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# HEPATIC TRANSPORT OF ORGANIC CATIONS: ACTIVE UPTAKE OF A QUATERNARY AMMONIUM COMPOUND, PROCAINE AMIDE ETHOBROMIDE, BY RAT LIVER SLICES

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**Abstract**—When rat liver slices were incubated aerobically at 37° with procaine amide ethobromide (PAEB), the compound readily entered the tissue and attained a concentration in tissue water about three times that in the suspending medium. Accumulation of PAEB in the slices occurred by a process that became saturated at high concentrations of the drug. In addition, accumulation was prevented by anaerobic conditions, by metabolic inhibitors such as iodoacetate or 2,4-dinitrophenol, and by certain quaternary amine compounds which presumably compete with PAEB for the uptake process. PAEB was bound to a small extent in homogenates of liver, but the characteristics of the binding were such that the binding could not account for the bulk of the accumulation of drug seen in tissue slices. It appears that the drug is accumulated by liver slices by an active transport process, and that this process is closely related to the secretory process for the biliary excretion of PAEB and certain other quaternary ammonium ions in the living animal.

RECENT studies of the biliary excretion of organic cations have disclosed that the liver possesses an active secretory process for certain quaternary ammonium ions.[1, 2] For example, the quaternary amine compound, procaine amide ethobromide (PAEB), upon intravenous administration in rats, is readily transported from blood to bile against a large concentration gradient. Transport occurs by a saturable process, and a number of quaternary ammonium compounds that are known to be excreted in bile depress transport, presumably by competing with PAEB for the transfer process.

In the present study the uptake of PAEB by rat liver slices is shown to have a number of characteristics in common with the active secretory process *in vivo*.

## METHODS

Male Sprague–Dawley rats (180 to 240 g) were decapitated and the livers immediately removed and placed in chilled beakers. Slices of liver, 0·5 mm thick, were prepared with a Stadie-Riggs microtome, and 10 to 20 slices (2·5 to 5 g) then suspended in 10 ml of Krebs-Ringer phosphate solution (pH 7·4) containing 1 g glucose/l. and various concentrations of procaine amide ethobromide-$^{14}$C-HBr (PAEB-$^{14}$C). In some experiments the following compounds were added to the incubation mixture in order to investigate their effect on the tissue uptake of PAEB-$^{14}$C: benzomethamine; mepiperphenidol (Darstine); oxyphenonium (Antrenyl); $N^1$-methylnicotinamide chloride

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(NMN); glycopyrrolate (Robanul: 1-methyl-3-pyrrolidyl-α-phenylcyclopentane-glycolate methobromide); 6-dimethylaminohexyl-trimethylammonium bromide hydrobromide monohydrate (SQ-3576); decamethonium bromide; or sulfobromophthalein sodium (Bromsulphalein). The mixtures, contained in 50-ml beakers, were shaken in a Dubnoff metabolic shaker (90 oscillations/min) at 37° in an atmosphere of oxygen. At various times, two liver slices and 0·2 ml of the suspending medium were removed. The slices were blotted on moist filter paper, weighed, and then digested in warm 5 N NaOH solution. The radioactivity of the tissue digests and suspending medium was measured by the liquid counting technique of Cotlove.[3] Total counts exceeded 5,000 and were at least 20 times the background. Results were expressed as a slice/medium (S/M) concentration ratio of PAEB, the concentration in the slice being calculated on the basis of its wet weight at the end of the incubation period.

The water content of liver slices, determined by heating the slices at 105° to constant weight, rose from a value of 70 per cent (range ±1) to a value of 77 per cent (range ±1) during the first 10 min of incubation; thereafter it remained constant.

For binding studies, homogenates of rat liver were prepared in cold Krebs-Ringer phosphate solution using a Tenbroeck all-glass homogenizer.

PAEB-$^{14}$C was synthesized by the New England Nuclear Corp. according to a method kindly supplied by the Squibb Institute for Therapeutic Research. One carbon atom of one of the N-ethyl groups was labeled.

