Taurocholate–Sodium Co-transport by Brush-Border Membrane Vesicles Isolated from Rat Ileum

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Uptake of taurocholate into brush-border membrane vesicles isolated from rat small intestine by a Ca^{2+}-precipitation method was investigated by using a rapid-filtration technique. Uptake of taurocholate by ileal brush-border membranes consisted of three phenomena: binding to the outside of the vesicles, transfer across the vesicle membrane and binding to the intravesicular compartment. The transport of taurocholate across the brush-border membranes was stimulated in the presence of Na^+ compared with the presence of K^+; stimulation was about 11-fold in the presence of a NaCl gradient ($Na_o > Na_i$), where the subscripts refer to 'outside' and 'inside' respectively, and 4-fold under equilibrium conditions for Na^+ ($Na_o = Na_i$). In the presence of a Na^+ gradient a typical 'overshoot' phenomenon was observed. Membranes preloaded with unlabelled taurocholate showed an accelerated entry of labelled taurocholate (tracer exchange) in the presence of Na^+ compared with the presence of K^+. The stimulation by Na^+ was observed only in membrane preparations from the ileum. Addition of monactin, an ionophore for univalent cations, decreased the Na^+-gradient-driven taurocholate uptake. The Na^+-dependent taurocholate transport showed saturation kinetics and the phenomenon of counterflow and was inhibited by glycocholate. Other cations such as Li^+, Rb^+ and Cs^+ could not replace Na^+ in its stimulatory action. When the electrical potential difference across the vesicle membrane was altered by establishing different diffusion potentials (anion replacement; K^+ gradient ± valinomycin) a more-negative potential inside stimulated Na^+-dependent taurocholate transport. These data demonstrate the presence of a rheogenic (potential sensitive) Na^+–taurocholate co-transport system in ileal brush-border membranes and support the hypothesis that the reabsorption of bile acids in the ileum is a secondary active uptake.

In the enterohepatic cycle, conjugated bile acids excreted into the duodenum by the liver are reabsorbed by specific transport systems in the distal ileum (Holt, 1964; Playoust & Isselbacher, 1964; Lack & Weiner, 1966; Schiff *et al.*, 1972; Lack & Weiner, 1973; Krag & Phillips, 1974), and then carried back to the liver by the portal blood (Weiner & Lack, 1968). In studies using intestinal epithelial cells (everted sacs, rings, slices and isolated cells) it has been demonstrated that the transport of bile acids in the ileum is active, i.e. occurs against a concentration difference, is saturable and depends on the presence of Na^+ in the luminal fluid (Holt, 1964; Playoust & Isselbacher, 1964; Glasser *et al.*, 1965; Dietschy *et al.*, 1966; Wilson & Dietschy, 1972; Schiff *et al.*, 1972; Wilson & Treanor, 1975). These findings suggested that bile acid transport is a secondary active uptake and might involve Na^+–bile acid co-transport across the brush-border membrane similar to the Na^+-dependent transport of amino acids, sugars and P_i

in the small intestine and renal proximal tubule (for review see Murer & Kinne, 1977). The experiments reported below were designed to look for a taurocholate–Na^+ co-transport system in isolated rat duodenal, jejunal and ileal brush border by studying the transport of bile acids into membrane vesicles.

In the present study, in addition to binding of taurocholate to the membranes (already observed by Wilson & Treanor, 1977) Na^+-dependent transport into the intravesicular space could be demonstrated which involves coupling of Na^+ and taurocholate fluxes via a Na^+–taurocholate co-transport system. This co-transport system could only be detected in ileal membranes, suggesting that it is involved in the Na^+-dependent taurocholate transport observed also only in this segment of the intact intestine.

While our work was in progress a study was published by Lack *et al.* (1977) on taurocholate uptake by guinea-pig small-intestinal brush-border vesicles. The results of these authors support the

notion of the presence of a taurocholate–Na^+ co-transport system not only in rat but also in guinea-pig small intestine.

Methods

Brush-border membranes of rat duodenal, jejunal and distal ileal segments were prepared from male Wistar rats of 180–220g body wt. by a modification of the procedure of Schmitz et al. (1973) as described by Lücke et al. (1978), by using mucosal scrapings instead of a suspension of epithelial cells. In essence, mucosal scrapings were homogenized in a hypo-osmotic medium; after addition of $CaCl_2$ (final concn. 10mM) the brush-border membranes were purified by differential centrifugation.

