Anion transport in basolateral (sinusoidal) liver plasma-membrane vesicles of the little skate (*Raja erinacea*)

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The mechanism(s) of [³⁵S]sulphate transport was investigated in basolateral liver plasma-membrane vesicles of the little skate elasmobranch, *Raja erinacea*. Imposition of an intravesicular alkaline pH gradient (pH 8.0 in/pH 6.0 out) stimulated sulphate uptake 5–10-fold compared with pH-equilibrated (pH 8.0 in = out) conditions and 2–3-fold over equilibrium sulphate uptake (overshoot). This pH-gradient-stimulated sulphate uptake was temperature-dependent, saturable with increasing concentrations of sulphate and could be inhibited by the protonophore carbonyl cyanide *m*-chlorophenylhydrazone and the anion-transport inhibitors 4,4'-di-isothiocyanostilbene-2,2'-disulphonic acid (DIDS) and probenecid. *cis*-Inhibition of pH-gradient-driven sulphate uptake was observed with sulphate, oxalate, cholate and bromosulphophthalein, but not with chloride and taurocholate. In addition, sulphate and oxalate *trans*-stimulated [³⁵S]sulphate uptake under pH-equilibrated conditions. Although also stimulated by an inside-alkaline pH gradient, transmembrane transport of [³H]cholate was not inhibited by DIDS, suggesting that its pH-gradient-driven uptake is not mediated by an anion-transport 'carrier'. In conclusion, these studies indicate that a basolateral plasma-membrane sulphate-transport system has evolved in skate hepatocytes and is similar to that in mammalian liver cells. This archaic anion-exchange system co-transporters certain organic anions such as oxalate and has developed early in vertebrate evolution.

INTRODUCTION

All vertebrate livers transport a wide variety of inorganic and organic anions from blood into bile. For many of these cholephilic compounds membrane transport systems have now been identified and characterized in sinusoidal and canalicular plasma membranes of mammalian hepatocytes (Boyer, 1986; Berk *et al.*, 1987). For example, in rat liver two basolateral (sinusoidal) organic anion-transport systems have been characterized in isolated plasma-membrane vesicles. The first system mediates Na⁺-gradient-driven (secondary active) hepatic uptake of taurocholate, the major bile salt in the rat (Inoue *et al.*, 1982; Van Dyke *et al.*, 1982; Duffy *et al.*, 1983). The second system represents a Na⁺-independent anion exchanger that has been described by us, using [³⁵S]-sulphate as tracer compound (Hugentobler & Meier, 1986). These studies indicated that the basolateral plasma-membrane domain of rat liver localizes a multi-specific hydroxyl/sulphate exchange system that can also transport organic anions such as the dicarboxylic acids oxalate and succinate. Furthermore, kinetic inhibition studies provided indirect evidence that the amphipathic anions cholate and bromosulphophthalein (BSP) may be taken up into hepatocytes by the same transport system.

Although bile flow in marine elasmobranch vertebrates such as the little skate (*Raja erinacea*) is about 100 times slower than in rodents (Boyer *et al.*, 1976a; Reed *et al.*, 1982), elasmobranch bile also is the major pathway for

elimination of amphipathic organic anions such as BSP or bile acids (Boyer *et al.*, 1976a,b; Grossbard *et al.*, 1987). Thus skate hepatocytes may, at least in part, possess the same plasma-membrane transport systems as mammalian liver cells. This assumption has been validated by the finding that basolateral plasma membranes of both rat and skate liver cells contain a similar bile-salt-binding polypeptide with an apparent *M_r* of 54000 (Kramer *et al.*, 1982; von Dippe & Levy, 1983; Fricker *et al.*, 1986). The present study now demonstrates that skate basolateral plasma-membrane vesicles also exhibit mediated hydroxyl/sulphate anion exchange, with properties that are very similar to those previously found in rat liver (Hugentobler & Meier, 1986). Thus the hepatic basolateral sulphate-anion-antiport system is similar to renal sulphate-transport processes (Renfro & Pritchard, 1982, 1983) and represents an archaic transport system that has evolved early during vertebrate evolution.

