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Metabolism of Thiamphenicol and Comparative Studies of Its Urinary and Biliary Excretion with Chloramphenicol in Various Species

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Following the administration of thiamphenicol (TP), the metabolites found in the urine and bile of rat, guinea pig and rabbit, and in the urine of human were unchanged TP, a hydrolysis product of TP, and a conjugate of TP with glucuronic acid. TP glucuronide was isolated from the guinea pig urine and bile, and identified.

The urinary and/or biliary excretion of TP metabolites in rat, guinea pig, rabbit and human was also studied by comparison with chloramphenicol (CP). The major route of the excretion of TP in human, rabbit and rat was by way of the kidneys, and the most of the drug was excreted in the unchanged form. In guinea pig, TP was excreted mainly into the bile and the most of the drug excreted was a glucuronide. On the other hand, CP was extensively conjugated and metabolized in all the animals used. In human, rabbit and guinea pig the major route of the excretion of CP was by way of the kidneys, and in rat by way of the bile and the excreted form was mostly a glucuronide.

Thiamphenicol (TP), synthesized by Cutler, *et al.*,[2] is an analog of chloramphenicol (CP) and has been used clinically as an effective antibacterial agent. It has been reported[3] that the compound was metabolized only to a slight degree in several animals. In the previous paper,[4] we reported that TP was conjugated with glucuronic acid to a not inconsiderable extent in rat and that the glucuronide was excreted mainly into bile. It is interesting to investigate the excretion of TP metabolites into urine and bile of several species. On the other hand, although CP metabolism in several animals has been well defined,[5] the biliary excretion of the metabolites in several animal species except rat[5c] has been known only to a slight extent. In the present report, isolation and identification of TP metabolites and comparative studies on the excretion of TP and CP metabolites in human, rabbit, guinea pig and rat were described.

Experimental[6]

Materials——Thiamphenicol (TP), kindly supplied by Eisai Co., Ltd., was recrystallized from water, mp 165—166°, and chloramphenicol (CP), mp 150—151°, was commercial product. D-*threo*-2-Amino-1-(4-methylsulfonyl)-1,3-propanediol (deacyl-TP) was prepared by the method of Cutler, *et al.*,[2] mp 141—143°. *p*-Methylsulfonylbenzaldehyde (MSBA) was prepared by the method of McChesney, *et al.*,[7] mp 155—156°.

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2) R.A. Cutler, R.J. Stenger and C.M. Suter, *J. Am. Chem. Soc.*, **74**, 5475 (1952).
3) E.W. McChesney, R.F. Koss, J.M. Shekosky and W.H. Deitz, *J. Am. Pharm. Assoc.*, **49**, 762 (1960).
4) T. Uesugi, M. Ikeda, Y. Kanei, R. Hori and T. Arita, *Biochem. Pharmacol.*, **23**, 2315 (1974).
5) *a)* A.J. Glazko, L.M. Wolf, W.A. Dill and A.C. Bratton, *J. Pharmacol. Exptl. Therap.*, **96**, 445 (1949); *b)* A.J. Glazko, W.A. Dill and M.C. Rebstock, *J. Biol. Chem.*, **183**, 679 (1950); *c)* A.J. Glazko, W.A. Dill and L.M. Wolf, *J. Pharmacol. Exptl. Therap.*, **104**, 452 (1952).
6) All melting points were uncorrected. Ultraviolet (UV) spectra were determined by a Hitachi Model EPS-3T spectrophotometer. Chemical assay of drugs was carried by a Hitachi Model 124 spectrophotometer.
7) E.W. McChesney, J.M. Shekosky, H.W. Eckert and R.F. Koss, *J. Am. Pharm, Assoc.*, **49**, 28 (1960).

Chloramphenicol glucuronide (CPG) was isolated by the method of Glazko, *et al.*[5] Azobenzenephenylhydrazinesulfonic acid diethanolamine salt (APHS reagent), mp 151—152°, was prepared by the method as reported previously.[8]

Human Studies——In a dose of 500 mg, powdered TP was given orally to each of 4 male human subjects (weighing 55 to 60 kg) after fasting overnight. The urine was collected for 24 hr after administration. After a rest period of 1 week, the human subjects were given orally powdered CP in a dose of 500 mg, and the urine was collected for 24 hr after administration.

