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MECHANISM OF GOITROGENESIS BY VERY LOW DOSES OF PROPYLTHIOURACIL AND THE ROLE OF IODINE INTAKE

By

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ABSTRACT

The effects of the daily feeding of 6 μg of propylthiouracil[1]/rat for about two weeks on the thyroid of animals maintained on different levels of iodine intake (from 0.5–3.6 μg/day/100 g BW) have been investigated. We have confirmed previous observations by other authors (*Yamada & Schichijo* 1962; *Greer et al.* 1962) showing that very low doses of PTU increase thyroid weight in rats on a low iodine supply without necessarily decreasing their rather low plasma PBI or their high thyroidal ^{131}I uptake, and that the same doses no longer have an effect on thyroid weight if the iodine intake is raised. In the present experiments this occurred when the iodine intake was raised to about 1.2–1.3 μg/day/100 g BW. As shown here, it is unlikely that these low doses of PTU block synthesis of the thyroid hormones. They do, however, slightly inhibit the extrathyroidal deiodination of T_4 and they probably trigger thyrotrophin (TSH) release from the pituitary gland. The present findings are compatible with the view that very small doses of PTU can be goitrogenic if the rats are on an iodine intake which is barely adequate for normal peripheral requirements, because these are increased chronically, even if

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[1]) The following abbreviations are used: 6-Propyl-2-thiouracil, PTU; 6-Methyl-2-thiouracil, MTU; Methyl-mercapto imidazole, MMI; L-thyroxine, T_4; Triiodo-L-thyronine, T_3; Monoiodotyrosine, MIT Diiodotyrosine, DIT; T_4*, T_3*, DIT* and MIT* for the radioactive compounds; Low iodine diet, LID; body weight, B.W.

slightly. Because of the low iodine stores the thyroid would then be unable to compensate for these increased hormone requirements. Even a small increase of available iodine may avoid this situation so that the effects of the low PTU doses are no longer detectable either in the plasma TSH activity or in the thyroid weight. The low PTU doses used here appear to hasten and aggravate a situation which would eventually develop from a more prolonged or a more severe degree of iodine deficiency only. The experimental situation induced by these low PTU doses might serve as an interesting model system for the study of simple goitre in areas in which iodine deficiency is not too extreme.

Thiouracils are known to have both *anti-* and *extra*-thyroidal effects involving the thyroid hormones (for a review see *Morreale de Escobar & Escobar del Rey* 1967). It has been proposed in this laboratory (*Escobar del Rey et al.* 1961, 1962; *Mouriz et al.* 1966) that the extrathyroidal actions of 6-Propyl-2-thiouracil on L-Thyroxine, reflected in a decreased rate of deiodination and decreased metabolic effectiveness of this hormone, are responsible for the rapid release of thyrotrophin (TSH) from the pituitary gland which follows the onset of its administration to rats. This increase in circulating TSH occurs before the blocking of hormonal synthesis by PTU could have influenced the thyroid-pituitary axis.

It appeared that such an assumption might be tested further if the *extra*-thyroidal actions of PTU could be dissociated from the *anti*-thyroidal actions and the response of the pituitary followed. An adequate experimental approach was offered by the finding that very low doses of PTU depress peripheral deiodination of T_4 in rats maximally (*Van Middlesworth et al.* 1960; *Morreale de Escobar & Escobar del Rey* 1962; *Mouriz et al.* 1966) whereas much higher doses are required for the maximal blocking of thyroid hormone synthesis (*Iino et al.* 1961). Existing data (*Yamada & Schichijo* 1962; *Greer et al.* 1962) indicated that the chronic administration of a few micrograms of MTU or PTU daily to rats on a low iodine intake resulted in an increased thyroid weight, without appreciably affecting the plasma PBI and the 24 h ^{131}I thyroidal »uptake«. Some changes were observed in the pattern of thyroidal ^{131}I distribution among iodoamino acids: a decreased proportion of the label was present as $T_3^* + T_4^*$ and the MIT^*/DIT^* and T_3^*/T_4^* ratios were increased. On the basis of these alterations of intrathyroidal ^{131}I distribution it was suggested that these low doses of thiouracils had induced goitre by interfering with the synthesis of adequate amounts of thyroid hormones. It was, however, difficult to reconcile this view with the concomitant observation that an increase in iodine intake (to about 3–10 μg/day) abolished the effects of the same low doses of PTU on thyroid weight and intrathyroidal ^{131}I distribution. Moreover, the above changes in the intrathyroidal ^{131}I distribution pattern could have been attributed just as well to the severe iodine deficiency and the

intense TSH stimulation of such glands (*Querido et al.* 1957; *Studer & Greer* 1965, 1967; *Greer et al.* 1967).

