Lack of Effect of Propylthiouracil and Methylmercaptoimidazole on Thyroglobulin Biosynthesis (43053)

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Abstract. **Experiments were performed both *in vivo* and *in vitro* to test a previous proposal that part of the antithyroid action of the thioureylene drugs, propylthiouracil (PTU) and methylmercaptoimidazole, can be attributed to inhibition of thyroglobulin (Tg) biosynthesis. Rat thyroid lobes were incubated in leucine-free Eagle's medium containing bovine thyroid-stimulating hormone and 0, 0.1–0.2, or 1 m*M* drug. After a 30-min preincubation, 5 μCi of [^{14}C]leucine were added and the incubation was continued for 4 hr. The soluble fraction was analyzed by sucrose density gradient centrifugation, and the fractions corresponding to the 19S Tg peak were pooled and assayed for ^{14}C. No inhibition of ^{14}C incorporation into 19S Tg was observed, even in thyroid lobes incubated in the presence of 1 m*M* methylmercaptoimidazole or 2 m*M* PTU. At the same time, ^{14}C incorporation into 19S Tg was completely inhibited when lobes were incubated in the presence of 0.1 m*M* puromycin. *In vivo*, rats received an injection of PTU (1 μmol/100 g body wt), followed 60 min later by an injection of 25 μCi of [^{14}C]leucine. Blood samples and thyroids were taken 5 hr after the [^{14}C]leucine injection. Serum thyroid-stimulating hormone was not significantly affected by the PTU injection. The thyroid-soluble fraction was analyzed by sucrose density gradient centrifugation. No significant differences between saline and PTU-injected groups were observed in [^{14}C]leucine incorporation into 19S Tg. We conclude from both our *in vitro* and our *in vivo* studies that PTU and methylmercaptoimidazole have no inhibitory effect on thyroglobulin synthesis in rat thyroids and that such inhibition does not play a significant role in the antithyroid action of these drugs.** [P.S.E.B.M. 1990, Vol 194]

The thioureylene drugs, propylthiouracil (PTU) and methylmercaptoimidazole (MMI), exert their antithyroid effect primarily through inhibition of thyroid peroxidase-catalyzed iodination of thyroglobulin (1). However, other antithyroid actions of these drugs have also been postulated. These include (i) a possible direct effect on the coupling reaction (2); (ii) an immunosuppressive effect (3, 4), which presumably reduces the formation of thyroid-stimulating immunoglobulins; (iii) the formation of a mixed disulfide with Tg (5, 6), which may interfere with the coupling reaction; and (iv) inhibition of the synthesis of Tg (7), which may lower formation of T_4.

The present communication is concerned with the question of possible inhibition of Tg synthesis by PTU and MMI. Such inhibition was reported by Monaco *et al.* (7) in experiments in which incorporation of [^{3}H] galactose and [^{14}C]leucine into the soluble protein of rat thyroid lobes was measured in the presence and absence of PTU and MMI. However, their findings were not in agreement with those reported earlier by Seed and Goldberg (8), who observed that incorporation of [^{14}C]valine into thyroglobulin in sheep thyroid slices was not affected in the presence of 1 m*M* PTU.

A continuing interest in this laboratory in the action of antithyroid drugs on thyroid function led us to examine further whether PTU and MMI inhibit Tg biosynthesis in rat thyroids. Experiments were performed both *in vitro* and *in vivo*.

Materials and Methods

Rats. Male Sprague-Dawley rats were obtained from Simonsen Laboratories. They were maintained for varying periods on a 12-hr light-dark cycle and fed

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the laboratory stock diet (Wayne Lab-Blox). At the time of use they weighed 180–250 g.

Incubation Medium. Minimal essential medium (Eagle), without L-leucine or L-glutamine, was obtained from Gibco Laboratories.

