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Biliary excretion of barbiturates

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Summary

1. Pentobarbitone and phenobarbitone are excreted into the bile of rats.
2. The concentration of pentobarbitone and/or its metabolites in the bile is 22-fold higher than in the plasma; the concentration of phenobarbitone and/or its metabolites in bile is 10-fold higher than in the plasma.
3. Probenecid decreases the excretion of the barbiturates into the bile.
4. Twenty-eight per cent of a 35 mg/kg dose of pentobarbitone and 18% of a 75 mg/kg dose of phenobarbitone is excreted in the bile in 6 hours.
5. Most of the pentobarbitone and phenobarbitone excreted into the bile is not in the form of the parent compound but rather as a more polar metabolite or metabolites.

Introduction

Barbiturates are generally considered to be removed from the body by renal excretion, either as the parent compound and/or as a metabolite. However, Maynert (1965) and Lous (1954) have reported that less than 50% of the administered dose of pentobarbitone and phenobarbitone was excreted in the urine, which leads one to suspect that barbiturates may also be excreted into the bile. Therefore, the purpose of this investigation is to determine if two barbiturates, pentobarbitone and phenobarbitone, are excreted into the bile of rats.

Methods

Animals and surgical procedure

Simonsen Sprague-Dawley male rats (250–350 g) were used throughout. The rats were anaesthetized with urethane (1,000 mg/kg, i.p.). The femoral vein and artery were cannulated with polyethylene tubing (PE-50) for administering the barbiturates and obtaining the blood samples, respectively. The bile duct was surgically exposed by a midline incision and cannulated with PE-10 tubing. Alteration in biliary flow due to hypothermia (Roberts, Klaassen & Plaa, 1967) was prevented by maintaining rectal temperature at 37° C with a heat lamp-temperature regulating device. Pentobarbitone sodium, obtained from Abbott Laboratories (North Chicago, Illinois), was mixed with 2-^{14}C-5-ethyl-5-(1-methylbutyl) barbituric acid (New England Nuclear, Boston, Mass.) to give a final specific activity of 36 μCi/g. Phenobarbitone sodium, obtained from Merck and Co., Inc. (Rahway, N.J.), was mixed with 2-^{14}C-5-ethyl-5-phenylbarbituric acid (New England Nuclear, Boston, Mass.) to give a final specific activity of 17 μCi/g. Probenecid was obtained from Merck Institute for Therapeutic Research (Rahway, N.J.).

Drug administration and sample collection

Probenecid (32 mg/kg) was administered intravenously immediately before the intravenous administration of pentobarbitone (35 mg/kg) or phenobarbitone (75 mg/kg). Blood samples (approximately 0·3 ml) were obtained from the cannulated femoral artery after 2, 15, 30, 45, 60 min and at hourly intervals for 6 h after administration of the barbiturate. Bile samples were taken at 15 min intervals for 1 h and at hourly intervals for the next 5 hours.

Quantitative methods

Bile volume for each collection period was measured with a graduated pipette. The total concentration of ^{14}C-pentobarbitone or ^{14}C-phenobarbitone and its metabolites in the plasma and bile was determined by adding 100 μl of plasma or 50 μl of bile to 1 ml of NCS tissue solubilizer (Nuclear Chicago Corporation, Des Plaines, Ill.) and allowing the mixture to stand overnight before addition of 10 ml of the scintillation medium. For measuring the amount of the barbiturates in the liver, 50–75 mg of liver were placed in a counting vial with 2 ml NCS and heated at 37° C in a shaking incubator overnight before addition of the scintillation medium. The scintillation medium consisted of 5 g of 2, 5-diphenyloxazole (PPO) and 0·5g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene dimethyl POPOP) per litre of toluene.

Qualitative methods

To determine if the ^{14}C-pentobarbitone and ^{14}C-phenobarbitone excreted into the bile were biotransformed or in the parent form, the following procedures were used: the thin layer chromatography method of Cochin & Daly (1963) for the separation of pentobarbitone and phenobarbitone and their metabolites; the paper chromatography method of Kuntzman, Ikeda, Jacobson & Conney (1967) for separation of pentobarbitone and its metabolites; the solvent extraction method of Brodie, Burns, Mark, Lief, Bernstein & Papper (1953) for the separation of pentobarbitone and its metabolites; and the extraction methods of Butler, Mahaffee & Waddell (1954) and of Waddell & Butler (1957) for the separation of phenobarbitone from its metabolites. To determine if the barbiturate metabolite may be a glucuronide, the barbiturates excreted in the bile were incubated with β-glucuronidase overnight and then chromatographed by the method of Cochin & Daly (1963).

