Biochemical Pharmacology, Vol. 21, pp. 363–377. Pergamon Press, 1972. Printed in Great Britain

BILIARY EXCRETION OF ^{35}S-LABELLED PROPYLTHIOURACIL, METHIMAZOLE AND CARBIMAZOLE IN UNTREATED AND PENTOBARBITONE PRETREATED RATS

P. D. PAPAPETROU, B. MARCHANT, H. GAVRAS and W. D. ALEXANDER
University Department of Medicine, Gardiner Institute, Western Infirmary, Glasgow, U.K.

(*Received* 31 *May* 1971; *accepted* 4 *August* 1971)

Abstract—The excretion of ^{35}S-labelled antithyroid drugs 4(6)-Propyl-2-thiouracil (PTU), methimazole and carbimazole in the bile of the rat was studied. Carbimazole had the highest biliary clearance and PTU the lowest. The total amount of ^{35}S-radioactivity excreted in the bile 5 hr after the i.v. injection of the drugs was 31·71 ± 8·16 per cent (mean ± S.E.) of the dose for carbimazole, 21·10 ± 2·80 per cent for methimazole and 8·20 ± 0·62 per cent for PTU in rats not pretreated with pentobarbitone.

The nature of the ^{35}S-compounds excreted in the bile was investigated using thin-layer chromatography. Methimazole and carbimazole gave the same number of metabolites and with exactly the same R_fs in four different solvent systems. 10·60 per cent of ^{35}S-radioactivity in the bile of rats injected with methimazole was due to free methimazole; about the same percentage of free methimazole was observed after carbimazole injection. There was no free carbimazole detectable in the bile. These findings suggest that carbimazole is metabolized through methimazole. None of the metabolites of the three drugs which appeared in the bile had R_f similar to free thiourea or to conjugates of thiourea appearing in the bile of rats injected with [^{35}S]thiourea. It is concluded that hepatic metabolism of the three drugs does not occur through thiourea.

The major metabolite of methimazole in the bile was a glucuronic acid conjugate hydrolysed by β-glucuronidase to a metabolite different from methimazole. The main metabolite of PTU was also a glucuronic acid conjugate which was hydrolysed by β-glucuronidase to a compound having the same R_fs as free PTU in four different solvent systems. It is concluded that while methimazole is conjugated with glucuronic acid in the liver of the rat after previous conversion to another compound, PTU is itself conjugated with glucuronic acid. A marked increase in the biliary excretion of ^{35}S-radioactivity occurred in rats injected with [^{35}S]methimazole and pretreated with pentobarbitone. The amount of radioactivity excreted in the bile within 5 hr after the injection was increased more than 100 per cent. The biliary clearance rate (BRC) and the bile/plasma ratio (B/P ratio) of the radioactivity were also significantly increased by the barbiturate. This was due mainly to increased biliary excretion of the glucuronide of methimazole. The effect of pentobarbitone on carbimazole was similar although less marked. The BCR of [^{35}S]PTU was also enhanced by pentobarbitone. This was due, however, solely to significantly higher biliary flow in the pretreated with pentobarbitone rats. The B/P ratio of radioactivity was not increased by the barbiturate.

ALTHOUGH antithyroid drugs have been widely used for over 25 years there is little information about their metabolites or metabolic pathways. We report here on the labelled metabolites of ^{35}S-antithyroid drugs excreted in the bile of rats. Considerable differences between the various drugs were found.

Since barbiturates are often prescribed for thyrotoxic patients and since they enhance hepatic metabolism of a wide variety of drugs[1] it was of interest to study the effect of pentobarbitone on the biliary excretion of the antithyroid drugs.

MATERIALS AND METHODS

Materials

Male Sprague–Dawley rats weighing 255–320 g were used. They were fed diet 41B and water *ad lib.* Some rats were pretreated with pentobarbitone (30 mg/kg, i.p., once daily for 4 days). ^{35}S-labelled PTU, methimazole and carbimazole were obtained from the Radiochemical Centre (Amersham) at a specific activity of 32 mc/m-mole, 38·2 mc/m-mole and 28·8 mc/m-mole respectively. The radiochemical purity was found to be greater than 98 per cent by thin-layer chromatography. Cellulose thin-layer chromatography sheets were purchased from Eastman Kodak Ltd.

Methods

All the rats (i.e. non pretreated and pentobarbitone pretreated) were anaesthetized with pentobarbital (60 mg/kg, i.p.) and maintained unconscious during the experiment with supplemental doses as needed. As soon as the animal was anaesthetized, tracheostomy was performed and the common bile duct was cannulated with a fine polyethylene cannula. At zero time (which was 30–120 min after the injection of the anaesthetic) the ^{35}S-antithyroid drug was injected into the exposed femoral vein and the collection of bile was started. The injected dose was 0·33 mg/kg for PTU and carbimazole and 0·25 mg/kg for methimazole. These doses are equimolar. The drug was injected in 0·20 ml normal saline pH 8–9 (tested with indicator paper) for PTU, normal saline for methimazole and normal saline with 10% alcohol for carbimazole. The solution was made up just before the injection. Bile was collected for 5 hr in 20-min time periods. In the middle of every other 20-min interval a blood sample (about 0·10–0·25 ml) was obtained from the tail and heparinized plasma was separated. The total volume of blood removed never exceeded 1·5 ml. All samples were kept in an ice bath during the collection and throughout the procedures. Bile and plasma (0·02–0·05 ml) were digested with 1 ml of hydroxide of hyamine (Packard) in liquid scintillation vials and made up with 10 ml of scintillator (Nuclear Enterprises NE 250). All samples were counted for ^{35}S-radioactivity in a tricarb-liquid scintillation spectrometer (Packard) and appropriate corrections for quenching made. The rest of the bile and plasma were kept at $-20°$ for thin-layer chromatography.

