

Divalent Anion Transport in Crustacean and Molluscan Gastrointestinal Epithelia

George A. Gerencser and Gregory A. Ahearn

Abstract A novel invertebrate gastrointestinal transport mechanism has been shown to couple chloride/sulfate exchange in an electrogenic fashion. In the lobster, *Homarus americanus*, the hepatopancreas, or digestive gland, exists as an outpocketing of the digestive tract, representing a single cell layer separating the gut lumen and an open circulatory system comprised of hemolymph. Investigations utilizing independently prepared brush-border and basolateral membrane vesicles revealed discrete antiport systems which possess the capacity to bring about a transcellular secretion of sulfate. The luminal antiport system functions as a high affinity, one-to-one chloride/sulfate exchanger that is stimulated by an increase in luminal hydrogen ion concentration. Such a system would take advantage of the high chloride concentration of ingested seawater, as well as the high proton concentrations generated during digestion, which further suggests a potential regulation by resident sodium-proton exchangers. Exchange of one chloride for one divalent sulfate ion provides the driving force for electrogenic vectorial translocation. The basolateral antiport system was found to be electroneutral in nature, responsive to gradients of the dicarboxylic anion oxalate, while lacking in proton stimulation. No evidence of sodium/sulfate cotransport, commonly reported for the brush border of vertebrate renal and intestinal epithelia, was observed in either membrane preparation. The two antiporters together can account for the low hemolymph to seawater sulfate levels previously described in decapod crustaceans. A secretory pathway for sulfate based upon electrogenic chloride-antiport may appear among invertebrates partly in response to digestion taking place in a seawater environment.

Keywords Antiporter · Gastrointestinal epithelia · Divalent anion exchangers · Electrogenic · Nonelectrogenic · Mollusk · Crustacean

G.A. Gerencser (✉)

Department of Physiology and Functional Genomics, College of Medicine, University of Florida, Gainesville, FL 32610-0274, USA

e-mail: ggerencs@ufl.edu

1 Introduction – Crustacean Sulfate Transport

The first half of this chapter describes a proposed transcellular secretory mechanism for the divalent anion sulfate which has been characterized in the hepatopancreas of a decapod crustacean, *Homarus americanus*. The driving force for the overall transcellular movement of sulfate is a sulfate/chloride antiporter present in the brush border membrane which operates in an electrogenic fashion, exhibiting a 1:1 coupling ratio. The brush border antiporter is regulated by external protons, a physiologically relevant characteristic due to the generally low luminal pH of the crustacean hepatopancreas during digestion/absorption functions. There are separate anion exchangers on the hepatopancreatic basolateral membrane which provides for sulfate entry into the epithelial cell from the hemolymph or, conversely, for the movement of intracellular sulfate across the basolateral membrane into the hemolymph. The different processes involved with sulfate secretion will be discussed with reference to the current models of sulfate transport in vertebrate tissues.

The chapter in this volume by Markavich will extensively cover sulfate transporters in vertebrate epithelial membranes; therefore, we will give a cursory presentation of this topic in an introductory format as a prelude to sulfate transporters in crustacean and molluscan epithelia.

2 Vertebrate Sulfate Transport

Most of our current knowledge of sulfate transport mechanisms has been accumulated from investigations among many vertebrate organisms, with insects being the only invertebrate group where sulfate regulation has been studied [1, 2]. Mosquito larvae, existing in sulfate-rich hyperosmotic lakes, appear to absorb sulfate through the gut wall and secrete the anion, via a saturable system against a concentration gradient, by the Malpighian tubules [1].

2.1 Intestinal and Hepatic Sulfate Transport

Sulfate is actively reabsorbed across the apical border of the small intestine of the rabbit [3–5], the rat [6], and the pig [7] by a Na-cotransport system. Intestinal brush border membrane vesicles have also been shown to possess pH-dependent sulfate uptake [8] and the anion exchange mechanism [9]. The only reported mechanism for sulfate transport across the intestinal basolateral membrane is anion exchange [4, 10–12].

Anion exchange of inorganic and organic anions with sulfate has also been described in liver sinusoidal and canalicular plasma membrane vesicles from the rat [13, 14] and sinusoidal basolateral membrane vesicles of the elasmobranch [15].

2.2 Renal Sulfate Transport

Sulfate reabsorption in the mammalian kidney has been observed by numerous investigations. Luminal uptake is predominately via the sodium-dependent pathway [16–18], with anion exchange exhibited in both perfused tubules [19] and membrane vesicles [20, 21]. The transport systems described for sulfate exit from renal epithelia involve a basolateral exchanger which can accept both inorganic and organic anions [10, 22–24].

Other vertebrate renal systems have been investigated with respect to sulfate regulation. In contrast to the mammalian reabsorption paradigm teleost fish exhibit a net secretory sulfate mechanism. The driving force for transepithelial sulfate movement appears to be proton-dependent sulfate cotransport at the basolateral membrane [25]. No effect of sodium on sulfate uptake was observed in basolateral or brush border membrane vesicles. The efflux of sulfate from flounder renal tubule occurred by the anion exchanger demonstrated on the brush border [26].

Sulfate transport in the chick renal tubule demonstrated characteristics of both the mammalian and teleost systems described above [27]. Brush border membrane vesicles were shown to maintain multiple pathways for sulfate transport. Sulfate uptake was stimulated by both sodium and proton cotransport and anion exchange with bicarbonate. Basolateral membrane vesicles did not respond to sodium or proton cotransport, but sulfate/bicarbonate exchange produced concentrative uptake. It appears that the avian renal tubule maintains the capacity for potential bidirectional sulfate transport.

3 Transmembrane SO₄/Cl Exchange in Vertebrate Epithelia

Among the many sulfate/anion exchange mechanisms described for epithelial cells, there exists a wide variety of specificities for acceptable substrates which can drive the antiporter. Sulfate exchangers are saturable with respect to external sulfate and internal anions. Most of the antiporters are suppressed by the classic disulfonic stilbene exchange inhibitors such as SITS (4-acetamido-4'-isothiocyano-2,2'-disulphonic stilbene) and DIDS (4,4'-diisothiocyano-2,2'-disulfonic acid). There are few studies which directly address both the coupling ratio of sulfate anion exchange and electrogenicity. Table 1 provides a comparison of apparent kinetic constants for sulfate antiport in brush border and basolateral membranes of several vertebrate species and the lobster.

