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SHORT COMMUNICATIONS

Concentrative transfer of an organic cation from blood into bile

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A NUMBER of organic acids, including fluorescein, bilirubin, the bile acids, and several sulfonic acid dyes, are secreted into bile in concentrations greatly exceeding their plasma concentrations. Although some of these anionic substances are present in bile partly as metabolically conjugated forms, there is a sufficient concentration of the unchanged compounds to suggest that their transfer occurs by an active transport process.[1, 2] The present report describes the concentrative transfer, from blood into bile, of an organic cation, procaine amide ethobromide (PAEB).*

Male Sprague–Dawley rats (230–280 g) were anesthetized with pentobarbital and ether, the bile duct was cannulated, and PAEB administered intravenously (5 mg/kg) over a 5-min period. The concentrations of the drug in bile and plasma were measured colorimetrically in 4% trichloroacetic acid (TCA) filtrates by diazotization and coupling of the aromatic amino group according to the method of Bratton and Marshall.[3]

Preliminary experiments disclosed that PAEB was highly concentrated in bile, and that, in addition to the unchanged drug, two conjugated forms[4] were present. One of the conjugates (I) could be hydrolysed to the parent compound at room temperature (23 °C) by adding HCl (final concentration of 1 N) to the TCA filtrate. The other conjugate (II) was more stable, and did not react with the Bratton and Marshall reagents unless previously heated at 100 °C for 30 min in the presence of 1 N HCl. Evidence that the PAEB in bile had not been metabolically altered, other than by conjugation of the aromatic amino group, was provided by paper chromatography of the TCA filtrates of bile, the

TABLE 1. CONCENTRATIONS OF PAEB AND ITS CONJUGATES IN BILE AND PLASMA AFTER INTRAVENOUS ADMINISTRATION IN RATS

Fluid	Concentration (μg/ml)	
	PAEB	Conjugates I and II combined
First half-hour period		
Bile	205 ± 16	234 ± 20
Plasma	2·1 ± 0·2	2·6 ± 0·2
Second half-hour period		
Bile	135 ± 12	247 ± 20
Plasma	1·9 ± 0·2	2·4 ± 0·2

Rats with ligated renal pedicles received 5 mg of PAEB per kg intravenously over a 5-min period; bile was then collected for one or two half-hour periods. The values for plasma indicate the concentration at the end of each half-hour period. Results are expressed as the mean ± the range of values in 8 or 16 animals.

filtrates to which HCl had been added, and the filtrates that had been heated in the presence of HCl. Single violet-colored spots with the same R_f-value as PAEB were obtained when chromatograms developed in an ethanol-ammonia solution[5] or in a butanol-acetic acid-water mixture[6] were sprayed with the Bratton and Marshall reagents.

* [2-(*p*-Aminobenzamido)ethyl] triethylammonium bromide, hydrobromide (procaine amide ethobromide) was kindly supplied by The Squibb Institute for Medical Research, New Brunswick, New Jersey.

For a quantitative study of the transfer of PAEB from plasma into bile, the drug was administered intravenously to two groups of 8 rats in which the renal pedicles had been ligated to help maintain a fairly constant plasma level of drug. In one group, bile was collected for 30 min, and plasma obtained at the end of the collection period. In the other group, bile was collected for two consecutive half-hour periods, and the plasma then obtained. The results (Table 1) show that during both half-hour periods, the concentration of unchanged PAEB in bile was about 80 times that in plasma; and the conjugates of the drug, presumably formed in the liver, were also more concentrated in bile than in plasma.

The secretion into bile of PAEB at high concentrations suggests that the liver may have a specialized transport process for organic cations as well as for organic anions. Preliminary studies with the tertiary amine derivative of PAEB and with three other organic cations, decamethonium, tetraethylammonium, and Darstine® (mepiperphenidol), suggest that only the Darstine® is concentratively transported from blood to bile.

Further work is in progress to characterize the hepatic transport process for organic cations.

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REFERENCES

1. I. SPERBER, *Pharmacol. Rev.* **11**, 109 (1959).
2. C. A. M. HOGBEN, *Ann. Rev. Physiol.* **22**, 381 (1960).
3. A. C. BRATTON and E. K. MARSHALL, JR., *J. Biol. Chem.* **128**, 537 (1939).
4. R. T. WILLIAMS, *Detoxication Mechanisms* p. 428. John Wiley and Sons, Inc., New York, 2nd edit. (1959).
5. H. M. BREGOFF, E. ROBERTS and C. C. DELWICHE, *J. Biol. Chem.* **205**, 565 (1953).

Differential binding and release of norepinephrine and tachyphylaxis

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THE phenomenon of tachyphylaxis to sympathomimetic amines has been a classical problem in pharmacology for many years. Burn and Rand have provided indirect evidence showing that certain sympathomimetic amines exert their effects by the liberation of norepinephrine from stores.[1] Recently, several investigators have shown that after the development of tachyphylaxis the actions of sympathomimetic amines can be restored by the administration of norepinephrine.[2, 3] We have previously demonstrated that the spontaneous release of H^3-norepinephrine from the heart is rapid at first and then becomes progressively slower.[4] This suggests that H^3-norepinephrine is taken up by a store with rapid turnover rate and then gradually enters stores with slower turnover. Blaschko and Welch observed that only one-fifth of the pressor amines in the undenatured granule of the adrenal medulla had an immediate physiological effect after its intravenous administration.[5] This communication will provide direct evidence showing two kinds of stores of norepinephrine, one in which it is easily released and another in which it is tightly bound. Tyramine produces tachyphylaxis by depleting the more easily releasable store of norepinephrine.

Previous work in this laboratory has shown that tyramine releases H^3-norepinephrine from the rat heart.[6] A significant reduction of endogenous catecholamines in the rat heart was also found after the administration of 10 mg of tyramine per kg (Fig. 1). Tyramine was given repeatedly at 15-min intervals beginning 15 min after the injection of H^3-norepinephrine. The hearts were assayed for H^3-norepinephrine[7] and endogenous catecholamines[8] 60 min after the injection of H^3-norepinephrine.