Thin-layer chromatography (TLC)

Bile (0·02–0·03 ml) was banded at the origin of a cellulose-coated sheet (Eastman) or silica gel-coated plate (0·25 mm, prepared from silica gel, Merck). The solvent systems used for the development of the chromatograms were the following: (1) Ethanol–IM ammonium acetate (E/AA 7·5:3 or 7:3·5) with cellulose sheets. The latter solvent gives a higher R_f for the $SO_4^{=}$ and the compounds between the origin and the $SO_4^{=}$ are better separated. (2) Chloroform–methanol–water (160:40:25) (C.M.W.) using the organic phase with silica gel plates. It was used as a second system for methimazole and carbimazole. (3) Butanol–water (86:14) (B.W.) with cellulose sheets and (4) Benzene–ether–methanol–acetic acid glacial (90:90:10:10) (B.E.M.A.) with silica gel plates. The systems chiefly used were E/AA 7·5:3 and E/AA 7:3·5. The developed chromatograms were scanned on a radiochromatogram scanner (Panax Ltd.) and the ^{35}S-peaks located. In some cases where the chromatograms were not very active autoradiography using X-ray films (Kodirex, Kodak) was carried out and the radio-

active spots were scraped and counted in scintillation vials with 10 ml of Toluene scintillator (Shephard). Plasma (0·10 ml) was mixed with 0·10 ml of absolute alcohol, centrifuged and the supernatant was used for TLC in E/AA.

Incubation with β-glucuronidase and sulphatase

Methimazole. The various compounds from previous chromatograms (in E/AA 7:3·5) of bile from rats injected with [^{35}S]methimazole were scraped separately and eluted in distilled water (10 ml). The water was evaporated to dryness in an Edwards Freeze drier. The dry compound was then dissolved in 0·2 ml phosphate buffer (0·1 M, pH 6·5) containing β-glucuronidase (Fishman bacterial preparation) or in acetate buffer (0·1 M pH 5·2) containing sulphatase (Sigma) in a final concentration of 1000 U/ml. Part of the same solutions of the radioactive compounds but without enzyme were incubated in parallel as controls at 37°. The result was assessed after 5 and 18 hr incubation by TLC.

TABLE 1. TOTAL AMOUNT OF ^{35}S-RADIOACTIVITY EXCRETED IN THE BILE IN 5 hr (% OF THE INJECTED DOSE)

	Number of rats	Radioactivity Mean ± S.E.	Significance
PTU Non pretreated	5	8·20 ± 0·62	
PTU pentobarb. pretreat.	2	13·72	
Methimazole Non pretreat.	8	21·10 ± 2·80	P < 0·0005
Methimazole pentobarb. pretreat.	4	46·65 ± 3·49	
Carbimazole Non pretreat.	6	31·71 ± 8·16	P < 0·05
Carbimazole pentobarb. pretreat.	4	44·21 ± 10·00	

PTU. The various compounds of PTU were also scraped from previous chromatograms (in E/AA 7:3·5) eluted in distilled water and evaporated as described above with methimazole. The dry compound was dissolved in 0·2 ml phosphate buffer containing β-glucuronidase. The same compounds were incubated in parallel in buffer only, as controls. A solution of the original [^{35}S]PTU in phosphate buffer containing β-glucuronidase was also incubated. The concentration of PTU in these samples was the same as in the bile samples containing the various compounds of PTU. The rest of the procedures were the same as with methimazole. The compound No. 6 of Table 2 was also incubated with bovine liver β-glucuronidase (Sigma). The eluted metabolite was redissolved in acetate buffer (0·1 M pH 5·0) containing 500 U/ml of the bovine β-glucuronidase and incubated for 2 hr at 37°. In certain samples saccharolactone (prepared by boiling potassium saccharate[2]) was added in a concentration 100 μg/ml in order to investigate whether the effect of the β-glucuronidase is inhibited by this substance.

TABLE 2. ^{35}S-COMPOUNDS OCCURRING IN THE BILE OF RATS INJECTED WITH [^{35}S]PTU

^{35}S-compound no.	R_f Ethan–Amm.Acet 7·5:3·0	R_f Ethan–Amm.Acet 7·0:3·5	% total radioactivity in the bile: Non pretreated	% total radioactivity in the bile: Pretreated with pentobarbitone	Nature of the compound
Origin			6·93	13·13	
1	0·08–0·10	0·08–0·10	15·93	9·77	Completely resistant to β-glucuronidase
2		0·14–0·16	4·27	3·80	
3	0·13–0·15	0·22–0·24	4·13	3·45	$SO_4^=$
4	0·20–0·22	0·29–0·31	5·72	5·49	
5	0·37–0·39	0·44–0·46	8·73	7·00	Glucuronide of No. 7?
6	0·52–0·55	0·58–0·60	22·78	30·07	Glucuronide of PTU
7	0·73–0·74	0·73–0·75	18·19	12·86	
8	0·84–0·86	0·85–0·87	13·32	14·43	Free PTU
Solvent front					

During the first 20 min period the concentration of radioactivity in the bile was lower than in the subsequent samples. This happened because it takes about 3 min for the first traces of radioactivity to appear in the bile after the i.v. injection and it reaches a plateau between 15–20 min after the injection. Only clearances from the 20th min and farther on were taken into consideration. The biliary clearance of ^{35}S-radioactivity was calculated in the conventional manner and expressed in millilitre of plasma per hour and per kilogram of body weight. For the 20 min bile samples to which no corresponding blood sample was taken off the plasma value was found by interpolation of the counted plasma samples.

