Biochemical Pharmacology, Vol. 26, pp. 617–623. Pergamon Press, 1977. Printed in Great Britain.

GLUCURONIDE CONJUGATION OF 6-*n*-PROPYL-2-THIOURACIL AND OTHER ANTITHYROID DRUGS BY GUINEA PIG LIVER MICROSOMES *IN VITRO*

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(*Received* 8 *March* 1976; *accepted* 27 *August* 1976)

Abstract—Guinea pig liver microsome UDP glucuronyl transferase and UDPGA were incubated with the radioactive antithyroid drugs 6-*n*-propyl-2-thiouracil (PTU), 1-methyl-2-mercaptoimidazole (methimazole, MMI) and 2-thiouracil (TU). Radioactive metabolites were produced with PTU and thiouracil and, in each case, were identified as the corresponding β-glucuronide conjugate. No measurable glucuronidation of MMI was observed. Kinetic studies with the microsomal preparation demonstrated a K_m value of 7.2×10^{-4} M for PTU and 6.7×10^{-3} M for thiouracil. Glucuronide conjugation of PTU was linear for 1 hr, declining thereafter while conjugation of phenolphthalein was linear for 2 hr. Conjugation of phenolphthalein by microsomes stored in 0.154 M KCl at −20° for 14 days was 41 per cent higher than in fresh microsomes, whereas conjugation of PTU was 67.4 per cent lower. PTU glucuronidation did not occur in the absence of UDPGA and was essentially linear with respect to enzyme concentrations. Under the same conditions, spontaneous *N*-glucuronidation of PTU by glucuronate was not measurable. The pH optimum for PTU glucuronidation was 8.0 and similar to the broad optimums of 7.3 to 7.9 for UDP glucuronyl transferases from a variety of sources rather than to non-enzymatic *N*-glucuronidation, which has a reported pH optimum of 3–4. The conjugating enzyme for PTU was located primarily in the guinea pig liver microsomes with this fraction exhibiting 75 per cent of the total activity of whole homogenates. PTU conjugation was inhibited by MMI but not by thiouracil, thiourea or 6-methyl-2-thiouracil. The results obtained demonstrate that β-glucuronide conjugation of the antithyroid drugs PTU and thiouracil, but not MMI, is readily catalyzed by a guinea pig liver microsomal UDP glucuronyl transferase *in vitro*.

Several metabolites have been observed in body fluids after the administration of the radioactive antithyroid drugs 6-*n*-propyl-2-thiouracil (PTU) or 1-methyl-2-mercaptoimidazole (methimazole, MMI), the current drugs of choice in the treatment of hyperthyroidism. Urinary metabolites of PTU which have been identified include sulfate [1], PTU glucuronide [1–4], *S*-methyl-PTU [1], and propyluracil [1]. The identity of PTU disulfide, which was suggested in analyses of highly alkalinized guinea pig urine [5], has not been confirmed. The major identifiable PTU metabolite in urine is PTU glucuronide, which has been reported to account for 16–60 per cent [1, 4] of the total radioactivity in this fluid after the administration of [^{14}C]PTU or [^{35}S]PTU. There is general agreement that PTU glucuronide is also the major metabolite in bile and plasma [1–5] and is accompanied by small amounts of sulfate and unaltered PTU. Glucuronides of PTU metabolites are also present in small amounts in bile.

The only metabolite of MMI positively identified is sulfate, which has been reported as a minor metabolite in bile [3], plasma [6], and urine [6] and as a major metabolite in the thyroid [6]. Several unidentified metabolites have been observed in bile and urine. Unlike PTU, no glucuronide of unaltered MMI has been reported but a glucuronide of an MMI metabolite is a major metabolite in bile [3].

The observations described above demonstrate that glucuronidation is a major pathway for the metabolism of the thionamide antithyroid drugs. However, enzymatic glucuronidation of these drugs has not been previously studied. The formation of glucuronides is usually catalyzed by UDP glucuronate glucuronyl transferase (UDP glucuronyl transferase, EC 2.4.1.17) localized in the microsomal cell fraction. Adult liver appears to contain the highest activity of this enzyme/mg of microsomal protein with guinea pig liver being an especially rich source [7, 8]. The primary objectives of the present study are to determine if glucuronidation of the antithyroid drugs PTU, MMI and thiouracil (TU) can be demonstrated with UDP glucuronyl transferase from guinea pig liver microsomes *in vitro* and to determine some of the properties of the enzyme system with respect to the antithyroid drug substrates.