We have therefore attempted to reproduce the findings reported by *Yamada & Schichijo* (1962) and *Greer et al.* (1962), and determine whether they were consistent with the following assumptions: a) The daily administration of a few micrograms of PTU does not block thyroid hormonal synthesis or release in rats on either a low or a normal iodine intake. b) It does, however, affect the extrathyroidal metabolism and effectiveness of T_4 sufficiently to trigger off a TSH-releasing response of the pituitary gland if peripheral amounts of the thyroid hormones are barely adequate for normal requirements. c) As a consequence of this, the thyroid is stimulated to secrete more hormone. Two major possibilities then arise. If the rats are on an adequate iodine intake the gland could easily compensate for the increased extrathyroidal requirements, since we have assumed in a) that thyroid hormone synthesis is not blocked and it is known (*Mouriz et al.* 1966) that the extrathyroidal effects of PTU on T_4 may be overcome by increasing the supply of the hormone. On the contrary, if the iodine intake were barely sufficient for minimal peripheral needs, the thyroid would be unable to maintain an increased secretion indefinitely. In such cases TSH levels would continuously be high enough to result in an increased thyroid weight.

MATERIALS AND METHODS

General procedure

Young male or female rats weighing either 70–90 or 110–130 g at the onset of the experiments were fed a medium residue low iodine diet of the Remington type. The iodine content of different batches of diet varied between 0.05 to 0.09 μg/g. Distilled water was used. When iodide supplementation was desired, the appropiate amount of KI solution was mixed with the food. After 5–7 days the diet of half the group was supplemented with 6 μg PTU/rat/day. The amount of daily ration was such that the animals could eat *ad libitum* with a minimum of waste. After 9–14 days on PTU, these animals and their respective control were injected intraperitoneally with 10 μc ^{131}I iodide either 3–4 or 18–24 h before being killed. The changes in thyroidal ^{131}I content were followed by *in vivo* counting of the neck under light ether narcosis. The procedure for isotopic equilibration of thyroidectomized rats with labeled T_4 and for the measurement of thyroidal ^{131}I disappearance rates in rats with $KClO_4$-blocked ^{131}I recycling have been described previously (*Escobar del Rey & Morreale de Escobar* 1961; *Escobar del Rey et al.* 1961, 1962). At the end of the experiment the animals were sacrificed under light ether narcosis by bleeding from the inferior vena cava after the injection of heparin.

Distribution of ^{131}I among thyroidal iodoamino acids

The glands were rapidly dissected, weighed and digested at 37° C with pancreatin (Viobin Co., Monticello, Ill.) for 18–24 h (*Tong & Chaikoff* 1958) or with pronase (Sigma Chemicals Co.) for 7–8 h (*Tong et al.* 1965). In both cases the digestion mix-

ture contained 0.01 M MMI. Aliquots were spotted on chromatographic strips along with stable carrier T_4, T_3, DIT, MIT, I^- and 10^{-3} M PTU. This procedure was followed to avoid artifactual deiodination during digestion and chromatography (*Morreale de Escobar et al.* 1963). Ascending chromatography in n-butanol-ethanol-1 N ammonia (5:1:2) separated both the iodotyrosines and the iodothyronines. Location of the radioactive spots was determined by radioautography. Iodide was identified by staining with palladium chloride and the iodoamino acids were visualized with diazotized sulphanilic acid. The appropriate spots were counted in a well-type NaI, thallium activated crystal scintillation counter.

Other determinations

All ^{127}I measurements were carried out by a modified Zak procedure (*Benotti & Benotti* 1963). Plasma TSH activities were evaluated either by the *McKenzie* (1958) *in vivo* or the *Kirkham* (1962) *in vitro* assay. In the latter case, 4-point assays were carried out using bovine international TSH standard as reference. Statistical evaluation of all data was carried out following standard procedures (*Snedecor* 1956).

RESULTS AND COMMENTS

1) *Effects of 6 μg PTU/rat/day on L-T_4 deiodination*

Twelve thyroidectomized rats were equilibrated isotopically by the daily intraperitoneal injection of 1.5 μg T_4, labelled with 1 μc ^{131}I-T_4 (as assessed on the 1st day). Once equilibrium was established, 6 rats were treated daily with 6 μg PTU/rat for 6 days, the other 6 rats serving as controls. There was a small but clearly detectable decrease of the urinary excretion of radioiodide from ^{131}I-T_4 in the rats receiving PTU: the mean (± SD) of all the data obtained in 6 animals during 6 days of such treatment was 34.4 ± 4.1 % of the ^{131}I dose, injected as the parent hormone, as compared to 40.6 ± 4.8 % for the control rats, $P < 0.001$.

2) *Effects of 6 μg PTU on the thyroidal ^{131}I release of rats on $KClO_4$*

Ten rats were injected with 10 μc ^{131}I-iodide after three days on LID. Twenty-four hours later they were given daily 160 mg $KClO_4$/rat by stomach tube and 1 μg T_4/rat until they were killed. The rate of disappearance of ^{131}I from the thyroidal region was determined by repeated *in vivo* counting during three days. The data were plotted on a semilogarithmic scale against the time after injection of the tracer, and the resulting slope was determined for each animal. Half the experimental group then received saline intraperitoneally, the other animals saline containing 6 μg PTU/rat. *In vivo* countings of the thyroid region were followed for another 24 hours. The slope of the semilogarithmic plot during the last 24 hours was determined and calculated as a percentage of the slope obtained before the saline or PTU injections. It increased in three out of the five rats receiving a single injection of 6 μg PTU. The mean values (± SD) for the 5 PTU-treated and the 5 saline injected rats

were 171 ± 70 % and 95 ± 7 % respectively, the difference being of borderline statistical significance ($P = 0.05$).