3.1 Luminal Sulfate Transport

Talor and coworkers [21] demonstrated the presence of an anion antiporter at the luminal membrane of the beef kidney epithelium by combined methods. First, they showed [³H]DIDS binding to the brush border membrane exhibiting kinetics

Table 1 Comparison of apparent kinetic characteristics of sulfate/anion antiporter in various animal epithelial systems

Antiporter	Epithelia	K_{in}	J_{max}	Reference
SO_4/HCO_3^-	Rat kidney BBM	0.4	1.1	[20]
SO_4/OH^-	Rabbit ileal BBM	0.48	4.1	[8]
SO_4/Cl^-	Lobster hepatopancreas BBM	0.27	11.0	[35]
SO_4/OH^-	Rat liver BLM	16	12.2	[13]
SO_4/HCO_3^-	Rabbit ileal BLM	0.12	539	[4]
SO_4/Cl^-	Rabbit ileal BLM	0.30	1.6	[11]
$SO_4/Oxalate$	Lobster hepatopancreas BLM	6.0	3.3	[38]

which suggested a single class of binding sites. Second, they observed stimulated sulfate uptake in the presence of outwardly directed chloride, bicarbonate, and hydroxyl ion gradients which were inhibited by DIDS and furosemide. On the basis of potassium/valinomycin-induced diffusion potentials, they concluded that the exchange process was electroneutral. It was suggested that the brush border antiporter may be involved in the secretory flux of sulfate [21]. At the brush border membrane of the rat kidney epithelium, a sulfate/bicarbonate antiporter has been shown which accepted chloride, nitrate, and hydroxyl ions [20] and which was inhibitable by both DIDS and SITS. Flounder renal brush border membrane vesicles demonstrated a concentrative sulfate/anion antiporter which was stimulated strongly by bicarbonate and to a lesser extent by chloride, thiosulfate, and thiocyanate [26]. A significant inhibition of sulfate/bicarbonate exchange occurred when challenged by DIDS and probenecid. Because of the fact that this antiporter did not respond to the manipulation of membrane potential, the authors proposed an exchange of one sulfate to two monovalent anions.

3.2 Basolateral Sulfate Transport

The rat renal sulfate/anion exchanger in the basolateral membrane showed a very broad range of substrate specificity with respect to inorganic and organic anions [23]. Stimulated uptake of sulfate occurred in the presence of chloride, bicarbonate, phosphate, thiosulfate, formate, acetate, lactate, p-aminohippuric acid, and oxalate. Probenecid was able to block the effects of the above anions. Pritchard and Renfro [22], working with the same tissue also observed sulfate anion exchange; although the carrier did not utilize chloride, it was found to be electroneutral and probenecid-sensitive. The chick renal tubule basolateral membrane also exhibited SITS-sensitive sulfate/bicarbonate exchange which was not *cis*-inhibited by chloride [27].

The rabbit ileum exhibited a carrier mediated sulfate/chloride exchange in the basolateral domain [11]. Among the anions tested oxalate was the most effective in *cis*-inhibition of the antiporter. The same membrane was shown to contain a sulfate/bicarbonate antiporter which also transported oxalate [24]. This appeared to be

an exchanger separate from the sulfate/chloride antiporter because chloride had no effect on sulfate/bicarbonate exchange [12].

Sulfate/oxalate exchange appeared to be a common mechanism in both sinusoidal and canalicular membrane vesicles of liver epithelia [13–15]. In rat sinusoidal membrane vesicles, sulfate uptake was stimulated by oxalate and succinate but not by chloride or bicarbonate [13]. In skate sinusoidal membrane vesicles, oxalate and sulfate *cis*-inhibited pH gradient stimulated sulfate uptake but chloride was ineffective [15]. In rat liver canalicular membrane vesicles, there was a sulfate/bicarbonate antiporter which was *trans*-stimulated by sulfate, oxalate, and thiosulfate, but not by chloride or other dicarboxylic acids [14]. All of the sulfate antiporters which accepted oxalate in liver membrane vesicles were suppressed by the anion exchange inhibitor DIDS.

4 Transmembrane Sulfate Antiport in Crustacean Hepatopancreatic Epithelium

The decapod hepatopancreas, also known as the digestive or midgut gland, is a multilobed structure located in the cephalothorax composed of many epithelial-lined blind-ended tubules. The gland exists as an outpocketing of the digestive tract in the pyloric region of the stomach and the anterior intestine [28–31]. Much research has been done which implicates the organ as a site for secretion of digestive enzymes, the site of final digestion of ingested materials, and the major site of nutrient absorption. Although the hepatopancreas has been largely known for its digestive and absorptive properties, it has been speculated that it may also play a role in excretion of ions or metabolic wastes [30, 32–34].

4.1 Electrogenic Sulfate/Chloride Exchange at the Luminal Membrane

Unlike sulfate transport mechanisms in vertebrate renal and intestinal brush border membranes, the lobster hepatopancreas was not shown to demonstrate a sodium/sulfate cotransport system [35]. However, recently, Gerencser and Levin [36] have demonstrated a nonelectrogenic $\text{Na}^{+-}/\text{SO}_4^{2-}$ symporter in the foregut apical membrane of the invertebrate *Aplysia californica*. Sulfate exchange via antiporters in the luminal membrane of lobster hepatopancreas was stimulated by chloride and sulfate, but not by bicarbonate gradients [35]. Because of the orientation of brush border membrane vesicles, the translocation of sulfate into the vesicular interior represented net sulfate movement into the hepatopancreatic epithelium. There are several lines of evidence which suggest that the physiological role of this antiporter is secretion of cytoplasmic sulfate into the lumen of hepatopancreatic tubules. First, the antiporter was found to respond strongly to the imposition

of a potassium/valinomycin-induced membrane potential. When the vesicular interior was positive, there was a stimulation of sulfate influx and an inhibition in the presence of a negative interior. On the basis of the negative transmembrane potential typical of intact epithelial cells [37], the movement of a net negative charge into an electronegative cytoplasm seems unlikely. A second line of evidence for the electrogenicity of this antiporter was provided when comparing chloride/sulfate exchange to sulfate/sulfate exchange under opposing membrane potentials. There was no effect of membrane potential on sulfate/sulfate exchange, while sulfate exchange with the monovalent anion reacted as described above. This suggested that the carrier protein operated in a 1:1 ratio regardless of substrate charge. Finally, radiolabeled chloride uptake into sulfate-loaded vesicles was stimulated in the presence of a negative interior when compared to short-circuited vesicles, which would imply the net movement of a negative charge out of the vesicle.

4.2 Modes of Chloride Transport

Previously described in the lobster hepatopancreatic epithelial basolateral membranes were a $\text{SO}_4^{2-}/\text{oxalate}^{2-}$ antiporter [38], a $\text{SO}_4^{2-}/\text{HCO}_3^-$ antiporter [39], and a $\text{Cl}^-/\text{HCO}_3^-$ antiporter [40] (Fig. 1). In support of the hypothesis that Cl^- could utilize any of the three documented antiporters, it was shown in recent studies [39, 40] that Cl^- could be driven to accumulate in the basolateral membrane vesicles (BLMV) via favorable gradients of either SO_4^{2-} , oxalate $^{2-}$, or HCO_3^- . Therefore, it is logical to assume that Cl^- appears to be a universal exchangeable anion for any of these three antiporters; therefore, one, two, or all three antiporters could provide the transport means for Cl^- homeostasis in the lobster.

