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ENZYMATIC *S*-METHYLATION OF 6-*n*-PROPYL-2-THIOURACIL AND OTHER ANTITHYROID DRUGS

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Abstract—Mouse kidney thiol transmethylase and *S*-adenosylmethionine were incubated with the radioactive antithyroid drugs, 2-thiouracil (TU), 6-propyl-2-thiouracil (PTU), methimazole (MMI), 6-methyl-2-thiouracil (6-methyl TU) or thiourea. Radioactive metabolites were produced with TU, PTU and 6-methyl TU and, in each case, were identified as the corresponding *S*-methyl derivatives. No measurable metabolism of MMI or thiourea was observed. Kinetic studies with the partially purified enzyme demonstrated K_m values for TU, PTU and 6-methyl TU of 1×10^{-3} M, $2{\cdot}5 \times 10^{-3}$ M and $1{\cdot}54 \times 10^{-3}$ M respectively. Extensive investigation with PTU demonstrated that methylation was to the sulfur rather than the nitrogen of the thiopyrimidine and that the pH optimum for PTU was 8·0. Methylation of PTU was proportional to enzyme concentration, with little spontaneous methylation occurring, and was not reversible. TU and 6-methyl TU inhibited PTU metabolism and were apparently competitive substrates. Thiouracil nucleoside and thiouracil nucleotide were not substrates for the enzyme. Studies with porcine thyroid peroxidase demonstrated that *S*-methylation of PTU, TU and MMI abolished the antiperoxidase activity observed with the parent compound. The results obtained demonstrate that *S*-methylation is a general pathway of metabolism for thiopyrimidine antithyroid drugs, but not for thiourea or MMI, which markedly decreases the antiperoxidase activity of the parent compound.

Interest in the metabolism of antithyroid drugs has recently been focused on 6-*n*-propyl-2-thiouracil (PTU) and 1-methyl-2-mercaptoimidazole (methimazole, MMI), the current drugs of choice in the treatment of hyperthyroidism. A study of the excretion of PTU and its metabolites in rat bile and urine demonstrated the presence of the *S*-methyl derivative of PTU in the urine of rats [1]. This metabolite accounted for 3·5 per cent of the total radioactivity in 24-hr urine after the administration of [^{14}C]- or [^{35}S]PTU, while unaltered PTU accounted for 42·3 per cent. *S*-methylation as a detoxification mechanism in mammals appears to have been first described by Sarcione and Sokal [2], who identified *S*-methyl-2-thiouracil (*S*-methyl-TU) in the urine of rats after the administration of the antithyroid drug, 2-thiouracil (TU). Approximately 8·3 per cent of the total radioactivity excreted in urine 24 hr after administration of [^{35}S]TU was *S*-methyl-TU and 80 per cent was unchanged TU.

The enzymatic *S*-methylation of thiopyrimidines and thiopurines was investigated by Remy-[3, 4], who found that a variety of mammalian tissues, particularly liver and kidney, contain a thiol transmethylase (EC 2.1.1.9, *S*-adenosylmethionine:thiol *S*-methyltransferase) that is specific for *S*-adenosylmethionine as the methyl donor but highly nonspecific with respect to the methyl acceptor. A wide variety of 2, 6 or 8 thio-substituted purines and, to a lesser degree, 2, 4 or 5 thio-substituted pyrimidines (including TU) serve as methyl acceptors. The thiopurine ribonucleosides and ribonucleotides are the preferred acceptors for both animal and bacterial thiol transmethylases [4].

Antithyroid drugs are usually administered for several months in the treatment of hyperthyroidism. Under these conditions, toxic effects may develop which include leukopenia and agranulocytosis. Dinning *et al.* [5, 6] and Skipper *et al.* [7] have presented evidence that the formation of leukocytes is related to the availability of methyl groups. Because of this relationship, Sarcione and Sokal [2] suggested that the high incidence of leukopenia produced by chronic TU therapy might be related to a depressed active-methyl pool resulting from methylation of the drug.