3) Effects of 6 μg PTU/rat/day on thyroid and pituitary function

a) *Rats on low iodine diet.* Fig. 1 exemplifies results obtained under these experimental conditions. The plasma TSH activity and the thyroid weight of the rats receiving 6 μg PTU/day increased, as compared to those of rats on the LID only. The plasma PBI, however, was not appreciably lower in the PTU-treated animals than in the controls; both values were subnormal. Though not shown in Fig. 1, the total thyroidal ^{127}I was lower in the rats on PTU (0.48 ± 0.01 μg/lobe) than in the controls (1.20 ± 0.27 μg/lobe), $P < 0.001$.

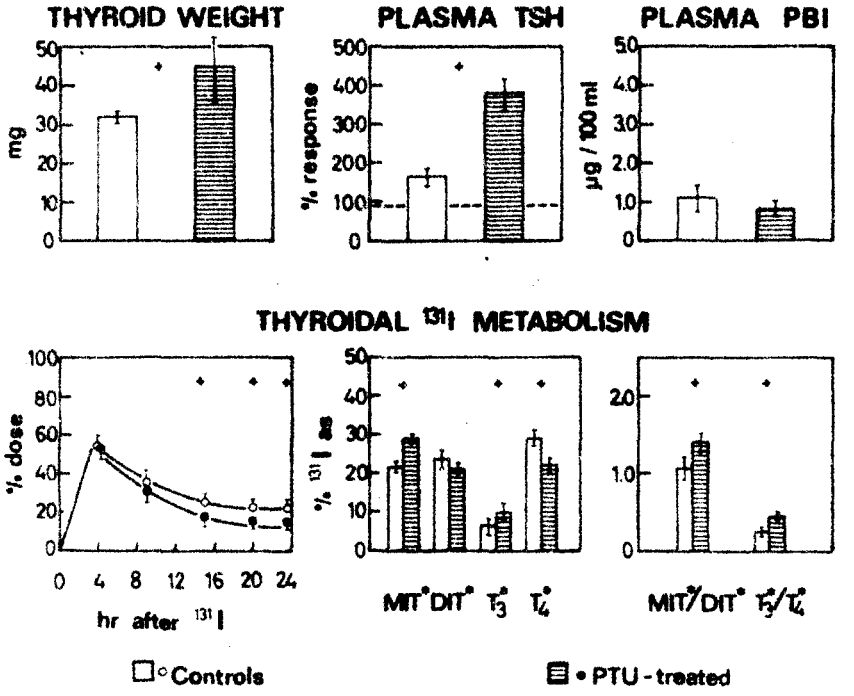

Fig. 1.

Effects of very low doses of PTU on several metameters of thyroid and pituitary function. Male rats, weighing 130–150 g were fed a diet with an iodine intake of about 0.5 μg/rat/day (LID). Five days later half the group also received 6 μg PTU/rat/day. A tracer dose of ^{131}I was injected 24 h before killing. Data are shown as means (± SD) of the values of 6 rats/group. In the case of the plasma TSH activities, obtained by the *McKenzie* (1958) assay, the data are mean responses (± SE) of the mice to pooled plasma samples. Plus signs (+) indicate when the difference between mean values of PTU-treated animals (● and hatched bars) and controls (○ and open bars) were statistically significant, for P equal to or less than 0.05.

The »uptake« was not depressed by the low PTU doses and the rate of disappearance of labelled compounds was accelerated. The pattern of ^{131}I distribution among thyroidal iodoamino acids was obtained 24 h after the injection of the label and, therefore, during this release phase. The MIT*/DIT* and T_3^*/T_4^* ratios both of control and PTU-treated rats were higher than usually found in animals on a normal iodine intake. For our colony these values are about 0.5 and 0.1, respectively, in agreement with those of other laboratories (e. g. *Studer & Greer* 1965). The proportion of the label present as $T_3^* + T_4^*$ in the glands from PTU-treated rats was decreased as compared to that of the controls ($P < 0.01$).

It was possible that the changes observed in the intrathyroidal distribution of ^{131}I after a large proportion of the label had left the gland would not have been the same if the samples had been obtained at a time nearer to that of maximal thyroidal ^{131}I content. This was investigated in several experiments. Typical results of one of them are shown in Fig. 2. When the intrathyroidal ^{131}I distribution pattern was determined 24 h after administration of the label, the results (Fig. 2) were similar to those already described for Fig. 1. However, a decrease in the proportion of $T_3^* + T_4^*$ was no longer observed when the glands were obtained nearer the time of maximal thyroidal ^{131}I content; the proportion of $T_3^* + T_4^*$ could actually be increased. The time of sacrifice did not appreciably alter the high T_3^*/T_4^* ratios of both control and PTU-treated groups.