Protein was determined after precipitation of the membranes with ice-cold 10% (w/v) trichloroacetic acid as described by Lowry et al. (1951), with bovine serum albumin (Behringwerke, Marburg, Germany) as standard. Maltase (EC 3.2.1.20) was used as marker enzyme for the microvillus membrane and was assayed as described by Haase et al. (1978). Activity of $(Na^+ + K^+)$-stimulated adenosine triphosphatase (EC 3.6.1.3), a marker of the basolateral plasma membrane, was measured as reported by Berner & Kinne (1976). The maltase activity of the brush-border membrane vesicles isolated from the ileal segment of of rat small intestine was enriched about 16-fold compared with the starting homogenate, whereas $(Na^+ + K^+)$-stimulated adenosine triphosphatase was not enriched in the final membrane fraction. A similar behaviour of marker-enzyme activities was obtained for the membranes isolated from the two other intestinal segments.

Uptake of labelled compounds by isolated brush-border membrane vesicles was measured by a rapid filtration technique as described previously (Berner et al., 1976; Evers et al., 1976). The exact compositions of the incubation media are given in the Figure legends. All experiments were performed in duplicate and were repeated at least three times with similar results.

Materials

Tauro[G-³H]cholic acid (specific radioactivity 3.39Ci/mmol) and D-[1-¹⁴C]mannitol (specific radioactivity 51.44mCi/mmol) were purchased from New England Nuclear Corp. (Boston, MA, U.S.A.). Taurocholic acid, sodium salt, and Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] were obtained from Serva (Heidelberg, Germany). Enzymes and substrates needed for maltase and $(Na^+ + K^+)$-stimulated adenosine triphosphatase assays were obtained from Boehringer (Mannheim, Germany). All other chemicals, of the highest purity available, were purchased from Merck (Darmstadt, Germany).

Results and Discussion

Taurocholate uptake by duodenal, jejunal and ileal brush-border membrane vesicles

In Table 1 the uptake of taurocholate by brush-border membrane vesicles isolated from rat duodenum, jejunum and distal ileum is compared. The initial rate of taurocholate uptake in the absence of Na^+ is highest in the jejunum, somewhat slower in the ileum and slowest in the duodenum. In the presence of Na^+ the ileal brush-border membranes show the highest uptake rate; the uptake by the duodenal and jejunal membranes is not affected when KNO_3 is replaced by $NaNO_3$. Thus, since our main interest was to study a potential Na^+–taurocholate co-transport system, only the ileal brush-border membranes were investigated in more detail.

Differentiation between facilitated diffusion, free diffusion and binding

In investigating the transport properties of a lipophilic substance such as taurocholate, the discrimination between facilitated diffusion of the substance across the membrane, simple diffusion and non-specific binding to the membrane is important. An argument for the presence of a facilitated diffusion mechanism for taurocholate is the demonstration of counterflow for Na^+-dependent taurocholate uptake. Preloading the membranes with unlabelled 0.2mM-taurocholate enhanced the subsequent uptake of labelled taurocholate about 1.4-fold compared with non-preloaded controls (Table 2). Furthermore, since facilitated diffusion of a solute, in contrast to binding and free diffusion, should be preferentially affected by temperature changes, the uptake of taurocholate in the presence of Na^+ and of K^+ was investigated at 0°, 15°, 37° and 45°C (Fig. 1). At 0°C no difference between uptake in the presence of K^+ and that in the presence of Na^+ was observed, whereas at 15°, 37° and at 45°C the taurocholate uptake was stimulated (2.2-, 6.2- and 8.1-fold) by a Na^+ gradient. At the same time the uptake of taurocholate in the presence of KNO_3 increased only slightly. The uptake of tauro-cholate in the presence of K^+ or Na^+ at 0°C after 20s is an estimate of initial binding of the substrate to the external membrane surface, and amounted to about 50pmol/mg of protein. A similar value for the external binding of taurocholate to the membranes was obtained by measuring the initial uptake of taurocholate in the presence of a Na^+ gradient and $HgCl_2$ at 37°C. As indicated in Table 3, addition of $HgCl_2$ caused a 95% inhibition of taurocholate up-take in the presence of a $NaNO_3$ gradient, as com-

Table 1. *Comparison between taurocholate uptake by brush-border membrane vesicles prepared from duodenum, jejunum and distal ileum*

The experiments were carried out in an incubation medium containing 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4), 100 mM-NaNO$_3$ or -KNO$_3$ as appropriate, and 0.075 mM-[^{3}H]taurocholate. Incubation temperature was 37°C. Values in parentheses represent percentages of equilibrium uptake.

