormone, as judged by increases in gland weight and ^{131}I uptake. Other tissues also showed no significant changes (Table 4). When propylthiouracil was administered alone there was a small increase in thyroid tyrosine concentration, and injection of TSH to such rats caused additional increase ($P < 0{\cdot}01$) (Table 4).

Effect of hypophysectomy. Hypophysectomy *per se* led to a slight but not significant reduction in tyrosine concentration that was somewhat further decreased by simultaneous T 4 treatment for 9 days. These data are expressed in terms of tyrosine to protein ratios in Table 5. However, only six of fifteen hypophysectomized rats survived the T 4 treatment. Tyrosine concentrations in kidney, liver and muscle were not altered from values presented in Table 2 (9-day treatment) by hypophysectomy or hypophysectomy plus T 4 treatment. Since direct inspection at the time animals were killed confirmed complete removal of the pituitary, it was of interest to speculate whether the low level of autonomous thyroid activity persisting after hypophysectomy was being further suppressed by a direct action of T 4 on the thyroid gland. In order to clarify this question a second group of hypophysectomized rats was studied.

In the second group, ten control, ten hypophysectomized and ten

TABLE 2. Free tyrosine concentrations in body tissues of thyroxine-treated rats

Treatment (no. of animals)	Average body weight change (g)	Free tyrosine concentration (μg/g)*				
		Kidney	Liver	Brain	Skeletal muscle	Heart
1(*a*) Thyroxine, 9 days (12)	−1	84·7 ± 20·6	39·9 ± 6·2	—	45·8 ± 15·9	—
(*b*) Control (12)	+16	92·3 ± 8·5	44·5 ± 25·2	—	44·8 ± 8·8	—
2(*a*) Thyroxine, 15 days (18)	+9	66·2 ± 26·4	25·9 ± 4·4	24·5 ± 5·1	—	33·1 ± 7·4
(*b*) Control (12)	+67	88·1 ± 26·5	26·7 ± 6·9	24·8 ± 5·6	—	35·0 ± 10·3
3(*a*) Thyroxine, 30 days (6)	+18	49·0 ± 13·3	38 1 ± 6·2	17·4 ± 4·1	32·0 ± 5·6	—
(*b*) Control (12)	+63	54·0 ± 12·7	38·8 ± 9·5	19·9 ± 3·1	27·0 ± 7·2	—

* Values shown in Tables 2 to 5 are means ± S.D.

No significant changes in tyrosine concentration ($P < 0·05$) were detected in the organs listed.

TABLE 3. Effect of thyroxine treatment on ^{131}I uptake, weight, free tyrosine and total protein concentration of thyroid gland

Treatment (no. of animals)	^{131}I uptake (% of control)*	Average weight (mg)	Free tyrosine (μg/g)	Protein (mg/g)	Tyrosine:protein ratio (μg tyrosine/mg protein)
1(*a*) Thyroxine, 9 days (9)	1·4	10·4 ± 1·8	76·2 ± 5·7†	179 ± 13·8	0·431†
(*b*) Control (10)	100	18·5 ± 2·6	113·3 ± 17·9	131 ± 14·3	0·862
2(*a*) Thyroxine, 15 days (18)	0·6	10·6 ± 1·2	64·9†‡	—	—
(*b*) Control (12)	100	16·2 ± 3·0	117·5‡	—	—
3(*a*) Thyroxine, 30 days (5)	0·6	10·5 ± 0·9	49·9 ± 8·5†	147 ± 10·1	0·336†
(*b*) Control (10)	100	16·4 ± 1·5	81·0 ± 13·8	131 ± 12·3	0·611

* Average uptake by control animals was 72% of the injected dose.

† Values expressed are significantly different ($P < 0·01$) from control values.

‡ Mean value of pooled samples analysed in duplicate.

TABLE 4. Effect of treatment for 6 days with TSH, propylthiouracil, and TSH plus propylthiouracil on free tyrosine in tissues

Treatment (no. of animals)*	^{131}I uptake (% of control)*	Weight of thyroids (mg)	Free tyrosine concentration ($\mu g/g$)				
			Thyroid	Kidney	Liver	Brain	Muscle
TSH (17)	177	17·2 ± 1·6†	110 ± 13·5	68·9 ± 15·5	34·9 ± 9·4	15·3 ± 3·5	30·0 ± 9·1
Control (18)	100	13·2 ± 0·9	107 ± 12·3	74·2 ± 23·4	38·5 ± 12·9	14·8 ± 2·9	24·7 ± 5·8
Propylthiouracil (10)†	—	9·9 ± 0·5	126 ± 8·8	49·6 ± 14·7	39·7 ± 6·1	—	—
Propylthiouracil plus TSH (10)‡	—	9·9 ± 0·7	159 ± 15·7†	71·2 ± 18·7	44·6 ± 8·4	—	—

* Average uptake by control animals was 30 % of injected dose.