b) *Effects of increasing the iodine intake.* In the experiments described above the iodine intake was low enough to be goitrogenic and also induce alterations in the intrathyroidal ^{131}I metabolism typical of iodine deficiency (*Querido et al.* 1957; *Studer & Greer* 1965, 1967; *Greer et al.* 1967). Fig. 3 shows some of the data obtained in an experiment carried out simultaneously on rats fed three different amounts of iodine with or without 6 μg PTU/rat. Even a slight increase in the iodine intake from 0.6-0.9 μg/rat/day decreased the thyroid weight of the control rats. It was also observed (data not shown in Fig. 3) that the thyroidal ^{127}I content and the plasma PBI increased somewhat, but they were still lower than normal (0.9 μg/gland and 1.6 μg/100 ml for the control rats on 0.9μg I/day). The 24 h intrathyroidal ^{131}I distribution pattern and the thyroidal ^{131}I release rates of control and PTU-treated rats were comparable in all respects to those described for the experiments illustrated in Figs. 1 and 2, both at the 0.6 and 0.9 μg I/rat/day intake levels.

When the iodine intake was raised to 2.6 μg the effects of these low PTU doses changed strikingly. The thyroid weight and the thyroidal ^{131}I release rate did not increase above the control values and the intrathyroidal ^{131}I distribution pattern was the same as that of the controls. But in these animals this pattern was not completely normal for either group: the increase in iodine intake had not been adequate to completely normalize the plasma PBI or the thyroidal ^{127}I

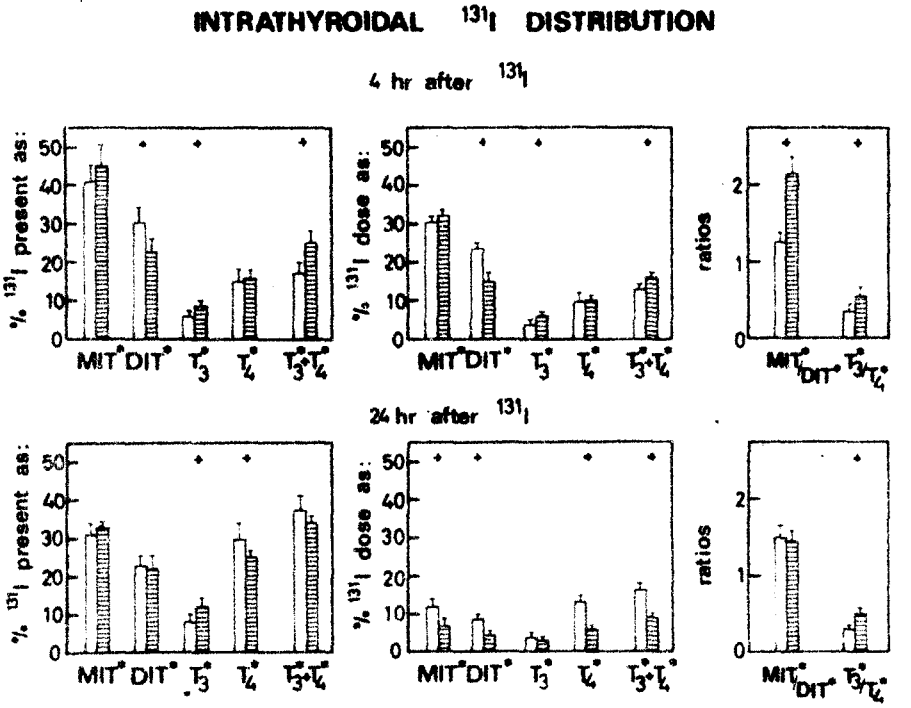

Fig. 2.

Intrathyroidal ^{131}I distribution of iodoamino acids at 4 and 24 h after the injection of a tracer dose of ^{131}I into male rats weighing 110–130 g and on an iodine intake about 0.6 μg/rat/day for a total of 16 days. Half of the group also received 6 μg PTU/rat/day for the last 11 days. The data are means ($\pm$ SD) of 6 rats/group. Meaning of + and symbols, the same as for Fig. 1. The values of other metameters of thyroid function for the control and PTU-treated rats were, respectively: weight, 33.2 $\pm$ 5.2 and 41.3 $\pm$ 7.0 mg ($P = 0.01$); ^{127}I content, 1.0 $\pm$ 0.3 and 0.4 $\pm$ 0.2 μg/gland ($P<0.001$); 4 h ^{131}I content, 70.5 $\pm$ 3.8 and 65.7 $\pm$ 8.7 % dose; 24 h ^{131}I content, 47.5 $\pm$ 4.1 and 29.2 $\pm$ 6.7 % dose ($P < 0.01$). The plasma PBI values were 1.4 $\pm$ 0.3 and 1.2 $\pm$ 0.2 μg/100 ml for controls and PTU-treated rats, respectively.

content, which were still only 1.8 μg/100 ml and 2.6 $\pm$ 0.5 μg/gland, respectively. The mean thyroidal ^{127}I content of the group on PTU was lower than that of the controls at all three iodine intake levels.

