BBA 73023

The ileal bile salt transport system: Effect of the charged state of the substrate on activity

Previous studies of substrate structure–activity properties of the intestinal (ileal) bile salt transport system demonstrated the importance of the net charge on the bile salt. Substitution of a positively charged quaternary grouping for the negatively charged sulfonate radical of taurocholate resulted in a compound which was not transported and which did not inhibit the transport of the natural analogue[1]. In addition when cholic acid was conjugated with amino acids containing two acidic groups, one obtained compounds which were very poorly transported. The question therefore arose whether the intestinal transport system might possess a requirement for a single negative charge, and that the small residual transport observed with the dibasic compounds represented the movement of the singly charged fraction present at the pH of the incubating media.

In order to test this hypothesis comparative incubations were performed in media of different pH values. A change in pH of the media calculated to increase the proportion of the singly charged bile salt ions would be expected to cause an increase of its transport when compared to substrate possessing only one charge. In the following experiments transport was measured by the everted gut sac technique of WILSON AND WISEMAN and utilized guinea pig ileum.

Transport of cholylaspartate is compared with the transport of glycocholic acid in Fig. 1A. Fig. 1B demonstrates experiments comparing the transport of taurocholate with *N*-cholylaminoethylphosphonic acid. The experimental conditions of these incubations are described in the legends of the figures. The syntheses of the dibasic bile acid derivatives have been previously described[1]. Glycocholate transport is depressed in media of lower pH, while the transport of the aspartic acid derivative is increased (Fig. 1A). The enhanced transport of cholylaspartate relative to glycocholate is given in the insert to the figure. The relative transport of the phosphonic acid derivative of cholate is similarly increased at lower pH (Fig. 1B).

Active transmucosal movement represents two sequential processes: Uptake by the mucosal cell and extrusion into the serosal space. The final serosal–mucosal ratios represent the over-all activity of these two events. One may study transport by measuring uptake by the intestinal epithelial cells (disappearance from the mucosal compartment in the everted gut sac preparation). Table I demonstrates that transport calculated on this basis also shows the same pH–activity relationships. The second dissociation constants for the dibasic bile acid derivatives have been determined by titration curves. The second pK values of these substances are 5.2 for cholylaspartic acid, and 6.1 for *N*-cholylaminoethylphosphonic acid. In each case one might expect more of the singly charged bile salts at pH 6.9 than at pH 7.8.

It has been shown that mutual inhibition of transport exists between different pairs of bile salts[3,4,1]. If the transport of the derived bile salts were limited exclusively to the singly charged molecule one would expect enhanced inhibitory potency at lower pH. Table II shows that this is the case. The inhibition of glycocholic acid and taurocholic acid by cholylaspartate is greater at the lower pH where transport of the dibasic substances is more optimal. The conditions of Expt. B shown in Table II