4.3 Electroneutral Sulfate/Oxalate Exchange at the Basolateral Membrane

In the lobster hepatopancreatic epithelial basolateral membrane, we observed another antiport mechanism which accepted the divalent carboxylic anion oxalate [38]. This exchange mechanism was electroneutral and transported one sulfate for one oxalate ion. There appeared to be a quite specific acceptance of substrates with respect to other carboxylic acids. Formate, succinate, oxaloacetate, α -ketoglutarate, and citrate did not stimulate sulfate uptake as they do in other sulfate exchange systems. The sulfate/oxalate antiport did not respond to the imposition of transmembrane pH gradients as observed in the brush border exchanger. The sulfate/oxalate exchanger was extremely sensitive to the anion antiport inhibitors SITS and DIDS [38].

It was also shown in a later study [41] that sulfate exhibited similar binding characteristics to the antiporter whether bound internally or externally. Similarly, oxalate had similar binding characteristics to the antiporter whether it was bound internally

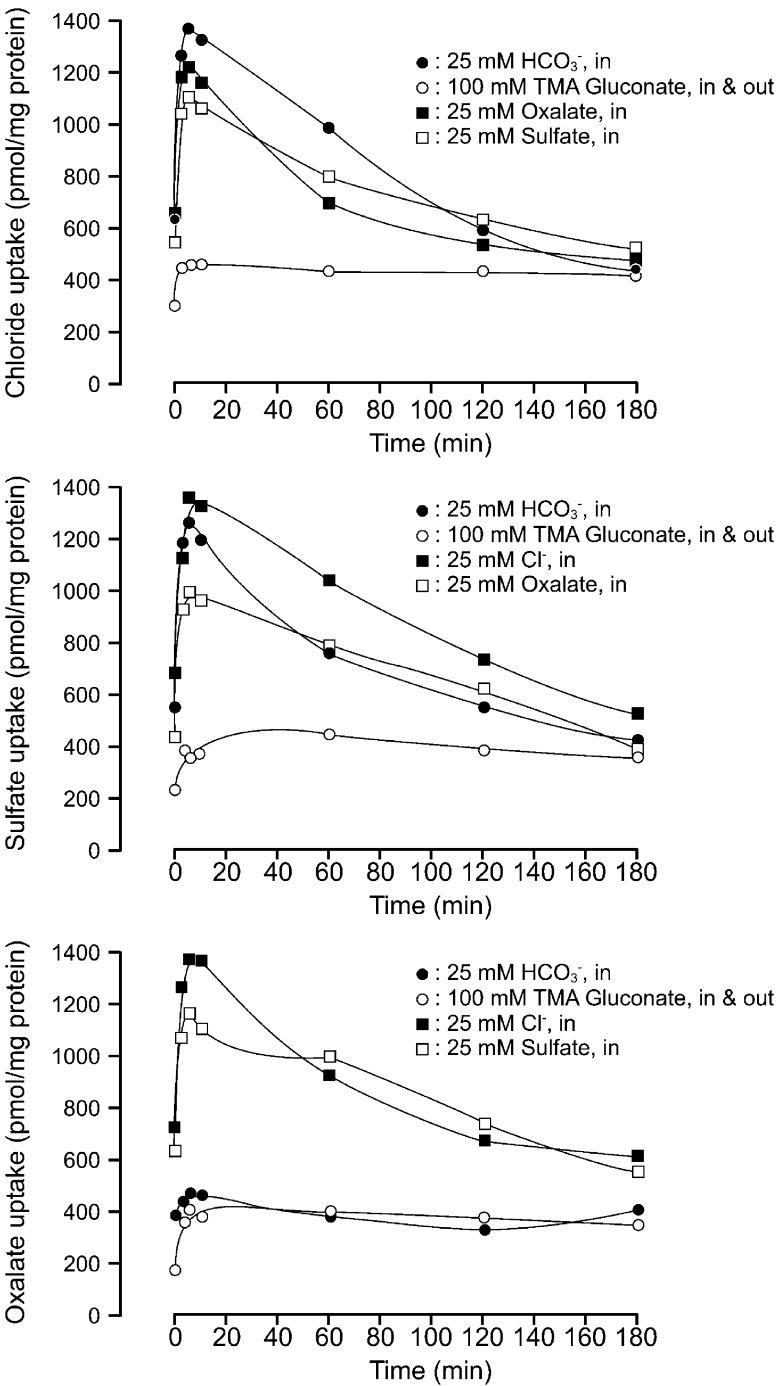


Fig. 1 Experimental protocols illustrating Cl⁻ as a ubiquitous substrate for either of three antiporters described in the BLM of lobster hepatopancreas

or externally. However, oxalate had a greater affinity for the antiporter than did sulfate. These results suggested that oxalate, not sulfate, regulates the antiporter transport rate because of its greater affinity to the transporter.

4.4 $\text{HCO}_3^-/\text{SO}_4^{2-}$ Exchange in the Basolateral Membrane (BLM)

Recently, a $\text{HCO}_3^-/\text{SO}_4^{2-}$ antiporter was described in BLMV of lobster hepatopancreatic epithelial cells [39]. The antiporter was electrogenic and shown to have a $\text{HCO}_3^-/\text{SO}_4^{2-}$ transport stoichiometry of 1:1 (Fig. 2). This mechanism was also inhibited by disulfonic stilbenes; however, it was stimulated by external protons. Speculatively, this antiporter could provide a cellular means for cytoplasmic pH regulation which could be further modified by extracellular pH (Fig. 3). At the same time, this antiporter could also provide intracellular uptake of sulfate. This event would be beneficial for cellular viability of cellular metabolic reactions such as: (1) sulfur conjugation [42], and/or (2) complexing with heavy metals as concretions [38] in intracellular membrane-bound vesicles. The sulfate may be needed to detoxify these heavy metals before they are transferred to the blood where they could have deleterious effects in other parts of the crustacean's body. Additionally, sulfate movement from the cytoplasm to the hepatopancreatic lumen via the sulfate/chloride exchanger located in the brush border membrane [35] could execute a secretory function [38, 42] as a means for sulfate excretion [32, 33]. However, it is incumbent to include the possibility of sulfate absorption by this brush border sulfate/chloride exchanger since it is a bidirectional transport device. Then accumulated intracellular sulfate could facilitate the absorption of bicarbonate across

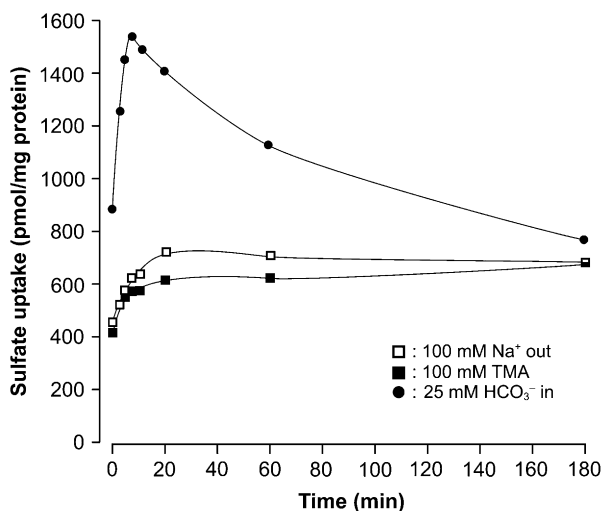


Fig. 2 Time course of bicarbonate-driven sulfate uptake by lobster hepatopancreatic BLMV