Animal Studies——Four male rabbits weighing 2.5—3.5 kg were used after fasting for 18 hr. The animals were anesthetized with sodium pentobarbital (40 mg/kg) and urethane (750 mg/kg). Through an abdominal incision, the common bile duct was cannulated with a polyethylene tubing (the inside diameter: 2.0 mm). After the incision had been closed, TP or CP dissolved in physiological saline was administered intravenously via the femoral vein in a dose of 12.50 mg/kg or 11.25 mg/kg, respectively. The bile was collected for 7 hr under anesthetization. The urine was collected for 7 hr from the urinary bladder through Nelaton's catheter. A thermister prove was placed on the surface of liver, and body temperature was maintained throughout the experiments at $38\pm1°$ with a heating lamp.

Ten male guinea pigs weighing 400—500 g were used after fasting for about 18 hr. The animals were anesthetized with sodium pentobarbital (40 mg/kg) and urethane (750 mg/kg). For the collection of bile, the common bile duct was cannulated with a polyethylene tubing (the inside diameter: 1.5 mm) through an abdominal incision. Urine was allowed to excrete naturally during an experimental period, and after the experiment the urinary bladder was removed and washed with water. In a dose of 100 mg/kg, TP or CP dissolved in 20% propylene glycol was administered intraduodenally to six of the animals. To maintain the physiological conditions as far as possible, the animal was warmed with a heating lamp and a blanket through the experimental period. Bile and urine were collected for 24 hr under anesthetization. To 4 animals, 100 mg/kg of TP or CP dissolved in physiological saline was administered intravenously *via* the femoral vein. Bile and urine were collected for 7 hr under anesthetization. Body temperature (liver surface) was maintained at $38\pm1°$ with a heating lamp.

Seven male rats weighing 340—360 g were used after fasting about 18 hr. The animals were anesthetized with ether. For the collection of bile, the common bile duct was cannulated with a polyethylene tubing (the inside diameter: 0.8 mm) through an abdominal incision. After the incision had been closed, the animals were put into restraining cages, and the end of the tube was inserted into a flusk to collect bile during 24 hr. Urine was collected in a polyethylene bag fitted beneath the restraining cage, and after the experiment the urinary bladder was removed and washed with water. TP or CP dissolved in 20% propylene glycol was injected intraduodenally to the animals in a dose of 100 mg/kg. The animal was warmed with a heating lamp and a blanket through the experimental period.

Thin-Layer Chromatography (TLC)——Metabolites were separated on thin-layer plates of silica gel (Merck, Kieselgel HF_{254}). The solvent systems employed were shown in Table I, and *Rf* values of TP and other relevant compounds were shown in Table II. Chromatograms were examined under ultraviolet light (254 mμ). TP and its metabolites quenched the background fluorescence of the plate and appeared as dark spots. TP glucuronide (TPG) gives a purple color with naphthoresorcinol. 2,4-Dinitrophenylhydrazine in 2N HCl (0.4 g/100 ml) was used to detect MSBA.

Identification Procedure of TP Metabolites——The following tests were used for identification of metabolites; the metabolites were eluted from the silica gel plates with MeOH and the eluate was concentrated and re-chromatographed in several solvent systems under comparison with the authentic compounds. The MeOH eluate was evaporated to dryness under reduced pressure, the residue was dissolved in a suitable amount of water and then treated as described below.

Detection of TP: One ml of sample solution was treated with 1 ml of 1N NaOH at room temperature for 60 min. The mixture was neutralized with 0.5 ml of 1 M NaH_2PO_4 solution. To the mixture, 5 ml of ethylene dichloride and 3 ml of 0.5% $NaIO_4$ solution were added. The mixture was shaken mechanically for 10 min and then centrifuged for 2 min. The organic layer containing MSBA was evaporated to dryness under reduced pressure and the residue was examined by TLC with solvent system C.

Detection of Deacyl-TP: Three ml of 1M phosphate buffer (pH 7) and 5 ml of ethylene dichloride were added to 1 ml of sample solution. To the mixture, 1 ml of 0.5% $NaIO_4$ solution was added and then the mixture was shaken mechanically for 10 min. The organic layer containing MSBA was examined by TLC with solvent system C.

