[Chem. Pharm. Bull. 22(2) 433—438 (1974)]

UDC 547.483.09 : 615.31.033

Studies on the Biliary Excretion Mechanisms of Drugs. III.[1] Active Transport of Glucuronides into Bile in Rats

Takashi Uesugi, Mariko Ikeda, and Yoshiaki Kanei

Department of Biopharmacy, Meiji College of Pharmacy[2]

(Received September 11, 1973)

Thiamphenicol glucuronide (TPG), chloramphenicol glucuronide (CPG) and phenolphthalein glucuronide (PPG), administered intravenously to rats with ligated renal pedicles, rapidly appear in the bile in high concentrations as the unchanged compounds. Between 4 and 30 min after administration, the bile-to-blood concentration ratios of the glucuronides are 33—122 for TPG, 35—142 for CPG and 6—101 for PPG. Furthermore, the transport process of the glucuronides is saturable and the excretion is depressed by probenecid. The results indicate that TPG, CPG and PPG are excreted into the rat bile by an active transport process.

It is known that certain endogenous compounds such as bilirubin[3] and thyroxine[4] and a large number of foreign compounds are excreted into the bile as glucuronides. However, relatively little is known about the biliary excretion mechanism of glucuronides except a few reports,[5,6] which may be due to their unstability and difficulty to obtain them in pure crystalline forms. Therefore, the biliary excretion mechanism of glucuronides has been speculated mostly from the studies of a number of organic acids. In this study, the biliary excretion mechanism of thiamphenicol glucuronide (TPG), chloramphenicol glucuronide (CPG) and phenolphthalein glucuronide (PPG) in rats was investigated in detail.

Methods

Procedure in Animals——Male Wistar rats weighing 340—360 g were anesthetized with sodium pentobarbital intraperitoneally (40 mg/kg). Through an abdominal incision, the renal pedicles were ligated to prevent renal excretion of drugs. The bile duct was cannulated with a polyethylene tubing (18 cm). A thermistor probe was placed on the surface of liver, and body temperature was maintained throughout the experiments at 38° with a heating lamp. After the incision had been closed, bile was collected for a 10 min period prior to an intravenous injection (femoral vein) of drugs. The drugs were injected over a 1.5 min period, and bile was collected for 2 min periods for 30 min, and then for 10 min periods for an additional 60 min.

Determination of TPG and CPG——Concentrations of TPG and CPG in bile were estimated by the methods as described in the previous paper[7,8] and of Glazko, *et al.*,[9] respectively. The glucuronide levels in blood were estimated by the same methods described above after deproteinization of the sample with 0.5N NaOH and 10% $ZnSO_4$–$7H_2O$. Probenecid and its metabolites did not interfere the determination of the glucuronides.

Determination of PPG——Bile and blood containing PPG were heated with 1 ml of 8N HCl in a boiling water bath for 40 min, and 3 ml of 2.5N NaOH was added to the mixture. After the mixture was cooled to room temperature, the phenolphthalein liberated by hydrolysis was extracted with 5 ml of benzene under mechanical shaking for 10 min. Four ml of the organic layer was transferred into a test tube containing

1) Part II: T. Uesugi, M. Ikeda, Y. Kanei, R. Hori, and T. Arita, *Biochem. Pharmacol.*, "in press".
2) Location: *1-35, Nozawa, Setagaya-ku. Tokyo.*
3) B.H. Billing, P.G. Cole, and G.H. Lathe, *Biochem. J.*, **65**, 774 (1957).
4) E.V. Flock and J.L. Bollman, *Biochem. J.*, **84**, 621 (1962).
5) P. Millburn, R.L. Smith, and R.T. Williams, *Biochem. J.*, **105**, 1275, 1289 (1967).
6) G.J. Mulder, *Biochem. Pharmacol.*, 22, 1751 (1973).
7) T. Uesugi, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **21**, 570 (1973).
8) T. Uesugi, M. Ikeda, K. Katayama, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), "submmitted".
9) A.J. Glazko, L.M. Wolf, and W.A. Dill, *Arch. Biochem. Biophys.*, **23**, 411 (1949).