***In Vitro* Experiments with [^{14}C]Leucine.** Thyroid lobes were carefully dissected after the rats had been exsanguinated from the abdominal aorta under ether anesthesia. Each incubation sample contained pooled thyroids from two rats (four lobes). Incubation was performed in 25-ml glass-stoppered flasks containing 5 ml of minimal essential medium, to which had been added 25 m*M* $NaHCO_3$, 2 m*M* glutamine, 0.1 μM KI, 5 milliunits/ml bovine thyroid-stimulating hormone (TSH), and either PTU, MMI, or deionized water. The incubation flasks were flushed with 95% O_2–5% CO_2 before the start of incubation and then placed in a shaking water bath at 37°C (100 oscillations/min). After a 30-min preincubation period, 5 μCi of [^{14}C]leucine (uniformly labeled, 270 mCi/mmol; ICN) were added and incubation was continued for 4 hr. The flasks were reflushed with O_2-CO_2 at hourly intervals.

Two separate experiments were performed. In the first, PTU and MMI were present at a concentration of 100 μM. In the second, the drug concentration was increased to 1 m*M* MMI or 2 m*M* PTU. Control samples contained an equal volume of deionized water. All samples (each containing pooled thyroids from two rats) were run in triplicate.

After the incubation, the thyroid lobes were rinsed twice in successive beakers of saline to remove adhering [^{14}C]leucine, blotted lightly on filter paper to remove excess liquid, weighed, and homogenized in 350 μl of 0.1 *N* KCl-0.02 *M* phosphate (pH 7.0) in a small, all glass, conical homogenizer (Kontes Glassware Co.) in an ice bath. An aliquot of the homogenate (20 μl) was counted for measurement of total ^{14}C uptake by the tissue. The remainder of the homogenate was centrifuged at 4°C in a Fisher microcentrifuge at 12,000*g* for 10 min. An aliquot (20 μl) of the supernatant was counted, and another aliquot (200 μl) was layered on top of a 10–40% linear sucrose gradient that had been prepared in 67 m*M* phosphate (pH 7.0). After centrifugation at 4°C for 16 hr in a Beckman SW60 Ti rotor at 30,000 rpm, fractions (eight drops, ~0.1 ml) were collected from the bottom of the tube (Beckman, 11 × 60 mm, Ultra-Clear) and assayed for ^{14}C in a liquid scintillation counter. The fractions corresponding to the 19S peak were pooled for measurement of [^{14}C] leucine incorporation into Tg. The position of the peak for 19S Tg was based on similarly treated samples containing ^{131}I-Tg derived from the thyroids of rats previously injected with ^{131}I.

***In Vitro* Experiments with ^{131}I.** Experiments with rat thyroid lobes were also performed to test inhibition of organic ^{131}I formation by PTU and MMI. The incubation conditions were exactly as described above, except that ^{131}I was added in place of [^{14}C]leucine, and PTU and MMI were added at only a single concentration (100 μM). Analysis was performed by paper chromatography, as previously described (9).

***In Vivo* Experiments with [^{14}C]Leucine.** Two separate experiments were performed, based on measurement of the incorporation of ^{14}C into Tg after injection of [^{14}C]leucine (ICN, uniformly labeled). Since only a very small fraction of the injected [^{14}C]leucine was taken up by the thyroid, it was necessary to inject a very large dose of [^{14}C]leucine into each rat (25 μCi). The high cost of this material severely limited the number of animals that could be used, and *in vivo* experiments were performed only with PTU.

In Experiment 1, six rats received three intraperitoneal injections of PTU (1 μmol/100 g body wt) over a period of 24 hr and six control rats were similarly injected with saline. Thirty minutes after the last injection, all animals were injected intraperitoneally with 25 μCi of [^{14}C]leucine (90 μmol). Thyroids were removed 5 hr later, after the rats had been exsanguinated from the abdominal aorta under ether anesthesia. Thyroid glands from two rats were pooled for homogenization, and the homogenate was treated as described above for the *in vitro* experiments.

In Experiment 2, 10 rats received a single injection of PTU (1 μmol/100 g body wt), followed 60 min later by an injection of 25 μCi of [^{14}C]leucine (136 μmol). Ten control rats were treated similarly, except that they received saline instead of PTU. Thyroids were removed 5 hr after the [^{14}C]leucine injection and treated exactly as in Experiment 1.

