Biochemical Pharmacology, Vol. 33, No. 20, pp. 3131–3136, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

DRUG ELIMINATION FUNCTION OF RAT SMALL INTESTINE: METABOLISM AND INTRALUMINAL EXCRETION

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(*Received* 14 *February* 1984; *accepted* 5 *April* 1984)

Abstract—The metabolic and excretory function of the small intestine was investigated after oral and intravenous administration of drugs having an aromatic amino group to rats. After administration of drugs into the intestinal loop at the initial concentration of 0.1 mM, significant excretion of their *N*-acetylated forms into the lumen was observed. The amount of *N*-acetyl forms excreted in the lumen were 39.3 ± 3.5, 63.5 ± 20.9 and 18.0 ± 13.8% of disappeared drugs from the lumen for *p*-aminobenzoic acid (PABA), *p*-aminosalicylic acid and sulfanilic acid, respectively. The excretion of *p*-acetamidobenzoic acid (Ac-PABA) after the absorption of PABA was reduced by the coadministration with salicylic acid, benzoic acid and 2,4-dinitrophenol. Salicylic acid noncompetitively inhibited the acetylation of PABA by the intestinal *N*-acetyltransferase. A good correlation was found between the intestinal *N*-acetyltransferase activities for drugs and the intraluminal excretion of *N*-acetyl derivatives after intestinal absorption of drugs. These results indicate that a drug having a higher susceptibility to intestinal *N*-acetyltransferase would undergo a greater excretion into the lumen in its *N*-acetyl form after intestinal absorption. After intravenous administration of PABA at a dose of 100 μmole/kg, 4.02 ± 0.51% of dose was excreted in the lumen as Ac-PABA in 30 min. On the other hand, a significantly smaller fraction (2.72 ± 0.68% of dose) was excreted in the lumen after intravenous injection of 100 μmole/kg of Ac-PABA. The larger excretion of Ac-PABA after administration of PABA indicates the contribution of intestinal metabolism on the transfer of PABA not only after oral, but also after intravenous administration.

The small intestine is usually considered to be an absorptive organ, but at the same time it can act as an excretory organ for some drugs. The mucosal epithelium is capable of transporting monoquaternary ammonium compounds [1–5] and cardiac glycosides [6] against a concentration gradient from the blood to the intestinal lumen. Likewise, it has been demonstrated that erythromycin [7], sulfanilic acid [8], acebutolol [9], hypoxanthine and xanthine [10] are secreted to the luminal side, thus revealing an excretory function of the intestine. Furthermore, presystemic elimination occurs when orally administered drugs are metabolized during their passage from the intestinal lumen to the systemic circulation [11]. In spite of their significant effects on drug disposition, precise quantitative descriptions of the intestinal drug elimination are almost lacking.

In our previous investigations, it has been found that the mucosal epithelium of rat small intestine readily catalyses the acetylation of *p*-aminobenzoic acid (PABA) and the product, *p*-acetamidobenzoic acid (Ac-PABA), is excreted in the intestinal lumen predominantly [12–14]. Such a pathway might be important not only as a route of elimination but also as an influence on the availability of the orally administered drugs.

The purpose of the present study is to characterize the metabolic and excretory function of the small intestine and to elucidate the role of intestinal elimination process in the drug disposition.

MATERIALS AND METHODS

Materials. *p*-Aminobenzoic acid, *p*-acetamidobenzoic acid and sulfanilic acid were obtained from Nakarai Chemicals Co. (Kyoto, Japan). Acetyl-CoA was purchased from P-L Biochemicals Ltd. (Northampton, U.K.). All other reagents used in these experiments were the finest grade available.

Absorption experiments. Male Wistar rats (170–230 g) were used under pentobarbital anesthesia. The loop of small intestine was prepared in the manner described previously [13] and the bile duct was ligated. Drugs were dissolved in isotonic buffer solution (NaH_2PO_4–Na_2HPO_4, pH 6.5) and 5 ml of drug solution was injected into the loop. After 5 or 10 min, the luminal contents were withdrawn and the intestinal lumen was washed with pH 6.5 isotonic buffer solution. The washings were combined with the luminal content and made up to 50 ml with the same buffer solution for the determination of the amount of drug disappeared from the lumen and metabolite excreted in the lumen.