In the present study, enzymatic methylation of currently important anti-thyroid drugs has been studied to determine if *S*-methylation is a general pathway of metabolism for these compounds and if there are any similarities between the incidence of leukopenia with these drugs and their metabolism by thiol transmethylase. In addition, some of the properties of the methylating system with respect to the antithyroid drug substrates have been investigated and the antiperoxidase activity of the parent drug has been compared with the respective *S*-methyl derivative to determine if this metabolic conversion alters biological activity.

MATERIALS AND METHODS

Materials. Authentic *S*-methyl TU, *S*-methyl-6-methyl TU, *S*-methyl PTU and *S*-methyl MMI were

synthesized by adaptations of the method described by Barret *et al.* [8]. *N*-methyl PTU was prepared by a modification of the procedure described by Anderson *et al.* [9] for the preparation of PTU in which *N*-methylthiourea was used instead of thiourea. A sample of the HCl salt of *S*-methyl MMI was also generously supplied by Eli Lilly & Co., Indianapolis, Ind. [2-^{14}C]PTU was obtained from Mallinckrodt/Nuclear at a specific activity of 3·78 μCi/μmole, [2-^{14}C]TU and [^{35}S]6-methyl-TU from Amersham–Searle Corp., at specific activities of 59 and 14·5 μCi/μmole, respectively, and [2-^{14}C]MMI from New England Nuclear Corp., at a specific activity of 3·8 μCi/μmole. Nonradioactive PTU, TU, 6-methyl TU and *S*-adenosylmethionine chloride, grade 1, were obtained from Sigma Chemical Co., and MMI was obtained from Eli Lilly & Co. Bio-Gel P-2, 200–400 mesh, was obtained from Bio-Rad Labs; cellulose No. 6064 and silica gel No. 6061 chromatograph sheets were obtained from Eastman Kodak, and silica gel-impregnated glass fiber thin-layer sheets ITLC-SG from Gelman Instrument Co.

Methylation of antithyroid drugs. Thiol transmethylase was freshly prepared from mouse kidneys by a modification of the method of Remy [3]. Male Swiss mice (22–25 g) were sacrificed by cervical dislocation and the kidneys immediately removed, cleaned, minced and then homogenized in 2 vol. of cold 0·154 M KCl in a glass homogenizer. The homogenate was centrifuged at 20,000 *g* for 30 min and the supernatant used as the source of enzyme. Incubation mixtures usually contained 0·5 ml of the enzyme (about 16 mg protein), 1 μmole *S*-adenosylmethionine (Cl salt), 1 μmole [^{14}C]-labeled antithyroid drug and 50 μmoles phosphate buffer, pH 7·4, in a final volume of 1·0 ml. Incubation was carried out with shaking at 37° for 4 hr and the reaction was stopped by the addition of 1·0 ml absolute ethanol. The mixture was centrifuged and the supernatant subjected to chromatographic analyses to measure *S*-methylation.

Separation and measurement of S-*methylated antithyroid drug.* Aliquots of the ethanol supernatant were chromatographed with authentic samples of the appropriate antithyroid drug and its *S*-methyl analog in various systems to achieve optimum separation. TU was separated from *S*-methyl TU by descending paper chromatography on Whatman No. 1 in *n*-butanol–3% boric acid (100:13), by thin-layer chromatography on silica gel-impregnated glass fiber sheets in hexane–acetone–ethanol (50:10:1·8) and on cellulose with *n*-butanol–glacial acetic acid–H_2O(40:10:50) and by column chromatography on 2 × 115 cm Bio-Gel P-2 columns eluting with H_2O. PTU was separated from *S*-methyl PTU by thin-layer chromatography on silica gel 6061 sheets with hexane–acetone–ethanol (60:20:2) or hexane–acetone (3:1) and on Silica gel-impregnated glass fiber sheets with hexane–acetone (5:1) and by column chromatography on Bio-Gel P-2 columns. MMI was separated from *S*-methyl MMI by thin-layer chromatography on silica gel-impregnated glass fiber sheets with hexane–acetone–triethylamine (50:10:5) and by column chromatography on Bio-Gel P-2 columns. 6-Methyl thiouracil was separated from *S*-methyl-6-methyl TU by descending paper chromatography on Whatman No. 1 sheets in *n*-butanol–3% boric acid (100:13) and by column chromatography on Bio-Gel P-2 columns.