Similar results were obtained in another experiment carried out simultaneously in animals on five different levels of iodine intake (Fig. 4). Again, PTU increased the plasma TSH activity and the thyroidal weight of rats on the lowest iodine intake, without any further decrease of the plasma PBI. Though not shown in Fig. 4, we found in animals on PTU, the usual decrease in the thyroidal ^{127}I content, increase in the thyroidal ^{131}I release rate and increased MIT*/DIT* and T_3*/T_4* ratios. Increasing the iodine intake abolished most

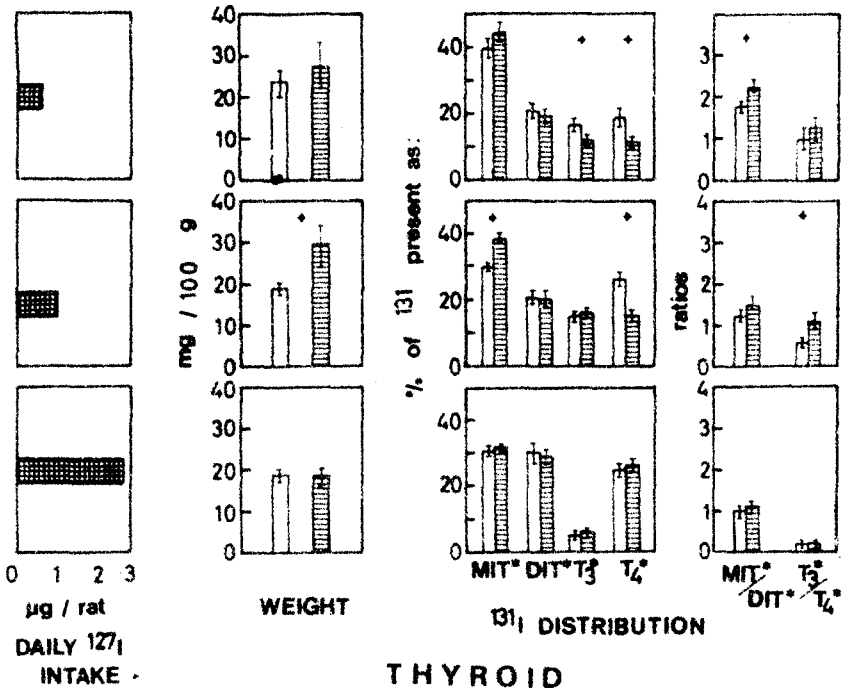

Fig. 3.

Effects of 6 µg PTU/rat/day given for the last 9 days on several metameters of thyroid function in rats maintained for a total of 16 days on three different levels of iodine intake (cross-hatched bars): 0.6, 0.9 and 2.6 µg/rat/day. ^{131}I distribution was determined at 24 h after ^{131}I. Female rats were used with an initial weight of 110–130 g. Data are mean (± SD) values of 6 rats/group. When the SD is not shown, this means that the determinations were carried out on pooled, and not on individual, samples. Meaning of symbols the same as for Fig. 1.

of these effects of PTU: the plasma TSH activity, the thyroid weight and the thyroidal ^{131}I release rate not being increased above control values. The pattern of intrathyroidal ^{131}I distribution among iodoamino acids was the same for PTU-treated and control animals and was normal for both groups. The thyroidal ^{127}I was always lower, however, for the rats receiving PTU than for their controls, though such differences could not always be validated statistically[2]. In this experiment an iodine intake of 2.0 µg/rat/day or higher brought the thyroidal ^{127}I and plasma PBI of control animals back to normal.

c) *Overall results.* A total of 12 experiments were carried out over a two

[2] In many cases it was not possible to assess statistical significance of the difference between PTU and control values because the estimations had not been carried out on individual, but on pooled, samples.

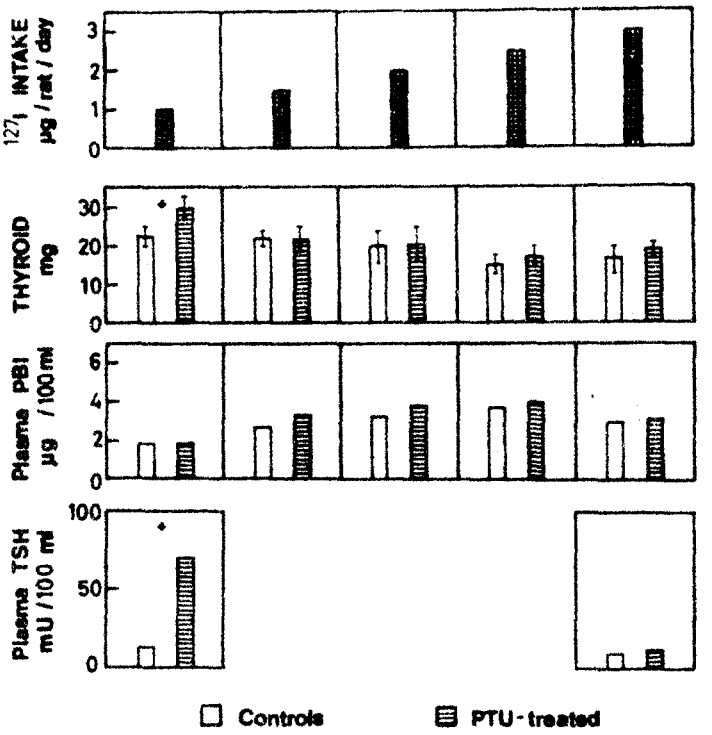

Fig. 4.