MATERIALS AND METHODS

All studies were performed at the Mount Desert Island Biological Laboratory, Salsbury Cove, ME, U.S.A., during July–August 1986.

Animals

Male skates (1.0±0.3 kg) were caught by net in Frenchman's Bay, off Bar Harbor, ME. They were

Abbreviations used: BSP, bromosulphophthalein; DIDS, 4,4'-di-isothiocyanostilbene-2,2'-disulphonic acid; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

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maintained in well-aerated tanks with running seawater at 15 °C and were used within 1–3 days of capture. Animals were anaesthetized before hepatectomy with pentobarbital sodium (2.5 mg/kg) injected via caudal veins.

Chemicals

[³⁵S]Sulphate (carrier-free) and [2,4-³H]cholic acid (25 Ci/mmol) were obtained from New England Nuclear (Boston, MA, U.S.A.). 4,4'-Di-isothiocyanostilbene-2,2'-disulphonic acid (DIDS), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and valinomycin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals and reagents were obtained from commercial sources, of the highest purity available.

Isolation of plasma-membrane subfractions

The procedure of Song *et al.* (1969) was used, with some modifications. All isolation steps were done at 0–4 °C. Livers (30–40 g) were rapidly removed from anaesthetized animals and chilled on ice. Portions (15–20 g) were cut into small pieces, washed in 3 × 100 ml of cold 1 mM-NaHCO₃ (pH 7.4), and homogenized in the same volume of 1 mM-NaHCO₃ in a Dounce homogenizer with a loose-fitting pestle (type A) (eight up-and-down strokes). The homogenate was further diluted to 900 ml with cold NaHCO₃ and filtered twice through two layers of 60-grade cheesecloth. The filtered homogenate was then centrifuged at 1500 *g* for 10 min. The resulting supernatant was discarded, and the 'crude nuclear pellet' resuspended in 5.5 vol. of 2.07 M-sucrose. A 15 ml portion of this suspension was overlaid with 10 ml of 1.42 M-sucrose and 10 ml of 1.25 M-sucrose, and the gradients were centrifuged at 74 300 *g* for 60 min in a Beckman SW 27 rotor. This procedure resulted in two membrane bands, at the 2.07 M-/1.42 M- and 1.42 M-/1.25 M-sucrose interfaces. The former subfraction was collected with a syringe, mixed with 5 vol. of 1 mM-NaHCO₃, and the material was centrifuged at 27 000 *g* for 10 min. The resulting pellets were combined and resuspended in a small volume of buffer suitable for the following experiments. Membranes were frozen with solid CO₂ and stored at –80 °C until used.

Protein and marker-enzyme analysis

The protein concentration of isolated membranes was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. 5'-Nucleotidase activity was determined as described by Avruch & Wallach (1971). The Mg²⁺-ATPase and the ouabain-sensitive Na⁺/K⁺-ATPase activities were determined by the coupled kinetic assay as modified by Scharschmidt *et al.* (1979). NADH- and NADPH-cytochrome *c* reductases were determined as described by Sottocasa *et al.* (1967).

Vesicle transport studies

For determination of pH-gradient-driven [³⁵S]sulphate uptake, freshly isolated membranes were vesiculated by repeated (10 times) passage through a 25-gauge needle in 'buffer 8.0', containing: 180 mM-sucrose, 40 mM-tetramethylammonium, 60 mM-K⁺, 0.2 mM-Ca²⁺, 5 mM-Mg²⁺, 110.4 mM-gluconate, 70 mM-Tris and 70 mM-Hepes, pH 8.0. In experiments where vesicles were preloaded differently, the composition of the membrane resuspension buffers is detailed in the corresponding Figure