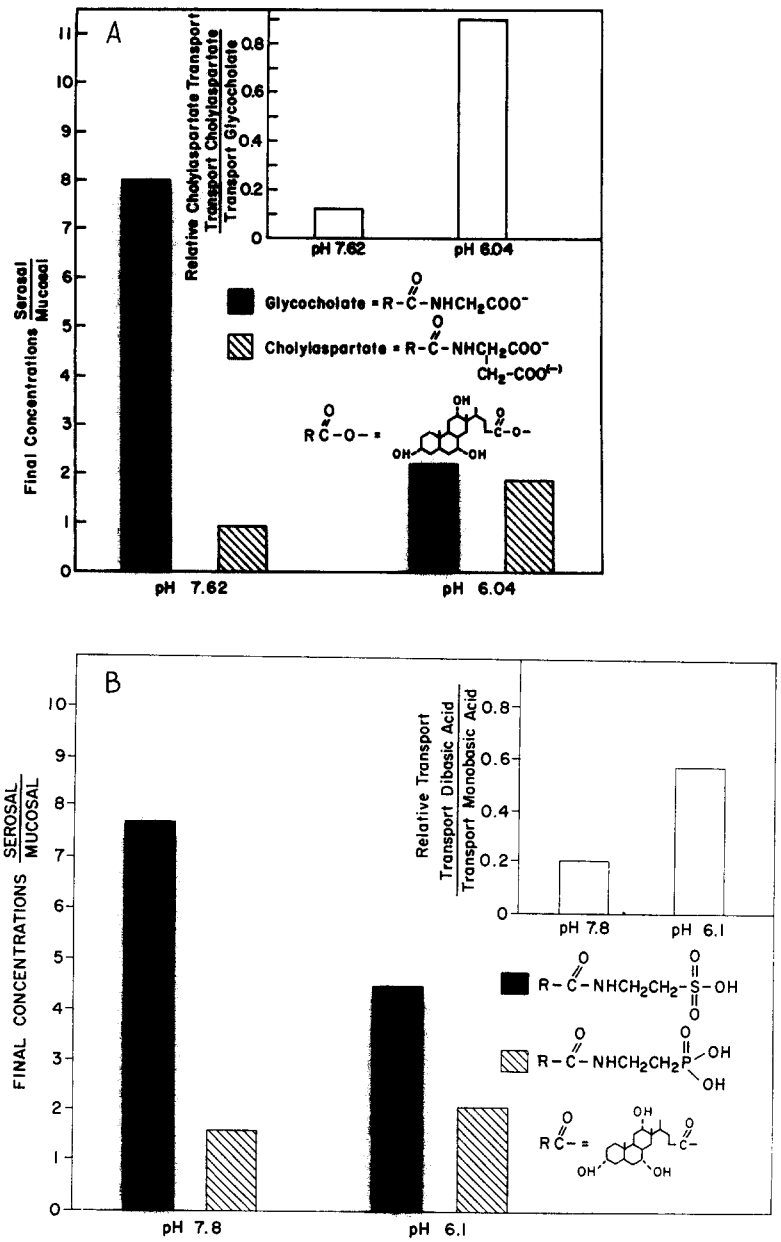

Fig. 1. Comparison of the transport of dibasic bile acids with that of their natural monobasic analogues in media of different pH. Each bar is the average value of 4 gut sac incubations. An entire experiment used 16 gut sacs from the ileums of 4 guinea pigs. It was essential that animals in excess of 480 g be used. The order of incubation of the gut sacs was staggered as described previously[2]. The incubation solutions were modified Krebs–Ringer phosphate media. Calcium was omitted and the phosphate buffer concentration was three times the conventional amount. Initial pH values were 8.0 and 5.7. At the end of the incubations the pH values were altered somewhat to the values given, presumably due to buffering by the tissue and metabolic products. All pH values given represent post-incubation determinations. Temperature 37°; time of incubation was 1 h; the gas phase was O_2.

TABLE I

EFFECT OF pH ON THE ACTIVE TRANSPORT OF GLYCOCHOLATE AND CHOLYLASPARTATE

Initially the concentrations of substrate in the serosal and mucosal compartment were equal: glycocholate, 0.24 mM; *N*-cholylaspartate, 0.14 mM; taurocholate, 0.14 mM; *N*-cholylaminoethylphosphonic acid, 0.14 mM. Vol. of the serosal compartment, 1.5 ml; vol. of the mucosal compartment, 10 ml. Regular Krebs–Ringer phosphate buffer with two times the concentration of buffer. Calcium omitted: temperature 37°; time of incubation, 1 h.

Expt.	*Substrate*	*Final pH*	*Amount* transported (μmoles)*	*Relative transport of the dibasic** bile acid derivatives*
A	Glycocholate	7.50	1.46	—
	Cholylaspartate	7.53	0.12	0.083
	Glycocholate	6.21	1.12	—
	Cholylaspartate	6.19	0.35	0.31
B	Taurocholate	7.60	0.51	—
	N-Cholylaminoethylphosphonic acid	7.61	0.12	0.24
	Taurocholate	6.24	0.39	—
	N-Cholylaminoethylphosphonic acid	6.25	0.25	0.65

* Amount of substrate leaving the mucosal compartment.

** Relative transport is defined as the amount of the dibasic substance leaving the mucosal compartment divided by the commensurate amount of transport of the monobasic analogue.