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Determination of acetylating activity in vitro. The enzyme activity was measured by the method described previously [12]. PABA, *p*-aminohippuric acid, *p*-aminosalicylic acid, sulfanilic acid, sulfisoxazole and sulfadimethoxine were examined as substrates. In the inhibition experiments, salicylic acid or benzoic acid was added to the substrate solution. The 9000 *g* supernatant fraction of the small intestinal mucosa was used as the enzyme source. The reaction mixture consisted of 0.2 ml of enzyme solution, 0.2 ml of aqueous acetyl-CoA (0.2 μmole) and 1.0 ml of substrate solution (pH 7.4, 0.01–0.1 mM). Control tubes contained no acetyl-CoA. The reaction was initiated by the addition of enzyme preparation and the tubes were incubated at 37° for a suitable time period (usually 3 min or longer). The reaction was then terminated by the addition of 1.0 ml of 30% trichloroacetic acid. The protein was removed by centrifugation and 2 ml of the clear supernatant solution were taken for the determination of non-acetylated amines. The extent of acetylation was obtained by subtracting the experimental reading from the control reading.

Intravenous administration experiments. Under pentobarbital anethesia, the carotic artery was cannulated with a polyethylene tubing (i.d. 0.5 mm, o.d. 0.8 mm, Dural Plastics, Australia) and then, heparin was administered intravenously (500 unit/kg). In some experiments, both renal arteries and veins were tied off. Drugs were dissolved in saline solution and were injected into a femoral vein (1 ml/kg). Blood samples (0.3 ml) were obtained periodically for 2 hr after drug administration and the plasma was separated immediately by centrifugation. The excretion of drugs and/or metabolites in the intestinal lumen was investigated by the method of small intestinal loop or by the single perfusion technique. In the case of the loop method, 5 ml of pH 6.5 isotonic buffer solution was injected into the small intestinal loop before drug administration i.v. The contents of the loop were recovered at intervals from 10 to 120 min after intravenous drug administration. In the case of single perfusion method, the small intestine was perfused with pH 6.5 buffer solution at a constant flow rate of 5 ml/min for 30 min. After intravenous administration of PABA (100 μmole/kg), each 10 ml fraction of the perfusate flowed out from the distal end of the small intestine was collected continuously and the amounts of PABA and Ac-PABA in the perfusate were determined.

Analytical methods. PABA and all other aromatic amines were diazotized, coupled with 2-diethylaminoethyl-1-naphthylamine and extracted with isoamyl alcohol after the addition of 1 to 2 g of sodium chloride. The optical density of the organic layer was determined at 560 nm [12]. The concentration of acetylated compounds was determined by the difference in total diazo reactants before and after the hydrolysis of the sample for 1 hr in a boiling water bath with 1 ml of 2N-HCl, except for *p*-aminosalicylic acid. Intact and total concentrations of *p*-aminosalicylic acid were determined by the method of Way *et al.* [15]. The protein concentration was determined by the method of Lowry *et al.* [16] with bovine serum albumin as the standard.

Data analysis. The plasma concentration and the intestinal excretion rate data were fitted to a two compartment open model and the pharmacokinetic parameters were determined by nonlinear least-squares regression [17]. The total body clearance of a drug was calculated by dividing the dose by the area under the plasma concentration versus time curve (AUC). Statistical analysis was performed using Student's *t*-test with $P = 0.05$ as the minimal level of significance.