legends. Frozen vesicle suspensions were quickly thawed in a 37 °C water bath, diluted to the desired protein concentration (~10 mg/ml), re-vesiculated by 20 passages through a 25-gauge needle, and routinely treated with valinomycin (5 µg/mg of protein) dissolved in dimethyl sulphoxide to control for the effects of membrane-potential changes. Uptake studies were performed at 20 °C by adding 90 µl of incubation medium to 10 µl of vesicle suspension. To create in-to-out hydroxylion gradients, the incubation medium ('buffer 6.0') contained 184 mM-sucrose, 40 mM-tetramethylammonium, 60 mM-K⁺, 0.2 mM-Ca²⁺, 5 mM-Mg²⁺, 110.4 mM-gluconate, 30 mM-Tris, 4 mM-Hepes and 90 mM-Mes, pH 6.0. Incubation media of different compositions are detailed in the corresponding Figure legends. Incubation media were supplemented with 2 × 10⁷ d.p.m. of [³⁵S]sulphate/ml or 10⁶ d.p.m. of ethanol-freed (by N₂ stream) [³H]-cholate/ml, and the desired substrate concentrations were adjusted with neutral di(tetramethylammonium) sulphate or Tris/cholate respectively. After incubation of the vesicles for the indicated time intervals, uptakes were terminated by addition of 3 ml of ice-cold stop solution, containing 204 mM-sucrose, 150 mM-K⁺, 0.2 mM-Ca²⁺, 5 mM-Mg²⁺, 150.4 mM-gluconate, 5 mM-sulphate, and 10 mM-Hepes/Tris, pH 7.5. Membrane-vesicle-associated ligand was separated from free ligand by immediate rapid filtration through a Millipore cellulose nitrate filter (0.45 µm pore size), which had been presoaked in cold deionized water. In cholate-uptake studies the filters were additionally rinsed with 1 mM-cholate before uptake, to decrease non-specific filter binding. After two washes with 3 ml of cold stop solution, the filters were dissolved in 5 ml of liquid-scintillation cocktail (Optifluor; Packard Instrument Co., Downers Grove, IL, U.S.A.), and the radioactivity was counted in a Packard Tri-Carb scintillation counter. Non-specific binding to filters and membranes was determined in each experiment by adding cold (0–4 °C) incubation and stop solution to 10 µl of membrane suspension. This membrane/filter blank was subtracted from all determinations. All experimental data were obtained from triplicate analyses of two or more separate membrane preparations.

RESULTS

Enzymic characterization of the isolated plasma-membrane subfraction

In the present investigation, plasma-membrane subfractions were isolated from skate liver by the method of Song *et al.* (1969), which has been previously used to prepare 'bile-canalicular-enriched' plasma membranes from rat liver. This procedure resulted in both 'light' and 'heavy' membrane subfractions, banding at the 1.25 M-/1.42 M- and 1.42 M-/2.07 M-sucrose interfaces respectively. The 'heavy' subfraction was used for most transport studies, for the following reasons: (a) 'heavy' membranes were 3–4-fold less enriched in Mg²⁺-ATPase and 5'-nucleotidase activities, indicating a lower content of bile-canalicular membranes, and (b) isolation of the 'heavy' subfraction was more reproducible and gave higher yields compared with 'light' membranes. As demonstrated in Table 1, the 'heavy' subfraction was 12-fold enriched over the homogenate in Na⁺/K⁺-ATPase activity, a marker for the basolateral (sinusoidal)

Table 1. Enzymic characterization of 'heavy' skate liver plasma membranes

Data represent means $\pm$ S.D., with the numbers of membrane isolations given in parentheses.