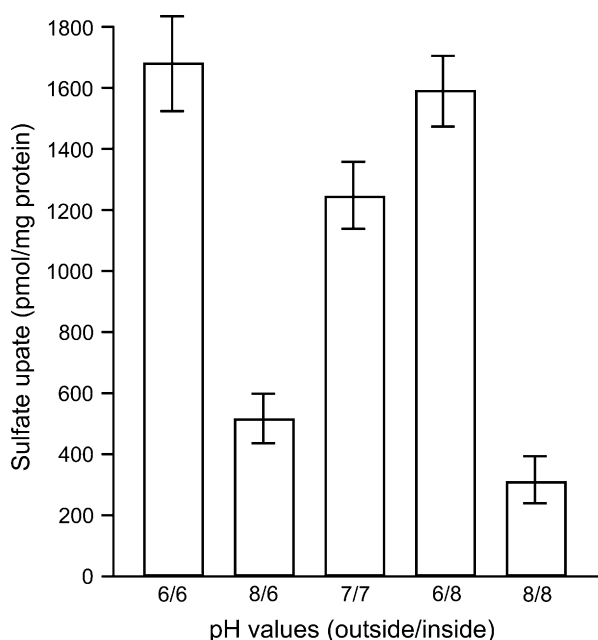


Fig. 3 Effect of various bilateral pH conditions and pH gradients on active uptake of sulfate into hepatopancreatic BLMV

the basolateral membrane via the sulfate/bicarbonate exchanger thereby providing a regulatory process for intracellular acid–base regulation.

4.5 Proton Regulation of Hepatopancreatic Chloride/Sulfate Exchange

As mentioned above, the luminal contents of the crustacean hepatopancreas have been recorded at a low pH value of 4 [30] due to the digestive processes of the organ. In the light of the reports of pH-dependent sulfate transport in different epithelial membranes [11, 25], and this natural proton gradient maintained across the hepatopancreatic brush border, it seemed likely that there might be a similar system in the lobster. Although no pH-dependent sulfate transport was shown in hepatopancreatic vesicles, there was a marked effect of low pH on chloride/sulfate exchange. In the presence of low extravesicular pH (high proton concentration), chloride/sulfate exchange was significantly enhanced. This effect was observed in the presence of both a transmembrane proton gradient and bilaterally equal pH conditions [42].

Regulation of membrane bound transport proteins by protons at either internal or external modifier sites has been described for several different cell types. Chloride/bicarbonate exchange in rabbit ileal brush border membranes was stimulated by a cytoplasmic pH modifier site which was presumed to be the effect

of internal hydroxyl ions [43]. In lobster basolateral membrane vesicles, chloride/bicarbonate exchange was stimulated by protons [39, 40, 42], but due to the response of this antiporter to transmembrane chloride and pH gradients, it was suggested that the process involved simultaneous movements of a proton with chloride. However, it was shown in the previous section that a newly discovered $\text{SO}_4^{2-}/\text{HCO}_3^-$ antiporter in the BLM of hepatopancreatic epithelial cells was stimulated by protons, but not by a proton gradient [39] (Fig. 3). Since this mechanism may be involved in both intracellular and extracellular pH regulation and the $\text{Cl}^-/\text{SO}_4^{2-}$ antiporter in nonpH related functions, the mode of proton regulation for $\text{HCO}_3^-/\text{SO}_4^{2-}$ and $\text{HCO}_3^-/\text{Cl}^-$ antiporters would be expected to be different. Lymphocytes also demonstrated an internal pH regulatory site which adjusted chloride/bicarbonate exchange in response to sodium/proton exchange [44]. A kidney cell line, Vero cells, showed a pH regulation of chloride antiport (either chloride/bicarbonate or chloride/chloride). This investigation performed on intact cells suggested that in response to internal hydroxyl ions chloride uptake was significantly enhanced [19]. Recently we observed the luminal membrane of lobster hepatopancreatic epithelium exhibits a sulfate antiporter which accepts only chloride, oxalate, or sulfate as the counter ion and it is stimulated by protons [45]. The relative specificity of this antiporter and its response to external proton concentration significantly differs from the basolateral membrane antiporter for sulfate [39] (Figs. 4, 5, and 6).

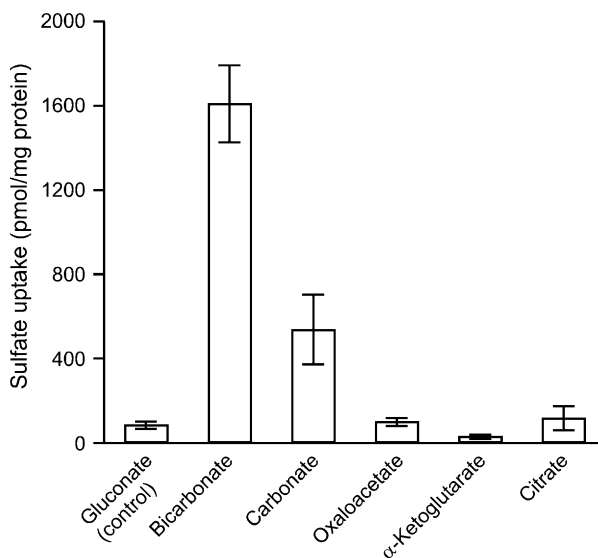


Fig. 4 Effect of internal organic anions on sulfate uptake into hepatopancreatic BLMV

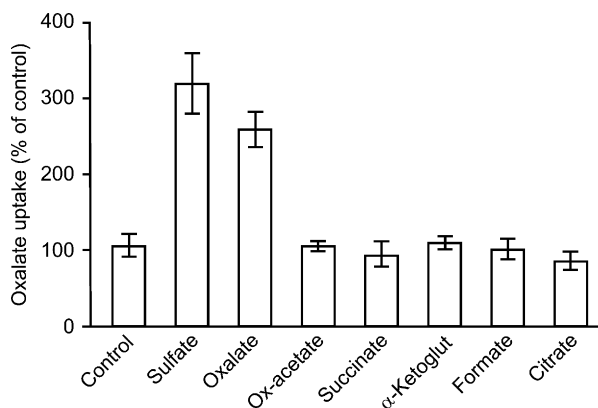


Fig. 5 Effect of internal organic anions on active oxalate uptake into hepatopancreatic Brush Border Membrane Vesicles (BBMV)