4 ml of buffer solution (0.5M Na_2CO_3–0.5N HCl, pH 10.7), and the tube was shaken mechanically for 5 min. After centrifugation, the absorbance at 552 mμ of the aqueous layer was measured against a blank prepared through the same procedure. The recovery of PPG was not disturbed by probenecid and its metabolites.

Materials——TPG, mp 190—192°, was isolated from guinea pig urine and bile. The entire procedure of isolation will be reported in the near future.[8] CPG was isolated from human urine by the method of Glazko, *et al.*[10] PPG and D-saccharic acid-1,4-lactone were obtained from Sigma Chemical Company. Probenecid, mp 194—196°, was used.

Result

Concentrative Transfer of the Glucuronides into Bile

The comparison between the concentrations of TPG, CPG and PPG in bile and blood was carried out at the various times after administration of the glucuronides. When the glucuronides were administered intravenously to rats with ligated renal pedicles, they readily appeared in bile in a high concentration. Bile was collected for 2 min each at 2, 10, 20 and 28 min after injection. One tenth ml of blood specimen was collected from the cannulated carotid at the end of the bile collection. Tables I, II and III show the concentrations of TPG, CPG and PPG in blood and bile. The results demonstrate that the concentrations of the glucuronides in bile greatly exceed those in blood at all experimental periods.

Table I. Concentrations of TPG in Blood and Bile of Rats after Injection of TPG

Time after inj., min	Concentration of TPG, μmoles/ml		Bile/Blood concentration ratio
	Blood	Bile	
2— 4	0.41±0.02	13.5±1.5	32.9
10—12	0.19±0.01	23.2±0.9	122.1
20—22	0.14±0.01	14.9±0.9	106.4
28—30	0.13±0.01	13.5±1.5	103.8

Rats with ligated renal pedicles received TPG (50 μmoles) *i.v.* Blood was obtained at the end-point of the bile collection periods, which were usually of 2 min duration. Results are expressed as mean± S.E. in 3 animals.

Table II. Concentrations of CPG in Blood and Bile of Rat after Injection of CPG

Time after inj., min	Concentration of CPG, μmoles/ml		Bile/Blood concentration ratio
	Blood	Bile	
2— 4	0.49±0.03	17.1±2.0	34.9
10—12	0.21±0.01	26.9±0.9	128.1
20—22	0.13±0.01	17.8±1.3	136.9
28—30	0.10±0.01	14.2±1.4	142.0

Rats with ligated renal pedicles received CPG 50 μmoles *i.v.* Blood was obtained at the end-point of the bile collection periods, which were usually of 2 min duration. Results are expressed as mean± S.E. in 3 animals.

Saturation of Biliary Excretion Process

The evidence that TPG, CPG and PPG are transported into bile by a saturable process was obtained from a study of the biliary excretion of the glucuronides at different dose levels. Figure 1 shows the biliary excretion rates (μmoles/2 min) of TPG after administration at

10) A.J. Glazko, W.A. Dill, and M.C. Rebstock, *J. Biol. Chem.*, **183**, 679 (1950).

Table III. Concentrations of PPG in Blood and Bile of Rat after Injection of PPG

Time after inj., min	Concentration of PPG, μmoles/ml		Bile/Blood concentration ratio
	Blood	Bile	
2— 4	0.66±0.05	4.2±0.33	6.4
10—12	0.39±0.04	28.5±1.21	73.1
20—22	0.28±0.02	28.2±0.64	100.7
28—30	0.24±0.02	23.7±0.68	98.8

Rats with ligated renal pedicles received PPG 50 μmoles *i.v.* Blood was obtained at the end-point of the bile collection periods, which were usually of 2 min duration. Results are expressed as mean± S.E. in 4 animals.