Enzyme	Specific activity (μ mol/h per mg)	Enrichment over homogenate (fold)	Recovery (homogenate = 100 %) (%)
Na ⁺ /K ⁺ -ATPase (11)	0.66 $\pm$ 0.32	12	6
Mg ²⁺ -ATPase (13)	2.45 $\pm$ 1.17	3.9	2
5'-Nucleotidase (2)	0.56	3.4	1.8
NADH-cytochrome <i>c</i> reductase (11)	2.34 $\pm$ 1.74	1.4	0.7
NADPH-cytochrome <i>c</i> reductase (9)	1.14 $\pm$ 1.32	1.3	0.7

plasma-membrane domain in rat liver (Blitzer & Boyer, 1978; Latham & Kashgarian, 1979; Meier *et al.*, 1984). In contrast, the relative enrichments of the canalicular markers Mg²⁺-ATPase and 5'-nucleotidase activities were only 4.2- and 3.4-fold respectively (Table 1). In addition, mitochondrial (NADH-cytochrome *c* reductase) and microsomal (endoplasmic reticulum; NADPH-cytochrome *c* reductase) membrane fragments were less than 2-fold enriched. Although not highly purified, the isolated plasma-membrane 'heavy' subfraction can be regarded to be relatively enriched in the basolateral (sinusoidal) domain of skate hepatocytes, and can be isolated rapidly within 3 h.

Sulphate-transport studies

In order to investigate whether skate liver basolateral plasma membranes contain a hydroxyl/sulphate anion-exchange system, we performed similar vesicle transport studies to those previously reported in rat liver (Hugentobler & Meier, 1986). Fig. 1 demonstrates that an intravesicular alkaline pH gradient (pH 8.0 in/pH 6.0 out) stimulated [³⁵S]sulphate uptake 5–10-fold compared with pH-equilibrated (pH 8.0 in = out) conditions, and 2–3-fold compared with equilibrium uptake values at 1 h (overshoot). Valinomycin had no effects on pH-gradient-dependent sulphate uptake, indicating that stimulation of sulphate uptake was not due to artificially induced ion-diffusion potentials. Furthermore, pH-gradient-driven [³⁵S]sulphate uptake rates were (a) temperature-sensitive (Fig. 1), (b) saturable with increasing concentrations of sulphate (apparent K_m 4.5 mM; V_{max} 22 nmol/min per mg) (Fig. 2), and (c) inhibited by the protonophore CCCP and the anion-transport inhibitors DIDS and probenecid (Table 2). Since the vesicles were voltage clamped with valinomycin, the inhibitory effects of CCCP most likely resulted from rapid dissipation of the pH gradient rather than a direct interaction of the protonophore with the sulphate-transport system. These findings strongly indicate that the skate liver contains a basolateral hydroxyl/sulphate exchange system and that its properties are similar to those observed in rat liver.

The above conclusion was further supported by *cis*-inhibition and *trans*-stimulation experiments. As demonstrated in Fig. 3, the addition of unlabelled sulphate, oxalate, cholate and BSP on the outside of the vesicles significantly inhibited pH-gradient-stimulated [³⁵S]sulphate uptake, but chloride and taurocholate had no effect. In addition, inclusion of unlabelled sulphate or

Fig. 1. pH-gradient-driven sulphate uptake in skate liver plasma-membrane vesicles

Vesicles were resuspended in 'buffer 8.0' (for composition see the Materials and methods section) and incubated either in the same buffer (no pH gradient; $\square$) or in 'buffer 6.0' (in-to-out OH⁻ gradient; $\bullet$). Valinomycin was used to short-circuit the membrane potential ($\circ$). Reactions were carried out at 20 °C ($\square$, $\bullet$, $\circ$) or 0 °C ($\blacksquare$). The assay concentration of [³⁵S]sulphate was 0.1 mM. The values represent means $\pm$ S.D. for six determinations in two separate membrane preparations.

oxalate within the vesicles *trans*-stimulated [³⁵S]sulphate uptake into pH- and voltage-clamped vesicles (Fig. 4). These results are again similar to our previous findings in rat liver (Hugentobler & Meier, 1986) and indicate that the identified anion-antiport system also transports dicarboxylic acids in addition to sulphate. Unfortunately, it was not possible to perform similar *trans*-stimulation experiments with millimolar concentrations of cholate and BSP, because of the well-known detergent effects and the high membrane binding of these amphipathic organic anions. Although the inhibition exerted by cholate and BSP on pH-gradient-driven [³⁵S]sulphate uptake (Fig. 3) was not evaluated kinetically, we finally