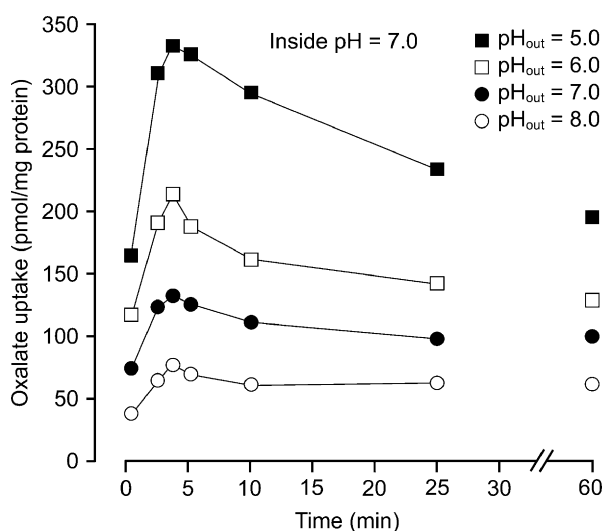


Fig. 6 Influence of external pH on active uptake of oxalate into hepatopancreatic BBMV

5 Transcellular Sulfate Transport in Lobster Hepatopancreas

The epithelial cells of the hepatopancreas operate in the secretion of digestive enzymes and the absorption of nutrients from the luminal contents [30]. There have been a variety of nutrient transporters described for the brush border of the lobster hepatopancreas which confirm the absorptive nature of the organ [45]. The lobster controls the concentration of sulfate in the hemolymph (18 mM) at a value lower than found in seawater which is 25 mM [46]. The lobster, as with other crustaceans, ingests seawater during both eating and drinking, which provides a high sulfate concentration in the hepatopancreas lumen [47, 48]. On the basis of our studies with

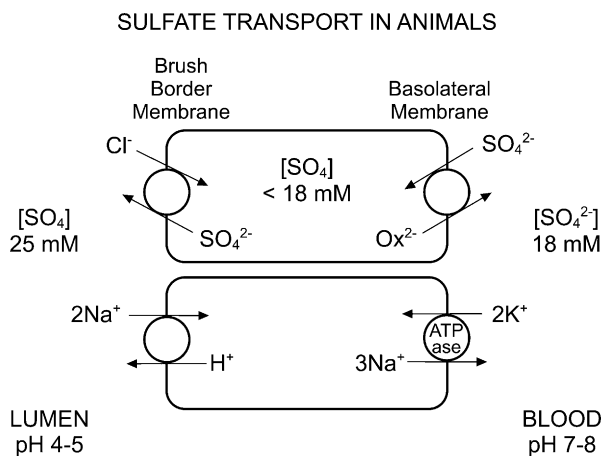


Fig. 7 Schematic diagram of hepatopancreatic epithelium illustrating the proposed model of sulfate secretion

sulfate transport in isolated membrane vesicles, we proposed a secretory pathway for this anion by the epithelial cells of the hepatopancreas (Fig. 7).

The initial step for sulfate movement from hemolymph to cytoplasm of the hepatopancreas cells occurs by an anion exchange system located in the basolateral membrane. The exchange of cytoplasmic oxalate for sulfate occurs by a saturable carrier-mediated mechanism which does not accept similar dicarboxylic acids. The cytoplasmic concentration of sulfate is unknown and assumed to be relatively low, so that the driving force for this antiporter would be the transmembrane sulfate gradient. The kinetic characteristics of the exchanger support this observation with the apparent binding constant for sulfate on the extracellular surface of the carrier being physiologically relevant ($K_m = 6.0 \text{ mM}$) to the blood concentration. The apparent binding coefficient for cytoplasmic oxalate is relatively low ($K_m = 0.18 \text{ mM}$), suggesting a high affinity of the antiporter for oxalate [41]. The physiological role of oxalate in the lobster is uncertain; it is possible that the anion, in the form of calcium oxalate, is involved with the sequestering of, or in the deposition of, calcium that occurs prior to molting and the formation of the new exoskeleton [38].

The sulfate/oxalate antiporter is an electroneutral carrier which exchanges one sulfate for one oxalate ion. The basolateral carrier also exhibits self-exchange and appears to accept chloride as a substrate. There is no H^+ stimulation of the carrier due to manipulations of transmembrane pH gradients. The carrier is very sensitive to the anion exchange inhibitors DIDS and SITS, which provides support for an antiport system [38].

The movement of sulfate from the hepatopancreas cytoplasm into the tubular lumen occurs by a separate antiporter present in the brush border membrane of the epithelial cells [35]. This electrogenic chloride antiporter appears to be the overall driving force for the transcellular movement of sulfate by this epithelium.

The combined transmembrane chloride gradient and the extrusion of a net negative charge from the cytoplasm of the epithelial cell would drive the carrier. When a potassium/valinomycin-induced membrane potential was established across the vesicle wall, sulfate uptake was stimulated by a positive, and inhibited by a negative electrical, interior. In contrast, when measuring chloride uptake into sulfate-loaded vesicles, there was enhanced chloride uptake due to an inside-negative membrane potential. On the basis of this observation and the normal gradients established in the lumen and the cytoplasm of hepatopancreas cells, the evidence suggests that the predominant movement of sulfate is out of the cell. The luminal membrane of the hepatopancreas lacks the sodium/sulfate cotransport system typical of vertebrate renal [16, 46] and intestinal [17] epithelia. Along with the absence of a sodium cotransport system, the brush border does not exhibit proton (hydroxyl)-stimulated transport such as that of the rabbit ileal brush border [8] or flounder basolateral renal tubule [25]. This was observed in the presence of either inward- or outward-directed pH gradients and under equilibrated sulfate conditions with a simultaneous inward-directed proton gradient [35].

It was interesting to find that chloride/sulfate exchange was enhanced by elevated external protons; the lower the extravesicular pH, the greater the sulfate uptake [35]. This was observed under pH gradient or equilibrated pH conditions. This is important when considering other physiological properties of the crustacean hepatopancreas. The gastrointestinal contents are acidic and are continuous with the hepatopancreas lumen, thereby providing an environment relatively high in protons during digestion and absorption. Until recently, the mechanism of tubular acidification was unclear. Recently, a sodium/proton exchanger was described in both the lobster and freshwater prawn hepatopancreatic brush border which could adequately account for observed luminal proton concentrations [47]. This acid environment would allow for increased activity of the electrogenic chloride/sulfate antiporter and play an important role in the regulation of sulfate [48] (Fig. 1).

The net secretion of sulfate by the lobster hepatopancreas represents an example of sulfate handling very different from that of vertebrate sulfate handling. Although there are sulfate anion exchange mechanisms present on the brush border of renal tubules [20, 21], the potent sodium cotransport system prevails driving tubule reabsorption. The antiporters in the lobster hepatopancreas have many properties in common with mammalian epithelia, yet the overall function appears to differ markedly. Sulfate secretion, also driven by anion exchange at the brush border, has been observed in the flounder renal tubules which also shares attributes of the lobster antiporter [49]. It appears that the lobster hepatopancreas acts in a manner similar to the teleost kidney with respect to the regulation of sulfate homeostasis.