Intestinal region	Salt gradient	Taurocholate uptake (pmol/mg of protein)		
		0.33 min	1 min	30 min (equilibrium)
Duodenum	NaNO$_3$	43 (5)	217 (26)	823
	KNO$_3$	60 (7)	318 (36)	870
Jejunum	NaNO$_3$	346 (34)	638 (62)	1013
	KNO$_3$	343 (35)	577 (59)	966
Ileum	NaNO$_3$	625 (107)	766 (131)	582
	KNO$_3$	149 (25)	247 (43)	574

Table 2. *Counterflow of Na$^+$-dependent taurocholate uptake into brush-border membrane vesicles (amount of taurocholate taken up during the first 0.33 min, 1 min and at equilibrium condition after 30 min)*

Membrane vesicles loaded with 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4) (control) and in addition with unlabelled 0.2 mM-taurocholate were incubated at 37°C in a medium containing 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4), 100 mM-NaNO$_3$ and 0.033 mM-[^{3}H]taurocholate.

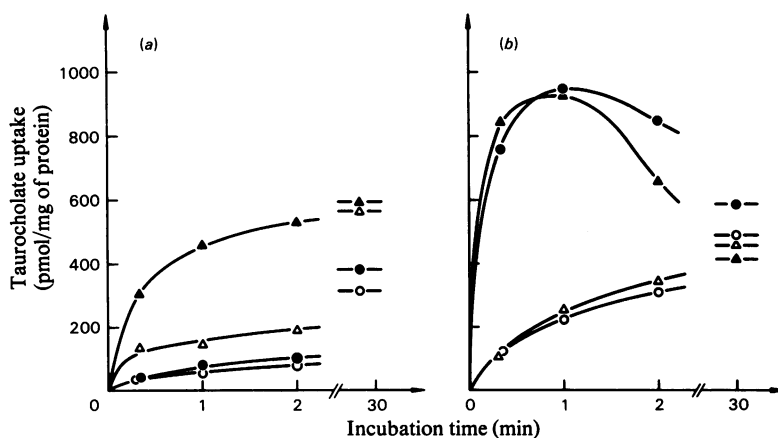| Conditions inside vesicles	Taurocholate uptake (pmol/mg of protein)		
	0.33 min	1 min	30 min (equilibrium)
Mannitol plus buffer	45	62	56
Mannitol plus buffer plus unlabelled taurocholate (0.2 mM)	63	103	57

Fig. 1. *Influence of incubation temperature on taurocholate uptake*

Brush-border membrane vesicles isolated from rat ileum were loaded with 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4) and incubated at 0°C or 15°C (*a*) and at 37°C or 45°C (*b*) in the same medium containing also 0.15 mM-[^{3}H]taurocholate and 100 mM-NaCl (*a*: ●, at 0°C; ▲, at 15°C; *b*: ●, at 37°C; ▲, at 45°C) or KCl (*a*: ○, at 0°C; △, at 15°C; *b*: ○, at 37°C; △, at 45°C). The low 'equilibrium' values at 0°C indicate that equilibrium was not reached at this time of incubation (30 min).

Table 3. *Effect of monactin and HgCl₂ on taurocholate transport into brush-border membrane vesicles (amount taken up during the first 0.33 min, 1 min and at equilibrium condition after 30 min)*

The experiments were carried out in an incubation medium as described in Table 1. Monactin (10 mg/ml in ethanol), when present, was 15 μg/mg of protein, and HgCl₂ was 10 μM. Incubation temperature was 37°C.