Samples subjected to analyses by thin-layer chromatography were applied at 2-cm intervals to the appropriate thin-layer sheet. The chromatograms were developed in the appropriate solvent and the authentic standards located with an ultraviolet lamp and lightly outlined with a soft pencil. The sheets were cut into 2-cm strips and $\frac{1}{4}$ inch segments cut out, placed in counting vials and the radioactivity was determined in a liquid scintillation counter. Similar analyses were carried out by paper chromatography with the spacing of samples and the size of strips being appropriately altered. In each case, self-absorption was determined and counting efficiency calculated.

Samples analyzed by column chromatography were applied to 2 × 115 cm Bio-Gel P-2 columns (200–400 mesh) previously equilibrated with H_2O. Elution was accomplished with H_2O. Fractions of 3·0 ml were collected and ultraviolet absorption was determined to locate the authentic standards. Aliquots of 50–100 μl were then analyzed for radioactivity in a Packard Tri-Carb liquid spectrometer.

Peroxidase assay. Porcine thyroid peroxidase was prepared as previously outlined [10] and assayed by a modification of the guaiacol test [11]. The assay medium contained 190 μmoles guaiacol, 108 μmoles Tris–HCl (pH 7·4), 1·0 mg of enzyme protein and various concentrations of antithyroid drug or the *S*-methyl derivative in a final volume of 3·0 ml. The reaction was initiated by the addition of 2 μmoles H_2O_2. The increase in absorbance at 470 nm was measured for 15 sec during the initial linear reaction using a Perkin–Elmer 124 recording spectrophotometer. Protein was determined by the method of Lowry *et al.* [12].

RESULTS

The antithyroid drugs PTU and MMI have replaced TU and 6-methylthiouracil (6-methyl TU) as the drugs of choice in the treatment of hyperthyroidism. Metabolism of TU, 6-methyl TU, PTU and MMI by mammalian thiol transmethylase, an enzyme previously reported to *S*-methylate TU [3, 4] was investigated to determine if other antithyroid drugs were substrates. The drugs were incubated with *S*-adenosylmethionine in the presence and absence of the enzyme. After incubation, the reaction mixtures were examined by column chromatography on 2 × 115 cm Bio-Gel P-2 columns with the results shown in Fig. 1A for TU, Fig. 1B for PTU and Fig. 1C for 6-methyl TU. In the presence of the enzyme, 11 per cent of the TU was converted to a radioactive metabolite which appeared to be *S*-methyl TU and a small amount was desulfurated to uracil. PTU and 6-methyl TU were also utilized as

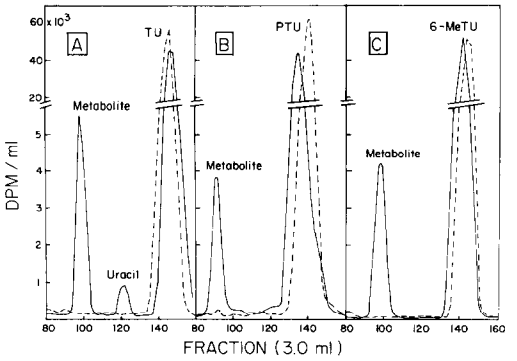

Fig. 1. S-methylation of thiopyrimidine antithyroid drugs. Radioactive antithyroid drugs were incubated for 4 hr in the presence and absence of mouse kidney thiol transmethylase, as described in Materials and Methods, and aliquots of the incubation mixtures were chromatographed on 2 × 115 cm Bio-Gel P-2 columns eluting with H_2O. The results obtained in the absence of enzyme are shown by the broken lines and those in the presence of enzyme by the solid lines utilizing [2-^{14}C]thiouracil (A), [2-^{14}C]6-propylthiouracil (B) or [^{35}S]6-methylthiouracil (C) as substrates.