For comparison of the rate of synthesis of 19S Tg in the two groups of rats, the ^{14}C activity in the 19S Tg fraction was calculated in two ways: (i) as a percentage of the ^{14}C activity applied to the sucrose gradient, and (ii) as a percentage of the total ^{14}C activity in the glands.

***In Vivo* Experiment with ^{131}I.** Although previous experiments in this laboratory (10) had demonstrated that the dosage of PTU used in the present study (1 μmol/100 g body wt) was extremely effective in blocking organic iodine formation, an additional experiment was performed to confirm this result. Four rats received a single injection of PTU (1 μmol/100 g body wt) and four control rats received a saline injection. Sixty minutes later the animals were injected with 30 μCi of ^{131}I, and thyroids were removed 5 hr later, after the animals had been exsanguinated. Thyroid glands from individual rats were homogenized in an ice bath in 400 μl of 0.1 *N* KCl-0.02 *M* phosphate (pH 7.0)-5 m*M* methimazole. An aliquot (25 μl) of the homogenate was counted for measurement of total ^{131}I uptake by the gland, and the remaining homogenate was centrifuged at 12,000*g* for 10 min at 4°C. An aliquot (25 μl) of the supernatant

was applied to a filter paper strip for separation of organic and inorganic ^{131}I, as previously described (9).

Serum TSH. Serum TSH was measured by radioimmunoassay standard technique using materials and protocols supplied by the NIDDK Rat Hormone Distribution Program. Results are expressed as ng/ml, using the RP-2 reference standard.

Results

Effect of PTU and MMI on *In Vitro* Tg Synthesis in Rat Thyroid Lobes. Figure 1 shows typical results obtained when rat thyroid lobes, incubated in the presence of [^{14}C]leucine, were analyzed by sucrose density gradient centrifugation. Results are shown for control lobes and for lobes preincubated with 1 m*M* MMI or 2 m*M* PTU. A peak for ^{14}C corresponding to the expected position of 19S thyroglobulin was observed in all samples, and the magnitude of the peak was similar for control and for drug-treated lobes.

Table I shows the percentage of the total counts eluted from the gradient that were contained in the 19S peak. No significant difference was seen between control lobes and lobes incubated in the presence of either 100 μM or 2 m*M* PTU or 100 μM or 1 m*M* MMI. Also shown in Table I are data demonstrating that PTU and MMI had no effect on the total uptake of [^{14}C]leucine or on the ^{14}C tissue:medium concentration ratio.