RESULTS AND DISCUSSION

Intestinal absorption of drugs and excretion of their metabolites: relationship with N-acetyltransferase activity. We previously found that PABA is rapidly absorbed from the rat small intestine, acetylated in the intestinal epithelial cells and the metabolite, Ac-PABA, is excreted into the intestinal lumen in a dose-dependent manner [13]. Table 1 shows the effect of some compounds on the absorption of PABA and excretion of Ac-PABA into the intestinal loop. When salicylic acid (0.5–5.2 mM) was administered with PABA (0.1 mM), the disappearance rate of PABA from the intestinal loop was unchanged, whereas the amount of Ac-PABA excreted in the lumen decreased significantly with increasing dose of salicylic acid. Benzoic acid and 2,4-dinitrophenol also showed a similar effect. The transport of PABA from the lumen to the epithelial cells across the brush border membrane seems to be not affected by these compounds, since no significant change of the disappearance rate of PABA was detected in these experiments. Ac-PABA found in the lumen after the oral dose of PABA was ascertained previously to

Table 1. Effect of various compounds on the absorption of PABA and excretion of Ac-PABA in lumen of rat small intestine *in situ*

Compounds	Concentration (mM)	% disappeared in 5 min	% of dose excreted in lumen as Ac-PABA	Number of experiments
None (control)		50.6 ± 3.3	20.0 ± 2.8	4
Salicylic acid	0.5	50.8 ± 2.0	14.2 ± 0.3*	3
	1.0	47.3 ± 1.3	10.9 ± 1.3***	4
	5.0	49.8 ± 3.8	6.3 ± 1.2****	4
Benzoic acid	5.0	51.3 ± 3.0	13.4 ± 0.9***	4
2,4-Dinitrophenol	0.05	49.0 ± 3.2	13.1 ± 2.1**	4
	1.0	46.3 ± 4.0	4.8 ± 0.9****	4

Initial concentration of PABA was 0.1 mM (pH 6.5, 5 ml). Results are given as the mean ± S.D.
* $P < 0.025$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$ when compared to control (Student's *t*-test).

Table 2. Absorption of drugs and excretion of their *N*-acetyl derivatives in lumen of rat small intestine *in situ*

Drug	Concentration (mM)	% disappeared	% of dose excreted in lumen as *N*-acetyl form
p-Aminosalicylic acid	0.1	27.2 ± 1.5	17.3 ± 5.8
	1.0	29.7 ± 1.9	7.0 ± 1.3*
	5.0	27.5 ± 2.6	1.9 ± 1.2**
Sulfanilic acid	0.1	9.2 ± 1.5	1.5 ± 0.9

Absorption period was 5 min for *p*-aminosalicylic acid and 10 min for sulfanilic acid. Results are given as the mean ± S.D. of 4 experiments.
* $P < 0.025$, ** $P < 0.005$ when compared to 0.1 mM *p*-aminosalicylic acid (Student's *t*-test).

be derived from the intestinal metabolism and the contribution of the intestinal microflora or the metabolism in the systemic circulation was negligible [13]. Hence, the intestinal metabolism and/or excretion processes may be altered by these compounds.

Panagopoulos *et al.* reported decreased acetylation of PABA in healthy volunteers also given salicylic acid, probably due to the inhibition of the metabolic enzyme [18]. Accordingly, the *N*-acetyltransferase activity of rat small intestinal mucosa towards PABA was determined. As shown in Fig. 1, salicylic acid inhibited the acetylation of PABA noncompetitively, and the inhibition constant of salicylic acid was 1.72×10^{-4} M. The acetylation of PABA was also suppressed in the presence of benzoic acid and 2,4-dinitrophenol. The acetylation rate of PABA at the initial concentration of 0.021 mM decreased from 1.58 nmole/min/mg protein to 0.52 nmole/min/mg protein in the presence of 5 mM benzoic acid. Thus, the decreased excretion of Ac-PABA after the absorption of PABA reflects the inhibition of the metabolism in the intestinal epithelium.