6 Sulfate and Phosphate Transport in Molluscan Epithelia

Gastrointestinal and renal transport of the divalent anion sulfate across epithelia has been investigated in various vertebrate groups including mammals [5, 20], teleost fish [25, 26], and the domestic chicken [27]. A number of mechanisms for

brush-border carrier-mediated sulfate transport across epithelial membranes have been proposed and include sodium/sulfate cotransport [5, 16], anion exchange [21, 25], and pH gradient-dependent transfer [8]. These processes contribute to transepithelial regulation of sulfate levels, and may affect acid–base balance and plasma osmolarity [48].

However, there are very few studies of sulfate transport across epithelia of invertebrates. A proton-stimulated sulfate/chloride exchanger has recently been described in apical membranes of lobster (*H. americanus*) hepatopancreatic epithelial cells (this chapter).

6.1 Sulfate Transport in *Aplysia* Gut

However, it was not until 1979 [50] that active absorption of sulfate in a molluscan epithelium was demonstrated. It was further shown, more recently, that active sulfate absorption by the *A. californica* foregut was sodium dependent and this action dependence was also concentration dependent [36]. The symporter for sodium and sulfate resided in the luminal membrane, had a stoichiometry of 2 sodium: 1 sulfate, and was not inhibited by phosphate, but was competitively inhibited by thiosulfate.

6.2 Regulation of Sulfate Transport in *Aplysia* Gut

In contrast to the wealth of knowledge of renal and intestinal apical sodium/sulfate symport mechanisms very little information is available in the literature about possible regulatory control mechanisms for renal and intestinal apical sodium/sulfate symport. Glucocorticoids have been shown to be inhibitory for renal reabsorption of sulfate in the chicken [27] and thyroid hormone has been shown to stimulate sodium/sulfate symport activity in rat kidney [51]. Furthermore, the intestinal transport of sulfate in the rabbit seems to be regulated by some secretory stimuli, such as theophylline or the heat-stable enterotoxin [3–5].

It was recently demonstrated that the sodium sulfate symporter located in the luminal membrane of *A. californica* foregut was up-regulated by triiodothyronine [52]. The gastropod, *A. californica*, has been shown to have a hormone equivalent to triiodothyronine [35] which strengthens the physiological significance of this finding. Recently we reported that cyclic guanosine monophosphate stimulated the sodium/sulfate symporter in the apical membrane of *Aplysia* foregut absorptive cells [53]. Therefore, triiodothyronine could stimulate the production of cGMP which would act as the secondary messenger of triiodothyronine in stimulating the Na/SO₄ symporter similar to what has been reported in amphibian tissue [54]. Also, it appears that the triiodothyronine stimulation of the sodium/sulfate symporter via induced protein synthesis for actinomycin D, puromycin, or cycloheximide thwarted the triiodothyronine effects on active absorption of sulfate [55]. This transport event

could be beneficial for viability of cellular metabolic reactions such as sulfur conjugation [20] complexing with heavy metals, as happens in lobster hepatopancreas [38]. Sulfate homeostasis in the *Aplysia* is, at least, partly maintained by this luminal T_3 -regulated sodium/sulfate symporter [36, 52] which is mediated through de novo protein synthesis [55]. In contrast it was demonstrated that the sodium/sulfate symporter was down-regulated by glucocorticoids [56]. The physiological significance of glucocorticoid inhibition of the sodium/sulfate symporter in the *Aplysia* foregut may be related to glucocorticoid inhibition of glucose uptake and utilization by cells in general. Since there is less glucose utilization by cellular metabolism, there would be less need for cellular sulfate for sulfonation reactions such as protein synthesis. Therefore, glucocorticoid inhibition of cellular sulfate uptake would work in tandem with its inhibition of cellular glucose uptake and utilization.

6.3 Phosphate Transport in *Aplysia* Gut

Gastrointestinal and renal transport of the anion phosphate across epithelial apical membranes has been investigated in various vertebrate groups including: mammals such as rabbit [57] and rat [58]; avian such as chicken [59], and other lower vertebrates [60]. Studies with intact vertebrate tissue preparations have documented that transepithelial inorganic phosphate (P_i) transport against an electrochemical potential difference in the small intestine is dependent on the presence of sodium (Na^+) [61]. In vertebrates, this process can contribute to the transepithelial regulation of P_i levels, and may affect acid–base balance and plasma osmolarity.

However, there is a dearth of studies regarding P_i transport across epithelia of invertebrates. In view of this vacuum of P_i transport information in invertebrates, the present chapter was written to describe the nature of the P_i transporter in the mucosa of *Aplysia* gut. Recently, Gerencser and colleagues [62] used isolated foregut from *A. californica* to characterize a Na^+/P_i symporter that is located in the mucosal membrane of the gut cells, is inhibited by arsenate and ouabain, and has a stoichiometry of 2 Na^+ :1 P_i .

6.4 Regulation of Phosphate Transport in *Aplysia* Gut

1. Hormonal regulation of P_i transport in vertebrate renal or gastrointestinal epithelia has shown an up-regulation of phosphate absorption by triiodothyronine [63] and a down-regulation of P_i absorption by glucocorticoids [64].
2. Similarly, the Na^+/P_i symporter in the invertebrate *Aplysia* foregut luminal membrane was up-regulated by T_3 [65] and down-regulated by glucocorticoids [66]. Additionally, it seems that the T_3 stimulation of Na^+/P_i symporter activity in the *Aplysia* foregut is via induced protein synthesis because actinomycin D, puromycin, or cycloheximide inhibited the T_3 effects on Na^+/P_i active absorption

[67]. Since the gastropod, *A. californica*, has been shown to possess a hormone equivalent to T_3 [35], based upon the presence and activity of iodinated thyronine-like compounds in the animal, these present observations strengthen the physiological significance of this finding. This event could be beneficial for cellular viability of cellular metabolic reactions such as phosphorylation. P_i homeostasis in *Aplysia*, is at least, partly maintained by T_3 regulation of the luminal Na^+/P_i symport transport mechanism [65].

6.5 Statement of Significance

The evolutionary ties of invertebrates to vertebrates intellectualizes the genetic bond between the two animal groups. This can facilitate, in the clinical setting, amelioration of protein-deficient diseases by drugs such as triiodothyronine which can aid in incorporation of peptide side-groups that contain sulfate and/or phosphate.

Acknowledgements This project was supported by the Eppley Foundation for Research, Inc. (G.A.G.) and by N.S.F. IBN 99-74569 (G.A.A.).