Results from five nonpretreated (N.P.) and two pentobarbitone pretreated (P.P.) rats injected with [^{35}S]PTU, eight N.P. and four P.P. rats injected with [^{35}S]methimazole and six N.P. and four P.P. rats injected with [^{35}S]carbimazole are reported in this study.

RESULTS

The clearance of PTU was the lowest of the three drugs in the N.P. rats. It was significantly lower ($P < 0{\cdot}005$, Wilcoxon test) than the clearance of methimazole during the first hour and it remained constant throughout the collection period (Fig. 1). The clearance is the product of the bile–plasma (B/P) ratio and biliary flow. The B/P ratio of PTU was the lowest of the drugs studied ($P < 0{\cdot}005$ between all B/P ratio points of the PTU curve and the smallest B/P ratio of methimazole) and remained constant throughout (Fig. 2). The biliary flow in the N.P. rats injected with PTU was also the lowest (Fig. 3) but not significantly and this cannot account for the significantly lower clearance of PTU.

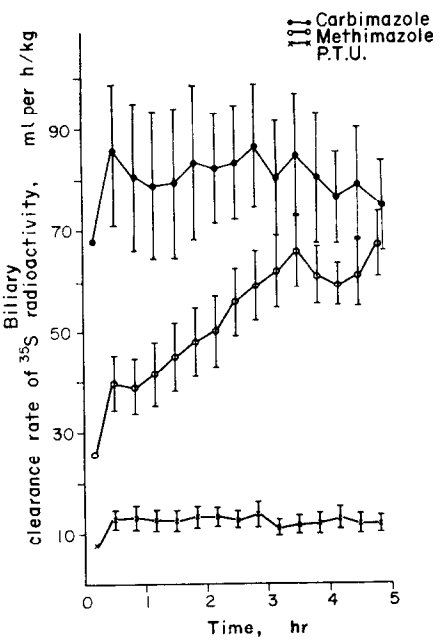

FIG. 1.

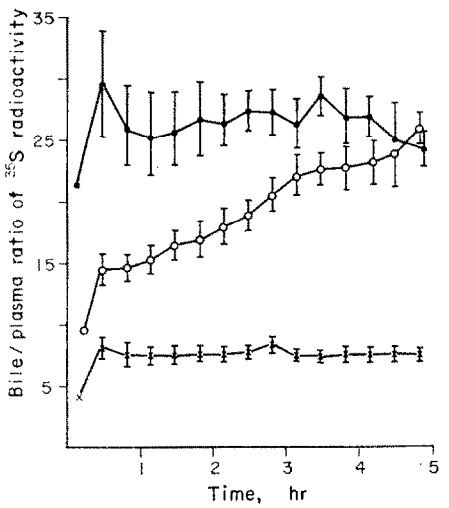

FIG. 2.

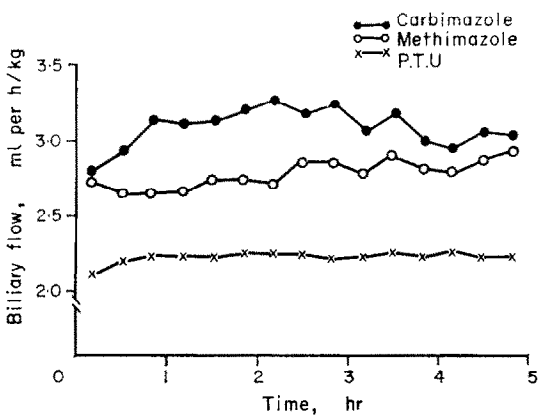

FIG. 3.

FIGS. 1, 2 and 3. Biliary clearance and B/P ratio of ^{35}S and biliary flow following injection of [^{35}S]PTU, methimazole or carbimazole in rats, which were not pretreated with pentobarbitone.

The clearance and B/P ratio of methimazole in the N.P. rats started rising from the beginning of the second hour. The clearance reached a plateau during the fifth hour while the B/P ratio continued to rise till the end (Figs. 1 and 2). The biliary flow in this group was fairly constant throughout (Fig. 3) and therefore the rise in clearance was due to the increasing B/P ratio of ^{35}S-radioactivity of methimazole.

The biliary clearance of [^{35}S]carbimazole in the N.P. rats gave the highest value which was significantly higher than the clearance of methimazole till the end of the third hour ($P < 0{\cdot}05$, Wilcoxon test) (Fig. 1). The B/P ratio of [^{35}S]carbimazole was significantly higher ($P < 0{\cdot}025$) than of methimazole up to the end of the fourth hour

(Fig. 2). The biliary flow in the group injected with carbimazole was slightly higher than in the methimazole group. Biliary clearance, B/P ratio and biliary flow were fairly constant throughout the collection time in the N.P. carbimazole injected group (Figs. 1, 2 and 3). The total amount of ^{35}S-radioactivity (expressed as per cent of the injected dose) excreted in the bile during the 5 hr after the injection of the drugs is shown in Table 1. The amount of PTU (8·20% ± 0·62 of the dose) (mean ± S.E.) was significantly lower ($P < 0{\cdot}025$) than of methimazole (21·20% ± 2·80) which was lower ($P < 0{\cdot}05$) than of carbimazole (31·71% ± 8·16) (Wilcoxon test).

Thin-layer chromatography results

PTU. Eight radioactive compounds appear in the bile of rats injected with [^{35}S]PTU (E/AA 7:3·5) (Table 2 and Fig. 4).* Serial bile samples from two N.P. rats injected with PTU were analysed. The proportion of the various metabolites remained practically the same throughout the time of the collection of the bile.