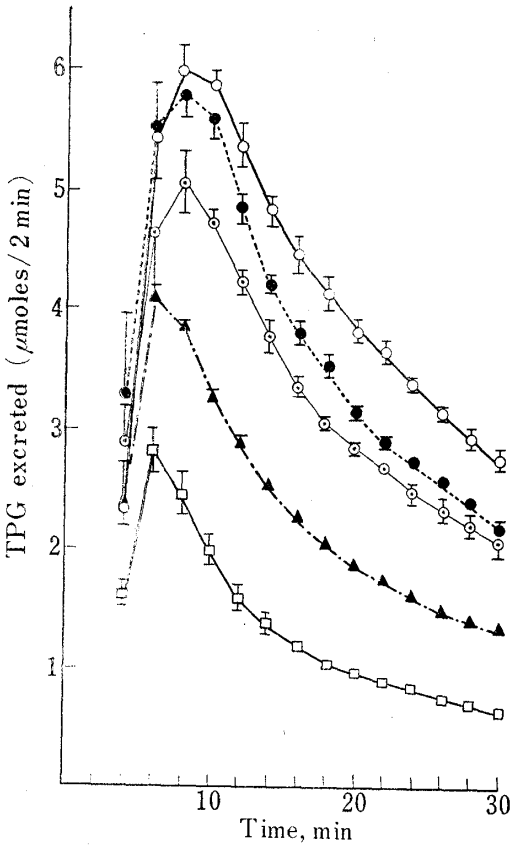

Fig. 1. Biliary Excretion of TPG in Rats with Ligated Renal Pedicles at Various Doses

TPG was administered intravenously. Bile was collected in 2 min periods for 30 min. Results are the means of 4 expriments and the vertical lines show the standard error of the mean.
○: 200 μmoles ●: 175 μmoles
⊙: 150 μmoles ▲: 100 μmoles
□: 50 μmoles

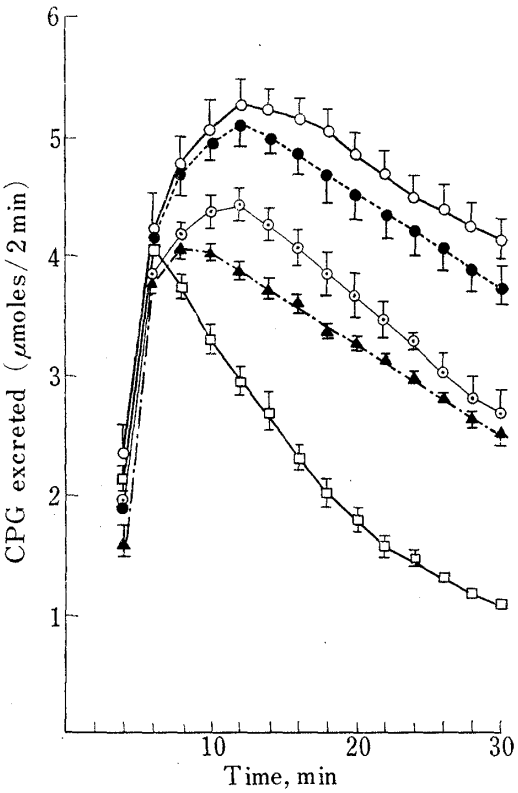

Fig. 2. Biliary Excretion of CPG in Rats with Ligated Renal Pedicles at Various Doses

CPG was administered intravenously. Bile was collected in 2 min periods for 30 min. Results are the means of 3—4 experiments and the vertical lines show the standard error of the mean.
○: 200 μmoles ●: 175 μmoles
⊙: 150 μmoles ▲: 100 μmoles
□: 50 μmoles

various doses ranging from 50 to 200 μmoles. Bile was collected for 2 min periods for 30 min. The maximal excretion rates observed at doses of 175 and 200 μmoles were essentially identical ($P>0.50$). Figure 2 shows the biliary excretion of CPG at various doses ranging from 50 to 200 μmoles. The maximal excretion rates observed at doses of 175 and 200 μmoles were essentially identical ($P>0.60$). Figure 3 shows the biliary excretion of PPG at doses of 50, 75 and 100 μmoles. The maximal excretion rates observed at doses of 75 and 100 μmoles were essentially identical ($P>0.60$).