MATERIALS AND METHODS

Materials. [2-^{14}C]PTU was obtained from Mallinckrodt/Nuclear at a specific activity of 3.78 μCi/μmole, [2-^{14}C]thiouracil from Amersham–Searle Corp. at a specific activity of 59 μCi/μmole and [2-^{14}C]MMI from New England Nuclear Corp. at a specific activity of 3.8 μCi/μmole. [2-^{14}C]PTU glucuronide was isolated and purified from rat bile or urine after the administration of [2-^{14}C]PTU as previously described [1]. Thiouracil, thiourea, PTU, 6-methyl-2-thiouracil (MTU), uridine-5′-diphosphoglucuronic acid (UDPGA), β-glucuronidase (type

B-10 from bovine liver), D-saccharic acid-1, 4-lactone, and phenolphthalein 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 and DEAE-Sephadex (A-25) from Pharmacia Fine Chemicals, Inc. Chromatographic sheets of cellulose (No. 6064) and silica gel (No. 6061) were obtained from Eastman Kodak and sheets of Baker-flex cellulose DEAE from J. T. Baker Co.

Glucuronidation of antithyroid drugs. Guinea pig microsomes were prepared by a modification of the method of Pogell and Leloir [9]. Young adult male guinea pigs (300–400 g) were fasted overnight and sacrificed by exsanguination while under light ether anesthesia. The livers were quickly removed and homogenized in 4 vol. of cold 0.154 M KCl in a Potter Elvehjem homogenizer. The homogenate was centrifuged at 2,000 *g* for 10 min, the pellet was discarded and the supernatant was centrifuged at 105,000 *g* for 60 min. This pellet was washed once with the original volume of KCl and centrifuged at 105,000 *g* for 30 min. The resulting pellet was brought up in 0.4 of the original homogenate volume with 0.154 M KCl. This preparation served as the source of UDP glucuronyl transferase and was used when freshly prepared or after lyophilization and reconstitution with H_2O. Lyophilized microsomes stored at −20° retained UDP glucuronyl transferase activity for several weeks and were frequently used for economy and convenience.

Incubation mixtures usually contained 0.1 μmole of the radioactive anti-thyroid drug (dissolved with warming in buffer), 20 μmoles of Tris–HCl buffer (pH 8.0), 0.2 μmole D-saccharic acid-1,4-lactone, 0.5 μmole UDPGA and 0.8 to 2.0 mg of microsomal protein in a final volume of 0.31 ml. Controls were identical except for the absence of UDPGA. Incubation was carried out in air, with shaking, at 37° for 1 hr unless otherwise indicated and the reaction was stopped by the addition of 2 vol. of 95% ethanol. The mixture was centrifuged and the supernatant lyophilized. The sample was reconstituted with 0.3 ml H_2O and subjected to chromatographic analyses.

When used as the substrate, phenolphthalein in 20% ethanol was added to a final concentration of 315 nmoles/0.31 ml in an incubation mixture identical to that used with antithyroid drugs and incubated for 1 hr unless otherwise indicated. The reaction was stopped in aliquots of 50–100 μl by the addition of 2.5 ml of 0.2 M glycine buffer, pH 10.4, and absorbance measured at 540 nm.

Radioactive antithyroid drugs were separated from the glucuronide product by column or thin-layer chromatography (t.l.c.). For column chromatography, aliquots of the reconstituted supernatant were applied to 2 × 115 cm Bio-Gel P-2 columns (200–400 mesh) previously equilibrated with H_2O. The columns were eluted with H_2O and fractions of 3.0 ml collected. Aliquots of 50–100 μl were then analyzed for radioactivity in a Packard Tri-Carb liquid spectrometer. This system achieved excellent separation and quantitation of PTU and thiouracil glucuronides and was the preferred method for both.