Incubation of the compound No. 6 with the bacterial β-glucuronidase (5 hr) resulted in 5–40 per cent hydrolysis in buffer-only samples and in 75–95 per cent hydrolysis in samples with the enzyme. The radioactivity which was liberated after the hydrolysis with β-glucuronidase was distributed in two positions in E/AA 7·5:3·0 chromatograms: 5–30 per cent was at R_f 0·74 corresponding to compound No. 7 and 70–95 per cent was at R_f 0·85 which is the R_f of free PTU in this system. This compound had the same R_f as PTU in three additional solvent systems, R_f 0·58 in C/M/W, R_f 0·31 in chloroform–isopropanol–ammonia (C/I/A) (45:45:10) and R_f 0·9 in B/W.

In the samples in which original [^{35}S]PTU was incubated in the presence of β-glucuronidase there was a 5–30 per cent transformation of PTU to compound No. 7. It was concluded, therefore, that hydrolysis of the compound No. 6 with β-glucuronidase liberated a compound having the R_f of free PTU in four solvent systems and that some of the PTU was converted to compound No. 7 during the incubation.

The difference between buffer-only samples and enzyme-containing ones was less marked at 18 hr as the hydrolysis was almost complete in both series.

A mean 90·2 per cent hydrolysis of the compound No. 6 occurred with the bovine β-glucuronidase, with liberation of Free PTU. There was a mean 62·7 per cent inhibition of β-glucuronidase activity in the samples containing saccharolactone.

The compounds No. 1 and 2 remained intact after 18 hr incubation with β-glucuronidase. The compound No. 5 was hydrolysed by β-glucuronidase to the compound No. 7. The scrapes of No. 5 were always contaminated with traces of the compounds No. 4 and 6 and the conclusion cannot be certain as with the result of the incubation of No. 6. No. 8 had the R_fs of PTU in E/AA, B/W and C/I/A.

Four of the radioactive compounds appearing in the bile of rats injected with [^{35}S]PTU, namely Nos. 3, 6, 7 and 8 of Table 2 appeared also in the blood of the same rats. No. 3 which is SO_4'' rose from 3·2 per cent at 10 min up to 6·6 per cent at the end of the fourth hour. Similarly, No. 8 (=PTU) fell from 46 to 33 per cent; No. 7 remained practically unchanged (42 per cent) and the compound No. 6 increased from 8·6 up to 18·4 per cent of the total ^{35}S in the plasma. The latter compound in the blood is a glucuronide of PTU.[3]

* The numbers of the compounds in the text correspond to the numbers of Table 2 for PTU and Table 3 for methimazole.

TABLE 3. ^{35}S-COMPOUNDS OCCURRING IN THE BILE OF RATS INJECTED WITH [^{35}S]METHIMAZOLE

^{35}S-compound no.	R_f Ethan–Amm.Acet. 7·5:3·0	R_f Ethan–Amm.Acet. 7·0:3·5	% total radioactivity in the bile: Non pretreated	% total radioactivity in the bile: Pretreated with pentobarbitone	Nature of the compound
Origin			5·98	3·27	
1	0·05–0·06	0·09–0·10	11·87	11·34	Conjugate (probably sulphate) of a compound with R_f 0·83 in E/AA and 0·02 in C/M/W
2		0·16–0·17	4·67	4·39	Conjugate (probably sulphate) of a compound with R_f 0·77 in E/AA and staying at origin in C/M/W
3	0·13–0·15	0·22–0·24	1·5	1·5	SO_4^*
4	0·25–0·27	0·36–0·37	31·60	50·96	Glucuronide of a compound with R_f 0·83 in E/AA and 0·03 in C/M/W
5	0·53	0·56–0·58	27·04	16·83	Unknown unstable conjugate of a compound with R_f 0·77 in E/AA and origin in C/M/W
6	0·76	0·78	6·74	3·41	
7	0·82–0·83	0·84	10·60	8·30	Methimazole + a second compound
Solvent front					

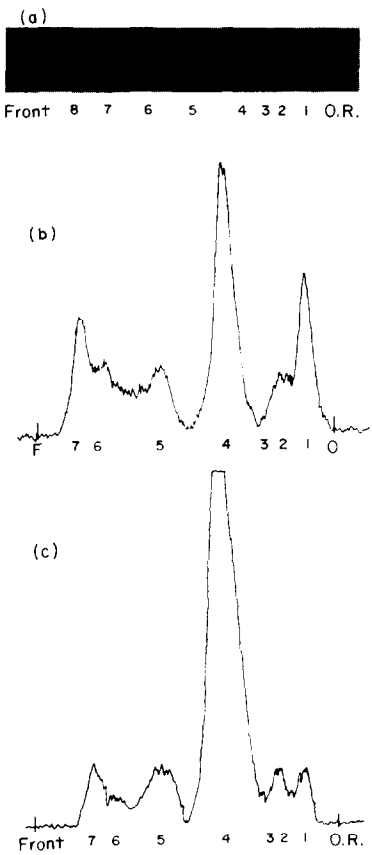

FIG. 4 (a) Autoradiogram of a chromatogram in E/AA 7:3·5 of bile from a rat injected with [^{35}S]PTU. The numbers of the bands correspond to the numbers in Table 2.
(b) Scan of a typical chromatogram (developed in E/AA 7:3·5) of bile from a rat injected with [^{35}S]methimazole. The numbers correspond to the compounds of Table 3.
(c) Scan of a typical chromatogram (E/AA 7:3·5) of bile from a rat injected with [^{35}S]carbimazole. Note the same number and position of the compounds as in (b).