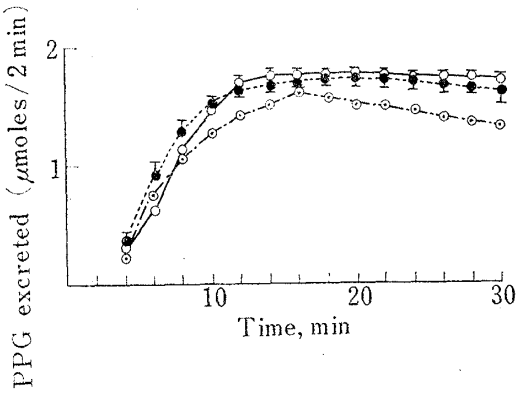

Fig. 3. Biliary Excretion of PPG in Rats with Ligated Renal Pedicles at Various Doses

PPG was administered intravenously. Bile was collected in 2 min periods for 30 min. Results are the means of 4 experiments and the vertical lines show the standard error of the mean.
○: 100 μmoles ●: 75 μmoles ⊙: 50 μmoles

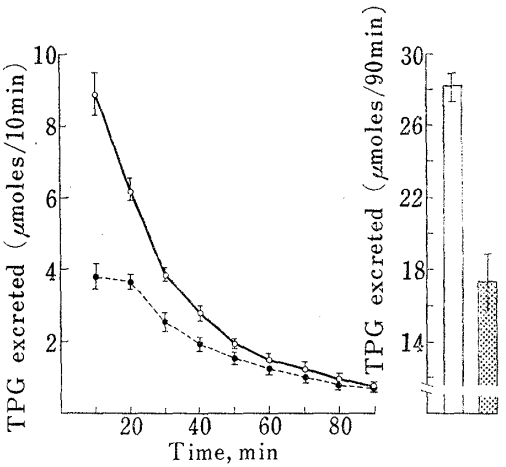

Fig. 4. Effect of Probenecid on the Biliary Excretion of TPG

Rats with ligated renal pedicles received TPG (50 μmoles), or TPG together with probenecid (50 μmoles) intravenously over a 1.5 min period. Bile was collected in 10 min periods for 90 min. Results are the means for 4 animals receiving TPG alone and 3 animals receiving both of the drugs.
○, □: :TPG alone
●, ▨: TPG together with probenecid

TPG and PPG were excreted in the rat bile as unchanged forms. On the other hand, only a few amount of a metabolite, aromatic amino derivative, was excreted when CPG was administered to rats intravenously; the proportion of dose excreted in 90 min was less than 1% at a dose of 100 μmoles.

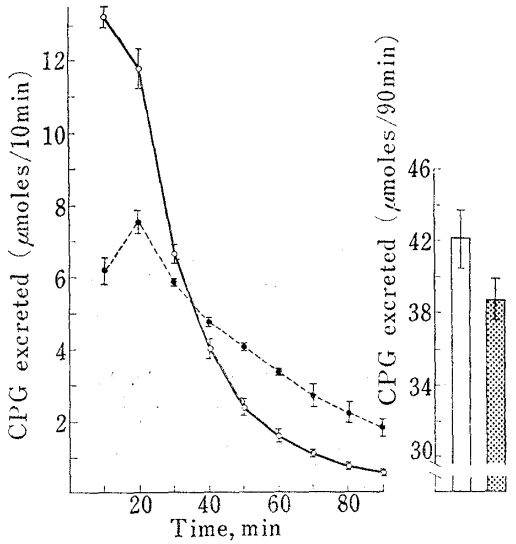

Fig. 5. Effect of Probenecid on the Biliary Excretion of CPG

Rats with ligated renal pedicles received CPG (50 μmoles), or CPG together with probenecid (50 μmoles) intravenously over a 1.5 min period. Bile was collected in 10 min periods for 90 min. Results are the means for 4 animals receiving CPG alone and 3 animals receiving both of the drugs.
○, □: CPG alone
●, ▨: CPG together with probenecid