Statistical analysis

The means were compared by the two tailed Student's t test with $P<0·05$ as the level of significance (Steel & Torrie, 1960).

Results

Figure 1 shows the disappearance from plasma, the concentration in bile and biliary excretion of pentobarbitone, and the bile flow of control rats and rats previously treated with probenecid. The biliary concentration of pentobarbitone reached a maximum of 880 μg/ml about 1 h after its administration, which is considerably slower than the time observed for other compounds that are excreted

into the bile (Klaassen, 1970). Pentobarbitone does not appear to be a choleretic agent in that no increase in bile flow was observed after its administration. Since the bile flow remained relatively constant, the biliary excretory pattern of pentobarbitone parallels the concentration in the bile. Over the 6 h collection period 28% (range 25–33%) of the administered dose of pentobarbitone was excreted into the bile.

Probenecid, which is actively secreted by the organic acid transport system of the liver (Guarino & Schanker, 1968) and competes with excretion of other organic acids for biliary excretion, was administered to determine its effect on the plasma disappearance and biliary excretion of pentobarbitone (Fig. 1). With probenecid, the rate of disappearance of pentobarbitone from plasma tended to decrease but the decrease was not statistically significant. A marked decrease in the biliary

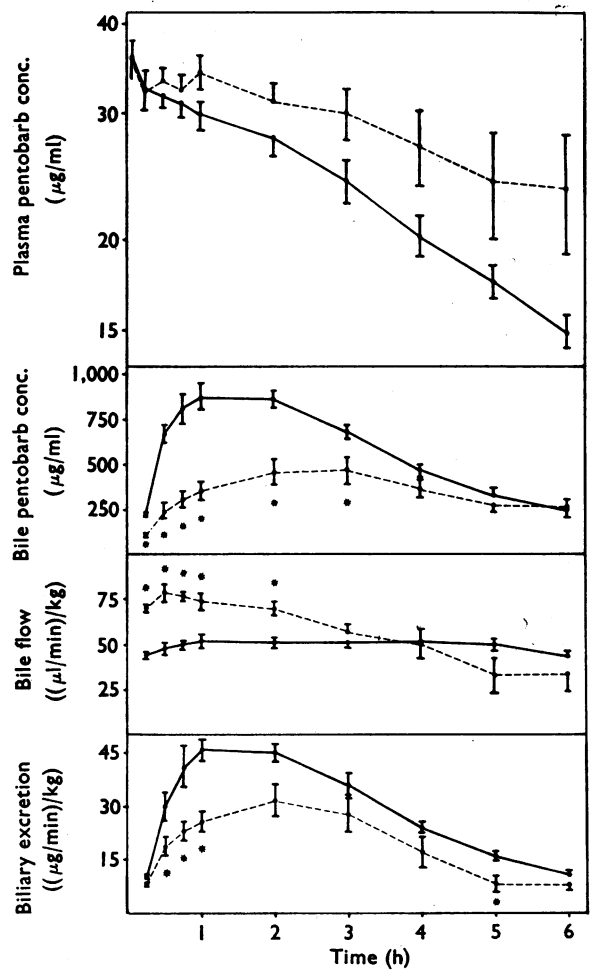

FIG. 1. Disappearance from plasma, biliary concentration and excretion of pentobarbitone (35 mg/kg), and bile flow in rats. Each point represents the mean ± S.E. from six to seven rats. The solid line represents the results from the rats which received only pentobarbitone and the broken lines from the rats that received both pentobarbitone and probenecid. The asterisk indicates that the value obtained in the rats receiving pentobarbitone and probenecid is significantly different from the rats receiving only pentobarbitone ($P<0.05$).

concentration of pentobarbitone was observed for 3 h after probenecid administration. Probenecid increased the biliary flow (Fig. 1); however there was still a decrease in the biliary excretion rate of pentobarbitone in the probenecid treated rats at the 30, 45 and 60 min collection periods.

Table 1 demonstrates the concentration of ^{14}C-pentobarbitone or its metabolites 1 h after its administration in plasma, liver and bile. The concentration in the liver is about 3 times higher and in the bile 22 times higher than the plasma. Thus, the greatest concentration gradient of pentobarbitone is from the liver to the bile rather than from the plasma to liver.