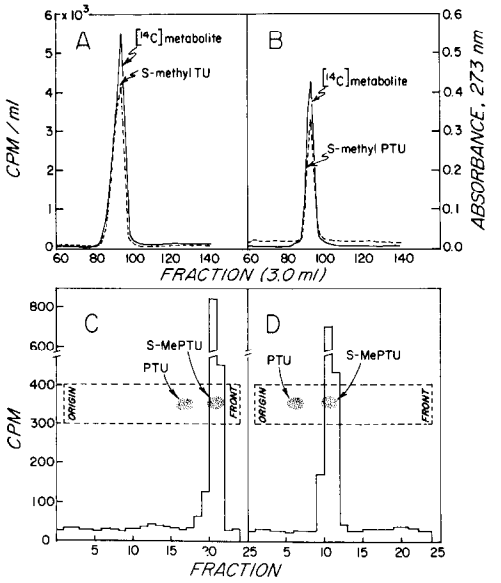

Fig. 2. The radioactive metabolites of TU and PTU from Fig. 1 were co-chromatographed on Bio-Gel P-2 columns with authentic S-methyl TU (A) and S-methyl PTU (B) respectively. The "cold" standards, measured by absorbance at 273 nm, are shown as broken lines, the radioactivity as solid lines. The radioactive PTU metabolite and "cold" authentic S-methyl PTU were also co-chromatographed on Silica gel 6061 TLC sheets developed with hexane–acetone–ethanol (C) and on Silica gel-impregnated glass fiber TLC sheets developed with hexane–acetone (D). The TLC strip showing the position of co-chromatographed standards is outlined with broken lines; the distribution of radioactivity is shown by solid lines.

substrates by the enzyme with a 5·7 per cent conversion to a radioactive metabolite being observed with PTU and 8·1 per cent with 6-methyl TU. In numerous experiments under the same conditions of incubation, thiourea and MMI were not measurably altered by the enzyme and do not appear to be substrates.

The fractions containing each radioactive metabolite from Fig. 1 were pooled, lyophilized and subjected to further analyses. Co-chromatography of the radioactive TU metabolite and authentic "cold" S-methyl TU on the Bio-Gel column (Fig. 2A) demonstrated that the elution properties of the radioactive metabolite were identical to those of authentic S-methyl TU. Identical chromatographic characteristics were also observed with various TLC and paper chromatographic systems (described in Materials and Methods), confirming the identification of S-methyl TU.

Co-chromatography of the radioactive PTU metabolite with authentic "cold" S-methyl PTU on 2 × 115 cm Bio-Gel P-2 columns (Fig. 2B), on silica gel 6061 sheets developed with hexane–acetone–ethanol (60:20:2, Fig. 2C) and on silica gel-impregnated glass fiber sheets developed with hexane–acetone (5:1, Fig. 2D) demonstrated that their chromatographic properties were identical. Similarly, identical chromatographic properties were observed with the radioactive 6-methyl TU metabolite and authentic "cold" S-methyl-6-methyl TU on Bio-Gel columns and paper chromatograms developed in butanol–boric acid. Thus the radioactive metabolites of TU, PTU and 6-methyl TU were identified as the S-methyl derivatives.

Since methylation of the ring nitrogen of purines by a kidney enzyme requiring S-adenosylmethionine has been described [13], experiments were conducted to establish clearly whether the PTU was metabolized to the S-methyl or N-methyl analog. In most chromatographic systems the two compounds were inseparable; however, partial separation was achieved with the TLC systems used in Fig. 2. The R_f values for S-methyl PTU and N-methyl PTU were 0·44 and 0·60, respectively, in the silica gel 6061 system, and 0·84 and 0·93 in the silica gel glass fiber system. Differences in the two compounds were more clearly distinguished after partial degradation with HCl and H_2O_2. A summary of the products formed from the radioactive PTU metabolite from Fig. 1, authentic S-methyl PTU and N-methyl PTU is shown in Table 1. HCl hydrolysis of both S-methyl PTU and the radioactive PTU metabolite formed propyluracil (PU), whereas N-methyl PTU was not altered by HCl. H_2O_2 had no effect on S-methyl PTU or the radioactive PTU metabolite, while desulfuration of N-methyl PTU occurred to form N-methyl-6-propyluracil (N-methyl PU). These results along with the chromatographic data demonstrate that PTU is converted to S-methyl PTU by the kidney enzyme and not N-methyl PTU.