In some experiments, the tubes corresponding to the radioactive glucuronide peaks were pooled and lyophilized. Samples to be further purified were dissolved in 1.0 ml of 0.1 M ammonium carbonate and applied to a 1 × 10 cm column of DEAE-Sephadex A-25 previously equilibrated with 0.1 M ammonium carbonate. The column was eluted with 0.1 M ammonium carbonate and 3.0-ml fractions were collected. The radioactive glucuronide peak was located by counting 0.1-ml aliquots. These fractions were then pooled and lyophilized to vaporize the ammonium carbonate.

Optimum separation of PTU and PTU glucuronide by thin-layer chromatography was accomplished using Eastman Kodak 6064 cellulose sheets eluted with ethanol–1 M ammonium acetate (75:10). Adequate separation was also achieved with sheets of DEAE-Sephadex developed with 0.05 M ammonium carbonate or NaCl and silica gel sheets developed with benzene–isopropanol (60:10). Separation of thiouracil and thiouracil glucuronide by thin-layer chromatography was excellent with DEAE-Sephadex sheets developed with 0.05 M NaCl and adequate with the cellulose and silica gel systems used with PTU. Details for analyses by thin-layer chromatography have been previously described [10].

β-Glucuronidase hydrolysis. β-Glucuronidase hydrolysis was conducted at pH 6.8 rather than 5.0 due to the instability of PTU glucuronide at the lower pH. The reaction mixture consisted of 3.75 μmoles of potassium phosphate buffer, pH 6.8, containing 4.1 μmoles chloroform; 2,500 Fishman units β-glucuronidase; and at least 25,000 cpm of the unknown sample in a final volume of 0.2 ml. D-Saccharic acid-1,4-lactone (0.5 μmole), a specific inhibitor of β-glucuronidase [11], was added to controls; the experimental vessels were identical except for the absence of the inhibitor. Incubation was at 37° for 1 2 hr; the samples were quickly frozen in an acetone–ethanol bath and lyophilized. The dry samples were dissolved in 50 μl H_2O, and 10 μl spotted on t.l.c. plates.

Protein determinations. Protein in all assays was determined by the method of Lowry *et al.* [12].

RESULTS

The ^{14}C-labeled antithyroid drugs PTU, MMI and thiouracil were incubated for 4 hr with freshly prepared guinea pig liver microsomes in the presence and absence of UDPGA. 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 PTU, Fig. 1B for MMI and Fig. 1C for thiouracil (TU). In the presence of UDPGA, PTU and thiouracil were utilized by the microsomal preparation to form radioactive metabolites. Since conversion did not occur in the absence of UDPGA, the metabolites were believed to be the respective glucuronides. The metabolites formed represented 4.7 per cent of the initial radioactive thiouracil and 14.1 per cent of the [^{14}C]PTU. Numerous experiments under identical incubation conditions were also carried out with MMI, and the incubation medium was examined in all the t.l.c. systems used to separate PTU and thiouracil from their glucuronides (see Materials and Methods) and by column chromatography on Bio-Gel P-2 and DEAE-Sephadex columns. Measurable formation of an MMI glucuronide could not be demonstrated in any system

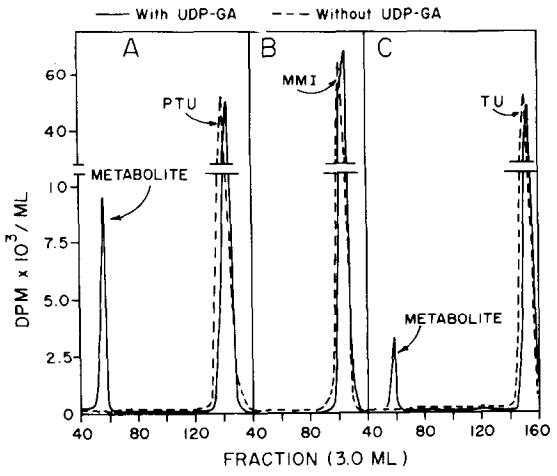

Fig. 1. Glucuronidation of ^{14}C-antithyroid drugs by guinea pig microsomes. The drugs were incubated for 4 hr in the presence (solid lines) and absence (broken lines) of UDPGA utilizing [2-^{14}C]PTU (A), [2-^{14}C]MMI (B) or [2-^{14}C]thiouracil (C) as substrates, described in Materials and Methods, and aliquots of the incubation mixture were chromatographed on 2 × 115 cm Bio-Gel P-2 columns eluting with water.