B.P.—*facing page* 370

Methimazole. The compounds appearing in the bile of rats injected with [^{35}S]methimazole are shown in Table 3 and Fig. 4. Serial bile samples from two N.P. rats were analysed. There was no marked change in the proportion of the various compounds with time. The compound No. 4 was hydrolysed 80 per cent by β-glucuronidase and 33 per cent in the samples without enzyme (18 hr). The difference was significant. The radioactive compound which occurred after the hydrolysis had an R_f 0·81–0·83 in E/AA 7·5:3 and R_f 0·03 in C/M/W. It may be concluded that No. 4 is very probably a glucuronide of a metabolite of methimazole (R_f of methimazole 0·47 in C/M/W) (Fig. 5).

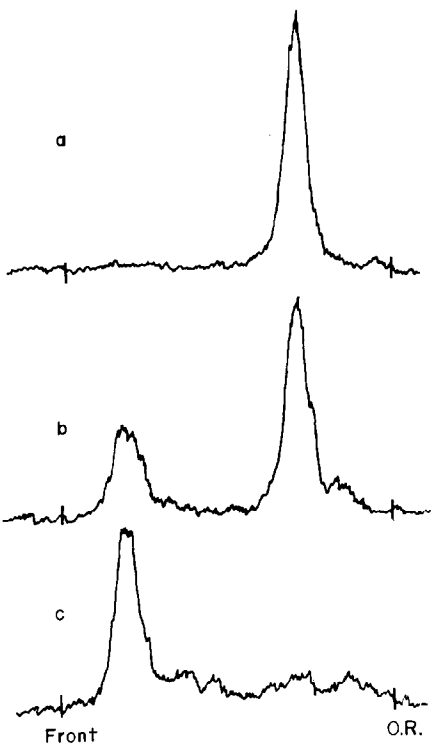

FIG. 5. The compound No. 4 (Fig. 4 and Table 3) of [^{35}S]methimazole obtained in pure form after elution etc. (a) before the incubation (b) after 18 hr incubation at 37° in phosphate buffer (c) after 18 hr incubation in the same buffer with β-glucuronidase.

No. 1 was hydrolysed 61 per cent by sulphatase and 46 per cent in the samples without sulphatase. The compound which resulted from this hydrolysis had R_f 0·83–0·84 in E/AA 7·5:3 and R_f 0·02 in C/M/W. It seems possible that No. 4 and No. 1 are both conjugates (glucuronide the first and sulphate the second) of the same metabolite of methimazole.

No. 5 is an unstable compound which changes rapidly (completely after 2·5 hr with or without β-glucuronidase) to another one with R_f 0·77 in E/AA 7·5:3 and origin in C/M/W. No. 2 was contaminated with No. 1 and No. 4 in chromatograms in E/AA 7·5:3 and no definite conclusion could be drawn. There is evidence that it

might be a sulphate conjugate of the metabolite to which No. 5 is hydrolysed. No. 7 has the following R_fs: E/AA 0·82–0·84, C/M/W 0·47, B/W 0·73–0·74, B/E/M/A 0·42, which are the R_fs of unconjugated methimazole in these solvent systems. Serial blood samples from two rats were analysed. Only $SO_4^{=}$ and free methimazole were detectable in the plasma. The $SO_4^{=}$ from 0·5 per cent at 10 min increased up to 4·90 per cent of the total plasma radioactivity at the end of the fifth hour.

Carbimazole. A typical chromatogram in E/AA 7:3·5 of the bile from a N.P. rat injected with [^{35}S]carbimazole is shown in Fig. 4. The number of the compounds and the R_fs in E/AA, C/M/W and B/E/M/A and B/W are the same as in the chromatograms of bile from rats injected with [^{35}S]methimazole. The compound No. 7 has the R_f of methimazole (0·47) and not of carbimazole (0·76) in C/M/W. TLC of unconcentrated plasma from rats injected with [^{35}S]carbimazole was not feasible because of the low specific activity of this drug at the time it was used.

Pretreatment with pentobarbitone

The biliary clearance of [^{35}S]methimazole was higher in the pentobarbitone pretreated than in the non pretreated rats ($P < 0{\cdot}005$, Wilcoxon test), up to the end of the fourth hour when the difference became not significant (Fig. 6). The B/P ratio of

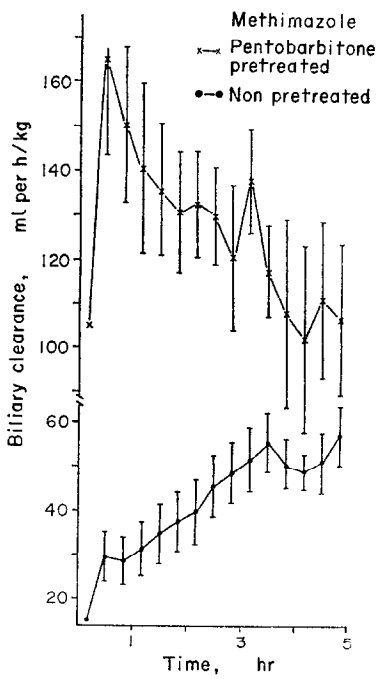

FIG. 6.