† P values < 0·01 when compared to control animals.

‡ Animals used in this experiment had an average body weight of 100 g. The thyroid glands had an average weight of 9·9 mg. Tyrosine content of various organs was similar to that of other control animals.

T 4-treated hypophysectomized rats were killed on days 2, 7 and 10 of the study. Tyrosine and RNA concentrations of the thyroids were measured. In general, the same type of data were obtained as is shown in Table 5, but by day 10 there was a significant decrease ($P < 0{\cdot}03$) of thyroid tyrosine in the hypophysectomized animals treated with T 4 compared to their controls (Table 6). In addition, it was found that the RNA content of the thyroids decreased in the hypophysectomized animals, but in the

TABLE 5. Effect of hypophysectomy and thyroxine administraton on ^{131}I uptake and tyrosine:protein ratios of thyroid gland

Treatment (no. of animals)	^{131}I uptake (% of control)*	Tyrosine:protein ratios (μg/mg $\pm$S.D.)
Hypophysectomy (11)	2·8	0·661 $\pm$ 0·018
Hypophysectomy plus thyroxine (6)	0·9	0·590 $\pm$ 0·021
Control (12)	100	0·862 $\pm$ 0·026

* Average uptake by control animals was 22% of injected dose. Statistical analysis between groups was not calculated because only six of the fifteen hypophysectomized rats treated with thyroxine survived.

TABLE 6. Effects of hypophysectomy and hypophysectomy plus thyroxine on tyrosine and RNA concentrations, and tyrosine:RNA ratios in thyroid gland

Treatment (no. of animals)*	Day 2 μg tyrosine / μg RNA	Ratio	Day 7 μg tyrosine / μg RNA	Ratio	Day 10 μg tyrosine / μg RNA	Ratio
Control (30)	$\frac{83{\cdot}5 \pm 9{\cdot}0}{948 \pm 7}$	0·008	$\frac{81{\cdot}4 \pm 3{\cdot}4}{1023 \pm 12}$	0·080	$\frac{70{\cdot}9 \pm 8{\cdot}0}{1016 \pm 24}$	0·070
Hypophysectomy (30)	$\frac{52{\cdot}6 \pm 6{\cdot}6\dagger}{819 \pm 11\dagger}$	0·064†	$\frac{41{\cdot}5 \pm 4{\cdot}4}{820 \pm 14\dagger}$	0·051†	$\frac{59{\cdot}5 \pm 3{\cdot}0}{891 \pm 20\dagger}$	0·067
Hypophysectomy plus thyroxine (30)	$\frac{54{\cdot}8 \pm 7{\cdot}5\dagger}{947 \pm 51\dagger}$	0·058†	$\frac{45{\cdot}8 \pm 4{\cdot}2}{1145 \pm 38\dagger}$	0·040†	$\frac{46{\cdot}8 \pm 3{\cdot}1\dagger}{1330 \pm 21\dagger}$	0·035†

* Ten animals in each group were sacrificed on three different days, which were respectively days 2, 7 and 10. Values given are μg/g thyroid $\pm$S.E.

† P values indicate significant differences; see text for actual numbers.

group which also received T 4 the content increased significantly above that of the hypophysectomized group ($P < 0{\cdot}05$). Thus, when concentrations of tyrosine were expressed as ratios of tyrosine concentration to RNA concentration, the ratios of the control animals were consistently greater than those of the hypophysectomized animals, which were in turn always higher than those of the hypophysectomized group treated with T 4 (Table 6).

RNA measurements. The demonstration of raised total protein concentrations (Table 3) in the thyroid gland after treatment with T 4 for 9 days suggests that a falling level of free tyrosine might have some functional significance and not be merely a reflexion of involutionary

changes. However, since the colloid of thyroid follicles contains protein in significant amounts, a better index of cellular mass is given by the RNA concentration within the gland (Matovinovic & Vickery, 1959). RNA assays were done in two groups of normal-T 4-suppressed (3 and 4 weeks) and TSH-stimulated (1 week) thyroid glands and in addition on two groups of glands of hypophysectomized animals, some of which were