Hydrolysis of TPG with β-Glucuronidase: TPG isolated from guinea pig urine and bile was dissolved in 5 ml of 0.2M acetate buffer (pH 5). The solution was incubated at 37° for 2 hr together with 1 ml of β-glucuronidase (13000 Fishman units/ml, Tokyo Zoki). After the incubation the mixture was extracted by mechanical shaking with 20 ml of ethyl acetate. The extract, after being concentrated under reduced pressure, was examined by chromatography with solvent system A and C.

8) T. Uesugi, R. Hori and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **21**, 570 (1973).

Table I. Solvent Systems for Thin-Layer Chromatography

Systems	Contents	Ratios
A	*n*-BuOH:H_2O:AcOH	13:2:1
B	$CHCl_3$:MeOH:28%NH_4OH	20:9:2
C	EtOAc	—
D	$CHCl_3$:MeOH:28%NH_4OH	10:6:1
E	$CHCl_3$:MeOH:AcOH:H_2O	20:6:1:1
F	*n*-BuOH:EtOH:28%NH_4OH:H_2O	4:4:1:2

Table II. Thin-Layer Chromatography of Compounds Related to TP

Compound	Solvent[a)]	*Rf*					
		A	B	C	D	E	F
TP		0.74	0.92	0.50	1.00	0.67	—
Deacyl-TP		0.25	0.75	—	0.88	0.23	—
TPG		0.14	0.15	—	0.26	0.30	0.44
MSBA		—	—	0.80	—	—	—

a) See Table I for composition of solvents.

Separatory Determination of TP Metabolites——TP metabolites in urine and bile of various species were determined by the methods as described below. The amount of TPG was given by the substraction of the amounts of unchanged TP and deacyl-TP from that of total TP as TP equivalent. Since deacyl-TP was observed to be present very slightly except in human urine, it was not measured in other species.

Determination of Total TP: In a glass-stoppered test tube, 2 ml of diluted urine or bile and 2 ml of 0.5N NaOH were placed. The tube was heated in a boiling water bath for 10 min, and then cooled to room temperature. To the tube 2 ml of 1N HCl was added. The acidic solution was washed by shaking with 5 ml of ethyl acetate for 5 min. After the mixture was separated into two layers, the organic layer was removed by aspiration. The aqueous layer was washed again by shaking with 4 ml of ethylene dichloride for 5 min. After centrifugation, 5 ml of the aqueous layer was transferred to a glass-stoppered test tube which contained 3 ml of 1M phosphate buffer (pH 10) and 5 ml of ethylene dichloride. Three ml of 0.5% $NaIO_4$ solution was added into the tube, and then the tube was shaken mechanically for 10 min to extract MSBA occurred. The MSBA in the organic layer was measured by the colorimetric method using APHS reagent as reported previously.[8)]

Determination of Unchanged TP: To 1 ml of diluted urine or bile in a 50 ml glass-stoppered centrifuge tube were added 20 ml of ethyl acetate and 2 ml of 5% $NaHCO_3$ solution. The tube was shaken for 10 min and centrifuged, and 18 ml of the organic layer was transferred to a tube which contained 3 ml of 0.5N HCl. The tube was shaken for 5 min and centrifuged. Fifteen ml of the organic layer was transferred to a 30 ml flask and evaporated to dryness under reduced pressure at 40°. The residue was dissolved in 5 ml of 0.2N NaOH and heated in a boiling water bath for 10 min. The alkaline solution was acidified with 1 ml of 2N HCl. The solution was washed with ethyl acetate and with ethylene dichloride, and then treated by the same procedure as for total TP assay.

Determination of Deacyl-TP: The determination procedure was the same as for total TP except that the initial alkaline hydrolysis was omitted. Five ml of diluted urine or bile was acidified with 1 ml of 1N HCl. The solution was washed with ethyl acetate and with ethylene dichloride, and then treated by the same procedure as for total TP assay. The recovery of deacyl-TP from the mixture containing the metabolites in the various concentrations was about 100%.