## RESULTS

### *Accumulation of PAEB by liver slices*

When rat liver slices were incubated with $5 \times 10^{-4}$ M PAEB, the drug readily entered the tissue and appeared to approach a steady-state S/M concentration ratio somewhat greater than 2·0 (Fig. 1, upper curve). A ratio of 2·1, attained after 4 hr, indicated a concentration of drug in tissue water 2·7 times that in the medium.

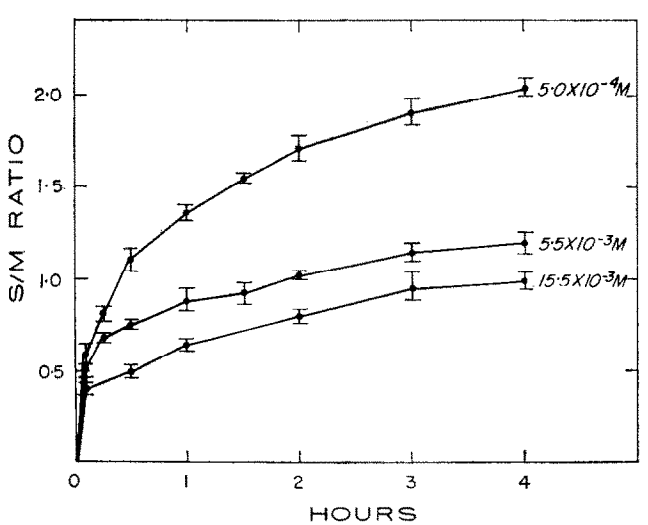


FIG. 1. Influence of concentration of PAEB on its uptake by liver slices. Each point is the mean of values obtained in 5 or 6 experiments. Brackets indicate the S.E.

It should be emphasized that these results do not indicate whether the drug is accumulated within the parenchymal cells or within the lumina of the bile canaliculi. If accumulation were to be confined to the tiny space of the canaliculi, the concentration gradient between canalicular fluid and the incubation medium would be many times greater than that indicated by the S/M ratio.

*Influence of concentration of PAEB on its uptake by liver slices*

A decrease in the S/M ratio of PAEB resulted when the concentration of drug in the incubation medium was raised. For example, on increasing the concentration 11-fold (Fig. 1, middle curve), the 4-hr S/M ratio declined from a value of 2·1 to a value of 1·2; and on increasing the concentration 31-fold (Fig. 1, lower curve), the S/M ratio declined to a value of about 1·0. These results suggested that the uptake of PAEB by liver slices occurs at least in part by a process that can be saturated.

*Effect of metabolic inhibitors and the absence of oxygen*

Evidence that the hepatic uptake of PAEB is dependent on cell metabolism was obtained from studies of the effect of anaerobic conditions and several metabolic inhibitors. In an atmosphere of 100% nitrogen, or in the presence of 0·001 M 2,4-dinitrophenol or iodoacetate, the 2-hr S/M ratio of the drug was depressed from a control value of 1·9 to values of about 1·1 (Fig. 2). The comparable degrees of depression

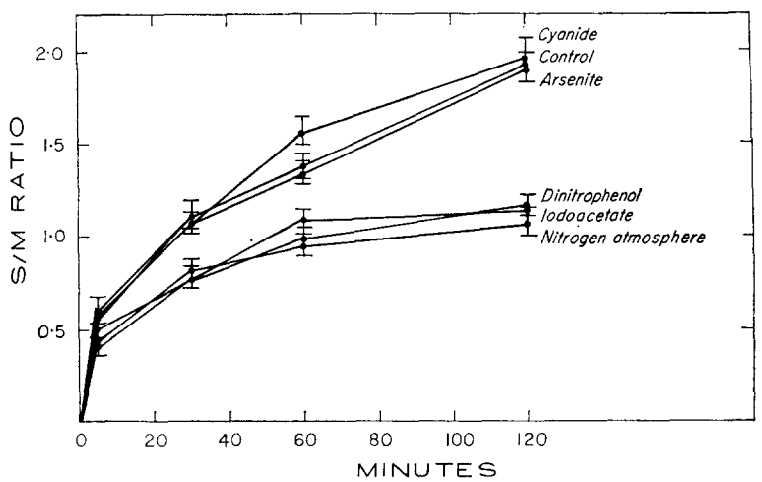


FIG. 2. Effect of metabolic inhibitors and the absence of oxygen on the uptake of PAEB by liver slices. The initial concentration of PAEB in the suspending medium was $5 \times 10^{-4}$ M. The concentration of the various inhibitors was $1 \times 10^{-3}$ M. Each point is the mean of values obtained in 4 to 6 experiments. Brackets indicate the S.E.

under the varied conditions suggested that energy processes necessary for transport had been blocked almost completely and that the drug was now entering the tissue predominantly by a process of diffusion.