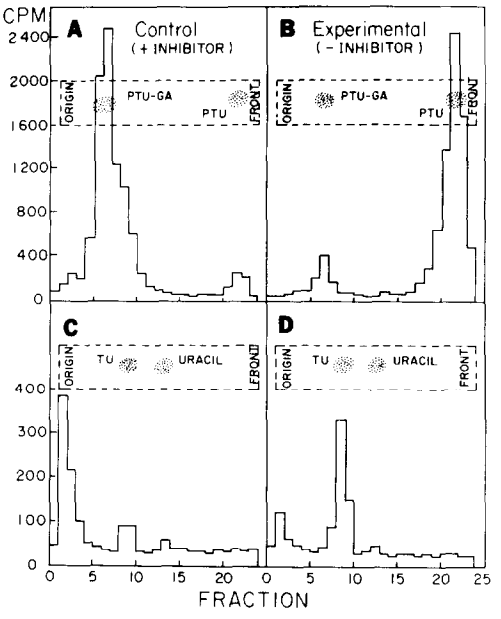

Fig. 2. β-Glucuronidase treatment of [^{14}C]PTU and [^{14}C]TU metabolites from Fig. 1. The metabolites were hydrolyzed for 2 hr with beef liver β-glucuronidase at pH 6.8 in the presence (control) and absence (experimental) of D-saccharic acid-1,4-lactone. Aliquots of the incubation mixtures containing the [^{14}C]PTU metabolite were cochromatographed with cold PTU glucuronide and PTU on t.l.c. cellulose sheets (A and B) developed in 1 M ammonium acetate–ethanol (10 : 75); aliquots with the [^{14}C]TU metabolite were cochromatographed with TU and uracil on t.l.c. DEAE cellulose sheets (C and D) developed in 0.05 M NaCl. The t.l.c. strip showing the position of the cochromatographed standards is outlined with broken lines; the distribution of radioactivity is shown by solid lines.

as illustrated in Fig. 1B for the Bio-Gel P-2 columns, indicating that MMI was not a substrate for UDP glucuronyl transferase.

The fractions containing the suspected PTU and thiouracil glucuronides were pooled, lyophilized and applied to 1.0 × 10 cm DEAE-Sephadex columns for further purification. The purified metabolites were then subjected to further analyses. Treatment of the suspected [^{14}C]PTU glucuronide with β-glucuronidase in the absence of the specific β-glucuronidase inhibitor D-saccharic acid-1,4-lactone liberated [^{14}C]PTU as shown in Fig. 2, panels A and B. Inhibition of β-glucuronidase hydrolysis in the presence of the inhibitor demonstrated that the conjugate was a β-glucuronide [11]. The identity of the [^{14}C]PTU product was confirmed by cochromatographing it with "cold" PTU on thin-layer sheets of DEAE, developed with 0.05 M ammonium carbonate, and silica gel, developed with benzene–isopropanol (60:10), and by cochromatographing on Bio Gel P-2 and DEAE-Sephadex columns. Similarly, treatment of the suspected [^{14}C]thiouracil glucuronide with β-glucuronidase liberated [^{14}C]thiouracil as shown in Fig. 2, panels C and D. The radioactive product of β-glucuronidase hydrolysis was cochromatographed with cold thiouracil and displayed identical chromatographic properties to it on cellulose and silica gel thin-layer sheets and on Bio Gel P-2 and DEAE-Sephadex columns confirming its identity as [^{14}C]thiouracil.

In the early stages of this study, phenolphthalein was assayed along with PTU as a substrate for guinea pig liver microsomes to provide a positive control for testing the viability of the preparations under various conditions. It soon became apparent that PTU and phenolphthalein were not conjugated in the same manner with respect to time, as demonstrated in Fig. 3. Conversion of phenolphthalein was linear for 2 hr but declined thereafter. The slower rate after 2 hr may be due primarily to substrate depletion since 180 of the initial 315 nmoles was used after 2 hr and 275 after 4 hr, the latter representing a conversion of 87 per cent. In contrast, formation of PTU glucuronide was linear for only 1 hr, during which over 50 per cent of the total glucuronide was synthesized. PTU glucuronidation declined after 1 hr and continued at a slower but linear rate for the remainder