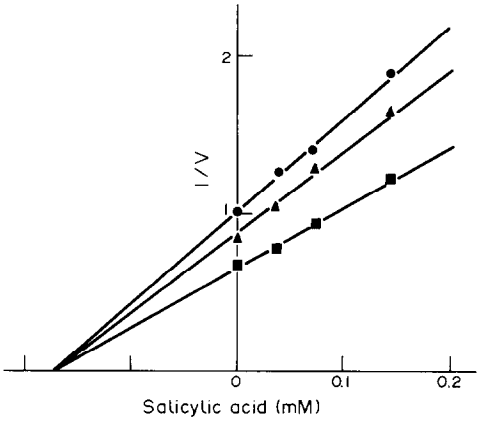

Fig. 1. Inhibitory effect of salicylic acid on the acetylation of PABA by rat intestinal *N*-acetyltransferase. PABA at the initial concentrations of 0.007 mM (●), 0.011 mM (▲) and 0.021 mM (■) with salicylic acid (0–0.143 mM) was incubated with 9000 *g* supernatant fraction of rat small intestinal mucosa and 0.2 μmole acetyl-CoA at 37°. The reaction was terminated in 3 min by the addition of 30% trichloroacetic acid. The acetylation rate of PABA (V) was expressed in nmole/min per mg of protein. Each point represents the mean of two experiments.

Table 2 shows the absorption of *p*-aminosalicylic acid and sulfanilic acid from the small intestine and the excretion of their *N*-acetyl derivatives in the intestinal lumen. Significant fractions of the drugs lost from the lumen were recovered in the lumen in their *N*-acetylated forms. When increasing doses of *p*-aminosalicylic acid were administered, its disappearance rate from the lumen was unchanged, whereas a dose dependency was observed for the excretion of its *N*-acetyl form into the lumen. It is noteworthy that more than 60% of *p*-aminosalicylic acid lost from the lumen was excreted in the *N*-acetylated form into the lumen at the initial concentration of 0.1 mM. A similar dose dependency was previously found for PABA [13]. The dose dependent excretion of the *N*-acetyl forms suggests the saturation of *N*-acetyltransferase activity.

As shown in Fig. 2, a significant correlation was found between the intestinal *N*-acetyltransferase activities for various drugs and the ratios of *N*-acetyl

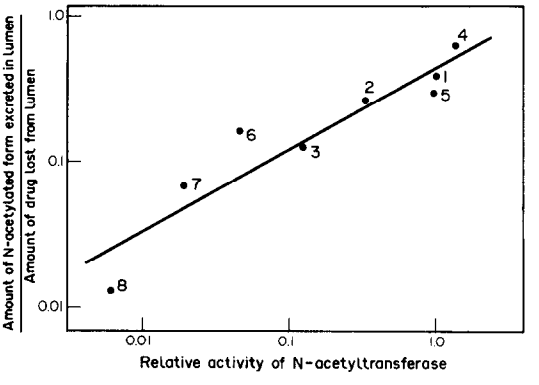

Fig. 2. Relationship between intestinal *N*-acetyltransferase activity against various drugs and excretion of their metabolites in intestinal lumen. *N*-Acetyltransferase activities of the rat small intestine for various drugs at the initial substrate concentration of 0.021 mM was expressed as the ratio to that of PABA (0.855 nmole/min per mg of protein). The intestinal excretion of metabolites was determined after the administration of drugs into the intestinal loop at the initial concentration of 0.1 mM. The solid line was drawn by regression analysis; $\ln y = 0.565 \ln x - 0.341$ ($r = 0.9362$). Data for 5, 7 and 8 were taken from Ref. 12 and 13. Key: 1, PABA; 2, PABA + salicylic acid (5 mM); 3, PABA + benzoic acid (5 mM); 4, *p*-aminosalicylic acid; 5, *p*-aminohippuric acid; 6, sulfanilic acid; 7, sulfisoxazole; 8, sulfadimethoxine.

derivatives excreted in the lumen to the amount of these drugs absorbed from the small intestine. The results indicate that a drug having a higher susceptibility to intestinal *N*-acetyltransferase would undergo a greater excretion into the lumen in its *N*-acetyl form after intestinal absorption.