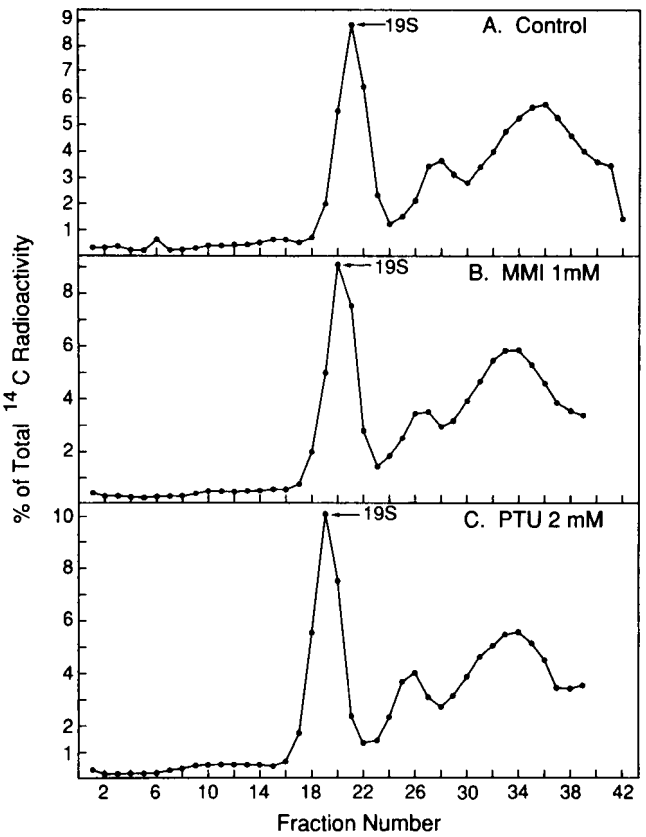

Figure 1. Sucrose density gradient patterns obtained with rat thyroid lobes incubated *in vitro* with [^{14}C]leucine. Rat thyroid lobes were incubated for 30 min in Eagle's medium containing no drug (A), 1 m*M* MMI (B), or 2 m*M* PTU (C). [^{14}C]Leucine was then added, and the incubation was continued for 4 hr. The thyroids were homogenized, centrifuged, and a portion of the supernatant was layered on a 10–40% sucrose gradient. After centrifugation, fractions were collected from the bottom of the tube and assayed for ^{14}C.

To demonstrate the validity of the procedure used for measuring Tg synthesis, the effect of 100 μM puromycin on the incorporation of [^{14}C]leucine into 19S Tg was determined. As shown in Figure 2, incorporation of [^{14}C]leucine into 19S Tg was completely inhibited under these conditions.

Effect of PTU and MMI on Iodination in Rat Thyroid Lobes. The results in Table II demonstrate that concentrations of PTU and MMI as low as 100 μM inhibited organic iodine formation more than 99% in rat thyroid lobes incubated under exactly the same conditions used for measuring protein synthesis.

Effect of PTU on Tg Synthesis *In Vivo*. In Experiment 1, rats received three injections of PTU (1 μmol/100 g/injection) over a period of 24 hr before they were injected with [^{14}C]lecuine. Control rats received saline instead of PTU. Typical sucrose density gradient patterns for the ^{14}C in the control and PTU-injected rats are shown in Figure 3. Two major peaks were observed, corresponding to 19S Tg and to 3-8S proteins. Table III shows the ^{14}C counting data for the PTU and saline-injected groups. Each group consists of three samples, and each sample represents results obtained with pooled thyroids of two rats. There was no significant difference between the PTU and saline-injected groups in total ^{14}C uptake by the thyroid. Also, there was no significant difference in cpm in the 19S peak, calculated either as percentage of total cpm applied to the sucrose gradient or as percentage of total cpm in the gland.

After Experiment 1 had been completed, we became aware of results previously reported by Larsen and Frumess (11), indicating that serum TSH levels in rats are elevated as early as 24 hr after injection of PTU. This raised the possibility that inhibition of Tg synthesis by PTU in Experiment 1 may have been masked by TSH stimulation of Tg synthesis. We, therefore, performed a second experiment in which PTU (1 μmol/100 g) was administered in a single dose and [^{14}C] leucine was injected 60 min later. The interval between the injection of [^{14}C]leucine and removal of the thyroid glands was again 5 hr, as in Experiment 1. The animals were thus exposed to PTU for a total of 6 hr. At this interval, Larsen and Frumess (11) observed no elevation in serum TSH.

The results of Experiment 2 are shown in Table III. The experiment was performed with 10 PTU- and 10 saline-injected rats, thus giving 5 PTU and 5 control samples after thyroids from 2 rats were pooled for analysis. Serum TSH was measured individually in all rats. There was no significant difference between the

Table I. Effect of Thioureylene Antithyroid Drugs on *In Vitro* Thyroglobulin Synthesis in Rat Thyroid Lobes

Drug	Drug concentration (*M*)	[^{14}C]Leucine uptake in 10 mg thyroid (% total [^{14}C]leucine in the medium)	T/M $\left[\frac{\text{cpm/g thyroid}}{\text{cpm/ml medium}}\right]$	19S Tg peak	
				% total counts in the gradient	cpm/mg thyroid
Deionized water	— (8)[a]	2.6 ± 0.3[b]	13.6 ± 1.4	22.1 ± 1.9	1.8 ± 0.3 × 10^3
PTU	1×10^{-4} (3)	2.4 ± 0.2	13.6 ± 0.9	23.3 ± 0.4	1.7 ± 0.2 × 10^3
PTU	2×10^{-3} (3)	3.2 ± 0.1	16.0 ± 0.4	24.4 ± 1.2	2.4 ± 0.2 × 10^3
MMI	1×10^{-4} (3)	2.8 ± 0.2	14.0 ± 1.0	24.9 ± 0.6	2.0 ± 0.1 × 10^3
MMI	1×10^{-3} (3)	3.0 ± 0.2	15.0 ± 0.9	24.5 ± 0.7	2.2 ± 0.1 × 10^3

[a] Number of samples. Each sample = two pooled glands.
[b] Values given are the mean ± SD.