Conditions in incubation medium	Taurocholate uptake (pmol/mg of protein)		
	0.33 min	1 min	30 min (equilibrium)
NaNO₃ gradient	440	447	333
NaNO₃ gradient plus monactin	112	163	272
NaNO₃ gradient plus HgCl₂	20	116	310

pared with controls without HgCl₂ in the medium. Therefore, since it is not likely that HgCl₂ affected non-specific binding of taurocholate to the membranes, it must be assumed that the almost complete inhibition by HgCl₂ represents an inactivation of a specific pathway for taurocholate. Addition of HgCl₂ at the concentrations used in the experiments had only a very little effect on the equilibrium uptake of taurocholate. Furthermore, as indicated by experiments with [¹⁴C]mannitol-pre-equilibrated membrane vesicles, the vesicular space was not decreased by the addition of HgCl₂. The last two experiments indicate that binding of taurocholate to the external membrane surface is rather low, i.e. below 10% of the equilibrium uptake. Therefore the values in the present paper have not been corrected for this component of taurocholate uptake.

Binding of taurocholate to the membranes was also analysed at equilibrium distribution of taurocholate. Fig. 2 demonstrates the influence of osmolarity of the medium on taurocholate uptake by isolated ileal brush-border membranes. The equilibrium uptake (after 30 min) of taurocholate was measured as a function of increasing osmolarity of the medium (by the impermeant cellobiose). Since only 60% of taurocholate uptake seems to respond to alteration of the intravesicular space by changing osmolarity of the medium, it must be assumed that only this part represents free taurocholate kept in the intravesicular space and that at least 40% of the total taurocholate uptake is bound either to the inner or to the external membrane surface of the membrane vesicles. This value is obtained by extrapolating the line given in Fig. 2 to infinite osmolarity (zero intravesicular space) and seems to be in discrepancy with the value estimated for initial binding (below 10%). However, experimental evidence indicates that the binding of taurocholate to the membranes after equilibrium uptake (40%) seems to occur on the inside of the vesicle rather than on its outside: addition of unlabelled glycocholate (1 mM) or taurocholate (1 mM) to the ice-cold buffer solution, in which the membranes
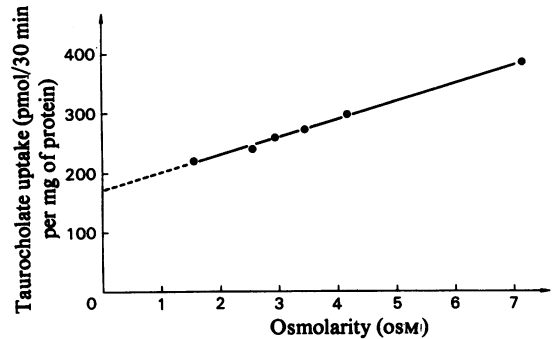

Fig. 2. *Influence of osmolarity of medium on taurocholate uptake by isolated brush-border membrane vesicles*

The uptake of 0.15 mM-[³H]taurocholate was determined in the presence of 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4), 10 mM-NaCl and sufficient cellobiose to give the indicated osmolarity. The values given represent equilibrium values obtained after 30 min of incubation at 45°C.

were diluted in a short contact time before they were collected rapidly on a cellulose nitrate filter, decreased the equilibrium values of tracer taurocholate to only about 10% of normal in the presence of glycocholate and 16% in the presence of taurocholate. These values are similar to those obtained for external binding. If taurocholate had been bound primarily to the outside of the membrane vesicles, a larger percentage of labelled taurocholate should have been displaced by unlabelled taurocholate or glycocholate during this procedure.

Effect of Na⁺ on taurocholate uptake

When ileal brush-border membrane vesicles were prepared in a NaCl-free medium and incubated in a NaCl-containing buffer (pH 7.4) at 37°C the uptake of taurocholate showed an 'overshoot' phenomenon

during the first 2 min and reached equilibrium after about 30 min (Fig. 3, upper curve). The 'overshoot' indicates an intravesicular accumulation of tauro-cholate and occurs because of the persistence of a Na^+ gradient when the intravesicular taurocholate has already reached the concentration in the incuba-tion medium (Murer et al., 1974). Owing to the relatively high amount of taurocholate bound at equilibrium to the inside of the vesicle, the actual 'overshoot' was underestimated. The initial uptake of taurocholate in the presence of a Na^+ gradient was stimulated about 11-fold, compared with its uptake in the presence of a KCl gradient after 20s of incuba-tion time.