Intestinal elimination of drugs after intravenous administration. In order to examine the effect of route of administration on the metabolism and excretion of drugs in the small intestine, drugs were administered to rats intravenously. PABA showed a biexponential disappearance from plasma following intravenous administration at a dose of 100 μmole/kg (Fig. 3). The mean elimination half life in the second phase was 20 min and the total body clearance of PABA was 39.3 ml/min/kg. The plasma concentration of Ac-PABA reached a peak level at 20 min after the administration of PABA and thereafter maintained at higher levels than those of PABA. Previously we confirmed that the main metabolic pathway for PABA in the rat small intestine is acetylation and no other metabolites than Ac-PABA could be found at low doses of PABA in the everted sac preparation [12]. In the case of the intravenous dose, the formation of other metabolites must be taken into consideration. Acetylation, glycine conjugation and glucuronidation are known as the major metabolic pathways for PABA [19]. In this study, the concentrations of PABA and Ac-PABA were determined by the diazotization method before and after acid hydrolysis, so that conjugates of PABA and Ac-PABA at their carboxyl groups might be included in the analytical results. However, PABA is excreted primarily as Ac-PABA at low doses and a competition for conjugation of PABA with glycine at the carboxyl group against acetylation at the amino group has been demonstrated. No glycine conjugate of Ac-PABA was found in rat urine after intraperitoneal administration of Ac-PABA at doses less than 140 μmole/kg [20]. Hence, the conjugates of PABA and Ac-PABA at their carboxyl groups could be considered to be minor metabolites in this study.

The intestinal excretion of PABA and Ac-PABA after intravenous administration of PABA was determined by the single perfusion technique (Fig. 4). The excretion rate of PABA rapidly decreased corresponding to the fast plasma elimination of PABA. On the other hand, the intestinal excretion rate of Ac-PABA showed a marked increase and at 5 min after the administration of PABA, it was already about 3-fold faster than that of PABA. The intestinal excretion of Ac-PABA reached its maximum rate 10 min before the attainment of the peak plasma level of Ac-PABA, which suggests that Ac-PABA excreted in the intestinal lumen would be partly derived from metabolism in the intestinal epithelial cells. The total amount excreted into the intestinal lumen was 4.68 ± 0.54% of dose in 30 min and 85.7 ± 1.1% of the total excreted was recovered as Ac-PABA in the lumen.

To clarify the contribution of intestinal metabolism to the excretion of Ac-PABA, Ac-PABA was directly administered to rats. When administered to normal rats intravenously at a dose of 100 μmole/kg, Ac-PABA showed a rapid elimination from plasma and the plasma concentration versus time curve was fitted to a two compartment open model (Fig. 5). The elimination half life in the second phase was 30 min and the total body clearance of Ac-PABA

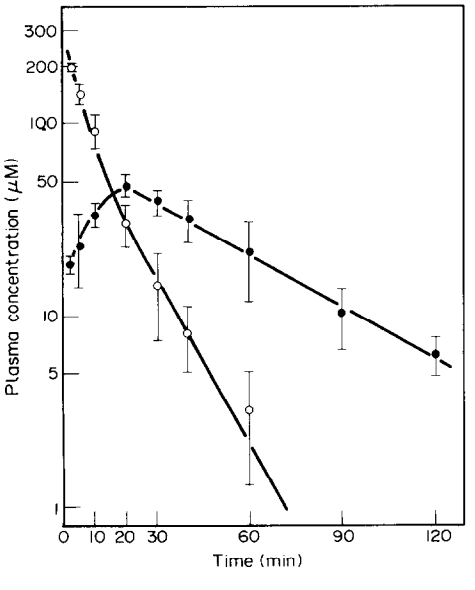

Fig. 3. Plasma elimination curves of PABA (○) and Ac-PABA (●) after intravenous administration of 100 μmole/kg of PABA to rats. Each point is the mean ± S.D. of three to four rats. The disappearance curve of PABA was calculated by the least squares method.

