Species Difference in the Plasma Disappearance and Biliary Excretion of Procaine Amide Ethobromide[1] (36381)

ANDREA HUNTER[2] AND CURTIS D. KLAASSEN[3]
(Introduced by J. Doull)
Department of Pharmacology, University of Kansas Medical Center, Kansas City, Kansas 66103

Characterization of the biliary excretion in rats of the organic base, procaine amide ethobromide (PAEB), has necessitated proposing a specialized transport system for the biliary excretion of basic compounds by the liver. PAEB appears readily in the bile of rats as the free compound and in a conjugated form, with the bile concentration of the conjugated or free approaching 80 times that of the plasma. The biliary excretion of PAEB has been shown to occur by a saturable process, to be depressed by other quaternary and tertiary bases, and not by acidic compounds such as sulfobromophthalein (1, 2). The purpose of the following study was to ascertain whether or not a species difference existed in the biliary excretion of the organic base PAEB, as has been demonstrated for a number of other organic compounds such as sulfobromophthalein (3) and indocyanine green (4).

Methods. Male Simonsen rats (weighing 260 to 370 g) and rabbits (weighing 1.6 to 2.2 kg) were anesthetized with urethan (900 mg/kg) while mongrel dogs (weighing 7.6 to 11 kg) were anesthetized with pentobarbital sodium (35 mg/kg). The femoral artery, vein, and bile duct were cannulated with the appropriate size of polyethylene tubing. The renal pedicles were ligated because the kidney will also excrete the PAEB. The animals were maintained at 37° with a heat lamp temperature regulator to prevent hypothermic alteration in hepatic function (5).

The PAEB was dissolved in normal saline and administered into the femoral vein during 1 min in doses of 5, 10, and 20 mg/kg to the rats 10 and 20 mg/kg to the rabbits and 10 mg/kg to the dogs.

Bile samples were collected every 10 min after PAEB administration; blood samples were taken from the cannulated artery at 2, 5, 10, 15 and 20 min after administration and then every 10 min for an additional 10, 100 and 220 min in the rat, rabbit and dog, respectively.

The animals were prepared as previously described in the experiments in which the PAEB was measured in the plasma, liver and bile, 20 min after administration. The PAEB (10 mg/kg) was administered via the femoral vein. A bile sample was collected for 10 min, starting 10 min after the PAEB was given, a blood sample was taken 20 min after PAEB administration, and the animals were also sacrificed at 20 min after administration of the PAEB, and the livers were excised.

PAEB was estimated by the colorimetric method of Bratton and Marshall (6). The concentration of unconjugated (free) PAEB and conjugated PAEB was measured in acid filtrates of bile, plasma, and liver homogenates before and after acid hydrolyses, repectively. In a number of pilot experiments it was found that the concentration of free and total PAEB in the plasma were identical, therefore only the free PAEB was measured in the experiments reported. The PAEB was extracted from the liver (Schanker, personal communication) in duplicate from 4 g pieces of liver and homogenized with a motor-driven Potter-Elvehjem homogenizer (Teflon pestle) in 4 ml of normal saline for 90 sec. After addition of 3.7 ml of 10% TCA and 0.5 ml

[1] Supported by U.S. Public Health Service Research Grant AM 14513.

[2] Supported by U.S. Public Health Service Training Grant GM 5T1403.

[3] Supported by U.S. Public Health Service Career Development Award, 1-KO4-GM-30996.

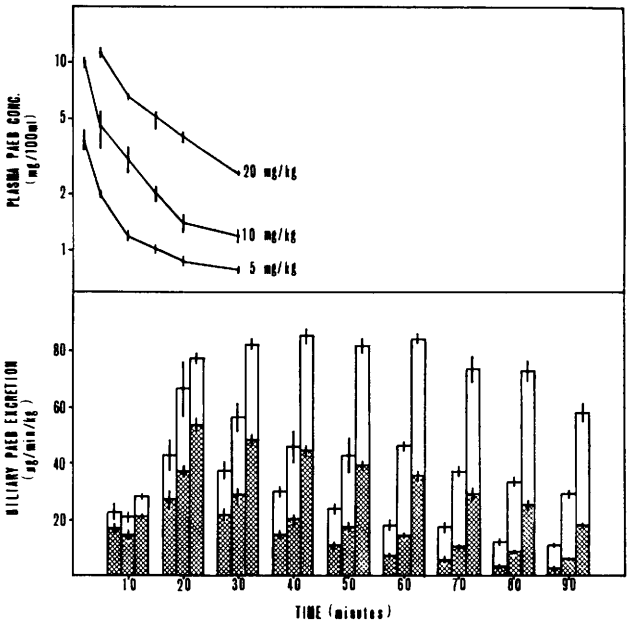

FIG. 1. Plasma disappearance and biliary excretion of PAEB in rats after the intravenous administration of 5, 10 and 20 mg/kg of PAEB. Each group of three bars represent the biliary excretion of PAEB at 5, 10 and 20 mg/kg from left to right. The shaded portion of each bar graph represents the amount of free PAEB and the unshaded portion the conjugated PAEB. Each value represents the mean ± SE of 6 rats.

of HCl, the sample was rehomogenized. The homogenate was then centrifuged and an aliquot of the supernate was analyzed for PAEB.