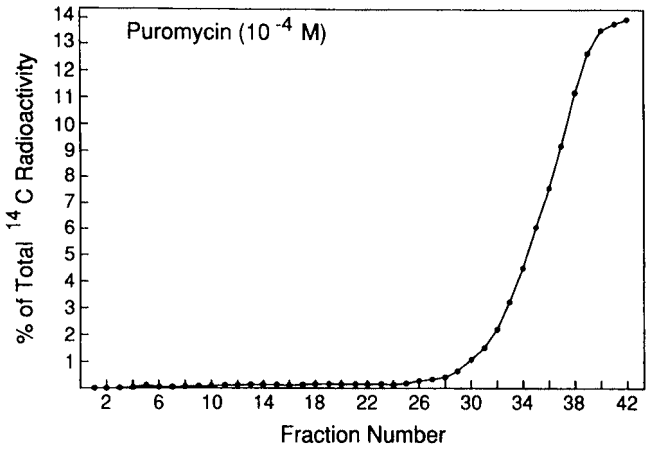

Figure 2. Sucrose density gradient pattern obtained with rat thyroid lobes incubated *in vitro* with [^{14}C]leucine. The procedure was as described in the legend to Figure 1, except that the rat lobes were preincubated with 0.1 m*M* puromycin.

serum TSH levels in the two groups. The thyroid results are very similar to those in Experiment 1, showing no significant differences between control and PTU-injected animals.

Effect of PTU on Organic ^{131}I Formation in Thyroid. As shown in Table IV, administration of a single dose of PTU (1 μmol/100 g) almost completely inhibited organic iodine formation in rat thyroids (97% inhibition), measured 5 hr after PTU injection. There can be little doubt, therefore, that PTU was a very effective antithyroid agent under the conditions used in Experiment 2. Our previous experiments (10) also make it very likely that PTU was a very effective inhibitor of iodination under the conditions of Experiment 1.

Discussion

The antithyroid drugs, PTU and MMI, are widely used in the treatment of hyperthyroidism. Although inhibition of iodination of thyroglobulin accounts for the primary mechanism of action of these drugs, other possible antithyroid actions of PTU and MMI have been suggested. One of these, the inhibition of Tg synthesis, has been examined in the present study.

In one of the earliest studies on the biosynthesis of Tg, Seed and Goldberg (8) reported that incorporation of [^{14}C]valine into 19S Tg in sheep thyroid slices was unaffected by 1 m*M* PTU. More recently, however, Monaco *et al.* (7) studied the effect of PTU (2 m*M*) and MMI (1 m*M*) on the incorporation of [^{14}C]leucine and [^{3}H]galactose into soluble protein of rat thyroid lobes incubated *in vitro*. Using a 2-hr preincubation period, followed by a 4-hr incubation in the presence of [^{14}C]leucine or [^{3}H]galactose, they observed approximately 75% inhibition of [^{3}H]galactose incorporation and somewhat less with [^{14}C]leucine (68% with MMI,

Table II. Effect of Antithyroid Drugs on *In Vitro* Iodination of Thyroglobulin in Rat Thyroid Lobes

Drug	Drug concentration (*M*)	Incubation time (hr)	^{131}I uptake in 10 mg thyroid (% total ^{131}I in the medium)	% thyroid ^{131}I in organic fraction	Organic ^{131}I in thyroid	
					% total ^{131}I in 10 mg thyroid	% control
Deionized water	— (6)[a]	4	7.9 ± 2.5[b]	75 ± 5	5.9 ± 1.7	100
PTU	10^{-4} (3)	4	3.5 ± 0.6[c]	0.43 ± 0.02[c]	0.015 ± 0.002[c]	0.26[c]
MMI	10^{-4} (3)	4	2.4 ± 0.2[c]	1.1 ± 0.32[c]	0.028 ± 0.010[c]	0.47[c]

[a] Number of samples.
[b] Values given are the mean ± SD.
[c] $P < 0.001$.