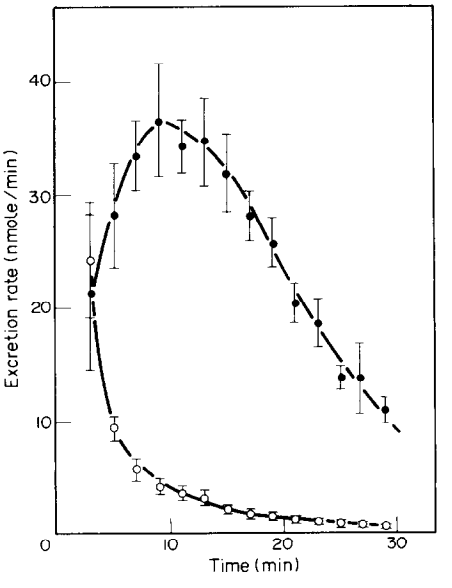

Fig. 4. Excretion of PABA (○) and Ac-PABA (●) in intestinal lumen after intravenous administration of 100 μmole/kg of PABA to rats. The small intestine was perfused with pH 6.5 buffer solution at a constant flow rate of 5 ml/min for 30 min. After the intravenous injection of PABA, 10 ml each of the perfusate flowed out from the distal end of the small intestine was collected continuously. Each point is the mean ± S.D. of four rats.

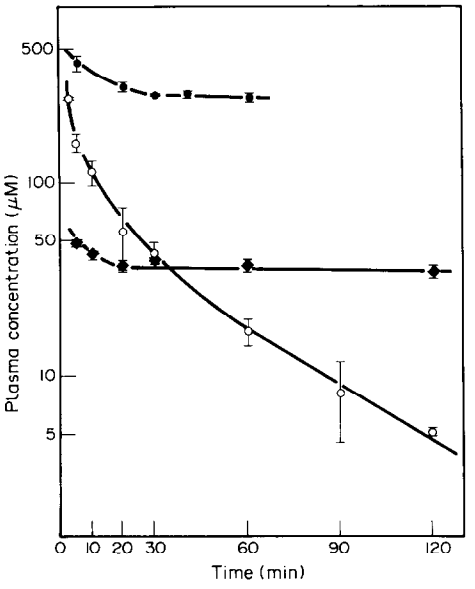

Fig. 5. Plasma elimination curves of Ac-PABA after intravenous administration of Ac-PABA to normal rats (○) or renal ligated rats (●, ◆). Ac-PABA was administered to rats at doses of 10 μmole/kg (◆) and 100 μmole/kg (○, ●). Each point is the mean ± S.D. of more than three rats.

was 21.3 ml/min/kg. The intestinal excretion of Ac-PABA assessed by the single perfusion technique was 2.72 ± 0.68% of dose in 30 min and was significantly lower than the amount of Ac-PABA excreted after the administration of PABA (4.02 ± 0.51% of dose, $P < 0.05$). The time course of the intestinal excretion rate followed a biexponential decline and the total amount of Ac-PABA excreted in the lumen was obtained by integrating the biexponential equation from zero to infinite time. The intestinal clearance of Ac-PABA calculated by dividing the total amount of Ac-PABA in the lumen by plasma AUC of Ac-PABA was 1.35 ml/min/kg, which was about 6% of the total body clearance of Ac-PABA. Thus, Ac-PABA in the systemic circulation would be excreted mainly by the kidney and the contribution of the small intestine to its elimination was not large. In fact, when Ac-PABA was injected to rats with ligated renal pedicles, plasma concentrations of Ac-PABA were maintained at a almost constant level after 20 min of the administration (Fig. 5).