Separatory Determination of CP Metabolites——CP metabolites in urine and bile of various species were determined by the modified method of Levine's,[9)] Maruyama's[10)] and Glazko's[11)] methods. For the reduction of nitro group of CP metabolites, 2% hydrosulfite solution was used. After removing the biological blanks in urine or bile with ethyl acetate, the aqueous layer was treated by 1% $NaNO_2$ and 1.5% ammonium sul-

9) J. Levine and H. Fishbach, *Antibiotics Chemotherapy*, **1**, 59 (1951).
10) M. Maruyama and Y. Suzuki, *Takamine Kenkyusho Nempo*, **10**, 158 (1958).
11) A.J. Glazko, L.M. Wolf and W.A. Dill, *Arch. Biochem.*, **23**, 411 (1949).

famate solution and colored with 0.2% Tsuda's reagent.[12] For a rapid assay the color development was carried out at 50° for exactly 30 min. The absorbance was read at 558 mμ against a blank prepared through the same procedure. For the extracting solvent for unchanged CP, ethylene dichloride was used. The urinary and biliary excretion of CP glucuronide (CPG) in several species is often 10-fold greater than that of unchanged CP.[4,5] Ethyl acetate has usually been used for the extracting solvent for unchanged CP. When ethyl acetate is used for the extraction of unchanged CP, a contamination of CPG can not been avoided. On the other hand, the use of ethylene dichloride eliminated this problem. The recovery of CP from bile of several animal species containing 500 or 1000 μg of CPG was about 100%. Total aryl amines were determined by the same procedure as for total CP except that the initial reduction with hydrosulfite was omitted. The amount of CPG was given by the substraction of the amount of unchanged CP and total aryl amines from that of total CP as CP equivalent.

***In Vitro* Glucuronide Formation**——Animals were decapitated, and the liver was removed and homogenized with cold isotonic KCl. An incubation mixture containing 10% homogenate (1 ml), 2 μmoles UDP-glucuronic acid, 0.66 μmole substrate and 200 μmoles tris(hydroxymethyl)aminomethane buffer (pH 7.4), in a final volume of 3 ml, was incubated for 20 min at 37°.

Isolation and Identification of TP Metabolites——Isolation of TP: The presence of unchanged TP in human and rabbit urine was definitely established by the isolation and crystallization. Five male human subjects each took 1 g of TP orally and their urine was collected for 12 hr. Approximately 4 liters of urine at pH 7 was extracted with 4 liters of ethyl acetate. The ethyl acetate extract was evaporated to dryness under reduced pressure at room temperature. The residue was extracted with 10 ml of hot ethylene dichloride and the solution was clarified with charcoal and allowed to stand overnight at 0°. The crystals separated were filtered and recrystallized from water to give colorless crystals, mp 165—166°. The substance produced no depression in melting point when mixed with TP. Unchanged TP was isolated also from rabbit urine. Urine was collected from 4 rabbits over a 12 hr period following oral administration of 250 mg of TP, and treated similarly as for human urine.

Identification of Deacyl-TP [D-*threo*-2-amino-1-(4-methylsulfonylphenyl)-1,3-propanediol]: This compound is readily obtained by acid or alkaline hydrolysis of TP. Urine and bile from rabbit, guinea pig and rat, and urine from human showed the presence of the compound. The compound was identified with authentic sample by comparing of the *Rf* values, as shown in Table II. Ultraviolet spectrum of the EtOH eluate of the spot had λ_{max}^{EtOH} at 224, 254, 261.5, 266 and 273 mμ which was same as that of authentic deacyl-TP. The compound was readily converted to MSBA by the detection method of deacyl-TP as shown in the preceding section.