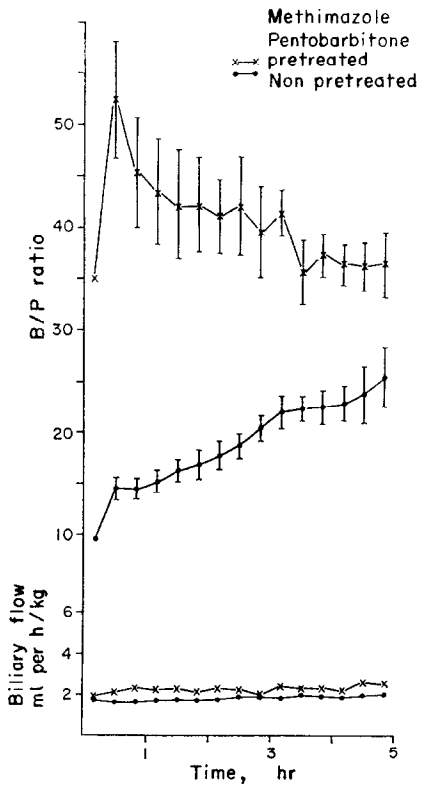

FIG. 7.

FIGS. 6 and 7. Biliary clearance and B/P ratio of ^{35}S and biliary flow following injection of [^{35}S]methimazole in untreated and pentobarbitone pretreated rats.

methimazole was also significantly higher in the P.P. throughout the collection period of the bile ($P < 0.005$) (Fig. 7). The biliary flow in the same group was only slightly higher than in the N.P. rats (Fig. 7) and therefore the higher biliary clearance of [^{35}S]methimazole in the P.P. rats compared to N.P. was due to a higher B/P ratio. The total amount of ^{35}S-radioactivity excreted within 5 hr in the bile of rats injected with methimazole was 21·10 per cent ± 2·80 (mean ± S.E.) of the injected dose in the N.P. and 46·65 per cent ± 3·49 in P.P. rats. The difference was significant ($P < 0.0005$, Wilcoxon test) (Table 1). The analysis of the bile from two P.P. rats by TLC showed that the ^{35}S-containing glucuronide which occurs after [^{35}S]methimazole (compound No. 4 in Table 3) was in higher concentration in the bile of P.P. rats (50·96 per cent of the radioactivity in the bile) compared to N.P. rats (31·60 per cent) (Table 3).

The biliary clearance of [^{35}S]carbimazole was higher in the P.P. than in the N.P. rats. The difference was significant up to the end of the second hour and became not significant afterwards (Fig. 8). The B/P followed the same pattern. It was significantly higher in the P.P. rats during the first 1·5 hr and not significant for the rest of the period of the collection of the bile. The biliary flow in the P.P. group was only slightly higher and therefore the higher biliary clearance of carbimazole in the P.P. compared to N.P. rats was due to a higher B/P ratio (Fig. 9). The total amount of ^{35}S-radioactivity excreted during the first 5 hr after the injection of carbimazole was 31·71 per cent ± 8·16 of the injected dose (mean ± S.E.) in the N.P. and 44·21 per cent ± 10·0 in the P.P. rats. This difference was significant ($P < 0.05$).

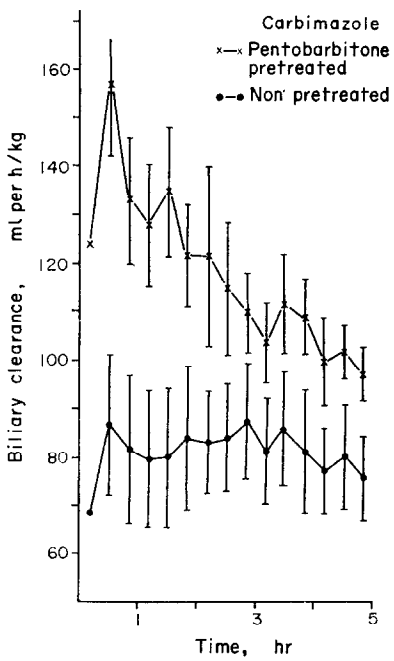

FIG. 8.

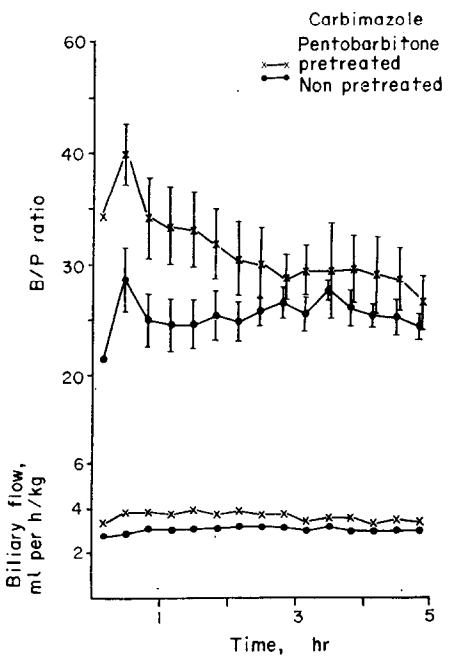

FIG. 9.

FIGS. 8 and 9. Biliary clearance and B/P ratio of ^{35}S and biliary flow following injection of [^{35}S]carbimazole in untreated and pentobarbitone pretreated rats.

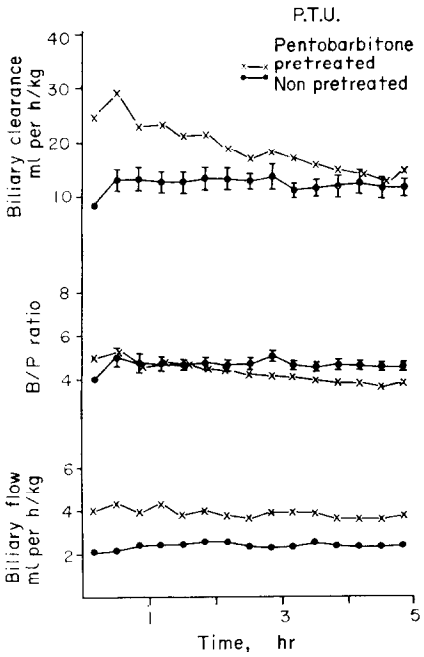

FIG. 10. Biliary clearance and B/P ratio of ^{35}S and biliary flow following injection of [^{35}S]PTU in untreated and pentobarbitone pretreated rats.