TABLE II

EFFECT OF pH ON THE INHIBITION OF BILE SALT TRANSPORT BY *N*-CHOLYLASPARTATE

Incubation was at 37° for 60 min. Krebs–Ringer phosphate solution was used without Ca^{2+} but with three times the conventional amount of phosphate buffer. All values are the mean of four gut sacs. Uninhibited transport is assigned 100%.

Expt.	*Final pH*	*Substrate*	*Initial concn. (mM)*	*Inhibitor*	*Concn. (mM)*	*Substrate* transported (μmoles)*	*Control activity (%)*
A	7.7	Glycocholate	0.24	None	—	1.55	100
	7.7	Glycocholate	0.24	*N*-Cholylaspartate	0.56	1.42	92
	6.1	Glycocholate	0.24	None	—	0.98	100
	6.1	Glycocholate	0.24	*N*-Cholylaspartate	0.56	0.58	59
B	7.8	Taurocholate	0.20	None	—	1.36	100
	7.8	Taurocholate	0.20	*N*-Cholylaspartate	0.28	1.38	102
	6.2	Taurocholate	0.20	None	—	1.06	100
	6.2	Taurocholate	0.20	*N*-Cholylaspartate	0.28	0.80	75
C	7.8	Taurocholate	0.20	None	—	1.51	100
	7.8	Taurocholate	0.20	*N*-Cholylaspartate	0.56	1.34	88
	6.1	Taurocholate	0.20	None	—	1.20	100
	6.1	Taurocholate	0.20	*N*-Cholylaspartate	0.56	0.77	64

* Removed from the mucosal compartment.

were adjusted so that there would be virtually no inhibition of transport at pH 7.8. This demonstrates that the lesser transport of the dibasic substances under conditions of high pH may not be ascribed to toxicity of these detergent-like substances because the transport of the naturally occurring bile salts was not adversely affected. The foregoing data are in accord with our hypothesis that the ileal transport system for bile salts is specific for cholanic acid derivatives containing a single negative charge on the side chain.

The work was supported by grants AM-09582 and HE-10595 from the National Institute of Health. The excellent technical assistance of Mrs. SARAH H. TAYLOR and CLEMENTINE S. SESSONS is acknowledged.

Department of Physiology and Pharmacology, LEON LACK
Duke University Medical Center,
Durham, N.C.
and Department of Pharmacology, I. M. WEINER
State University of New York,
Upstate Medical Center,
Syracuse, N.Y. (U.S.A.)

1 L. LACK AND I. M. WEINER, *Am. J. Physiol.*, 210 (1966) 1142.
2 L. LACK AND I. M. WEINER, *J. Pharmacol. Exptl. Therap.*, 139 (1963) 248.
3 L. LACK AND I. M. WEINER, *Federation Proc.*, 22 (1963) 1334.
4 P. R. HOLT, *Am. J. Physiol.*, 210 (1966) 635.

Received May 23rd, 1967

Biochim. Biophys. Acta, 135 (1967) 1065–1068

BBA 73024

Release of lipopolysaccharide during the preparation of cell walls of *Pseudomonas aeruginosa*

At alkaline pH values EDTA has a potent bactericidal action against *Pseudomonas aeruginosa*[1] and causes release of lipopolysaccharide from the isolated cell wall of the organism[2]. During a study of the probable connection between the two effects it was desirable to estimate the contribution made by the cell wall to the mass of the whole cell. For Gram-negative bacteria a figure of 20% or less is generally accepted[3], although a doubtfully high value of 76–78% was indicated by early work[4] on *P. aeruginosa*. These values have generally been obtained either directly, from the yields of cell walls, or by calculation, using nitrogen analyses of soluble and insoluble fractions of cells.

In the present work, analyses of washed whole cells and of isolated cell walls of *P. aeruginosa* (NCTC 1999) have been made for compounds believed to be uniquely or predominantly present in either the lipopolysaccharide or the glycosaminopeptide fraction of the cell wall. Walls were prepared from cells grown for 24 h at 37° on Tryptone glucose extract agar (Oxoid) and disintegrated using a Braun MSK