Fig. 2. Kinetics of pH-gradient-driven sulphate uptake in skate liver basolateral plasma-membrane vesicles

Vesicles were resuspended in 'buffer 8.0', and initial velocities (10 s) of pH-gradient-driven sulphate uptake were determined by incubating the vesicles in 'buffer 6.0' in the presence of increasing concentrations of sulphate (0.1–20 mM) and valinomycin (5 μ g/mg of protein). Kinetic values were linearized by plotting the data according to Hanes (inset), with the curve fitted by linear-regression analysis: $K_m \sim 4.5$ mM; $V_{max} \sim 22$ nmol/min per mg. Data represent the means of four uptake measurements.

Table 2. Effects of CCCP, DIDS and probenecid on pH-gradient-driven sulphate uptake in skate liver basolateral plasma-membrane vesicles

Absolute data represent means $\pm$ s.d., with the total numbers of uptake measurements given in parentheses. CCCP is a protonophore, and DIDS is an anion-exchange inhibitor.

Inhibitor	Concn.		Sulphate uptake	
			(pmol/15 s per mg of protein)	(%)
None (control)		(9)	169 $\pm$ 17	100
CCCP	0.02 mM	(6)	36 $\pm$ 27	21
DIDS	0.05 mM	(6)	102 $\pm$ 24	60
	0.50 mM	(6)	21 $\pm$ 10	12
Probenecid	0.50 mM	(6)	88 $\pm$ 20	52
	1.00 mM	(6)	35 $\pm$ 19	21
	5.00 mM	(6)	3 $\pm$ 3	2

attempted to substantiate our previous conclusions from rat liver that cholate is also a co-substrate of the basolateral hydroxyl/sulphate exchanger (Hugentobler & Meier, 1986) by determining the pH-gradient-dependency and DIDS-sensitivity of [3 H]cholate uptake into the skate liver vesicles.

Fig. 3. *cis*-Inhibition of pH-gradient-stimulated sulphate uptake

Vesicles were resuspended in 'buffer 8.0' and incubated for 15 s in 'buffer 6.0' containing 0.1 mM-[3 S]sulphate and the indicated concentrations of various inorganic and organic anions. Under all conditions equilibrium (e.g. 60 min)-sulphate uptakes were identical, indicating preservation of membrane integrity. All vesicles were voltage-clamped with valinomycin. Data represent the means $\pm$ s.d. for four determinations in two separate membrane preparations. Levels of significance of differences from controls were calculated by Student's *t* test; n.s., not significant; **P* < 0.001.

Vesicle uptake of cholate

Fig. 5 demonstrates that an inside-alkaline pH gradient (pH 8.0 in/pH 6.0 out) stimulates [3 H]cholate uptake above the equilibrium values at 1 h (overshoot) and above uptake values in the presence of pH-equilibrated conditions. The differences between cholate uptake at pH 6.0 in = out and pH 8.0 in = out can be explained by pH-dependent membrane binding of cholate (Accantino & Simon, 1976; Hugentobler & Meier, 1986). Although extravesicular sulphate (20 mM) significantly inhibited pH-gradient-driven cholate uptake, the extent of inhibition was smaller than theoretically expected for competition of sulphate and cholate for the same carrier. Furthermore, in contrast with hydroxyl/sulphate exchange (Table 2), pH-gradient-driven cholate uptake was not inhibited by DIDS (Fig. 5). This latter finding suggest that pH-gradient-driven cholate uptake is not mediated by anion exchange. Rather, cholate most probably inhibits pH-gradient-stimulated sulphate uptake (Fig. 3) by some other mechanism(s), possibly by dissipation of the transmembrane pH gradient, which would then decrease the driving force for sulphate uptake.