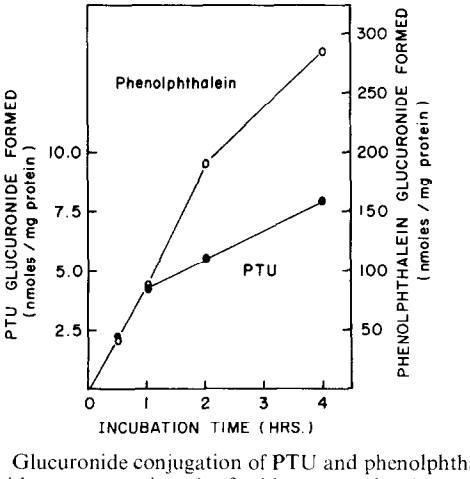

Fig. 3. Glucuronide conjugation of PTU and phenolphthalein, with respect to time, by freshly prepared guinea pig liver microsomes.

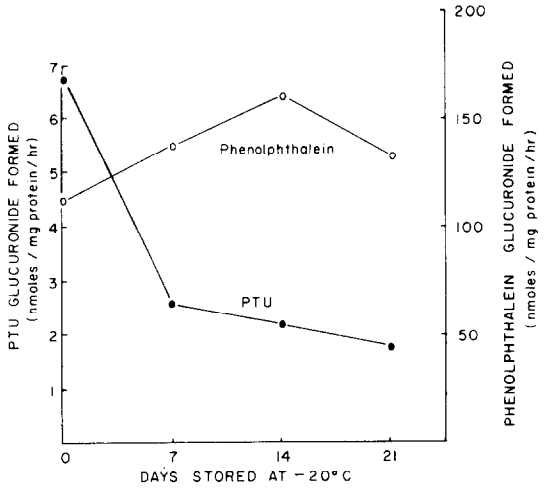

Fig. 4. Stability of conjugating activity for PTU and phenolphthalein by microsomes stored frozen at −20°. Glucuronidation was measured for 1 hr at 37°.

of the 4 hr of incubation. Substrate depletion is an unlikely factor in the declining rate of PTU conversion, since the total substrate utilized represented only 8 per cent of the total 100 nmoles initially available.

On the basis of the results obtained in Fig. 3 which demonstrate that glucuronidation of PTU was linear for only 1 hr, subsequent experiments were all conducted using an incubation time of 1 hr.

Further evidence that PTU and phenolphthalein were conjugated differently was obtained with frozen guinea pig liver microsomes. A freshly prepared microsomal fraction in 0.154 M KCl was assayed for activity with PTU and phenolphthalein as substrates. Several 2.0-ml aliquots of the same preparation were frozen at −20°. At various time intervals, a frozen aliquot was thawed and again assayed with both PTU and phenolphthalein. The results are presented in Fig. 4. Conjugating activity with phenolphthalein as a substrate was greater in the frozen microsomes for the 21 days examined than in the original fresh microsomes with activity being 121.2, 141.0 and 117.4 per cent of the fresh after storage at −20° for 7, 14 and 21 days respectively. This activation of UDP glucuronyl transferase is probably similar to that observed after detergent, sonication or phospholipase treatment

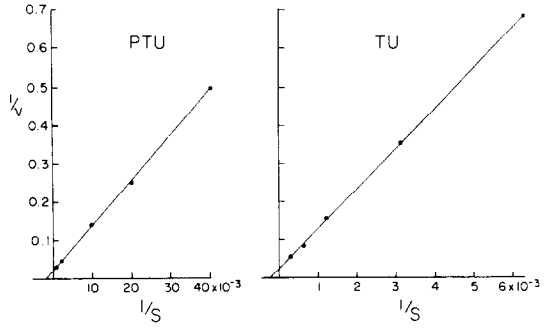

Fig. 5. Lineweaver–Burk plot of glucuronidation of PTU and TU by guinea pig liver microsome UDP glucuronyl transferase. Glucuronidation was measured after 1 hr of incubation with UDPGA at 1.61 mM; v represents nmoles glucuronide formed/mg of protein/hr.