Several procedures were performed to determine if the ^{14}C-pentobarbitone excreted into the bile was a metabolite or in the parent form. With the thin layer chromatography method of Cochin & Daly (1963) little or none of the radioactivity migrated with pentobarbitone, but remained at the origin. With the paper chromatographic method of Kuntzman *et al.* (1967) the radioactivity did not migrate with the parent pentobarbitone or with 5-ethyl-5-(3-hydroxy-1-methylbutyl)-barbituric acid, but also remained at the origin. With the extraction procedure of Brodie *et al.* (1953) little or none of the radioactivity was extracted with the pentobarbitone. After the bile was treated with β-glucuronidase, the radioactivity still remained at or near the origin. These data indicate that the radioactivity excreted into the bile after ^{14}C-pentobarbitone administration is not in the form of the parent compound, of a simple glucuronide or of the simple hydroxylated compound, but of a more polar compound.

Figure 2 demonstrates the results of a similar experiment in which phenobarbitone was used instead of pentobarbitone. The biliary concentration of phenobarbitone reached a maximum of 980 μg/ml 45 min after its administration, but was almost as high at the 30 min interval. This concentration is very similar to the 880 μg/ml seen after pentobarbitone. The plasma concentration of phenobarbitone was much higher than that of pentobarbitone because a larger dose of phenobarbitone was given and its disappearance is slower. The data also confirm the previous observation (Klaassen, 1971) that phenobarbitone does not cause an increase in bile flow as it is excreted into the bile. The biliary excretory rate of phenobarbitone reached a maximum of (50 μg/min)/kg which is very similar to the (45 μg/min)/kg observed for pentobarbitone. Over the 6 h period, 18% (range 14–23%) of the administered phenobarbitone was excreted into the bile. This is less than the 28% observed for pentobarbitone. However, this difference appears to be due to the larger dose of phenobarbitone used, since a larger absolute amount of phenobarbitone was excreted.

The effect of probenecid on the plasma disappearance and biliary excretion of phenobarbitone is also shown in Fig. 2. Probenecid did not significantly alter the

TABLE 1. *Concentration gradients from plasma to bile*

	Pentobarbitone	Phenobarbitone
Plasma conc. (μg/ml)*	38±2†	101±2
Liver conc. (μg/ml)	107±1	197±7
Bile conc. (μg/ml)	823±53	1053±141
Liver/plasma conc.	2·9±0·2	1·9±0·1
Bile/plasma conc.	22±2	10±1

* Concentration of pentobarbitone, phenobarbitone or equimolar concentrations of their metabolites.
† Mean±S.E. of four rats.

disappearance of phenobarbitone from plasma but did decrease the biliary concentration of phenobarbitone for 4 h after phenobarbitone administration. Probenecid significantly enhanced the bile flow; however there was still a decrease in the biliary excretion of phenobarbitone in the probenecid treated rats 30, 45 and 60 min after its administration.

Table 2 demonstrates the concentration of ^{14}C-phenobarbitone or its metabolites 1 h after its administration in plasma, liver and bile. The concentration in the liver is about twice and in the bile 10 times higher than the plasma. Thus the greatest concentration gradient of phenobarbitone, as with pentobarbitone, is from the liver to the bile rather than from the plasma to the liver.

The ^{14}C radioactivity excreted into the bile after ^{14}C-phenobarbitone appears to be in the form of a metabolic product. With the thin layer chromatograph method of Cochin & Daly (1963) little or none of the radioactivity migrates with phenobarbitone and even after treatment with β-glucuronidase the radioactivity remained