Fig. 4. *trans*-Stimulation of [35 S]sulphate uptake by sulphate and oxalate

Basolateral vesicles were resuspended in medium containing 180 mM-sucrose, 40 mM-tetramethylammonium, 60 mM- K^+ , 0.2 mM- Ca^{2+} , 5 mM- Mg^{2+} , 110.4 mM-gluconate, 10 mM-Hepes/Tris, pH 7.5, with either no addition ($\square$) or 5 mM-sulphate ($\bullet$) or -oxalate ($\circ$). [35 S]Sulphate uptake was initiated by the addition of 190 μ l of anion-free resuspension buffer to 10 μ l of vesicle suspension (100 μ g of protein), resulting in 20:1 in-to-out, sulphate or oxalate gradients. Extravesicular sulphate concentrations were adjusted to 0.25 mM for all incubations. All determinations were performed in the presence of equilibrated K^+ (60 mM in = out), of the potassium ionophore valinomycin (10 μ g/mg of protein) and of the protonophore CCCP (10 μ M), in order to avoid the secondary formation of transmembrane potential and/or pH gradients. Data represent means $\pm$ S.D. of three determinations in two membrane preparations.

DISCUSSION

The present study demonstrates an anion-exchanger (antiport) at the basolateral plasma membrane of skate liver that can facilitate transport of inorganic sulphate across the blood-faced membrane domain of skate hepatocytes. The properties of this antiport are very similar to those of the basolateral sulphate-anion-exchange system previously characterized in rat liver vesicles (Hugentobler & Meier, 1986). Thus, in both skate and rat liver plasma-membrane vesicles, sulphate uptake can be stimulated by an inside-alkaline pH gradient. This pH-gradient-driven sulphate uptake is inhibited by DIDS and probenecid, suggesting the presence of hydroxyl/sulphate exchange rather than proton/sulphate co-transport, although these two possibilities cannot be clearly differentiated from each other under the experimental conditions used. Furthermore, oxalate is a co-substrate for the basolateral sulphate transporter in both rat and skate liver, but chloride and taurocholate are not, since they do not *cis*-inhibit or *trans*-stimulate sulphate uptake (Figs. 3 and 4). The similar properties of the antiport in the two species suggest that hepatic uptake of sulphate is mediated by identical transport systems in mammalian and marine

Fig. 5. pH-gradient-dependent uptake of [3 H]cholate in skate liver basolateral plasma-membrane vesicles

Vesicles were resuspended in 'buffer 8.0' ($\circ$, $\bullet$, $\square$, $\triangle$) or 'buffer 6.0' ($\blacksquare$). The same buffer systems were also used to investigate [3 H]cholate (10 μ M, 20 $^{\circ}$ C) uptake under either pH-equilibrated ($\triangle$, pH 8.0 in and out; $\blacksquare$, pH 6.0 in and out) or pH-gradient ($\circ$, pH 8.0 in/pH 6.0 out) conditions. In the latter case 20 mM-di(tetramethylammonium) sulphate ($\bullet$) or 1 mM-DIDS ($\square$) was also included in the incubation medium. All vesicles were voltage-clamped with valinomycin (5 μ g/mg of protein). Values are means $\pm$ S.D. of quadruplicate determinations in one representative out of three experiments.

vertebrates. Thus hepatic basolateral sulphate-anion exchange must have evolved before the evolution of animal life from sea to land.

Marine species continuously ingest seawater that is highly enriched in sulphate (sulphate concn. $\sim$ 25 mM). Despite this continuous sulphate load, the plasma concentration of sulphate in marine animals such as teleosts is usually low ($\sim$ 1 mM) (Renfro & Pritchard, 1982), and is thought to be primarily maintained by efficient renal excretion of sulphate (Berglund & Forster, 1958). Indeed, high-capacity sulphate-transport systems have been characterized in basolateral and brush-border membrane vesicles from renal tubules of the southern flounder, *Paralichthys lethostigma* (Renfro & Pritchard, 1982, 1983). In these studies, basolateral transport of sulphate could also be stimulated by a trans-membrane pH gradient and inhibited by DIDS (Renfro & Pritchard, 1983). Furthermore, the basolateral teleost kidney transport system exhibited an apparent K_m for sulphate of approx. 2.5 mM, which is close to the affinity of the elasmobranch liver basolateral transport system (K_m 4.5 mM; Fig. 2). Although sulphate concentrations of elasmobranch bile are not known, the present findings suggest that transcellular excretion of sulphate from blood into bile might contribute to the regulation of plasma sulphate concentration in the fish. However, the slow flow rates of bile in elasmobranchs argue against