The intestinal excretion of Ac-PABA in renal ligated rats was determined by the loop method. The concentration of Ac-PABA in the lumen increased gradually with time after the intravenous injection of 10 μmole/kg of Ac-PABA and the concentration ratios of Ac-PABA in the intestinal lumen to that in the plasma were 0.109 ± 0.025, 0.213 ± 0.010, 0.283 ± 0.048 and 0.463 ± 0.057 at 10, 30, 60 and 120 min, respectively. Turnheim and Lauterbach [3] investigated the *in vivo* secretion of *N*-methylscopolamine, *N*-methylnicotinamide and tetraethylammonium by the small intestine of guinea pigs with ligated renal pedicles. They found the ratios of the concentrations of the unmetabolized quaternary ammonium compounds in the lumen to those in the plasma were greater than 1.0 and suggested the presence of actively secreting system for monoquaternary ammonium compounds [3]. The transport system for Ac-PABA seems to be different from the active secretion because the concentration ratio of Ac-PABA was less than 1.0 in this study.

Table 3 shows the intestinal excretion of drugs and/or their *N*-acetyl forms after intravenous administration to renal ligated rats. Compared with the administration of Ac-PABA, higher luminal concentration of Ac-PABA was found after the administration of PABA in spite of significantly lower plasma levels of Ac-PABA. These results also confirm the contribution of intestinal metabolism to the transfer of PABA from plasma to the intestinal lumen.

Sulfanilic acid was also found to be excreted in the lumen both intact and in the *N*-acetyl form after intravenous administration. Sulfanilic acid gives *N*-acetylsulfanilic acid as its sole metabolite after administration to rats [21]. The ratio of *N*-acetylated to intact sulfanilic acid was markedly higher in the lumen than in the plasma, which suggests the contribution of the intestinal metabolism to the intestinal excretion of sulfanilic acid in the same manner as for PABA.

The intestine has the ability to metabolize drugs by numerous pathways involving both phase I and phase II reactions [22]. The metabolites formed in the epithelial cells could be transferred to both directions to the lumen or to the mesenteric blood. In the present study, significant amounts of *N*-acetyl

Table 3. Excretion of drugs and their *N*-acetyl derivatives in the intestinal lumen after intravenous administration to rats with ligated renal pedicles

Drug	Dose (μmole/kg)	Luminal concentration (μM)		Plasma concentration (μM)	
		Drug	*N*-acetyl form	Drug	*N*-acetyl form
PABA	10	0.4 ± 0.1	11.5 ± 1.5	5.5 ± 0.8	26.5 ± 5.0
	100	5.9 ± 1.2	105.6 ± 24.9	100.7 ± 18.7	248.1 ± 19.9
Ac-PABA	10	8.2 ± 0.4	—	39.2 ± 1.2	—
	100	84.6 ± 16.1	—	279.2 ± 4.4	—
Sulfanilic acid	10	1.4 ± 0.3	1.9 ± 0.5	34.4 ± 1.7	2.0 ± 2.0
	100	15.8 ± 6.8	9.7 ± 2.6	349.2 ± 8.6	19.2 ± 8.3

Five milliliters of pH 6.5 isotonic buffer solution was injected into the small intestinal loop before drug administration. At 30 min after the intravenous administration of a drug, a blood sample was collected and the contents in the loop were recovered. Results are given as the mean ± S.D. of 4 experiments.

forms were found in the lumen after the absorption of aromatic amines from the intestine. Previously we found the intestinal absorption rates of *N*-acetyl derivatives are much slower than those of parent drugs [13]. Such a change of absorption rate by acetylation seems to be rational for the excretory function of the intestine. The excretion of metabolites into the lumen after intestinal absorption has been reported for some other drugs, such as salicylamide [23], 1-naphthol [24] and perazine [25]. Intestinal metabolism and excretion appears to depend on the activity of the metabolizing enzymes, the dose, the transfer rate of a drug into the epithelial cells from the lumen or from the blood, the presence of metabolic inhibitors and the route of drug administration. The intestine may play an important role in the overall metabolism of drugs especially when they are administered orally since they must pass through the enzyme-containing epithelial cells of the intestinal mucosa during the absorption to the systemic circulation.

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