The biliary clearance of PTU was also higher in the P.P. than in the N.P. rats. However, the biliary flow was significantly higher in the former group while the B/P ratio of PTU was the same in both groups. Thus the higher clearance of PTU in P.P. compared to N.P. rats was due solely to the higher biliary flow and not to a higher B/P ratio (Fig. 10). Analysis by TLC of the bile in two P.P. rats demonstrated that the proportion of the various metabolites of PTU was roughly the same in P.P. and N.P. animals (Table 2).

DISCUSSION

Of the three antithyroid drugs studied here, PTU showed the lowest and carbimazole the highest biliary excretion in N.P. rats. Millburn *et al.*[4] found that compounds with molecular weight less than 325 ± 50 are little excreted in the bile of the rat. Although the molecular weight of methimazole is the smallest among the three drugs studied here its excretion is intermediate. This is probably related to different polarity.[4]

The biliary clearance of methimazole in N.P. rats started rising from the beginning of the second hour. This rise was due to an increasing B/P ratio and not to alteration of the biliary flow. This phenomenon may be an effect of the anaesthetic pentobarbital.

Ether could not be used as anaesthetic for these experiments because it has a toxic effect on the excretion of methimazole and possibly on the other two drugs. Figure 11 shows the biliary clearance of methimazole in a rat to which ether was given for 4 min when the anaesthesia with pentobarbitone became light and the animal started moving around. A dramatic fall in the biliary clearance of methimazole was noticed while the biliary flow rate remained steady. The concentration of the glucuronide of

methimazole in the bile samples after ether was found much less than in the previous samples. It seems that ether inhibits the formation of the glucuronide of methimazole in the liver.

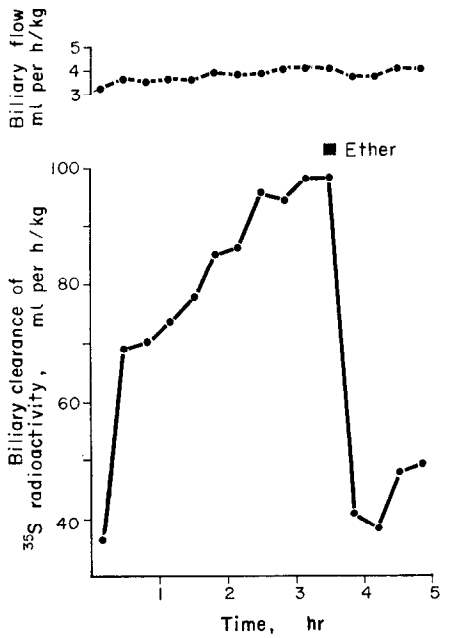

FIG. 11. The effect of 4 min ether administration on the biliary clearance of ^{35}S after [^{35}S]methimazole injection. Biliary flow remained constant while clearance fell markedly.

The main compound of PTU excreted in the bile is a glucuronide of the drug. The conjugate was detected also in the blood of the same animals with interrupted enterohepatic circulation (8–18 per cent of the radioactivity in the plasma) and in the urine of man.[3] It is probably formed in the kidney and in other organs known to form glucuronides, e.g. the gut.[5] It may be that the glucuronide of PTU appearing in the bile is not synthesised entirely in the liver but is partly extracted from the blood.

The main compound appearing in the bile of rats injected with [^{35}S]methimazole is also a glucuronide but not of methimazole. Hydrolysis of this compound with β-glucuronidase gave a ^{35}S-containing metabolite which in E/AA has the same R_f as [^{35}S]methimazole but in C/M/W has a very different R_f 0·02. (The R_f of methimazole is 0·47 in C/M/W.) It seems that another compound (quantitatively second in importance) of methimazole in the bile is a sulpho-conjugate of the same metabolite. This metabolite also occurs unconjugated in the bile: the peak which appears at the position of methimazole in E/AA (R_f 0·82–0·83) was always smaller when the same samples were subjected to TLC in C/M/W. Obviously, part of the methimazole peak (R_f 0·82 in E/AA) is due to this metabolite. Whether the formation of this compound precedes the conjugation with glucuronic and sulphuric acid or follows it cannot be answered from the data of this study. In contrast to the glucuronide of PTU, that of methimazole is not present in the blood or in the urine of the rat.[6]

Carbimazole is hydrolysed to methimazole in human plasma *in vitro*.[7] After i.v. administration of [^{35}S]carbimazole to rats we found free methimazole in the bile, but not carbimazole. The radioactive compounds which appear in the bile of rats injected with [^{35}S]carbimazole have the same R_fs in E/AA, B/W, C/M/W and B/E/M/A as those obtained after administration of [^{35}S]methimazole to rats (Fig. 4). These findings are in agreement with the results of Marchant *et al.*[8] who have demonstrated [^{35}S]methimazole in the urine, thyroid and plasma after administration of [^{35}S]carbimazole to rats and man. It seems very likely that the hepatic metabolism of carbimazole occurs after prior conversion to methimazole. However, the pattern of the biliary clearance and B/P ratio of [^{35}S]carbimazole was different from that of [^{35}S]methimazole in the N.P. rats of this study. While clearance and B/P ratio of carbimazole remained constant throughout it was significantly higher than that of methimazole during the first 3 hr. It is probable that although carbimazole is metabolized in the liver of the rat through methimazole it is taken up by the liver as carbimazole from the blood during the first 1–2 hr after the i.v. injection. Unfortunately, TLC of the plasma from the rats of this study was not feasible because of the low specific radioactivity of the carbimazole when used needing amounts of plasma bigger than the 0·10 ml obtained from these rats.