this possibility. Rather, this antiport may function to concentrate sulphate in the liver for the purpose of conjugating ligands to water-soluble less-toxic sulphate metabolites. For example, elasmobranch bile contains largely sulphated bile alcohols rather than bile acids, as commonly found in mammalian bile (Karlagnis *et al.*, 1982). Sulphate availability may therefore be important in biliary excretion in this species.

Alternatively, the characterized anion-exchange system may represent a multispecific transport system that primarily transports organic anions other than sulphate. The finding that sulphate-anion-exchange systems can transport a wide variety of endogenous and exogenous organic anions has been previously documented in basolateral membrane vesicles of rat kidney cortex (Löw *et al.*, 1984; Ullrich *et al.*, 1985a,b), rat intestine (Weinberg *et al.*, 1986) and rat liver (Hugentobler & Meier, 1986). In the last study, pH-gradient-driven sulphate uptake was competitively inhibited by cholate and BSP, suggesting co-transport of all three anions by the same transport system. Although the kinetics of inhibition exerted by cholate and BSP on sulphate uptake (Fig. 3) was not analysed in the present study, the fact that pH-gradient-driven cholate uptake was not inhibited by DIDS (Fig. 5) suggests that cholate is not a co-substrate for the hydroxyl/sulphate anion-exchange system in skate hepatocytes. Since cholic acid represents a weak organic acid, with an apparent pK_a of approx. 5, the data rather suggest that the pH gradient stimulates non-ionic diffusion of cholate into the vesicles. This interpretation is further corroborated by our preliminary findings that an inside-alkaline pH gradient also induces a transient intravesicular accumulation of cholate in artificial liposomes (Cafilisch *et al.*, 1987). Thus *cis*-inhibition of pH-gradient-driven sulphate uptake by cholate (Fig. 3), as well as inhibition of pH-gradient-driven cholate uptake by sulphate (Fig. 5), might have resulted from competition of both anions for the driving force (pH gradient) rather than for a common carrier. However, we cannot definitely exclude that, at a more physiological pH, anionic cholate may be taken up into hepatocytes via an anion-exchange mechanism. In fact, there is strong evidence that bile-salt and sulphate co-transport is mediated by a common anion-exchange mechanism in rat intestinal basolateral membrane vesicles (Weinberg *et al.*, 1986). Thus further studies will be required to investigate definitively whether anion exchange can account for Na^+ -independent transport of bile salts into skate hepatocytes (Smith *et al.*, 1987).

Finally, it has to be emphasized that the demonstration of pH-gradient-dependent sulphate (anion) uptake in membrane vesicles does not necessarily imply that the transmembrane pH difference is also the driving force for sulphate (anion) uptake in the intact cell. In fact, unless concomitant Na^+ /proton exchange maintains a high local in-to-out hydroxyl ion gradient, the intracellular pH, which is generally more acidic, would counteract sulphate (anion) uptake through exchange with intracellular hydroxyl anions. However, for broad substrate specificity, the direction of net ion fluxes via anion exchange would finally depend on the transmembrane distribution of any co-transported substrate. For example, basolateral efflux of intracellularly generated end products of intermediary metabolism could be coupled to hepatocellular uptake of metabolic substrates or bile components. Further studies will be required to delineate

more exactly the substrate specificity and physiological driving forces for sulphate and organic anion uptake into both skate and mammalian hepatocytes.

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