Results. The plasma disappearance and biliary excretion of PAEB in the rat after the intravenous administration of 5, 10 and 20 mg/kg is depicted in Fig. 1. A definite dose response in the removal of PAEB from the plasma was observed. With the lowest dose, 5 mg/kg, the PAEB rapidly disappeared from the plasma, while with the 10 mg/kg dose the slope of the disappearance curve was less and with the highest dose, 20 mg/kg, the slope was much less.

For all three doses of PAEB, the biliary excretion of PAEB appeared to peak during the 20-min collection period and then decreased. A dose-response relationship for the biliary excretion of PAEB was also observed; that is as the dose was increased a greater increase in the biliary excretion of PAEB was observed. The total (free plus conjugated) PAEB excreted into the bile reached a maximum at 20 min after administration and then gradually declined for the 5 and 10 mg/kg doses: with the 20 mg/kg dose of PAEB, a maximal biliary excretion of PAEB was also observed 20 min after administration but then it remained relatively constant for the next hour, suggesting that the transport system may have been saturated.

Figure 2 demonstrates the plasma disappearance and biliary excretion of PAEB in the rabbit. The PAEB disappeared from the plasma of the rabbits quite rapidly as was observed with the rats. The excretion of PAEB into the bile of the rabbits reached a maximum 20 min after administration and then decreased quite rapidly. Most of the PAEB excreted into the bile of the rabbits was in an unconjugated form, especially during the early time intervals.

The plasma disappearance and biliary excretion of PAEB by the dog is demonstrated in Fig. 3. PAEB disappeared from the plasma of the dog very slowly. One hour after the 10 mg/kg dose of PAEB the plasma level was still 5 mg/100 ml. The excretion of PAEB into the bile of the dog also proceeded

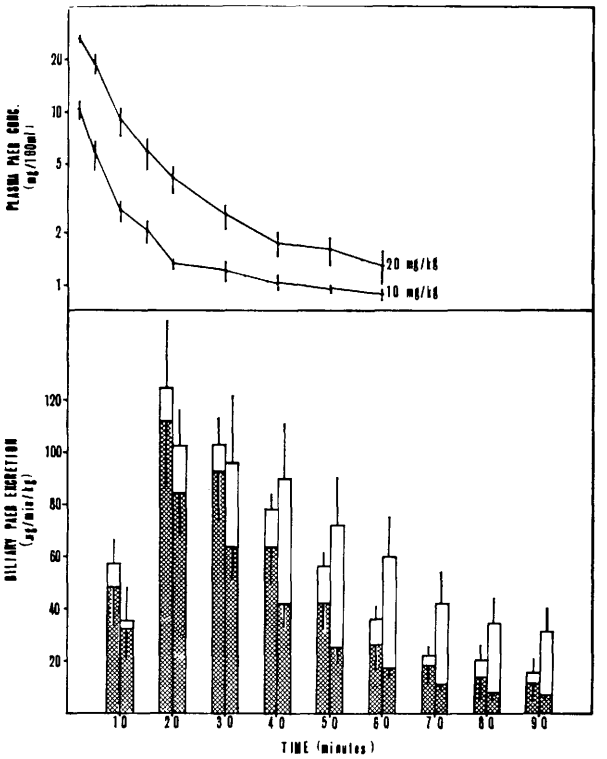

FIG. 2. Plasma disappearance and biliary excretion of PAEB in rabbits after the intravenous administration of 10 and 20 mg/kg of PAEB. The left bar graph of each pair represents the biliary excretion of 10 mg/kg of PAEB and the right bar graph the biliary excretion of 20 mg/kg. The shaded portion of each bar graph represents the amount of free PAEB and the unshaded portion the conjugated PAEB. Each value represents the mean ± SE of 4 rabbits.

at a very slow rate. Most of the PAEB excreted into the bile by the dog was in an unconjugated form. A 20 mg/kg dose of PAEB was also administered to the dogs, but this dose was toxic and the dogs did not survive.