References

1. Maddrell, HP and Phillips, JE. Active transport of sulphate ions by the Malpighian tubules of larvae of the mosquito *Aedes campestris*. J. Exp. Biol., 1975; 62:367–378.
2. Maddrell, HP and Phillips, JE. Induction of sulphate transport and hormonal control of fluid secretion by Malpighian tubules of larvae of the mosquito *Aedes taeniorhynchus*. J. Exp. Biol., 1978; 72:181–202.
3. Smith, PL, Orella, SA and Field, M. Active sulfate absorption in rabbit ileum: dependence on sodium and chloride and effects of agents that alter chloride transport. J. Membr. Biol., 1981; 63:199–206.
4. Langridge-Smith, JE and Field, M. Sulfate transport in rabbit ileum: characterization of the serosal border anion exchange process. J. Membr. Biol., 1981; 63:207–214.
5. Langridge-Smith, JE, Sellin, JH and Field, M. Sodium influx across the rabbit ileal brush border membrane: sodium and proton dependence, and substrate specificities. J. Membr. Biol., 1983; 72:131–139.
6. Cardin, CJ, and Mason, J. Sulphate transport by rat ileum: effect of molybdate and other anions. Biochim. Biophys. Acta, 1975; 394:46–54.
7. Wolfram, S, Grenacher, B and Scharer, E. Transport of selenate and sulphate across the intestinal brush-border membrane of pig jejunum by two common mechanisms. J. Exp. Physiol., 1988; 73:103–111.
8. Schron, CM, Knickelbein, RG, Aronson, PS, Della Puca, J and Dobbins, JW. pH gradient-stimulated sulfate transport by rabbit ileal brush-border membrane vesicles: evidence for SO_4 -OH exchange. Am. J. Physiol., 1985; 249:G607–G613.
9. Knickelbein, RG., Aronson, PS and Dobbins, JW. Substrate and inhibitor specificity of anion exchangers on the brush border membrane of rabbit ileum. J. Member Biol., 1985; 88:199–204.
10. Hagenbuch, B., Stange, G and Murer, H. Transport of sulphate in rat jejunal and rat proximal tubular basolateral membrane vesicles. Pflugers Arch, 1985; 405:202–208.
11. Schron, CM, Knickelbein, RG, Aronson, PS and Dobbins, JW. Evidence for carrier-mediated Cl - SO_4 exchange in rabbit ileal basolateral membrane vesicles. Am. J. Physiol., 1987; 253:G404–G410.

12. Knickelbein, RG and Dobbins, JW. Sulfate and oxalate exchange for bicarbonate across the basolateral membrane of rabbit ileum. *Am. J. Physiol.*, 1990; 259: G807–G813.
13. Hugentobler, G and Meier, PJ. Multispecific anion exchange in basolateral (sinusoidal) rat liver plasma membrane vesicles. *Am. J. Physiol.*, 1986; 251:G656–G664.
14. Meier, PJ, Valantinas, J, Hugentobler, G and Rahm, I. Bicarbonate sulfate exchange in canalicular rat liver plasma membrane vesicles. *Am. J. Physiol.*, 1987; 253: G461–G468.
15. Hugentobler, G, Fricker, G, Boyer, JL and Meier, PJ. Anion transport in basolateral (sinusoidal) liver plasma-membrane vesicles of the little skate (*Raja erinacea*). *Biochem. J.*, 1987; 247:589–595.
16. Lucke, H, Stange, G and Murer, H. Sulphate-ion/sodium-ion co-transport by brush-border membrane vesicles isolated from rat kidney cortex. *Biochem. J.*, 1979; 182:223–229.
17. Schneider, EG, Durham, JC and Sacktor, B. Sodium-dependent transport of inorganic sulfate by rabbit renal brush-border membrane vesicles. Effects of other ions. *J. Biol. Chem.*, 1984; 259:14591–14599.
18. Turner, RJ. Sodium-dependent sulfate transport in renal outer cortical brush border membrane vesicles. *Am. J. Physiol.*, 1984; 247:F793–F798.
19. Brazy, PC. and Dennis, VW. Sulfate transport in rabbit proximal convoluted tubules: presence of anion exchange. *Am. J. Physiol.*, 1981; 241:F300–F310.
20. Pritchard, J B. Sulfate-bicarbonate exchange in brush-border membranes from rat renal cortex. *Am. J. Physiol.*, 1987; 252:F346–F356.
21. Talor, Z, Gold, RM., Yang, WC and Arruda, JAL. Anion exchanger is present in both luminal and basolateral renal membranes. *Eur. J. Biochem.*, 1987; 164:695–702.
22. Pritchard, JB. and Renfro, JL. Renal sulfate transport at the basolateral membrane is mediated by anion exchange. *Proc. Natl. Acad. Sci. (USA)*, 1983; 80:2603–2607.
23. Low, I, Friedrich, T and Burckhardt G. Properties of an anion exchanger in rat renal basolateral membrane vesicles. *Am. J. Physiol.*, 1984; 246:F334–F342.
24. Kuo, SH and Aronson, PS. Oxalate transport via the sulfate/HCO₃ exchanger in rabbit renal basolateral membrane vesicles. *J. Biol. Chem.*, 1988; 263:9710–9717.
25. Renfro, JL and Pritchard, JB. H⁺-dependent sulfate secretion in the marine teleost renal tubule. *Am. J. Physiol.*, 1982; 243:F150–F159.
26. Renfro, JL and Pritchard, JB. Sulfate transport by flounder renal tubule brush border: presence of anion exchange. *Am. J. Physiol.*, 1983; 244:F488–F496.
27. Renfro, JL, Clark, NB, Metts, RE and Lynch, MA. Sulfate transport by chick renal tubule brush-border and basolateral membranes. *Am. J. Physiol.*, 1987; 252: R85–R93.
28. Loizzi, RF. Interpretation of crayfish hepatopancreatic function based on fine structural analysis of epithelial cell lines and muscle network. *Z. Zellforsch.*, 1971; 113:420–440.
29. Van Weel, PB. Hepatopancreas? *Comp. Biochem. Physiol.*, 1974; A47: 1–9.
30. Gibson, R and Barker, PL. The decapod hepatopancreas. *Oceanogr. Mar. Biol. Annu. Rev.*, 1979; 17:285–346.
31. Dall, W and Moriarty, DJW. Functional aspects of nutrition and digestion. In: Mantel LH, ed., *The biology of crustacea. Internal anatomy and physiological regulation*. Vol. 5, Academic Press, New York, 1983; 215–261.
32. Gifford, CA. Some aspects of osmotic and ionic regulation in the Blue crab *Callinectes sapidus*, and the Ghost crab, *Ocypode albicans*. *Publ. Inst. Mar. Sci. Univ. Tex.*, 1962; 8:97–125.
33. Dall, W. Osmoregulation in the lobster *Hornarus americanus*. *J. Fish Res. Board Can.*, 1970; 27:1123–1130.
34. Miller, DS and Hollida, CW. Organic cation secretion by *Cancer borealis* urinary bladder. *Am. J. Physiol.*, 1987; 253:R153–R159.
35. Cattey, MA, Gerencser, GA and Ahearn GA. Electrogenic H⁺-regulated sulfate-chloride exchange in lobster hepatopancreatic brush-border membrane vesicles. *Am. J. Physiol.*, 1992; 262:R255–R262.