of microsomal enzyme which is attributed to effects on microsomal membrane conformation [13, 14]. Regardless of the mechanism, the conjugating activity of the microsomes was not only stable for the phenolphthalein substrate after freezing but actually increased. In contrast, the conjugating activity with PTU as the substrate was very labile. Storage of microsomes at −20° for 7 days decreased enzyme activity for PTU to 37.8 per cent of that in the fresh microsomes compared to 121.2 per cent for phenolphthalein. At 14 days, activity for PTU had declined further to 32.6 per cent of the initial value while activity for phenolphthalein increased to 141 per cent. These results demonstrate that UDP glucuronyl transferase activity for PTU, unlike that for phenolphthalein and many other substrates [9], is not stable in frozen guinea pig liver microsomes and that conjugating activity for phenolphthalein is unrelated to the ability of the enzyme to conjugate PTU.

The results presented in Fig. 1 suggested that glucuronidation occurred more readily with PTU than with thiouracil. Kinetic studies with the microsomal preparation (Fig. 5) demonstrated apparent K_m values for PTU and thiouracil of 7.2×10^{-4} and 6.7×10^{-3} M, respectively, while the V_{max} for the two drugs was essentially the same. Since the enzyme appeared to have a higher affinity for PTU and this drug is one of the current antithyroid drugs of choice in the treatment of hyperthyroidism, further studies of glucuronidation were carried out primarily with PTU.

PTU and thiouracil possess several potential sites at which glucuronide conjugation may occur including the nitrogen in the heterocyclic ring. Conjugation to a nitrogen moiety usually occurs enzymatically but Bridges and Williams [15] have also found that a number of drugs combine spontaneously with glucuronate in aqueous solution to form N-glucuronides. Consequently the enzyme requirements for PTU glucuronide formation were examined with the results shown in Fig. 6. PTU glucuronide synthesis, which was not measurable in the absence of UDPGA (Fig. 1A) or enzyme (Fig. 6), was essentially linear with respect to enzyme concentration, demonstrating that PTU conjugation required microsomal enzyme and was proportional to the enzyme protein.

Dutton [7] has cautioned against assuming that glucuronidation was enzymatic when UDPGA and tissue are required, since hydrolysis of UDPGA by tissue enzymes could release glucuronate and this glucuronate may participate in non-enzymatic N-conju-

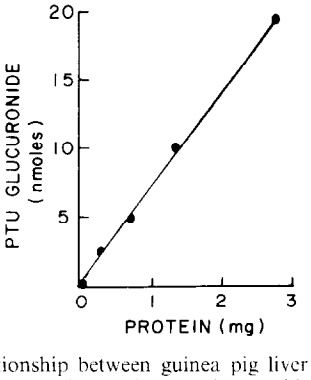

Fig. 6. Relationship between guinea pig liver microsomal enzyme concentration and PTU glucuronide formation.

gation. UDPGA degradation to glucuronate occurs readily with rat liver microsomes but Pogell and Leloir [9] reported that destruction of UDPGA was not measurable when UDPGA was incubated with guinea pig liver microsomes, suggesting that glucuronide formation from UDPGA would be minimal in our guinea pig liver microsome preparations. However, non-enzymatic conjugation of PTU by glucuronate was investigated under the same incubation conditions, omitting enzyme and UDPGA, in which microsomal conjugation of PTU was observed. [^{14}C]PTU incubated with 0.1 or 0.5 μmole glucuronate (compared to 0.5 μmole UDPGA in the microsomal system) for 1–4 hr was not conjugated at a measurable rate (data not shown). The sensitivity of the chromatographic assay system allows detection of 0.25 nmole [^{14}C]PTU glucuronide, which is about 5 per cent of the amount formed with microsomal enzyme in 1 hr. These results appear to exclude spontaneous glucuronidation of PTU as a possible explanation of PTU conjugation.

Additional evidence that glucuronidation of PTU is enzymatic was obtained from experiments in which the optimum pH for utilization of PTU as a substrate was determined (Fig. 7). Bridges and Williams [15] in a study of the non-enzymatic *N*-glucuronidation of a number of drugs *in vitro* reported the pH optimum to be 3–4 with little or no *N*-glucuronide synthesis occurring above pH 7.0. The glucuronidation of PTU was found to have a pH optimum of 8.0. This value closely resembles the broad 7.3 to 7.9 optimum for UDP glucuronyl transferase activity [7, 8] in different preparations but is vastly different from the pH 3–4 optimum for non-enzymatic *N*-glucuronidation.