In previous reports, extensive nonenzymatic transfer of methyl groups from S-adenosylmethionine to sulfhydryl compounds has been observed [4, 14, 15]. The

Table 1. Summary of results to distinguish between *S*-methyl PTU and *N*-methyl PTU.

Sample	Treatment*	Product†
S-methyl-PTU	HCl	PU
	H_2O_2	None
N-methyl-PTU	HCl	None
	H_2O_2	*N*-methyl-PU
PTU metabolite	HCl	PU
	H_2O_2	None

* Samples were hydrolyzed with 1 N HCl for 10 min at 100° or oxidized with 0·3% H_2O_2 for 30 min at room temperature.

† Identification was based on chromatographic properties relative to those of authentic standards in the TLC systems used in Fig. 2.

results presented in Fig. 1 suggest that the *S*-methylation of TU, PTU and 6-methyl TU was not spontaneous, since the *S*-methyl metabolite was not observed in the absence of enzyme. In addition, *S*-methyl PTU formation (Fig. 3) was essentially linear with respect to enzyme protein, demonstrating that the conversion is indeed enzymatic.

The pH optimum for PTU was determined to be 8·0, which is slightly higher than the 7·4 value for TU found by Remy [3].

The results presented in Fig. 1 suggested that TU might be a better substrate for the thiol transmethylase than PTU or 6-methyl TU. Kinetic studies with the partially purified enzyme (Fig. 4) demonstrated K_m values for TU, PTU and 6-methyl TU of 1×10^{-3} M, $2{\cdot}5 \times 10^{-3}$ M and $1{\cdot}54 \times 10^{-3}$ M respectively. The value for TU compares favorably with the value of $1{\cdot}7 \times 10^{-3}$ M reported by Remy [3] for the crude enzyme. The differences in K_m values were not related to substrate depletion by other reactions, since the only measurable alteration of PTU and 6-methyl TU by this enzyme preparation formed the *S*-methyl metabolite. Desulfuration of TU by the enzyme preparation did occur but caused a loss of only about 1 per cent of the parent compound. In addition, the formation of

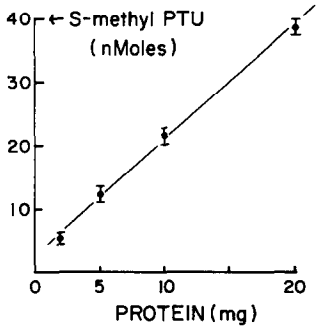

Fig. 3. Relationship of enzyme concentration to the amount of PTU *S*-methylated. The results presented represent the means ± S.E. of six determinations.

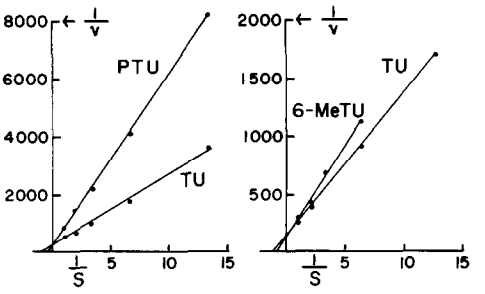

Fig. 4. Kinetics of mouse kidney thiol transmethylase metabolism of TU, PTU and 6-methyl TU.

neither *S*-methyl TU nor *S*-methyl PTU was reversible. Experiments with *S*-methyl TU or *S*-methyl PTU demonstrated that measurable demethylation did not occur under the same conditions in which methylation was measured. Demethylation of the *S*-methyl derivatives of TU, PTU and MMI by a rat liver microsomal demethylase was also undetectable, although the enzyme preparation produced substantial demethylation of puromycin (data not shown).

Several investigators [3, 15] have demonstrated that thiopurine nucleosides and nucleotides are methylated by thiol transmethylase. Thiopyrimidine nucleoside and nucleotide are also metabolites of thiouracil [16–19] in several systems. However, numerous experiments with radioactive thiouridine and thio-UMP failed to demonstrate any *S*-methylation under conditions yielding 10–12 per cent *S*-methylation of TU (data not shown). In related experiments, it was also found that *S*-methyl TU, unlike TU [17], was not a substrate for UMP pyrophosphorylase, and no evidence for the formation of an *S*-methyl TU nucleotide was obtained.