In contrast to the above results, 0·001 M cyanide or arsenite did not inhibit the uptake of PAEB.

*Effect of other quaternary ammonium compounds*

A number of quaternary ammonium compounds inhibited the hepatic uptake of PAEB, while others did not (Fig. 3). For instance, benzomethamine, Darstine, glycopyrrolate, and oxyphenonium, in concentrations ten times that of PAEB, depressed the 2-hr S/M ratio of PAEB from a control value of 1·9 to values of 0·9 to 1·1. In contrast, decamethonium, NMN, and SQ-3576 did not depress the S/M ratio of the compound.

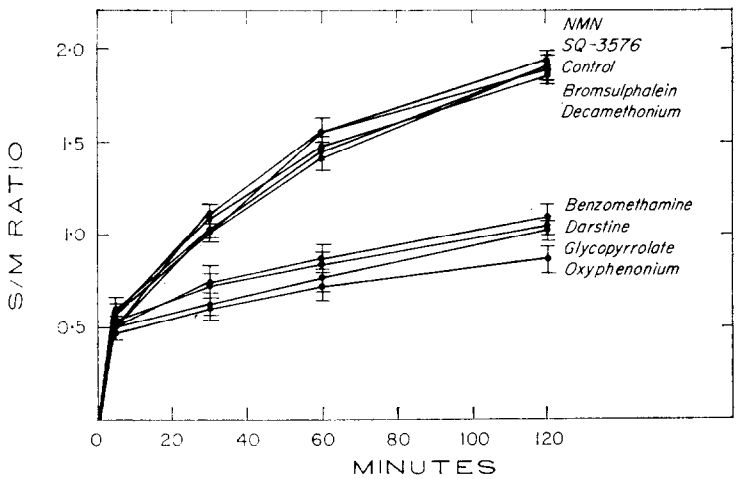


FIG. 3. Uptake of PAEB by liver slices in the presence of various quaternary ammonium compounds and sulfobromophthalein. The initial concentration of PAEB in the suspending medium was $5 \times 10^{-4}$ M. The concentration of other substances was $5 \times 10^{-3}$ M. Each point is the mean of values obtained in 4 to 6 experiments. Brackets indicate the S.E.

*Hepatic uptake of PAEB in the presence of sulfobromophthalein*

Sulfobromophthalein, an acidic compound known to be taken up in considerable amounts by liver slices,[4-6] was tested for a possible inhibitory effect on the uptake of PAEB. In a molar concentration ten times that of the PAEB, the acid had no significant effect on the uptake of the quaternary amine compound, suggesting that the two substances are taken up by different processes (Fig. 3).

*Studies of the binding of PAEB to homogenates of rat liver*

Although the foregoing data suggested that PAEB is accumulated in liver slices by an active transport process, the possibility remained that a part of the accumulation results from tissue binding. Binding was determined by subjecting 40 per cent homogenates of rat liver containing PAEB ($5 \times 10^{-4}$ M) to ultrafiltration through cellophane (Visking) according to the method of Rehberg.[7] The results of three determinations indicated that the drug was bound in the homogenate to the extent of 5 per cent (range $\pm 2$).

To estimate the extent of binding to tissue components in an undiluted state, 40% homogenates containing PAEB ($5 \times 10^{-4}$ M) were centrifuged for 15 hr at 30,000 rpm in a Spinco model L ultracentrifuge (rotor No. 30; average rotational centrifugal force 78,000 $\times$ *g*). The resulting concentration of drug in the water of the particulate

material was 1·36 times that in the supernatant fluid. To investigate the nature of the binding, this experiment was repeated in the presence of several different substances. As shown in Table 1, 0·001 M dinitrophenol or iodoacetate had no effect on the binding of PAEB, the particulate/supernatant concentration ratio of the drug ranging from 1·33 to 1·37. In contrast, 0·0055 M benzomethamine or NMN depressed the ratio to a value of 1·09.