Stimulation of anion flux by the Na^+ gradient does not necessarily mean a flux coupling via a co-trans-port system. In general coupling between the fluxes of cations and anions can also be due to an 'electrical coupling', following the principle of overall electro-neutrality of transmembrane fluxes. A distinction between these two possibilities was made by the following experiment. Addition of monactin, an electrogenic ionophore for univalent cations (Henderson et al., 1969), prevents the intravesicular accumulation ('overshoot') of taurocholate in the presence of a $NaNO_3$ gradient (concn. in medium > concn. in vesicle) (Table 3). In all experiments where monactin was used ($n = 4$) the equilibrium value of the incubation medium with monactin was about 20% lower than of the controls without monactin (all media received the same amount of ethanol, i.e. 1%). This might be due to a non-specific effect of monactin on the intravesicular space (destruction) after 30 min of incubation time. However, such a non-specific action of monactin cannot explain the monactin-induced decrease (175%) in taurocholate transport in the presence of a $NaNO_3$ gradient during the early time-points of incubation. Since monactin increases the cation-conductance of the membrane (Henderson et al., 1969), a Na^+-gradient-dependent diffusion potential should be increased initially rather than decreased by the addition of the ionophore. There-fore, if the Na^+-gradient-dependent movement of taurocholate across the membrane would be caused primarily by the diffusion potential and not by direct coupling, an increased uptake rate of taurocholate in the presence of monactin should be observed. Its decreased uptake in the presence of monactin (Table 3) is strong evidence for a co-transport system for Na^+ and taurocholate in brush-border membrane vesicles isolated from rat ileum.

A direct effect of Na^+ on the transport system for taurocholate is also indicated by tracer-exchange experiments (Fig. 4): the vesicles were preloaded with Na^+ or K^+ and in addition with unlabelled tauro-cholate, after which the uptake of labelled taurocho-late was measured. The tracer taurocholate influx, i.e. tracer exchange, in the absence of salt and tauro-cholate gradients proceeded more rapidly (about 4-fold) in the presence of Na^+ than in the presence of K^+. Since there was no Na^+ gradient as driving force, an 'overshoot' of taurocholate could not be detected.
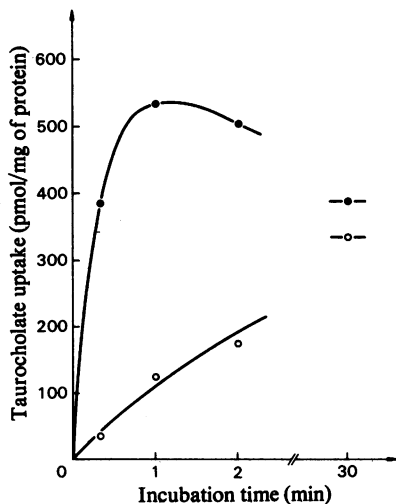

Fig. 3. *Effect of Na^+ and K^+ gradients on taurocholate uptake by rat ileal brush-border membrane vesicles*
Membrane vesicles were loaded as described in Fig. 1 and incubated at 37°C in the same medium containing also 0.075 mM-[³H]taurocholate and 100 mM-NaCl (●) or 100 mM-KCl (○).
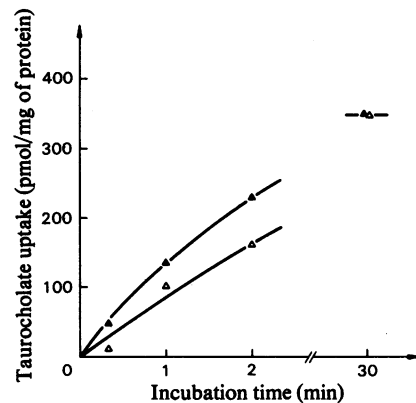

Fig. 4. *Effect of Na^+ on the taurocholate transport by ileal brush-border membrane vesicles (tracer exchange)*
Membrane vesicles loaded with 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4), 0.075 mM-taurocholate (unlabelled) and 100 mM-NaCl (▲) or 100 mM-KCl (△) were incubated at 37°C in the same medium containing also [³H]taurocholate.