Effects of 6 µg PTU/rat/day given for the last 11 days on metameters of thyroid and pituitary function in rats on five different levels of iodine intake (cross-hatched barst) for a total of 17 days. Female rats were used with an initial weight of 70–90 g. Data are means (± SD) of values of 5–6 rats/group. For the TSH activities, the *Kirkham* (1962) assay was used. For the meanings of symbols and of the lack of indication of the SD, see legends to Figs. 1 and 3.

year span using different batches of LID. There were differences in the age and sex of the animals, duration of treatments, etc. All the data have, however, been used in an attempt to obtain an overall picture of the effects of low doses of PTU in rats on varying iodine intakes. We have tried to make the data more comparable by i) expressing thyroid weight and iodine intake per 100 g BW, ii) calculating the mean value of a given metameter of PTU-treated rats as a percentage of the mean value of the corresponding control group or iii) both. Statistical evaluation of trends that were observed in individual experiments could then be carried out and interrelationships studied between different metameters of thyroid function.

1) Thyroid weights: Fig. 5 shows the result of plotting the mean thyroid weights of groups of PTU-treated and of control rats against the logarithms

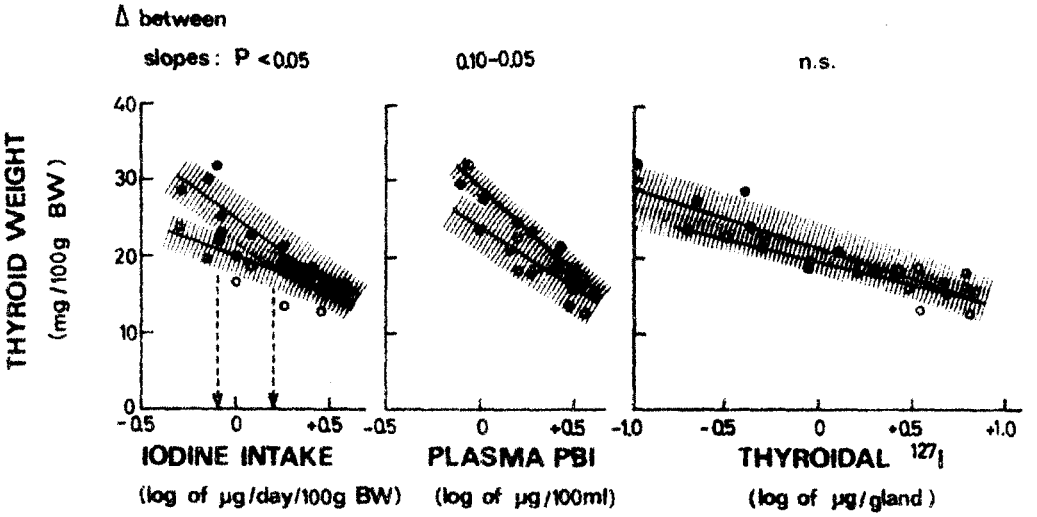

Fig. 5.

Relationships between the mean thyroid weight and the iodine intake, plasma PBI or total thyroidal ^{127}I for rats on different levels of dietary iodine, supplemented (●) or not (○) with 6 µg PTU/rat/day. Each point is the mean for 5–6 rats. The arrows indicate the logs. of 0.85 and of 1.2 µg I/100 g body weight.

of their daily iodine intakes, the plasma PBI or the total thyroidal ^{127}I content. The lines of best fit were calculated by the method of least squares, using data from PTU-treated and from control groups independently. The resulting slopes were compared statistically.

The mean thyroid weight increases as the thyroidal ^{127}I content decreases in the same way whether or not the animals receive the low PTU doses. This is not the case when thyroid weight is plotted against the log. of the plasma PBI or of the iodine intake. In the case of the PBI, a tendency was observed for the data from groups of rats on PTU to fall on a line with a steeper slope than those from control animals. Though the probability that both slopes were the same was, however, still 10 %, this observation may at least be taken to indicate that for plasma PBI values below 1.5–2.0 µg/100 ml approximately, the rats on the low PTU doses are more likely to have a larger thyroid than those not receiving the drug, even when their plasma PBI is the same. In the case of the thyroid weight against the iodine intake, two different slopes were obtained, P being somewhat lower than 0.05. From Fig. 5 it is apparent that below an intake of about 0.85 µg I/100 g BW, there is a 95 % probability that the mean thyroid weight of rats receiving the low doses of PTU in any experiment is higher than the mean value for the corresponding control group

and the difference statistically significant. This probability decreases as the iodine intake increases[3]. Above 1.2–1.3 μg/100 g BW[4] it is quite unlikely that 6 μg PTU/rat/day for a few weeks result in an increased thyroid weight.