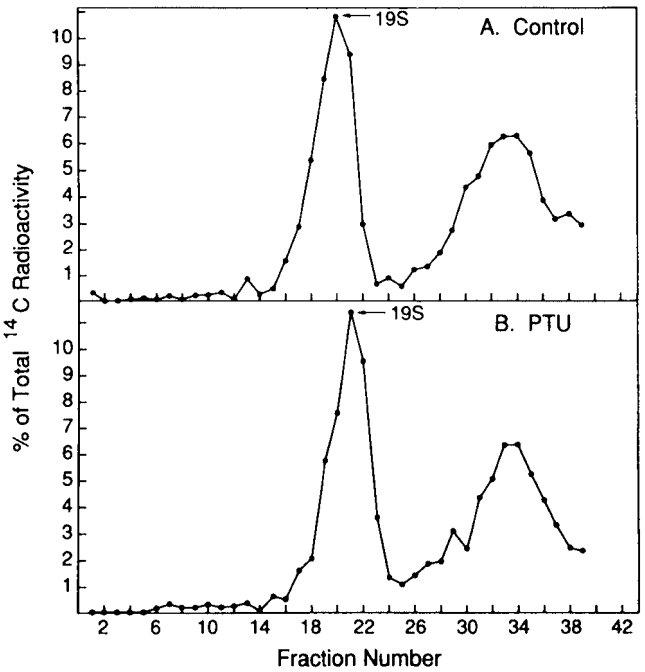

Figure 3. Sucrose density gradient patterns obtained with rat thyroids labeled *in vivo* with [^{14}C]leucine. Rats received three injections of PTU or saline over a period of 24 hr. Thirty minutes after the last injection, they were injected with [^{14}C]leucine, and 5 hr later the thyroids were homogenized and analyzed by sucrose density gradient centrifugation as described in the legend of Figure 1.

53% with PTU). They concluded that the inhibitory effect of these drugs on thyroid hormone formation is due not only to inhibition of iodination but also to depression of Tg synthesis.

Our results, reported in the present communication, do not support the findings of Monaco *et al.* (7). We observed no inhibition of [^{14}C]leucine incorporation into 19S Tg in rat thyroid lobes preincubated for 30 min with 1 m*M* or 0.1 m*M* MMI, or with 2 m*M* or 0.1 m*M* PTU, followed by a 4-hr incubation with [^{14}C] leucine in the continued presence of the drugs. The higher concentrations of PTU and MMI used in our study correspond to those employed by Monaco *et al.* (7). These concentrations are at least 10-fold greater than those required for complete inhibition of iodination (Table II).

We used a shorter preincubation period (30 min) than did Monaco *et al.* (2 hr). However, the total incubation time in the presence of the drugs was 4.5 hr in our study compared with 4–6 hr in their study. Moreover, we showed that, with puromycin, a 30-min preincubation was sufficient to inhibit Tg synthesis completely.

It should be noted that even the lower concentrations of PTU and MMI used in our *in vitro* experiments (100 μM) are still many fold greater than the serum levels attained during clinical therapy. Cooper *et al.* (12, 13) reported that the peak level of PTU in the serum of hyperthyroid patients receiving 150 mg/day averaged 4.5 μg/ml (2.6 μM), and the peak serum level of MMI in patients receiving 30 mg daily averaged 0.78 μg/ml (0.7 μM).

We also tested the effect of PTU on the synthesis of 19S Tg in the thyroids of rats *in vivo*. In previous studies (9), we showed that PTU is a more potent antithyroid drug than MMI in the rat. Since the high cost of the necessarily large amount of [^{14}C]leucine required in these experiments greatly limited the number of rats that could be used, our *in vivo* experiments were performed only with PTU. In the first experiment, the rats received three injections of PTU over a period of 24 hr before receiving an injection of [^{14}C]leucine. Although no differences in incorporation of ^{14}C into 19S Tg were observed between PTU-injected and control animals, this experiment was complicated by the possibility that serum TSH may have been elevated in the PTU-treated group (11). A second experiment was performed, therefore, with rats that received only a single injection of PTU, followed 60 min later by injection of [^{14}C]leucine. In both experiments the rats were killed 5 hr after the injection of [^{14}C]leucine. Serum TSH was measured in the second experiment,