Cellular localization of UDP glucuronyl transferase activity for PTU by differential centrifugation was examined. Approximately 75 per cent of the total activity exhibited by the whole liver homogenate was found in the microsomal fraction (data not shown).

Evidence for the multiple nature of UDP glucuronyl transferase has been carefully examined by Dutton [7, 8], who cautiously concludes that many UDP glucuronyl transferases may exist. Even so, single enzymes would be expected to have a limited specificity, and the utilization of a substrate may be inhibited by closely related substances. The effects of other closely related antithyroid drugs on the glucuronidation of PTU were examined with the results presented in Table 1. Thiouracil, a weak substrate for the enzyme, produced a slight but statistically insignificant decrease in PTU utilization while MMI, which was not used as a substrate at a measurable rate, significantly inhibited PTU conjugation. The mechanism of the MMI effect is not clear, since it is not acting as a competitive substrate. However, this compound is a relatively strong reducing agent and may alter microsomal membrane conformation, the mechanism by which many substances affect UDP glucuronyl transferase activity *in vitro* [13, 14].

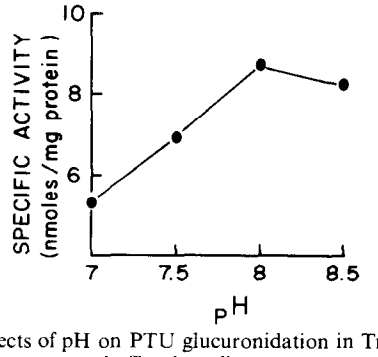

Fig. 7. Effects of pH on PTU glucuronidation in Tris–HCl buffered media.

Table 1. Effects of antithyroid drugs on PTU glucuronidation *in vitro**

Addition (0.5 μmole)	PTU glucuronide formed (nmoles/mg protein)	
None	4.91 ± 0.24	
Thiouracil	4.32 ± 0.35	$P < 0.2$
6-Methyl-thiouracil	4.60 ± 0.31	
Thiourea	4.50 ± 0.32	
MMI	3.88 ± 0.22	$P < 0.02$

* Incubation was with 0.1 μmole of PTU substrate/0.31 ml for 1 hr. The antithyroid drugs were dissolved, with warming, in buffer and appropriate buffer was added to controls. The results shown are means ± S.E. of eight determinations. P values were all obtained in comparisons with controls.

DISCUSSION

The data presented demonstrate that the antithyroid drugs PTU and thiouracil are substrates for a guinea pig liver microsomal enzyme which converted them to the corresponding glucuronide. Since conjugation did not occur in the absence of UDPGA or of microsomal enzyme and the product of the reaction was a β-glucuronide, the catalytic enzyme appeared to be UDP glucuronyl transferase. MMI was not a substrate for the enzyme and was not metabolized at a measurable rate.

Papapetrou *et al.* [3] noted that the major metabolite in bile after MMI administration was a glucuronide conjugate of an MMI metabolite. Since a glucuronide of unaltered MMI was not observed, they concluded that MMI is conjugated only after previous conversion to another compound. The present work fully supports this view since MMI was not a substrate for the conjugating enzyme. This is the second instance in which an enzyme readily utilizing PTU and thiouracil as substrates does not metabolize MMI. Lindsay *et al.* [10] recently observed that PTU, thiouracil and 6-methyl-2-thiouracil, but not MMI, were *S*-methylated by mouse kidney thiol transmethylase. These differences demonstrate that the metabolic pathways for peripheral metabolism of PTU differ substantially from those of MMI.