2) Thyroidal metabolism of ^{127}I and ^{131}I: Table 1 shows that the low doses of PTU used for the present study not only did not depress the high 3–4 h thyroidal ^{131}I »uptake«, but in general actually increased it somewhat above the control value, whether or not the group had developed goitre[5]. In contrast, the data of thyroidal ^{131}I content at 18–24 h after injection of the tracer fell into two distinct populations depending on whether or not the thyroid weight of the PTU-treated rats had increased above control values. The difference between both populations was so clearcut that an acceleration of the release rate of PTU-treated animals over that of the corresponding control group sufficed to predict an increase in thyroid weight before the animals were killed. The total thyroidal ^{127}I content had decreased in all groups on PTU, but this change was proportionately more intense for those developing goitre.

Fig. 6 shows that the mean MIT*/DIT* and T_3*/T_4* ratios have the same dependence on the mean total thyroidal ^{127}I content whether or not PTU was administered. Relatively small decreases in the total thyroidal ^{127}I content may be accompanied by a sharp increase in the MIT*/DIT* and, particularly, the T_3*/T_4* ratios, when the thyroidal iodine stores are already low (below 1–2 μg/gland approximately[4]). In contrast, at higher values of total thyroidal ^{127}I stores, a much larger variation may hardly affect these ratios.

3) Plasma PBI: Table 1 indicates that there are two distinct populations of data depending on whether or not the thyroid weight of PTU-treated rats had increased above the control value. In the first case there was often a decrease of the plasma PBI relative to the already low value of the group not receiving the drug. This usually occurred when the iodine intake was very low (0.6 μg/rat/day or less[5]). Contrary to this, in the groups in which PTU treatment was not followed by an increase in thyroid weight, there was a clearcut tendency for the PBI to be somewhat higher than that of the corresponding

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[3] Preliminary experiments would indicate that if the iodine intake is extremely low (about 0.5 μg/rat/day or lower) this would also happen because the thyroid weight of the *control* animals increases quite rapidly.

[4] Naturally, these values and others given in the present paper are likely to be different in different laboratories, since they would probably be affected by the strain and age of the animals, the composition of the diet as regards factors other than the iodine content, season, temperature, etc.

[5] In order to simplify the following comments and discussion, we have divided animals on PTU into those »developing goitre« and those who did not, depending on whether or not thyroid weights were higher than those of the corresponding controls, though many of the latter groups (on LID) had themselves a larger than normal thyroid gland.

Table 1.

Statistic evaluation of results from 12 different experiments, each consisting of a group of rats on 6 μg PTU/rat/day and a group of controls on the same iodine intake. Data are means (± SD) of the mean value of a given metameter of thyroid function for a PTU-treated group, expressed as a percentage of the mean value of the same metameter for the corresponding control group. The number of such mean values used for the calculations is given in brackets; Goitre: + and Goitre: — indicate when the PTU-treated group had, or not, a larger thyroid than the corresponding control group; when $P > 0.05$, this is indicated by n. s.

	A Goitre: +	Δ from 100 % *P*	B Goitre: —	Δ from 100 % *P*	Δ: A–B *P*	A + B All experiments	Δ from 100 % *P*
Thyroid							
^{127}I content	33 ± 14 (6)	< 0.001	82 ± 18 (6)	< 0.05	< 0.001	60 ± 36 (12)	< 0.01
3–4 h ^{131}I »uptake«	101 ± 6 (6)	n. s.	113 ± 14 (6)	n. s.	n. s.	109 ± 14 (12)	< 0.05
18–24 h ^{131}I content	62 ± 18 (6)	< 0.01	104 ± 14 (6)	n. s.	< 0.001		
Plasma PBI	70 ± 22 (6)	< 0.05	115 ± 13 (6)	< 0.05	< 0.01		

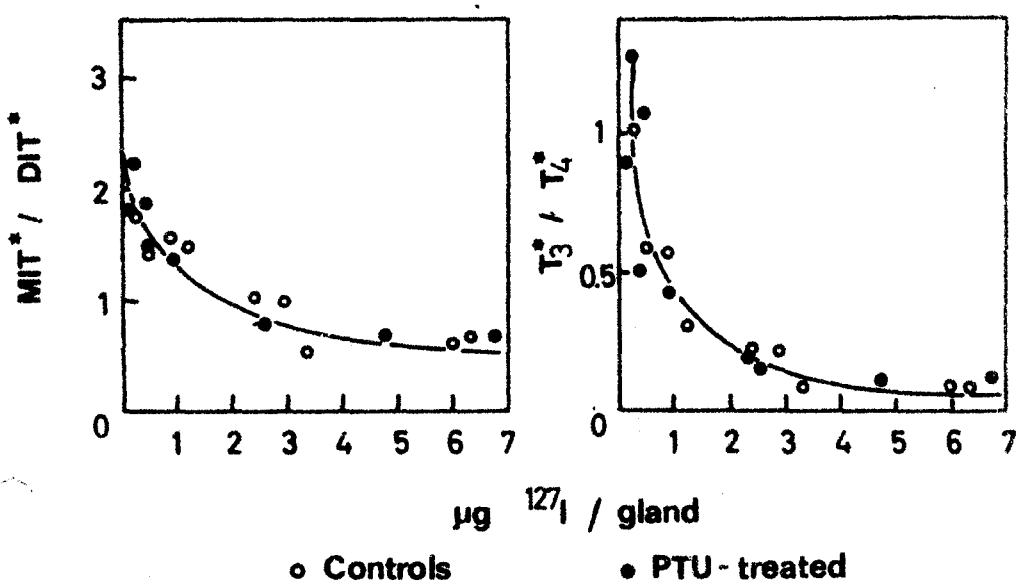

Fig. 6.
Mean MIT*/DIT* and T_3*/T_4* ratios, obtained 18–24 h after ^{131}I injection, as a function of the mean total thyroidal ^{127}I content of rats on different levels of dietary iodine, either supplemented (●) or not (o) with 6 µg PTU/rat/day.

controls, though such a difference might not have been validated statistically for individual experiments[2].