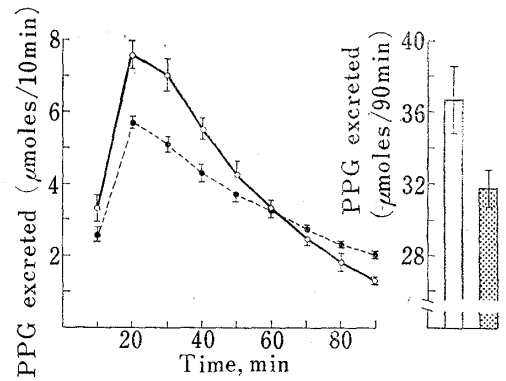

Fig. 6. Effect of Probenecid on the Biliary Excretion of PPG

Rats with ligated renal pedicles received PPG (50 μmoles), or PPG together with probenecid (50 μmoles) intravenously over a 1.5 min period. Bile was collected in 10 min periods for 90 min. Results are the mean for 4 animals receiving PPG alone and 3 animals receiving both of the drugs.
○, □: PPG alone
●, ▨: PPG together with probenecid

Depression of Biliary Excretion of TPG, CPG and PPG by Probenecid

Probenecid is a strong inhibitor of the hepatic as well as renal transport of organic anions. In order to investigate whether probenecid inhibits the excretion of these glucuronides, the following experiment was performed. Each glucuronide (50 μmoles) was administered together with probenecid (50 μmoles) intravenously and bile was collected for 10 min periods for 90 min.

As shown in Figures 4, 5 and 6, probenecid markedly depressed the biliary excretion of TPG, CPG and PPG. The maximal excretion rate of TPG was depressed from 8.9±0.6 to 3.8±0.4 μmoles/10 min, and the proportion of the dose excreted in 90 min declined from 56% to 35%. The maximal excretion rate of CPG was depressed from 13.3±0.3 to 7.5±0.3 μmoles/10 min and the proportion of the dose excreted in 90 min declined from 84% to 77%. The maximal excretion rate of PPG was also depressed from 7.6±0.4 to 5.7±0.2 μmoles/10 min and the proportion of the dose excreted in 90 min declined from 73% to 64%. Although the inhibitory effect of probenecid on the biliary excretion of the glucuronides was marked in the early stage of the excretion, the effect may become less in the latter stage and the excretion rates of CPG and PPG in this stage were increased more than those in the early stage.

Discussion

Several evidences suggest that TPG, CPG and PPG are excreted into bile by an active transport process: these glucuronides were transferred from blood to bile against a large concentration gradient; in addition, their transport process is saturable; their excretion is depressed by probenecid.

The transport maxima (Tms) in the biliary excretion of the glucuronides were observed when administered thiamphenicol (TP) or chloramphenicol (CP), which may be resulted from a saturation of conjugating process.[11] The Tms obtained when administered TP or CP (about 5.1 μmoles/10 min for TP and about 8.6 μmoles/10 min for CP) are very low in comparison with those obtained when administered TPG or CPG (about 22.6 μmoles/10 min for TPG and about 25.5 μmoles/10 min for CPG). From these results, it appeared that the rate-limiting step in the biliary excretion of TP and CP which are converted to glucuronides before they are excreted was not due to the transport process into bile of the formed glucuronides but to the conjugating process. In a study of the biliary excretion of phenolphthalein (PP), 4-methylumbelliferone and 8-hydroxychinoline, Mulder[6] suggested that the rate-limiting step in the biliary excretion of the compounds was not due to the conjugating process but to the transport process of the formed glucuronides. We anticipate that the Tms of the glucuronides of such compounds may be nearly equal to or less than those obtained when such aglycones were administered. Actually, it was observed that the Tm of PPG obtained when administered PP was nearly equal to that obtained when administered PPG in renal pedicles ligated rats in our laboratory (unpublished results).