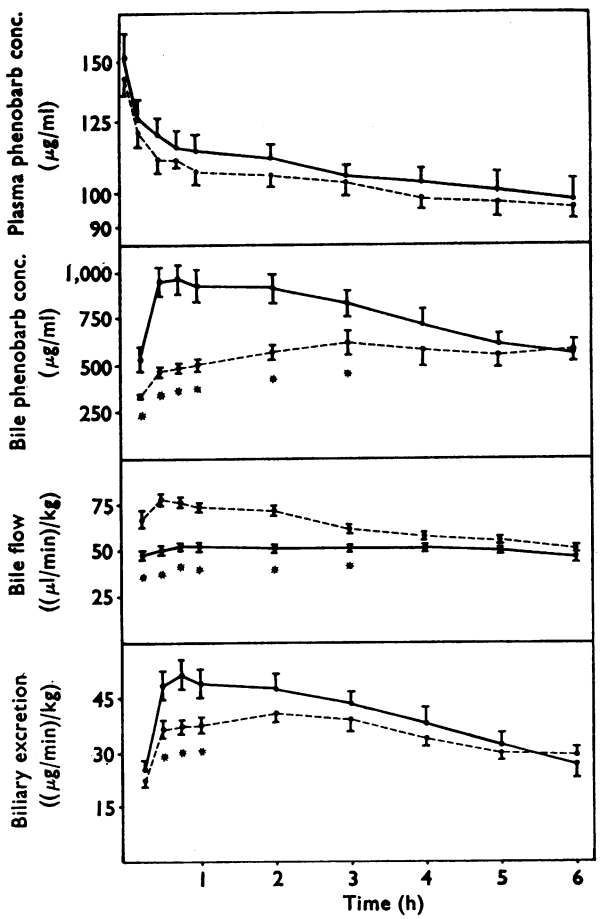

FIG. 2. Disappearance from plasma, biliary concentration and excretion of phenobarbitone (75 mg/kg), and bile flow in rats. Each point represents the mean ± S.E. of six to seven rats. The solid line represents the results from the rats which received only phenobarbitone and the broken lines from the rats that received both phenobarbitone and probenecid. The asterisk indicates that the value obtained in the rats receiving phenobarbitone and probenecid is significantly different from the rats receiving only phenobarbitone ($P<0.05$).

at or near the origin. Also with the extraction procedure of Butler *et al.* (1954) and Waddell & Butler (1957) little or none of the radioactivity was extracted into the organic phase. Thus, the ^{14}C is not in the form of phenobarbitone, a simple glucuronide or the simple p-hydroxylated product, but rather a more polar compound.

Discussion

Barbiturates are usually considered to be eliminated from the body by renal excretion. The study described here demonstrates that the liver also has the capacity to excrete barbiturates. This transport appears to occur by the active transport system that secretes organic acids, since the apparent bile/plasma concentration gradient of 10 and 22 for phenobarbitone and pentobarbitone, respectively, was observed and that probenecid competed for the excretion of the barbiturates. Since there appears to be only one hepatic transport system for the excretion of organic acids, barbiturates probably should be used with caution as an anaesthetic when examining the biliary excretion of organic acids that might have a relatively low affinity for the transport system.

Pentobarbitone and phenobarbitone are excreted into the bile as polar metabolites. Therefore, they probably do not go through an enterohepatic circulation. Quantitatively, it appears that the biliary excretion of barbiturates is significant since 28% of a 35-mg/kg dose of pentobarbitone and 18% of a 75-mg/kg dose of phenobarbitone was excreted into the bile within 6 h of its administration.

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REFERENCES

BRODIE, B. B., BURNS, J. J., MARK, L. C., LIEF, P. A., BERNSTEIN, E. & PAPPER, E. M. (1953). The fate of pentobarbital in man and dog and a method for its administration in biological material. *J. Pharmac. exp. Ther.*, **109**, 26–34.

BUTLER, T. C., MAHAFFEE, C. & WADDELL, W. J. (1954). Phenobarbital: studies on elimination, accumulation, tolerance, and dosage schedules. *J. Pharmac. exp. Ther.*, **111**, 425–435.

COCHIN, J. & DALY, J. W. (1963). The use of thin-layer chromatography for the analysis of drugs. Isolation and identification of barbiturates and nonbarbiturate hypnotics from urine, blood and tissues. *J. Pharmac. exp. Ther.*, **139**, 154–159.

GUARINO, A. M. & SCHANKER, L. S. (1968). Biliary transport of probenecid and its glucuronide. *J. Pharmac. exp. Ther.*, **164**, 387–395.

KLAASSEN, C. D. (1970). Effect of phenobarbital on the plasma disappearance and biliary excretion of drugs in rats. *J. Pharmac. exp. Ther.*, **175**, 289–300.

KLAASSEN, C. D. (1971). Studies on the increased biliary flow produced by phenobarbital in rats. *J. Pharmac. exp. Ther.*, **176**, 743–751.

KUNTZMAN, R., IKEDA, M., JACOBSON, M. & CONNEY, A. H. (1967). A sensitive method for determination and isolation of pentobarbital-C^{14} metabolites and its application to *in vitro* studies of drug metabolism. *J. Pharmac. exp. Ther.*, **157**, 220–226.

LOUS, P. (1954). Plasma levels and urinary excretion of three barbituric acids after oral administration to man. *Acta Pharmac. Tox.*, **10**, 147–165.

MAYNERT, E. W. (1965). The alcoholic metabolites of pentobarbital and amobarbital in man. *J. Pharmac. exp. Ther.*, **150**, 118–121.

ROBERTS, R. J., KLAASSEN, C. D. & PLAA, G. L. (1967). Maximum biliary excretion of bilirubin and sulfobromophthalein during anesthesia-induced alteration of rectal temperature. *Proc. Soc. exp. Biol. Med.*, **125**, 313–316.

STEEL, R. G. D. & TORRIE, J. H. (1960). *Principles and Procedures in Statistics.* New York: McGraw-Hill Book Company.

WADDELL, W. J. & BUTLER, T. C. (1957). The distribution and excretion of phenobarbital. *J. clin. Invest.*, **36**, 1217–1226.

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