36. Gerencser, GA and Levin, R. Sodium-sulfate symport by *Aplysia californica* gut. *Zool. Sci.*, 2000; 17(5):27–32.
37. Gerencser, GA. Transport across the invertebrate intestine. In: R. Gilles, M. Gilles-Baillien, eds., *Transport processes, iono- and osmoregulation*. Springer, Berlin Heidelberg New York, 1985; 25–64.
38. Gerencser, GA, Cattey, MA and Ahearn, GA. Sulfate-oxalate exchange in lobster hepatopancreatic basolateral membrane vesicles. *Am. J. Physiol.*, 1995; 38(3): R572–R577.
39. Gerencser, GA, Ahearn, GA and Cattey, MA. Sulfate/bicarbonate antiport by lobster hepatopancreatic basolateral membrane vesicles. *J. Exp. Zool.*, 1999; 284:158–167.
40. Gerencser, GA, Ahearn, GA, Robbins, F and Cattey, MA. Chloride transport by lobster hepatopancreas is facilitated by several anion antiport mechanisms. *Comp. Biochem. Physiol.*, 2000; 125A:223–228.
41. Gerencser, GA, Burgin, C, Robbins, F and Ahearn, GA. The oxalate/sulfate antiporter in lobster hepatopancreas: internal and external binding constants. *J. Exp. Biol.*, 2000; 203:1497–1502.
42. Gerencser, GA, Ahearn, GA and Cattey, MA. Antiport-driven sulfate secretion in an invertebrate epithelium. *J. Exp. Zool.*, 1996; 275:269–276.
43. Mugharbil, A, Knickelbein, RG, Aronson, PS and Dobbins, JW. Rabbit ileal brush-border membrane $\text{Cl}^-/\text{HCO}_3^-$ exchanger is activated by an internal pH-sensitive modifier site. *Am. J. Physiol.*, 1990; 259:G666–G670.
44. Mason, MJ, Smith, JD, Garcia-Soto, JD and Grinstein, RJ. Grinstein Internal pH-sensitive site couples $\text{Cl}^-/\text{HCO}_3^-$ exchange to Na^+/H^+ antiport in lymphocytes. *Am. J. Physiol.*, 1989; 256:C428–C433.
45. Ahearn, GA, Gerencser, GA, Thamotharan, M, Behnke, RD and Lemme, TH. Invertebrate gut diverticula are nutrient absorptive organs. *Am. J. Physiol.*, 1992; 26: R472–R481.
46. Lucke, H, Stange, G and Murer, H. Sulfate-sodium cotransport by brush-border membrane vesicles isolated from rat ileum. *Gastroenterology*, 1981; 80:22–30.
47. Ahearn, GA, Franco, P and Clay, LP. Electrogenic $2 \text{Na}^+/\text{H}^+$ exchange in crustaceans. *J. Membr. Biol.*, 1990; 116:215–226.
48. Robertson, JD. Ionic regulation in some marine invertebrates. *J. Exp. Biol.*, 1949; 26: 182–200.
49. Gerencser, GA, Robbins, F, Zhang, J and Ahearn, GA. Electrogenic proton regulated oxalate/chloride exchange by lobster hepatopancreatic brush border membrane vesicles. *J. Exp. Biol.*, 2004; 207(4):571–578.
50. Gerencser, GA. Metabolic dependence of active sulfate transport in *Aplysia californica* intestine. *Comp. Biochem. Physiol. A.*, 1979; 63:519–522.
51. Tenehouse, HS, Lee, J, and Harvey, N. Renal brush border membrane Na-sulfate cotransport: stimulation by thyroid hormone. *Am J. Physiol.*, 1991; 261: F420–F4326.
52. Gerencser, GA, Levin, R, Zhang, J. Sulfate absorption in *Aplysia californica* gut: thyroid hormone stimulation. *Can. J. Zool.*, 2002; 80(6):964–966.
53. Gerencser, GA, Levin, R, and Robbins, F. Sulfate absorption in *Aplysia californica* gut: intracellular regulation by cyclic guanosine monophosphate. *Can. J. Zool.*, 2001; 79:1–3.
54. Sidlowski, H, Frieden, B. T_3 induces cGMP in tadpole. *Biosci. Rep.*, 1982; 2:569–571.
55. Gerencser, GA, Loo, SY, Zhang, J. Thyroid hormone-induced sulfate absorption in *Aplysia californica* gut is mediated by protein synthesis. *Can. J. Physiol. Pharm.*, 2003; 81(4):405–408.
56. Gerencser, GA, Zhang, J, Levin, R. Sulfate absorption in *Aplysia californica* gut: glucocorticoid inhibition. *Can. J. Zool.*, 2002; 80:2037–2040.
57. Ahearn, GA and Murer, H. Functional roles of Na^+ and H^+ in SO_2^{-4} transport by rabbit ileal brush border membrane vesicles. *J. Membr. Biol.*, 1984; 78: 177–186.
58. Berner, WR, Kinne, R, Murer, H. Phosphate transport into brush border membrane vesicles isolated from small intestine. *Biochem. J.*, 1976; 160: 467–474.

59. Sacktor, B, Cheng, L. Sodium gradient dependent phosphate transport in renal brush border membrane vesicles. Effect of an intravesicular to extravesicular proton gradient. *J. biol. Chem.*, 1981; 256:8080–8084.
60. Danisi, G and Murer, H. Inorganic phosphate in small intestine. In *Handbook of Physiology*, Vol. 2 Edited by Field, M and Frizzell, R. New York Oxford University Press. 1991; 232–336.
61. Gerencser, GA. Effects of amino acids on chloride transport in *Aplysia* intestine. *Am. J. Physiol.*, 1981; 240:R61–R69.
62. Gerencser, GA, Levin, R and Zhang J. Sodium phosphate symport by *Aplysia californica* gut. *Zool. Sci.*, 2002; 19:163–166.
63. Tenenhouse, HS, Lee, J and Harvey, N. Renal brush border membrane Na – sulfate cotransport: Stimulation by thyroid hormone. *Am. J. Physiol.*, 1991; 261:F420–F4326
64. Taylor, Z, Gold, RM, Yang, WC and Arruda, JAL. Anion exchanger is present in both luminal and basolateral renal membranes. *Eur. J. Biochem.*, 1987; 261: F420–F4326.
65. Gerencser, GA, Levin, R and Zhang, J. Phosphate absorption of *Aplysia californica* gut: thyroid hormone stimulation. *Can. J. Physiol. Pharm.*, 2002; 80(6):604–607.
66. Gerencser, GA, Loo, SY, Robbins, FW and Zhang, J. Phosphate absorption in *Aplysia californica* gut is mediated by protein synthesis. *Can. J. Physiol. Pharm.*, 2003; 81(4): 405–408.
67. Gerencser, GA, Cornette, KM and Zhang, J. Thyroid-hormone-induced phosphate absorption in *Aplysia californica* gut is mediated by protein synthesis. *Can. J. Physiol. Pharm.*, 2003; 81(4):409–412.



<http://www.springer.com/978-1-60327-228-5>

Epithelial Transport Physiology

Gerencser, G.A. (Ed.)

2010, XI, 488 p., Hardcover

ISBN: 978-1-60327-228-5

A product of Humana Press