Effect of the membrane potential on Na+-dependent taurocholate uptake

In the translocation of solutes across biological membranes an important question to answer concerns rheogenicity (charge transfer) during transport. Since in most cells, including epithelial cells of the small intestine, the electrical potential difference across the plasma membrane is cell-interior negative, this potential difference might represent an important component to the driving force for rheogenic solute uptake (Rose & Schultz, 1970, 1971). Rheogenic Na+-dependent transport of sugars and amino acids was demonstrated in studies with brush-border membrane vesicles isolated from small intestine and kidney proximal tubule (for review see Murer & Kinne, 1977). Therefore the effect of artificially imposed diffusion potentials on the Na+-dependent taurocholate uptake was analysed. The diffusion potentials were modified in the presence of cation gradients by means of anion replacement or by the use of ionophores. Table 4 shows the effect of anion replacement on taurocholate transport. In the presence of a Na+ gradient, replacement of Cl− by the more permeant anions SCN− and NO3− stimulated the initial uptake

of taurocholate. If under the same conditions the nearly impermeable anion SO_4^{2-} was used instead of Cl−, the rate of uptake was markedly decreased. Table 5 shows the effect of valinomycin, an ionophore that increases the K+ conductance of membranes (Henderson *et al.*, 1969; Pressmann, 1968), on taurocholate transport in the presence of a K+ gradient directed from the inside to the outside of the vesicles ($K_o < K_i$). Addition of valinomycin stimulated the uptake of taurocholate into the vesicles after 20s by 1.4-fold compared with the uptake without valinomycin. These experiments demonstrate that experimental treatments that lead to an increased electrical diffusion potential across the brush-border membrane (vesicle interior negative) are able to stimulate the Na+-dependent transport of taurocholate.

This phenomenon could be explained by the assumption that during taurocholate–Na+ co-transport, one taurocholate anion is transported together with at least two cations (at least one of them being Na+), whereby the Na+-dependent taurocholate transport becomes rheogenic. Such a rheogenicity of anion–Na+ co-transport processes would not be unique for the intestine, but is also observed for phosphate transport (Hoffmann *et al.*, 1976) at low pH,

Table 4. *Effect of anion replacement on taurocholate uptake into brush-border membrane vesicles (amount taken up during the first 0.33 min, 1 min and at equilibrium condition after 30 min)*

The experiments were carried out in an incubation medium as described in Table 1. The different salt gradients are given in the Table. Incubation temperature was 37°C.

Salt in incubation medium	Taurocholate uptake (pmol/mg of protein)		
	0.33 min	1 min	30 min (equilibrium)
0.1 M-NaNO3	191	280	183
0.1 M-NaSCN	160	218	173
0.1 M-NaCl	126	155	151
0.05 M-Na2SO4 + 0.05 M-mannitol	109	145	160

Table 5. *Effect of valinomycin on taurocholate transport in K+-preloaded membranes*

The membranes were prepared in buffer containing 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4) and, in addition, 50 mM-potassium cyclamate. Taurocholate uptake was initiated by adding 1 vol. of K+-preloaded membranes to 11 vol. of incubation medium with sodium cyclamate (50 mM) instead of potassium cyclamate and [3H]taurocholate (0.075 mM) substrate. Valinomycin, when present, was 15.6 μg/mg of protein. Incubation temperature was 37°C.

Conditions in incubation medium	Taurocholate uptake (pmol/mg of protein)		
	0.33 min	1 min	30 min (equilibrium)
Sodium cyclamate gradient	321	440	523
Sodium cyclamate gradient plus valinomycin	436	491	523

Table 6. *Effect of cation replacement on taurocholate uptake into brush-border membrane vesicles (amount taken up during the first 0.33 min, 1 min and at equilibrium condition after 30 min)*

The experiments were carried out in an incubation medium containing 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4), 0.1 mM-[³H]taurocholate and different salt gradients as given in the Table. Incubation temperature was 25°C.

Salt in incubation medium	Taurocholate uptake (pmol/mg of protein)		
	0.33 min	1 min	30 min (equilibrium)
0.1 M-LiCl	38	72	635
0.1 M-NaCl	395	509	588
0.1 M-KCl	52	58	466
0.1 M-RbCl	28	70	484
0.1 M-CsCl	43	101	499

for lactate transport (Barac-Nieto *et al.*, 1978) and for the transport of acidic amino acids (J. Seeger, H. Murer & R. Kinne, unpublished work) in renal brush-border membranes.

Further characteristics of the Na⁺-dependent transport system for taurocholate

Table 6 shows the effects of various cations on taurocholate transport by brush-border membrane vesicles. In comparison with KCl, only Na^+ showed a stimulatory effect on taurocholate uptake: Li^+, Rb^+ and Cs^+ were without effect. This experiment indicates that the Na^+–taurocholate co-transport system has a higher specificity for Na^+ than the intestinal Na^+-dependent co-transport systems for sugars, amino acids and P_i, where a significant stimulation of transport by Li^+ was observed (Sigrist-Nelson *et al.*, 1975; Berner *et al.*, 1976).