Comparison of the plasma disappearance and biliary excretion of the 10 mg/kg dose of PAEB by the dog, rat and rabbit is depicted in Fig. 4. The disappearance of PAEB from the plasma of the rat and rabbit was very similar, while it was much slower for the dog. The rate of excretion of PAEB into the bile of the dogs was also much slower than for the other two species. More of a difference was observed between the rat and rabbit to excrete PAEB into the bile than was observed in their ability to clear it from the plasma. During the early time intervals the rabbit excreted the PAEB into the bile at a rate almost twice that of rats. However, it should be pointed out that the major difference depicted in Fig. 4 is the relative inability of the dog to excrete PAEB into the bile.

The concentration of PAEB was measured in the plasma, liver and bile 20 min after administration of 10 mg/kg PAEB, in an attempt to determine if the difference between the rat and rabbit compared to the dog was in the passage of the PAEB from the plasma into the liver or from the liver into the bile. The results are shown in Table I. As has been previously demonstrated (1), the liver of the rat is capable of accumulating PAEB. In these experiments the highest hepatic concentration of free PAEB was seen in rats and the highest biliary concentration of free PAEB was observed in rabbit bile. An apparent overall concentration gradient of free PAEB from plasma to bile was observed

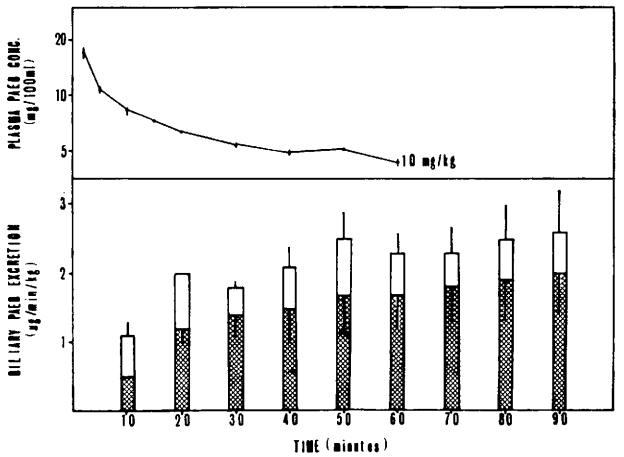

FIG. 3. Plasma disappearance and biliary excretion of PAEB in dogs after the intravenous administration of 10 mg/kg of PAEB. The shaded portion of each bar graph represents the amount of free PAEB and the unshaded portion the conjugated PAEB. Each value represents the mean ± SE of 4 dogs.

in all three species, but the gradient was much less for the dog than the other two species. In the passage of free PAEB from the plasma into the liver, an apparent concentration gradient was observed for the rat and rabbit but not for the dog. In the passage of free PAEB from liver into bile an apparent concentration gradient was observed for all three species.

Table II illustrates the total (free plus conjugated) PAEB concentrations in the plasma, liver and bile and the concentration gradients 20 min after administration. The dog had the highest concentration of PAEB in the plasma of the three species. The highest hepatic concentration of PAEB was in the rat liver, while the highest biliary concentration of PAEB was observed in rabbit bile. Again, an apparent overall concentration gradient from plasma to bile was observed in all three species, but being much less in the dog than the other two species. An apparent concentration gradient from plasma to liver was observed for the rat and rabbit but not for the dog. A concentration gradient of PAEB from liver to bile appears to exist in all three species.

Discussion. A very definite species difference exists in the ability to remove the prototype used to study the organic base transport system of the liver, PAEB, from the plasma into the bile. The rat and rabbit are very efficient in transporting the PAEB from the plasma into the bile, while the dog almost entirely lacks this ability.

There is considerable evidence to support

TABLE I. Free PAEB Concentrations in Rat, Rabbit and Dog 20 min After Administration.

	Rat (5)[a]	Rabbit (6)	Dog (3)
Plasma conc (μg/ml)	9.92 ± 0.35[b]	12.5 ± 1.6	68.4 ± 4.0
Liver conc (μg/g)	69.6 ± 0.06	22.5 ± 6.5	25.1 ± 1.2
Bile conc (μg/ml)	660 ± 34	1860 ± 32.8	282 ± 83
Bile/plasma	66.6 ± 2.6	156 ± 28.7	3.96 ± 0.92
Liver/plasma	7.01 ± 0.51	1.88 ± 0.61	0.37 ± 0.04
Bile/liver	9.88 ± 1.10	141 ± 61	11.8 ± 4.1

[a] Number of animals.
[b] Mean ± SE.