Remy [3, 4] observed that thiol transmethylase was nonspecific with respect to the methyl acceptor and that the rate of methylation of one substrate was competititvely inhibited by the presence of a second substrate. Thus many thiol-substituted purines and pyrimidines were inhibitors because they were actually competitive substrates. The effects of the presence of TU and other antithyroid drugs on the *S*-methylation of PTU were determined with the results shown in Table 2. Both TU and 6-methyl TU produced marked inhibition of PTU utilization, but neither MMI nor thiourea had any measurable effect. Since only those antithyroid drugs which are substrates for the enzyme are inhibitors, it is highly probable that they too are acting as competitive substrates.

It has been pointed out [4] that methylation generally reduces or abolishes biological effects of purines and pyrimidines. The *S*-methylation of TU first observed by Sarcione and Sokal [2] was considered to be a detoxification reaction; Remy [4] also classifies *S*-methylation as detoxification. However, specific assays of biological activity by the *S*-methyl analogs of the antithyroid drugs do not appear to have been

Table 2. Effects of other antithyroid drugs on S-methylation of PTU

Inhibitor*	S-methyl PTU formed (nmoles/mg protein)	% Inhibition
None	2·74 ± 0·12	
TU	0·77 ± 0·03	71·9
N-Methyl-TU	0·71 ± 0·06	74·1
MMI	2·57 ± 0·12	6·2
Thiourea	2·76 ± 0·12	0

* Inhibitor concentration was 5 μmoles/ml and that of PTU was 1 μmole/ml. The results presented are means ± S.E. of eight determinations.

reported. A comparison of the inhibitory effects on thyroid peroxidase by TU, PTU, MMI and their respective S-methyl derivatives is shown in Table 3. All three antithyroid drugs are potent inhibitors of thyroid peroxidase, the primary site of their action in the thyroid gland, but no measurable inhibition was observed with even the highest concentration of any of the S-methyl derivatives. These results demonstrate that S-methylation of the antithyroid drugs abolishes or drastically reduces the biological activity of the compounds and suggests that the term "detoxification" can be correctly applied to S-methylation of TU and PTU.

DISCUSSION

The data presented demonstrate that the antithyroid drugs TU, 6-methyl TU and 6-propyl TU, but not thiourea and MMI, are substrates for thiol transmethylase, indicating that the enzyme is not specific for all thiourylene compounds. However, it does appear that S-methylation is a general pathway of metabolism for the thiopyrimidine antithyroid drugs, with TU being transformed most readily followed by 6-methyl TU then PTU. The amounts of S-methyl TU and S-methyl PTU, 8·3 and 3·5 per cent respectively, appearing in rat urine after administration of the parent compound indicate that, while S-methylation is an important pathway for the metabolism of these compounds, it is not the major pathway in this species. However, the activity of thiol transmethylase in rat kidney and liver, which also S-methylates antithyroid drugs, is much lower than in mouse tissues, suggesting that species variations in the importance of this metabolic pathway may exist.

S-methylation of 2-thiopyrimidines differs in several respects from S-methylation of 6-thiopurines. 2-Thiouracil is the only thiopyrimidine anti-thyroid drug which has been shown to be metabolized to a nucleoside and nucleotide [16–19]. Neither 2-thiouridine nor 2-thio UMP was measurably S-methylated by the enzyme, while TU was an excellent substrate. In addition, S-methyl TU was not a substrate for UMP pyrophosphorylase, excluding this pathway as a mechanism for the formation of an S-methyl thiopyrimidine nucleotide. In contrast, thiopurine ribonucleosides and ribonucleotides are better methyl acceptors than the free bases [4]. Thus, while thiopurines readily form S-methyl nucleotides, thiopyrimidines do not.

The results with thyroid peroxidase suggest that S-methylation of thiopyrimidines abolishes their antithyroidal actions. However, S-methylation of 6-mercaptopurine nucleotide forming 6-methylmercaptopurine nucleotide results in an approximate 10-fold increase in its inhibitory properties on phosphoribosylpyrophosphate amidotransferase, [20] indicating that S-methylation does not necessarily abolish all biochemical activities.