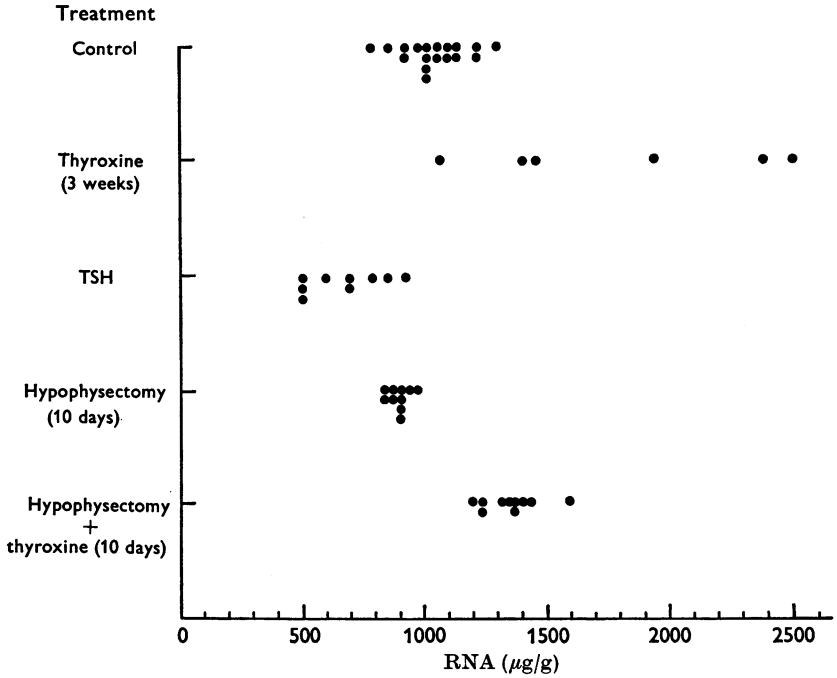

Fig. 1. Scatter diagram of individual RNA concentrations representative of different treatment groups. Control values are individual values from several groups.

treated with T 4 and one group of which was presented in the section on hypophysectomy and Table 6. The RNA results of the four groups are shown in Fig. 1.

The results of these determinations were provocative, but cannot be explained in the light of present knowledge. The various treatments produced a pattern of RNA alteration which was qualitatively similar to the previously described changes of protein content of the gland. RNA concentration (μg/g wet weight) was below normal in the TSH-stimulated group (TSH, 673 ± 53; N, 1028 ± 98; $P < 0{\cdot}01$) whose glands had not enlarged significantly. RNA increased in the glands of the T 4 treated group (T 4, 1746 ± 64; N, 1106 ± 120; $P < 0{\cdot}01$). It was lowered by hypophysectomy when compared to control animals and raised to control

levels in the group of hypophysectomized animals treated with T 4 for 10 days. The difference from control levels was significant ($P < 0{\cdot}01$) for all the treated groups (Table 6; Fig. 1).

The increase in thyroidal RNA produced by thyroxine is puzzling. It would not be expected for thyroxine to have a greater effect on thyroid weight than on relative cell mass while the rats were fed a low iodine diet. Such changes were seen by Matovinovic & Vickery (1959) in guinea-pigs on an *ad libitum* diet. Hypophysectomy and thyroxine produce similar morphological changes in the thyroid gland yet produce opposite changes in RNA concentration. Finally, thyroxine given to hypophysectomized animals does not increase the relative volume of cells but does raise RNA concentration. These observations suggest that thyroxine has a direct effect on thyroidal RNA independent of morphological changes and may be worthy of additional study.

Differences in experimental design (diet administered, species of animals used, dosage of TSH and duration of treatment) may account for the fact that we found decreased concentrations of RNA after TSH administration whereas other workers (Fiala, Sproul & Fiala, 1957; Matovinovic & Vickery, 1959) found the concentrations to be increased.

DISCUSSION

As yet no satisfactory explanation has been found for the increased levels of free tyrosine found by Levine *et al.* (1962) in the plasma of hyperthyroid patients and by Rivlin & Levine (1963) in animal plasma. Melmon *et al.* (1964) have shown that the elevation is specific for tyrosine and does not seem to result from a general increase in protein break-down or from increased absorption of amino acids. Enzymic changes induced by thyroid hormone probably do not account for the plasma tyrosine changes (Litwack, 1956; Levine *et al.* 1962; Davis, 1963; Rivlin & Levine, 1963). Concerning the possibility that thyroid overactivity affects tissue transport of tyrosine, the evidence from the present work is inconclusive, since no consistent change in tissue concentration of free tyrosine has been demonstrated, except in the thyroid gland.

Although the changes in thyroid tyrosine are probably insufficient to produce elevated plasma tyrosine levels, studies of tyrosine turnover rates in the gland are required to settle this point. Also, the possibility of altered transport of tyrosine in other tissues is by no means ruled out by the present findings. Very small losses of tyrosine by a large mass of tissue (e.g. muscle) or by several different tissues could raise the plasma tyrosine levels significantly, but the resulting small changes in tissue levels may have been obscured by the wide scatter of values obtained from individual animals.