TABLE 1. BINDING OF PAEB TO LIVER HOMOGENATES IN THE PRESENCE OF OTHER SUBSTANCES

Homogenates of liver (40 per cent) containing $^{14}$C-labeled PAEB ($5 \times 10^{-4}$ M) together with another substance were centrifuged for 15 hr at 78,000 × *g*. The concentration of PAEB in the particulate water and in the supernatant fluid was measured, and the results were expressed as a concentration ratio. Results are given as the mean value ± the range of values for three separate determinations.

| Substance added | Molar concentration of substance | PAEB in particulate water / PAEB in supernatant fluid |
|---|---|---|
| None | | 1·36 ± 0·2 |
| 2, 4-Dinitrophenol | 0·0010 | 1·37 ± 0·2 |
| Iodoacetic acid | 0·0010 | 1·33 ± 0·1 |
| Benzomethamine | 0·0055 | 1·09 ± 0·1 |
| $N^1$-Methylnicotinamide | 0·0055 | 1·09 ± 0·1 |

## DISCUSSION

The uptake of PAEB by liver slices appears to involve two processes: active transport, and simple diffusion. The existence of an active transport process is suggested by several lines of evidence. First of all, the drug is transferred from the suspending medium into the slice against a concentration gradient. Moreover, the transfer process becomes saturated at high concentrations of the drug. In addition, uptake of the drug is strongly inhibited by anaerobic conditions, by metabolic inhibitors such as iodoacetate or dinitrophenol, and by certain quaternary ammonium compounds which presumably compete with PAEB for transport into the slice. However, the drug is also taken up by simple diffusion as shown by the almost constant decline in the S/M ratio under widely different inhibitory conditions. Thus in a nitrogen atmosphere, or in the presence of metabolic inhibitors or certain quaternary ammonium compounds, the uptake of PAEB is always depressed to a steady-state S/M ratio of about 1·1.

Although 0·001 M iodoacetate or dinitrophenol blocks the accumulation of PAEB almost completely, comparable concentrations of cyanide or arsenite do not. Similar results were obtained by Farah *et al.*[8] in their study of the uptake of NMN by kidney slices. They reported that 0·015 M cyanide was required to inhibit the uptake by 50%, whereas only 0·0002 to 0·0005 M iodoacetate or dinitrophenol was required for the same degree of inhibition. Similarly, Cross and Taggart[9] reported that cyanide and arsenite were considerably less potent than dinitrophenol and iodoacetate in depressing the uptake of *p*-aminohippurate by kidney slices.

PAEB is bound to hepatic cell constituents, but the extent of binding is too low to account for the degree of accumulation seen in liver slices. Furthermore, the binding

is not depressed by iodoacetate or dinitrophenol, substances which block the accumulation of PAEB in slices; moreover, NMN depresses the binding but does not prevent accumulation in slices.

The uptake of PAEB by liver slices is depressed by benzomethamine, Darstine, glycopyrrolate, and oxyphenonium, quaternary ammonium compounds that are known to inhibit the active secretion of the drug into bile.[2] In contrast, the acidic dye, sulfobromophthalein, and the quaternary ammonium compounds, decamethonium and SQ-3576, neither inhibit the biliary excretion of PAEB nor depress the uptake by slices. Only one of the compounds studied, NMN, fails to show this parallelism; NMN has a small but significant inhibitory effect on the biliary excretion of PAEB,[2] but has no apparent effect on the uptake of the drug by liver slices. It appears that the affinity of NMN for the transport process is too low to be detected in the system *in vitro*.

In conclusion, PAEB is accumulated in liver slices by a process closely related to the biliary secretion process of the intact animal. This relationship is analogous to that seen between the renal tubular secretion of certain compounds and their accumulation in kidney slices.[8–11]

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