The R_fs of [^{35}S]thiourea are 0·66–0·67 in E/AA 7·5:3 and 0·45–0·46 in B/W. There was no detectable compound with similar R_fs in the bile of rats injected with [^{35}S]PTU, methimazole or carbimazole. In a pilot experiment [^{35}S]thiourea was injected i.v. into rats. Forty per cent of ^{35}S-radioactivity in the bile appeared as free thiourea (checked in E/AA and B/W). None of the hydrolysed compounds of PTU and methimazole gave (after hydrolysis) a compound having the R_fs of thiourea. If thiourea occurred as a product of the hepatic metabolism of these drugs it would appear as free thiourea in the bile. In fact, the radioactive compounds (apart from free thiourea) which are excreted in the bile after injection of [^{35}S]thiourea have R_fs in E/AA 7·5:3, 0·87, 0·43, and 0·03 which are different from the R_fs of all metabolites of the three drugs in this system. It can be concluded that the antithyroid drugs PTU, methimazole and carbimazole are not metabolized in the liver of the rat at least significantly through thiourea.

All the rats in this study received pentobarbitone as the anaesthetic agent. Some blurring of the results is thus inevitable since it is likely that the anaesthetic pentobarbital influenced the B/P ratio, at least for methimazole, in the non pretreated rats. However, it is clear that pretreatment with barbiturate made the bile of the rat a major route for the excretion of methimazole. If this happens in man it would be of importance since barbiturates are often given to thyrotoxic patients during treatment with methimazole.

The increase in the biliary clearance of [^{35}S]methimazole caused by pentobarbital was due to an elevated B/P ratio and not to increased biliary flow. Moreover, in the bile of P.P. rats the compound No. 4 appeared markedly increased compared to N.P. rats (Table 3). This compound is a glucuronic acid conjugate of a ^{35}S-containing metabolite of methimazole as mentioned previously. It is possible that the conjugation with glucuronic acid follows the conversion of methimazole to the above metabolite. Which of these two steps is enhanced by the barbiturate cannot be answered from the data of this study. Both are theoretically possible. Barbiturates can increase the synthesis of D-glucuronic acid and probably of UDP-D-glucuronic acid, in the liver of

the rat.[9] The latter is the donor of glucuronic acid during the biosynthesis of various glucuronides.[10] Barbiturates also enhance the excretion of bilirubin as glucuronide in the bile.[11] However, a more common action of barbiturates in the liver seems to be the induction of hydroxylation of many drugs which afterwards are conjugated with glucuronic acid or sulphuric acid.[12] This may be the mode of action of pentobarbitone on the hepatic metabolism of [^{35}S]methimazole described here. The biliary clearance of PTU was also increased by pentobarbitone but this was due entirely to an increased biliary flow. The B/P ratio of PTU was the same in P.P. and N.P. rats. The increase in the biliary flow may be caused by barbiturates and this phenomenon seems to be independent of the enzymatic induction caused by these drugs.[13] As a result of increased biliary flow after barbiturates, increased biliary excretion of some drugs which are excreted intact in the bile may occur.[14] The increase of biliary excretion of PTU caused by pentobarbitone in the rats of the present study is probably of this nature.

The excretion of [^{35}S]carbimazole in the bile of the rat was also enhanced by pentobarbitone but this increase was less dramatic than of methimazole.

The biliary clearance and B/P ratio in rats pretreated with pentobarbitone was highest at the earliest time after the injection and fell steadily throughout the collection period of the bile. This happened with all three drugs. No satisfactory explanation can be given for this phenomenon.

Acknowledgements—We should like to thank Dr. E. B. Astwood and Dr. W. Fishman for helpful comments.

REFERENCES

1. R. KUNTZMAN, *Ann. Rev. Pharmac.* **9,** 21 (1969).
2. G. A. LEVVY, *Biochem. J.* **52,** 464 (1952).
3. B. MARCHANT, W. D. ALEXANDER, J. W. K. ROBERTSON and J. H. LAZARUS, *Metabolism* (in press) (1971).
4. P. MILLBURN, R. L. SMITH and R. T. WILLIAMS, *Biochem. J.* **105,** 1275 (1967).
5. G. J. DUTTON, in *Glucuronic Acid* (Ed. G. J. DUTTON) p. 220. Academic Press, New York (1966).
6. B. MARCHANT and W. D. ALEXANDER, submitted for publication (1971).
7. J. B. STENLAKE, W. D. WILLIAMS and G. G. SKELLERN, *J. Chromatog.* **53,** 285 (1970).
8. B. MARCHANT, W. D. ALEXANDER, J. H. LAZARUS, J. LEES and D. CLARK, submitted for publication (1971).
9. J. J. BURNS, A. H. CONNEY, P. G. DAYTON, C. EVANS, G. R. MARTIN and D. TALLER, *J. Pharmac. exp. Ther.* **129,** 132 (1960).
10. G. J. DUTTON, in *Glucuronic Acid* (Ed. G. J. DUTTON) p. 215. Academic Press, New York (1966).
11. C. CATZ and S. J. YAFFE, *Am. J. Dis. Child.* **104,** 516 (1962).
12. H. REMMER, *Am. J. Med.* **49,** 617 (1970).
13. C. D. KLAASSEN, *J. Pharmac. exp. Ther.* **168,** 218 (1969).
14. C. D. KLAASSEN, *J. Pharmac. exp. Ther.* **175,** 289 (1970).