Since PTU metabolism by guinea pig microsomes was not measurable when UDPGA was absent from the incubation medium and hydrolysis of PTU glucuronide released unaltered PTU, conjugation of an existing group in the PTU molecule must have occurred. PTU possesses four groups which may participate in direct glucuronide conjugation. These are the SH group at C-2, the enolic OH group at C-4 and either of the heterocyclic NH groups in the thiourylene moiety. These groups represent some of

the more uncommon sites of glucuronidation. Glucuronidation of heterocyclic nitrogen is especially rare and the authors are aware of only one report of conjugation at this site and that involves glucuronidation of the heterocyclic N of sulfisoxazole [16]. The *N*-glucuronides are unique in that they are generally not hydrolyzed by β-glucuronidase [15, 17]. The only report of β-glucuronidase hydrolysis of an *N*-glucuronide appears to be that of Bridges *et al.* [18], who found that sulfadimethoxine N^1-glucuronide was a very poor substrate for the enzyme but measurable hydrolysis did occur after 24–96 hr with 15,000–30,000 Fishman units of β-glucuronidase. The PTU glucuronide formed in the present experiments was almost completely hydrolyzed by 2500 Fishman units β-glucuronidase in 1–2 hr, suggesting that the site of glucuronidation was to a group other than the heterocyclic nitrogen.

Glucuronide conjugation generally produces a strongly acidic derivative which is more water-soluble at a physiological pH and is usually more rapidly excreted than the parent compound. In addition, the pharmacological activity of a drug is usually eliminated or greatly diminished by glucuronidation. Direct evidence that glucuronide conjugation of PTU drastically alters its antithyroidal activity has been presented by Lindsay *et al.* [19], who found that PTU glucuronide was approximately 10 per cent as active as PTU as an inhibitor of thyroid peroxidase.

Both PTU and MMI molecules contain the thiourylene group (=N—C(=S)—N=), which accounts for three of the four sites available for conjugation. The thionamide (—C(=S)—N=) portion of this group is essential for the antithyroid activity of the thiourylene drugs. Substitution on the 2-sulfur atom abolishes antithyroidal action [10, 20, 21]. Substitution on either of the nitrogen in the thiourylene group of thiohydantion reduced antithyroid activity but *N*-methylation of thiopyridone increased the antithyroid effect [21]. Methyl substitution on the N-1 of PTU abolished its inhibitory effects on thyroid peroxidase although *N*-acetylation produced no alteration of its inhibitory effect [19].

If conjugation to a site on the thionamide moiety occurs, it is more likely to be to the *S* than to the *N* since conjugation to a heterocyclic *N* is especially rare and the PTU glucuronide is easily hydrolyzed by β-glucuronidase in contrast to *N*-glucuronides, which are resistant to hydrolysis by this enzyme. In addition, *S*-glucuronidation is more likely to account for the loss of biological activity produced by conjugation and the absence of MMI utilization by the enzyme than is *N*-glucuronidation. The potential for involvement of the *S* of PTU in enzyme reactions has been demonstrated with a thiol transmethylase which *S*-methylated PTU [10]. A qualitative difference in the reactivity of the *S* of PTU and *S* of MMI was also observed with the thiol transmethylase, since MMI was not a substrate for the enzyme.

Unfortunately, direct evidence for glucuronidation of the *S* of PTU has not been obtained, and although this appears to be a very likely site for conjugation, the enolic OH of PTU cannot be ruled out. MMI does not contain an enolic OH at C-4, and conjugation at this site could also account for the difference in metabolism of PTU and MMI by guinea pig liver UDP glucuronyl transferase.

PTU is approximately ten times more potent (compared to thiouracil) in rats than in man [21] and this difference may be related to glucuronide conjugation, which is a major pathway in both. Marchant *et al.* [6, 22] concluded that the rat and man have a vastly different ability to conjugate PTU and have suggested that this could contribute to the lesser effectiveness of PTU in man [22].

Urinary excretion of PTU glucuronide results in elimination of the conjugate from the body. In contrast, the PTU glucuronide excreted in bile is almost completely reabsorbed after first undergoing hydrolysis by β-glucuronidase in the gut [23], and fecal excretion is very low [1, 4]. Enterohepatic circulation of PTU in the rat is well established, while its existence in man has not been studied. Therefore, it is possible that efficient enterohepatic circulation rather than a limited ability to conjugate PTU may account for the persistence of PTU in the rat, with a half-life of 4–6 hr [2, 4] compared to man with a half-life of 1.1 to 2.5 hr [24, 25], and may be a primary factor in the greater effectiveness of PTU in rat than in man.

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