DISCUSSION

The present data agree with previous results of *Yamada & Schichijo* (1962) and *Greer et al.* (1962) regarding the possible effects of very low doses of PTU on the thyroid; they once more confirm *Astwood*'s observation (1945) that chronic ingestion of very small doses of thiouracils may be either innocuous or goitrogenic according to the iodine intake. We also show that the response (or lack of response) of the thyroid to the low doses of PTU reflects the level of circulating TSH activity.

We believe our data are compatible with the assumptions outlined in the introduction.

a) The decreased proportion of T_3* + T_4* 18–24 h after ^{131}I injection found in glands of PTU-treated rats developing goitre was always associated with an increase in the rate of disappearance of labelled compounds from the thyroid. The proportion of T_3* + T_4* did not increase in the same animals before this phase of ^{131}I metabolism. It was unchanged in the rats on PTU

who did not have an increased rate of thyroidal ^{131}I release and did not develop goitre.

The high T_3^*/T_4^* ratios of PTU-treated rats developing goitre and of their controls on the same iodine intake were independent of the interval after ^{131}I injection at which the glands were obtained. These ratios became normal for both control and PTU-treated groups when the lowering of intrathyroidal ^{127}I stores caused by PTU did not bring the glands into an iodine-deficient state (i. e., below 1–2 μg I/gland approximately).

We believe that these observations strongly support the conclusion that the changes induced by PTU in the intrathyroidal distribution of ^{131}I among iodoamino acids in rats on low iodine intake are not due to blocking of iodothyronine synthesis, but are a consequence of iodine deficiency and TSH stimulation (*Querido et al.* 1957; *Studer & Greer* 1965, 1967). Both are more severe in the PTU-treated rats than in the controls on the same iodine intake. If this interpretation is accepted, there is no longer an incompatibility between the different effects exerted by the same low doses of PTU in rats on a low and those on a more normal iodine intake: with the latter both the severe iodine deficiency and the intense TSH stimulation are no longer present.

b) It has been shown that 6 μg PTU/rat/day interfere with the peripheral deiodination of T_4, though less intensely than the higher doses (*Jones & VanMiddlesworth* 1960; *Escobar del Rey & Morreale de Escobar* 1961). The higher plasma PBI of PTU-treated than of control rats when the iodine intake is normal, also suggest an extrathyroidal effect of the low PTU doses. Again this appears to be less intense than that obtained with higher doses (*Jones & VanMiddlesworth* 1960; *Escobar del Rey & Morreale de Escobar* 1961).

This slight interference with the extrathyroidal metabolism of T_4 might, however, be sufficient to trigger off TSH release if the extrathyroidal pool of thyroid hormones were just adequate for normal peripheral requirements. This situation would be likely in rats which were already on a low iodine intake for a week or more before the onset of treatment with PTU. The increase in thyroidal ^{131}I release in rats on $KClO_4$ observed in several animals after a *single* dose of 6 μg PTU points to a triggering of TSH release. It moreover appears from the present data that the plasma PBI of the rats on the low doses of PTU had to be maintained at a slightly higher level than that of the untreated controls to ensure a normal thyroid weight. Both effects are more clearly demonstrable with high doses of PTU (*Van Middlesworth et al.* 1959; *Jagiello & McKenzie* 1960; *Escobar del Rey et al.* 1962) and are believed to involve extrathyroidal effects of this drug on T_4 (see review by *Morreale de Escobar & Escobar del Rey* 1967).

c) Once the thyroid is stimulated to secrete more hormones to compensate for the slightly increased peripheral requirements, its ability to do so would depend on the availability of iodine to the gland. From the present data it

would appear that peripheral needs are met as long as the thyroid is able to maintain a slightly increased plasma PBI. If this is no longer possible because of the inadequate supply of iodine, TSH secretion is maintained continuously high enough to result in an increased thyroid weight.

As a conclusion, we believe that the small doses of PTU used by us and by others (*Yamada & Schichijo* 1962; *Greer et al.* 1962) are goitrogenic when combined with moderate iodine deficiency, because they increase extra-thyroidal requirements for the thyroid hormones: they merely hasten and aggravate a situation which would eventually result from a more prolonged or a more severe degree of iodine deficiency only.

The experimental results which may be obtained by combining different degrees of iodine deficiency and low doses of PTU recall the observation that in some areas of endemic simple goitre where iodine deficiency is not too extreme, it is frequently found that some inhabitants develop goitre and others do not, without a difference being detected among them either in their high thyroidal ^{131}I uptakes and low normal plasma PBI (*Means et al.* 1963; *Follis et al.* 1963).

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