Studies on thyroidal tyrosine suggest that the level of the amino acid in the gland may be under hormonal control. Following treatment with thyroxine for various periods there was a significant fall both in thyroid weight and in free tyrosine concentration in the gland. Treatment with TSH is known to increase gland weight and uptake of ^{14}C tyrosine (Klitgaard, Palay & Meade, 1963; Poffenbarger, Powell & Deiss, 1963; Raghupathy, Tong & Chaikoff, 1963). In addition, TSH may also stimulate the rate of the deiodination of intraglandular iodotyrosines. Saisson & Rosenberg (1963) have shown that TSH-induced deiodination may be enhanced by simultaneous administration of PTU.

In the present studies tyrosine concentration in the gland did not increase until iodination was blocked with PTU. Since T 4 decreases thyroidal free tyrosine and TSH in the presence of PTU does the opposite, it is suggested that at least part of the effect of T 4 on concentrations of tyrosine in the thyroid was mediated through suppression of TSH secretion. In support of this concept was the finding that hypophysectomy produced changes in tyrosine in the same direction as thyroxine administration. The significant increase of thyroid-free tyrosine in animals treated with PTU alone may have been due to the decrease of endogenous T 4 production and subsequent stimulation of endogenous TSH release.

There is evidence in this study, as in those of Halmi, Granner, Albert & Doughman (1959), Khazin & Reichlin (1961) and Granner, Curtis, Scranton & Halmi (1962, 1963), that thyroid hormones may have an effect on thyroid gland function beyond their ability simply to inhibit release of TSH. The present experiments differ from those of Granner *et al.* (1962, 1963) and Poffenbarger *et al.* (1963) in that the effect of T 4 was evaluated in hypophysectomized animals. In the T 4-treated, hypophysectomized animals, there was a significant decrease of free tyrosine and increase of RNA content when compared to their untreated controls.

Free tyrosine in the thyroid gland may be concentrated from the blood by active transport, which was shown by Raghupathy *et al.* (1963) to occur *in vitro*, or it may be accumulated as a hydrolytic product within the gland. Pitt-Rivers & Cavalieri (1963) have stated that the free monoiodotyrosine, which may contribute to the second or perchlorate-nondischargeable iodide pool in the thyroid, could arise either from iodination of free tyrosine or from hydrolysis of iodinated peptides or protein. Experiments with thyroid homogenates, cells or partially purified peroxidase systems from thyroid glands (Fawcett & Kirkwood, 1953; Serif & Kirkwood, 1956; Alexander, 1959; Pastan, 1961; Klebanoff, Yip & Kessler, 1962; De Groot & Davis, 1962) show rapid iodination of added free tyrosine. De Groot & Davis (1962) have shown that varying the tyrosine concentrations from 1 to 0·1 μmole in an *in vitro* system containing the equivalent of 8 mg thyroid

tissue (our calculations) will reduce the ability of the enzyme to iodinate free tyrosine by about 60%. Calculations from our data on free tyrosine concentration in the thyroid show that, under normal conditions, 8 mg tissue would contain about 0·2–0·4 μmole tyrosine, and after T 4 administration this would be reduced by as much as 60%. Therefore, it seems reasonable that some or all of the free MIT and DIT in the intact gland may arise from iodination of the free amino acid and that this process could be slowed by decreasing tyrosine concentration during T 4 administration. The implication of these findings remains to be determined, since free iodinated tyrosines probably are not incorporated into hormone (Alexander 1964; Cartouzou, Aquaron & Lissitzky, 1964).

Hormone synthesis in the gland could possibly be affected by changes in the 'physiologic' concentrations of tyrosine if its incorporation into protein or peptide (and thus thyroid hormone) were dependent on tyrosine concentrations. If so, it would be logical to expect that TSH could increase hormone production in the thyroid by increasing the supply of tyrosine (Raghupathy *et al.* 1963), and that the thyroxine produced might in turn alter further synthesis by reducing intraglandular free tyrosine as well as suppressing TSH release. Such an hypothesis is quite tentative and may be tenuous in view of the estimated tyrosine content of thyroglobulin ranging from 104 to 120 residues/mole. The iodotyrosine content is about 15 residues/mole and the thyroxine content about 3 residues/mole. Therefore 80% of the tyrosine in thyroglobulin is uniodinated and hormone-induced tyrosine changes may have to be profound before synthesis of thyroglobulin is altered. The hypothesis is however worthy of testing if a suitable model could be devised.

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