Isolation of TPG: Urine and bile were collected from a series of guinea pigs over an 8 hr period following both intravenous and intraduodenal administrations of 50 mg of TP. The samples were combined and evaporated to dryness under reduced pressure at 30°. Two handred ml of MeOH was added to the residue and the precipitated inorganic material was removed by filtration. The MeOH solution was concentrated under reduced pressure at 30°, banded on thin-layer plates of silica gel and developed in solvent system A. The band corresponding to the glucuronide (identified by examining the plate under ultraviolet light and spraying with naphthoresorcinol as described in the preceding section) was scraped from the plates, suspended in MeOH and filtered. The sirupy residue was dissolved in 50 ml of water. The solution was brought to pH 7 with NH_4OH and treated with saturated basic lead acetate solution until no further precipitation occurred. The precipitates were collected and washed with water, and then with MeOH. The lead salt was suspended in 50 ml of 1% $NaHCO_3$ solution and treated with H_2S. After removal of PbS by filtration, the filtrate was evaporated to dryness under reduced pressure at 30° to give crude sodium TP glucuronide. The salt in MeOH was re-banded on thin-layer plates and developed in solvent system E. The band corresponding to the glucuronide was scraped from the plates, suspended in MeOH (100 ml) and filtered. The eluate was evaporated to dryness under reduced pressure to give the sodium salt of TP glucuronide. This material was recrystallized from MeOH containing EtOH to give colorless crystals, mp 190—192.° UV λ_{max}^{MeOH} mμ: 224, 255, 261.5, 266 and 273. *Anal.* Calcd. for $C_{18}H_{22}O_{11}NSCl_2Na$: C, 39.00; H, 4.00; N, 2.53. Found: C, 38.68; H, 4.27; N, 2.63. The compound obtained above gave a purple color with naphthoresorcinol and yielded glucuronic acid[13] and TP after hydrolysis with 1N HCl at 100° for 30 min or with β-glucuronidase.

Results

TP Metabolites in Urine and Bile of Various Species

In the previous section, the metabolites of TP in rat, guinea pig, rabbit and human were investigated. It was observed that TPG was excreted into the urine and bile of rat and guinea

12) K. Tsuda and S. Matsunaga, *Yakugaku Zasshi*, 62, 362 (1942).
13) M. Ishidate and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), 5, 515 (1957).

pig in large quantities and could be isolated from the urine and bile of guinea pig as pure crystalline form. It could be established by TLC and other methods that a small amount of TPG was excreted in the urine of human and in the urine and bile of rabbit. It is interesting from the therapeutic point of view that the excretion of TPG in human was much lower than that of CPG (see next section). Deacyl-TP was detected in the human urine and in the urine and bile of other animal species by TLC and other detection methods. Unchanged TP was detected in the urine and bile of all animals studied and isolated as crystalline form from the urine of human and rabbit.

Urinary Excretion of TP and CP in Human

In Table III, the amounts of the metabolites of TP and CP recovered in the urine in 24 hr after oral administration of TP or CP, 500 mg, were shown. It was observed that the amount of TP recovered was less than that of CP. The similar results were obtained in rat and guinea pig after intraduodenal administration as shown in the later sections, and may be due to the difference in intestinal absorption of TP and CP. The partition coefficients of TP and CP between organic solvents and phosphate buffer (0.2M, pH 7.4), are shown in Table IV and it is clear that CP is about 10—12 times more soluble in organic solvents than TP.

Table III. Urinary Excretion of TP, CP and Their Metabolites in Man[a]

TP:			
% of dose excreted in 24 hr[b]			
Total TP	Unchanged TP	Deacyl-TP	TPG
57.7(43.4—72.6)	55.1(41.1—70.2)	2.1(1.7—2.3)	0.5(0.0— 1.0)
CP:			
% of dose excreted in 24 hr[b]			
Total CP	Unchanged CP	Aryl amines	CPG
86.2(80.0—90.7)	12.9(10.1—15.7)	5.1(1.3—9.0)	68.2(63.8—76.7)

a) TP or CP, 500 mg, was given orally.
b) Results were shown as the mean for four men with the ranges in parentheses.

Table IV. Partition Coefficients[a]

Substance	K_{CHCl_3}[b]	$K_{Benzene}$[b]
TP	0.0308	0.0044
CP	0.2974	0.0527

a) Five ml of drug solution (2×10^{-4}M) was shaken with 5 ml of organic solvent at 37°, and drug content in organic and aqueous layers was determined after equilibrium was reached.
b) $K=\frac{\text{conc. of drug in organic layer}}{\text{conc. of drug in aqueous layer}}$

The most pronounced difference in the excretion of TP and CP was observed in the amount recovered of their metabolites: during 24 hr after a single dose of TP, about 4.5% of the total amount recovered in urine was excreted as its metabolites and only about a quarter of them was the glucuronide; about 85% of the total amount recovered of CP in urine was excreted as its metabolites and most of the metabolites was the glucuronide.