The uptake of taurocholate was saturable in the presence of a Na^+ gradient (Fig. 5, upper curve). In the presence of a K^+ gradient, a smaller and not saturable uptake (Fig. 5, straight line) was observed, which might indicate uptake by simple diffusion. Therefore it was assumed that the carrier-mediated Na^+-dependent taurocholate transport represents the difference between the uptake in the presence of Na^+ minus the uptake in the presence of K^+ (Fig. 5, lower curve). This Na^+-dependent component of taurocholate uptake was saturable in the range 2–4 mM. All other experiments described in the present paper have been carried out in a concentration range between 0.33 and 0.15 mM. As shown by the saturation experiment, the free diffusion of taurocholate at these concentrations is rather low (3–27% of total uptake). The values presented in all the experiments were therefore also not corrected for this diffusional and binding component.

Glycocholate, an analogue of taurocholate, was able to inhibit the uptake of taurocholate in a concentration of 0.1 mM in the presence of a Na^+ gradient to
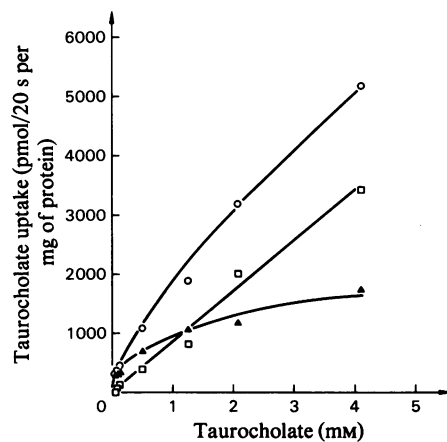

Fig. 5. *Saturation of taurocholate uptake*

The incubation medium contained 100 mM-mannitol, 20 mM-Hepes/Tris (pH 7.4), 100 mM-NaNO₃ (○) or 100 mM-KNO₃ (□) and [³H]taurocholate at the indicated concentrations. The specific Na^+-dependent [³H]taurocholate uptake (▲) is obtained by uptake in the presence of a NaNO₃ gradient (○) minus uptake in the presence of a KNO₃ gradient (□). The amount taken up by the vesicles at 37°C after 20 s is given.

about 50% of the initial uptake (Table 7). Glycocholate, at a concentration of 0.5 mM, had simultaneously a strong destructive (detergent) effect on the vesicles, which was indicated by decreased equilibrium uptake (50%) of taurocholate in the presence of glycocholate compared with its absence (data not shown).

Conclusions

In accordance with the results of Lack *et al.* (1977) the results presented above lead to the conclusion that taurocholate transport across rat ileal brush-border membranes is mediated by an electrogenic Na^+

Table 7. *Inhibition of taurocholate uptake by glycocholate*

The experiments were carried out in an incubation medium as described in Table 1, except for replacement of NO_3^- by Cl^-. Unlabelled glycocholate, when present, was 0.1 mM. Incubation temperature was 37°C.

Conditions in incubation medium (gradient: medium > vesicle)	Taurocholate uptake (pmol/mg of protein)		
	0.33 min	1 min	30 min (equilibrium)
NaCl plus taurocholate	126	155	151
KCl plus taurocholate	22	56	155
NaCl plus taurocholate plus glycocholate	65	102	162
KCl plus taurocholate plus glycocholate	23	57	123

co-transport system. Assuming that this system is present only at the luminal membrane the trans-epithelial transport of taurocholate in the ileum could be imagined to proceed in the following way: transport across the luminal membrane and intracellular accumulation is mediated by the Na^+–taurocholate co-transport system and driven by the electrochemical potential difference for Na^+. Efflux from the cell at the basolateral (contraluminal) cell side occurs by a Na^+-independent system driven by the chemical gradient for taurocholate and the electrical potential difference. The electrochemical potential difference for Na^+ across the luminal membrane is maintained by the action of the ($Na^+ + K^+$)-stimulated adenosine triphosphatase located in the basolateral membrane. Thus taurocholate transport could be defined as a secondary active transport.

The physiological importance of this transport system is underlined further by the observation that it is present only in microvillus membranes from the ileum, where also in studies *in vivo* active reabsorption of taurocholate is observed (Krag & Phillips, 1974).

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