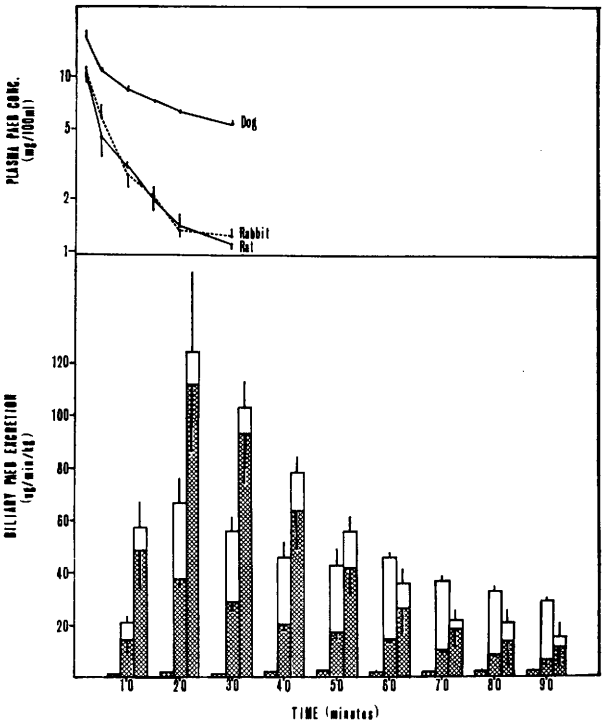

FIG. 4. Comparison of the plasma disappearance and biliary excretion of PAEB by dogs, rats and rabbits. Each group of three bars represents the biliary excretion of the dog, rat and rabbit from left to right. The shaded portion of each bar graph represents the amount of free PAEB and the unshaded portion the conjugated PAEB. Each value represents the mean ± SE of 4–6 animals.

the view that the hepatic removal of compounds from the plasma is dependent upon the simultaneous operation of a number of hepatic processes, such as uptake into a storage compartment, metabolic transformation and excretion into the bile. Therefore, a decreased capacity of one or more of these three parameters is probably responsible for the very low capacity of the dog to clear PAEB from the plasma.

It does not seem likely that a difference in the ability to conjugate PAEB is the reason why the dog has such a low capacity to excrete PAEB, because most of the PAEB excreted into the bile by the rat and rabbit, especially during the early time intervals, is in the free form. In an attempt to determine if a decreased uptake into the liver cell or a decreased excretory rate of PAEB into the bile may be the main reason why PAEB is

TABLE II. Total PAEB Concentrations in Rat, Rabbit and Dog 20 min After Administration.

	Rat (5)[a]	Rabbit (6)	Dog (3)
Plasma conc (μg/ml)	9.92 ± 0.35[b]	12.5 ± 1.6	68.4 ± 4.0
Liver conc (μg/g)	111 ± 8.6	55.8 ± 10.5	22.6 ± 1.3
Bile conc (μg/ml)	1026 ± 63.4	2426 ± 245	318 ± 26
Bile/plasma	103 ± 6.4	211 ± 32	4.74 ± 0.59
Liver/plasma	11.2 ± 1.4	4.06 ± 0.76	0.30 ± 0.04
Bile/liver	9.45 ± 1.0	50.6 ± 9.8	14.0 ± 0.40

[a] Number of animals.
[b] Mean ± SE.

cleared so slowly from the plasma of the dog, the concentration gradient from plasma to liver and from liver to bile was determined for all three species 20 min after administration of PAEB. An apparent concentration gradient from liver to bile exists in all three species, but the gradient from plasma to liver exists only for the rat and rabbit and not for the dog. Therefore, it would appear that the liver of the dog does not have the capacity to concentrate the PAEB over a concentration present in the plasma, and that this relative inability of the liver to take PAEB into the hepatocyte probably is a major reason why the PAEB disappears so slowly from the plasma of the dog.

Summary. The dog, in comparison to the rat and rabbit, has a very limited capacity to transfer procaine amide ethobromide from plasma to bile. It would appear that the relative inability of the dog to transfer the PAEB from the plasma into the parenchymal cell plays a major role in producing this effect.

1. Schanker, L. S., and Solomon, H. M., Amer. J. Physiol. **204**, 829 (1963).

2. Nayak, P. K., and Schanker, L. S., Amer. J. Physiol. **217**, 1639 (1969).

3. Klaassen, C. D., and Plaa, G. L., Amer. J. Physiol. **213**, 1322 (1967).

4. Klaassen, C. D., and Plaa, G. L., Toxicol. Appl. Pharmacol. **15**, 143 (1969).

5. Roberts, R. J., Klaassen, C. D., and Plaa, G. L., Proc. Soc. Exp. Biol. Med. **125**, 313 (1967).

6. Bratton, A. C., and Marshall, E. K., Jr., J. Biol. Chem. **128**, 537 (1939).

Received Dec. 16, 1971. P.S.E.B.M., 1972, Vol. 139.