Urinary and Biliary Excretion of TP and CP in Rabbit

Figure 1 shows the amounts of the metabolites of TP and CP recovered in the urine and bile after intravenous administration of TP (12.50 mg/kg) or CP (11.25 mg/kg) in rabbit

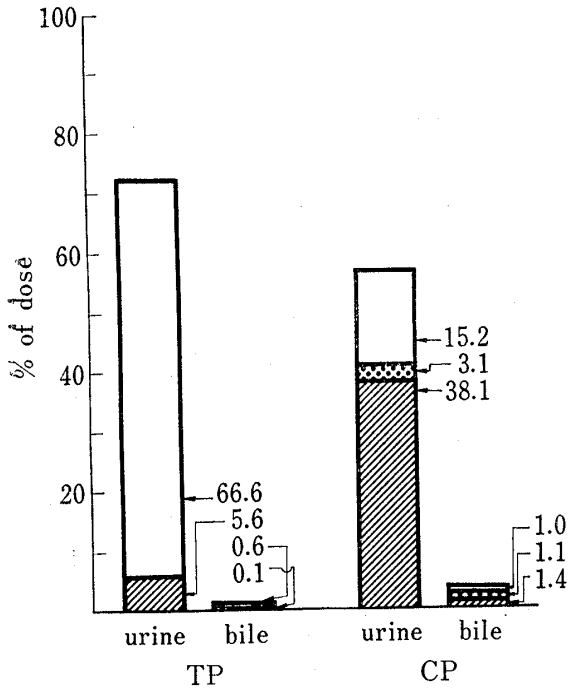

Fig. 1. Urinary and Biliary Excretion of TP, CP and Their Metabolites after Intravenous Administration of TP and CP to Rabbit

□: unchanged, ▨: glucuronide, ▩: aryl amines
Results were shown as the mean for 2 animals.
dose: TP-12.0 mg/kg, CP-11.25 mg/kg; total amount recovered in 7 hr: TP-72.9%, CP-59.9%

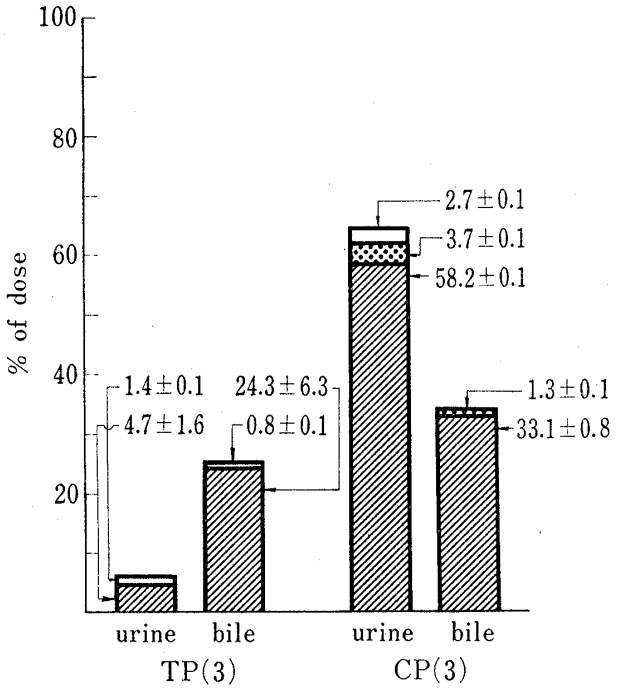

Fig. 2. Urinary and Biliary Excretion of TP, CP and Their Metabolites after Intraduodenal Administration of TP and CP to Guinea Pig

Results were shown as the mean ± SE with number of animals in parentheses. See explanation for bar graph of Fig. 1. dose: 100 mg/kg; total amount recovered in 24 hr: TP-31.2%, CP-99.0%

in 7 hr. Total amount recovered in urine and bile was about 73% for TP and about 60% for CP, and the percentage of glucuronide to total amount recovered was about 8% for TP and about 66% for CP, respectively. The poor excretion of TPG was also observed in rabbit as well as in human. In another experiments, the ability of the rabbit liver homogenates to conjugate TP and CP was determined. The glucuronide conjugating activity (μmole/g wet liver weight) of the rabbit liver was 0.083 for TP and 0.912 for CP. From these results it is considered that the poor ability of human and rabbit to form the glucuronide from TP may be not due to the nature and amount of the conjugating enzyme but to the physicochemical properties of the compound. Furthermore, the poor excretion of TP and CP into the bile was observed in this animal. The recovery of TP into the bile was only 1% of dose and most of it was unchanged TP, and only about 3% of dose of CP was excreted into the bile and about two-thirds of it was the metabolites.