Although the Tm of PPG which remained a constant value for a certain period was obtained at a relatively low dose, relatively high dose was required to yield the Tms for TPG and CPG: the Tms of these two glucuronides did not remain a constant value by a high dose as 200 μmoles. The distribution ratios between organic solvent and phosphate buffer, pH 7.4, of these glucuronides are shown in Table IV. The binding ratios to rat liver homogenate and the uptake by rat liver slices of these glucuronides are shown in Table V. These results may suggest that PPG has a higher rate of penetration into the liver than other two glucuronides, and that a high concentration of PPG in the liver enough to yield the Tm may be brought about by its relatively low dose. The Tms of these three glucuronides might be due to the transport process from the liver cell to the lumen of the bile canaliculus and affected by the

11) T. Uesugi, M. Ikeda, Y. Kanei, R. Hori, and T. Arita, *Biochem., Pharmacol.*, "in press".

Table IV. Distribution Ratios of Glucuronides between Chloroform and Phosphate Buffer, pH 7.4

Glucuronide	$C_{chloroform}/C_{buffer}$
TPG	0.0016
CPG	0.0043
PPG	0.0076

A solution (5 ml) of glucuronide in 1/15M phosphate buffer, pH 7.4, was shaken with 5 ml of chloroform for 45 min at 25°. The glucuronide remaining in the aqueous layer was determined.

Table V. Binding with Rat Liver Homogenate and Uptake by Liver Slices of Glucuronides

Glucuronide	Binding ratio with liver homogenate, %	Uptake by liver slices, %	
		10 min	30 min
TPG	1.08	0.00	0.40
CPG	8.30	3.47	5.11
PPG	38.3	10.1	15.3

Binding with liver homogenate: Three ml of 1/10M phosphate buffer, pH 7.4, containing 2 μmoles glucuronide was mixed with 1 ml of 50% liver homogenate containing *d*-saccharic acid-1,4-lactone (2 μmoles), and ultrafiltered for 3 hr in Visking tube at 4,200 rev/min at 4°. The external solution was determined for glucuronide.

Uptake by liver slices: Rat liver slices in Krebs–Ringer solution, pH 7.4, containing *d*-saccharic acid-1,4-lactone (2 μmoles) were incubated initially for 10 min at 37°. Glucuronide (2 μmoles) were then added and the incubation was continued for an additional 10 or 30 min. The supernatant solution after incubation was determined for glucuronide. All values were the average of 3—4 experiments.

affinity of the glucuronides for intrahepatic tissue components and/or by other factors.

Since probenecid is widely used to inhibit the transport of some organic anions, it is important to note that, at least in the rat, the compound is mostly biotransformed and then conjugated with glucuronic acid. Guarino, *et al.* found that the metabolites of probenecid in the rat bile were the unchanged drug, N-depropylated drug and three glucuronides,[12a] and that the greater part of an injected dose appeared in the bile as the glucuronides.[12b] Thus, the depression of the biliary excretion of TPG, CPG and PPG by probenecid might be due to a competition between these glucuronides and the glucuronides of probenecid.

Acknowledgments We wish to thank Professor Takaichi Arita of Hokkaido University and Professor Ryohei Hori of Hiroshima University for helpful advice and discussion.

12) *a*) A.M. Guarino, W.D. Conway, and H.M. Fales, *Eur. J. Pharmacol.*, **8**, 244 (1969); *b*) A.M. Guarino and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **164**, 387 (1968).