Leukopenia is a serious toxic effect produced by all of the clinically useful antithyroid drugs, since it is often a forerunner of agranulocytosis [21]. Both TU and PTU accumulate in bone marrow and leukocytes [22–24]. Furthermore, TU *in vitro* depresses rabbit bone marrow and polymorphonuclear leukocyte respiration [25] and inhibits reticulocyte ripening [26]. Reed and Tepperman [27] showed that leukocytes harvested from PTU-treated rats consisted of a higher percentage of immature forms than normal and suggested that PTU might affect marrow cell maturation.

Little is known about the mode of action of antithyroid drugs on the blood-forming organs. Reed and Tepperman [27] suggested that PTU might act as a uracil antimetabolite affecting marrow cell maturation, while

Table 3. Inhibition of thyroid peroxidase by antithyroid drugs and their S-methyl derivatives

	Thyroid peroxidase activity*				
	Inhibitor concn (× 10^{-5} M)				
Inhibitor	100	50	10	5	1
PTU		0·20 ± 0·02	1·19 ± 0·08	1·57 ± 0·07	
S-methyl PTU	2·05 ± 0·11	2·01 ± 0·09	1·99 ± 0·12		
TU			0·38 ± 0·03	0·79 ± 0·05	1·64 ± 0·07
S-methyl TU		2·03 ± 0·09	2·08 ± 0·11	2·04 ± 0·06	
MMI		0·24 ± 0·01	0·80 ± 0·01	1·15 ± 0·02	1·84 ± 0·01
S-methyl MMI		2·10 ± 0·02	2·11 ± 0·03	2·12 ± 0·02	

* Activity is expressed as ΔE at 470 nm/mg protein/min and was 2·07 ± 0·05 in controls without inhibitor. The results presented are means ± S.E. of six determinations.

Sarcione and Sokal [2] suggested that the high incidence of leukopenia associated with thiouracil administration might be due to a reduction of the methyl donor pool as a consequence of thiouracil methylation. Administration of diets deficient in sources of labile methyl groups produces leukopenia in rats [5, 6] and methionine antagonists enhance the antileukemic effects of some drugs in mouse leukemia [7]. On the other hand, administration of an active methyl source may reverse the leukopenia, including that caused by thiourea + thyroxine [28]. Methylation reactions in developing leukocytes and leukemic cells are elevated, with methylation of RNA being higher in immature and in leukemic leukocytes than in normal adult leukocytes [29]. Levels of *S*-adenosylmethionine are also elevated in leukemic leukocytes [30].

The results presented in this report demonstrate a marked similarity between the capacities of the antithyroid drugs to undergo *S*-methylation by mammalian thiol transmethylase and their capacities to cause leukopenia and agranulocytosis in human patients. Thiouracil was most readily methylated, as demonstrated by experiments *in vivo* and *in vitro*, followed by 6-methyl TU then PTU. Thiourea and MMI were not methylated. The incidence of leukopenia in the extensive survey reported by Vanderlaan and Storrie [21] was 4·5, 2·2, 1·1, 0·76 and 0·84 per cent, respectively, and that for agranulocytosis 1·07, 0·54, 0·44, 0·19 and 0·12 per cent, respectively, for TU, 6-methyl TU, PTU, thiourea and MMI. In this series, the highest incidence of both effects was observed with those antithyroid drugs undergoing *S*-methylation and the lowest by the drugs not *S*-methylated. It is also pertinent that patients who develop leukopenia or agranulocytosis due to PTU administration may often be given MMI without ill effects and vice versa [31] suggesting that the etiologies of the toxic effects are different.

The effects of antithyroid drugs on tissue pools of labile methyl donors and on reactions *in vivo* dependent upon labile methyl donors have not been determined and a direct relationship between antithyroid drug–thiol transmethylase interaction and leukopenia has not been established. However, the present demonstration that the antithyroid drugs which are *S*-methylated are those producing the highest incidence of leukopenia strengthens the suggestion that a relationship between these two effects may exist.

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