Urinary and Biliary Excretion of TP and CP in Guinea Pig

The amounts of the metabolites of TP and CP recovered in the urine and bile in 24 hr after intraduodenal administration of TP or CP, 100 mg/kg, in guinea pig are shown in Fig. 2. Total amount recovered of TP in urine and bile was about 31% and that of CP about 99%. The percentage of the glucuronide to the total amount recovered was about 93% for TP and about 92% for CP, respectively.

The amount recovered after intravenous administration of TP, 100 mg/kg, was also determined (Fig. 3). The amount recovered in 7 hr was 84% and the percentage of the glucuronide to the total amount recovered was about 86%.

From these results it is concluded that the differences in the recoveries of TP and CP observed in human and guinea pig were due to the difference in the absorption rates from gastrointestinal tract of TP and CP. It is considered, however, that such a low recovery

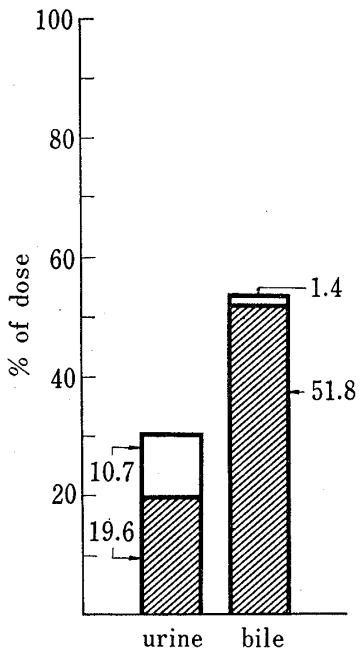

Fig. 3. Urinary and Biliary Excretion of TP and Its Metabolites after Intravenous Administration of TP to Guinea Pig

Results were shown as the mean for 2 animals. See explanation for bar graph of Fig. 1. dose: 100 mg/kg; total amount recovered in 7 hr: 83.5% of the dose

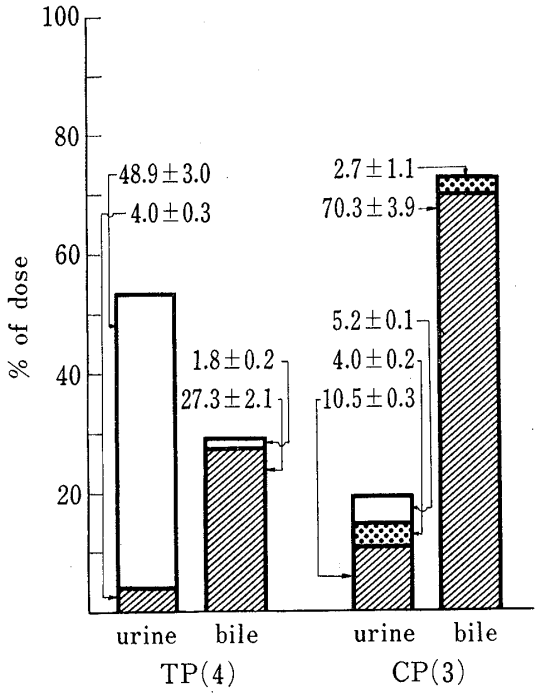

Fig. 4. Urinary and Biliary Excretion of TP, CP and Their Metabolites after Intraduodenal Administration of TP and CP to Rat

Results were shown as the mean ± SE with number of animals in parentheses. See explanation for bar graph of Fig. 1. dose: 100 mg/kg; total recovered in 24 hr: TP-82.0%, CP-92.7%

of TP in guinea pig may be also influenced by the food residues, since, in spite of the fact that the animals had been fasted for 18 hr prior to experiment, very bulky food residues remained in all levels of their gastrointestinal tracts.

A considerable amount of TP and CP was excreted into the bile of guinea pig. Most of the TP and CP metabolites excreted into the bile were the glucuronides, and very little was unchanged drugs. About 97% of the amount recovered into the bile of TP and CP was in the form of a glucuronide (Fig. 2 and 3).