Table III. Effect of PTU on Thyroglobulin Synthesis in Rat Thyroids *In Vivo*[a]

Experiment	Treatment	[^{14}C]Leucine uptake/ 10 mg thyroid (% injected dose)	cpm in 19S peak		Serum TSH[b] (ng/ml)
			% total cpm applied to gradient	% total cpm in gland	
1	Saline, 3 injections over 24 hr (3)	$6.2 \pm 2.5 \times 10^{-3}$	37.8 ± 3.0	22.3 ± 3.0	—
	PTU, 3 injections over 24 hr (3)	$7.9 \pm 0.25 \times 10^{-3}$	32.3 ± 1.5	21.3 ± 0.52	—
2	Saline, single injection (5)	$6.0 \pm 0.36 \times 10^{-3}$	41.3 ± 2.9	29.5 ± 3.9	697 ± 197
	PTU, single injection (5)	$6.1 \pm 1.4 \times 10^{-3}$	41.1 ± 7.0	31.1 ± 9.3	874 ± 329

[a] Each PTU injection was 1 μmol/100 g. In Experiment 1, the rats received 25 μCi of [^{14}C]leucine intraperitoneally 30 min after the last PTU injection. In Experiment 2, the [^{14}C]leucine was injected 60 min after the single injection of PTU. The thyroids were removed 5 hr after the [^{14}C] leucine injection. The number of samples is shown in parentheses. Each sample represents the pooled thyroids from two rats. Values are mean ± SD.

[b] $P > 0.1$, unpaired Student's *t* test.

Table IV. Effect of PTU on Organic ^{131}I Formation in Rat Thyroids *In Vivo*[a]

Treatment	^{131}I uptake/mg thyroid (% injected dose)	% thyroid ^{131}I in organic fraction	Organic ^{131}I in thyroid	
			% injected ^{131}I/mg	% control
PTU, single injection (4)	0.026 ± 0.0055	42.7 ± 6.9	0.011 ± 0.0014	2.9
Saline, single injection (4)	0.39 ± 0.051	97.4 ± 0.14	0.38 ± 0.048	100

[a] Rats received either saline or PTU (1 μmol/100 g) by intraperitoneal injection. After 60 min they were injected with 30 μCi of ^{131}I, and the thyroids were removed 5 hr later. The number of samples is shown in parentheses. Values are mean ± SD.

and no significant difference was observed between control and PTU-injected rats. There was also no difference in the incorporation of ^{14}C into 19S Tg, indicating that PTU had no effect on the synthesis of the polypeptide backbone of 19S Tg. Under the same conditions, PTU inhibited organic ^{131}I formation by 97%.

We have been unable, therefore, to confirm the observations of Monaco *et al.* (7). The source of the discrepancy between their *in vitro* findings and ours with rat thyroid lobes is not readily apparent. In any case, the drug concentrations used to obtain their results are 40- to 100-fold greater than the serum levels attained in patients undergoing therapy with PTU or MMI. Even had we obtained inhibition of Tg synthesis with such high concentrations of PTU and MMI, we would have viewed the results with suspicion. We have, in addition, found no evidence of inhibition of Tg biosynthesis in rat thyroids *in vivo* after administration of a dose of PTU that inhibited iodination by 97% Monaco *et al.* (7) also reported results of *in vivo* experiments. However, they used rats that received PTU or MMI in the drinking water for 20 days, conditions that lead to markedly elevated levels of serum TSH. Labeling experiments could not be used under these conditions, and they based their conclusions on the effect of the antithyroid drugs on the relative proportions of soluble and particulate Tg. These measurements provide, at best, only indirect information on the effect of PTU and MMI on Tg biosynthesis.

Our results, both *in vitro* and *in vivo*, lead us to conclude that the antithyroid action of PTU and MMI does not involve inhibition of Tg biosynthesis.

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