Urinary and Biliary Excretion of TP and CP in Rat

In Fig. 4, the amounts of the metabolites of TP and CP recovered in the urine and bile in 24 hr after intraduodenal administration of TP or CP, 100 mg/kg, in rat are shown. Total amount of TP recovered in the urine and bile was about 82% and that of CP was about 93%. The percentage of glucuronide to the total amount recovered was about 38% for TP and 87% for CP, indicating that CP was also conjugated with glucuronic acid to a larger extent than TP in rat. The ability of the rat liver homogenates to conjugate TP and CP was also determined. The rate of CP conjugation (0.245 μmole/g wet liver weight) was about 4.4 times higher than that of TP conjugation (0.056 μmole/g wet liver weight).[4]

The amount of TP excreted into the bile was about 29% of dose and that of CP about 73%, and the percentage of the glucuronide to the amount recovered in the bile was about 94% for TP and about 96% for CP, respectively.

Discussion

Metabolism of TP was studied in human, rabbit, guinea pig and rat. TP is excreted as two metabolites, TPG and deacyl-TP, and unchanged form in all the species. TPG can

be isolated as a pure crystalline form from the urine and bile of guinea pig which excretes efficiently TP as its glucuronide. Deacyl-TP is detected in the urine and bile of all the species by TLC and other chemical methods.

Pronounced differences were found in the excretion of the metabolites of TP and CP in human urine (Table III); only about 4.5% of the total TP amount recovered was excreted as the metabolites, while about 79% of the total CP amount recovered was excreted as the metabolites. The results may be interesting from the therapeutic point of view. The antibacterial active form of TP recovered in the urine is greater than that of CP, so that the former may be considered advantageous in treatment of urinary tract infection.[14]

It was observed that human and rabbit had high glucuronide conjugating ability for CP. However, these species excreted only a few amount of TPG. It has been well known that these species have high glucuronide conjugating ability for other many compounds such as acetaminophen and bilirubin. Thus, the possibility is considered that the poor ability of these species to form TPG may be not due to the nature and amount of the conjugating enzyme but to the physico-chemical properties of the compound, such as the perminability in tissues and the affinity to the enzyme.

The other interesting result in the present studies is the quantitative differences in the urinary and the biliary excretion of TP and CP in rabbit, guinea pig and rat. Milluburn, *et al.*[15] reported that for appreciable biliary excretion the compound should be highly polar and of molecular weight not less than 325 ± 50 or could be converted into such a compound by metabolism. TP and CP do not fit these criteria for their polarity. Indeed, unchanged TP and CP were more largely excreted into the urine than the bile of rabbit, guinea pig and rat, and most of TP and CP excreted into the bile, except TP in rabbit, were their glucuronides which fit in these criteria. It is also interesting to compare the excretion ratios of their glucuronides between in the urine and the bile in these species. In rabbit which has a high ability to form CPG, most of CPG was excreted into the urine. In guinea pig which has a high ability to form both TPG and CPG, most of TPG was excreted into the bile and most of CPG was excreted into the urine. It is known in general that all of glucuronides do not undergo the active excretion by the kidney tubules, and that glucuronides of relatively high molecular weight may not be actively excreted by the kidneys, and are cleared into the urine by glomerular filtration, whereas glucuronides of relatively low molecular weight are frequently actively excreted by the kidney tubules.[16] For example, sulfadimethoxine-N^1-glucuronide (mol. wt., 486) is excreted into the urine by glomerular filtration in dog[17] and *o*-aminobenzoic acid glucuronide (mol. wt., 313) undergoes active excretion in the same animal.[18] According to this suggestion, TPG (mol. wt., 532) and CPG (mol. wt., 499) might be excreted into the urine by glomerular filtration. However, any report on the species differences in the urinary excretion of glucuronides has not yet been shown. On the other hand, it has been known that the species differences are found in the biliary excretion of glucuronides,[19] and that several glucuronides are actively excreted into the bile in rat.[20] In the previous paper,[4] it was indicated that the glucuronide conjugating process of TP and CP was a rate-limiting step in the biliary excretion of the drugs in rat. However, the biliary excretion mechanism

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of glucuronides in other species has not also been known. From these results, it is suggested that the quantitative differences in the urinary and biliary excretion of TP and CP in these species may be not only due to the glucuronide conjugating ability but also due to the differences in the urinary and biliary excretion mechanisms.

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