ELSEVIER

Biochimica et Biophysica Acta 1282 (1996) 115–123

BBA Biochimica et Biophysica Acta

Sulfate transport in human placental brush-border membrane vesicles

Steven M. Grassl *

Department of Pharmacology, State University of New York Health Science Center, 766 Irving Avenue, Syracuse, NY 13210, USA

Received 10 November 1995; accepted 15 February 1996

Abstract

Membrane transport pathways for transplacental transfer of sulfate were investigated by assessing the possible presence of a bicarbonate-coupled anion exchange mechanism for sulfate in the maternal facing membrane of human placental epithelial cells. The presence of a SO_4^{2-}/HCO_3^- exchange mechanism was determined from $^{35}SO_4^{2-}$ tracer flux measurements in preparations of purified brush-border membrane vesicles. Under 10% CO_2/90% N_2 the imposition of an outwardly directed bicarbonate gradient (pH_o 6/pH_i 7.5) stimulated sulfate uptake to levels approximately 4-fold greater than observed at equilibrium. Maneuvers designed to offset the development of ion gradient-induced diffusion potentials (valinomycin, $[K^+]_o = [K^+]_i$) significantly reduced bicarbonate gradient-induced sulfate uptake but concentrative accumulation of sulfate persisted. Early time point determinations performed in the presumed absence of membrane potential suggest the reduced level of bicarbonate gradient-induced sulfate uptake resulted from a more rapid dissipation of the imposed bicarbonate gradient. Concentrative accumulation of sulfate was not observed in the presence of a pH gradient alone under 100% N_2, suggesting a preference of bicarbonate over hydroxyl ions as substrates for exchange. Static head determinations of opposing sulfate and bicarbonate gradients resulting in zero net flux of sulfate suggests the anion exchange mechanism mediates the electroneutral exchange of 2 bicarbonate or 1 carbonate for each sulfate. Sulfate uptake was increased with increasing intravesicular concentrations of carbonate at constant bicarbonate but was constant with increasing intravesicular concentrations of bicarbonate at constant carbonate suggesting carbonate as a substrate for anion exchange. The mechanism mediating bicarbonate gradient-induced sulfate uptake was sensitive to inhibition by stilbene derivatives, furosemide, bumetanide and probenecid. Substrate specificity studies suggest possible interactions of the anion exchange mechanism with salicylate, butyrate, thiosulfate, sulfite, selenate, chromate and oxalate. The results of this study provide evidence for the presence of a bicarbonate-coupled anion exchange mechanism as an electroneutral pathway for sulfate transport across the maternal-facing membrane of human placental epithelial cells.

Keywords: Sulfate transport; Transplacental transfer; Bicarbonate gradient; Brush-border membrane vesicle

1. Introduction

The human placenta performs an important function in sustaining normal growth and development of the fetus by serving as an interface for net transfer of nutrients from the maternal to fetal blood supply. Net transplacental transfer of metabolites from the maternal to the fetal circulation occurs at the cellular level as a result of the polarized distribution of transport processes expressed and targeted to the apical and basal membrane of syncytiotrophoblast cells. In keeping with the general features of epithelial function the apical or maternal surface of the syncytiotrophoblast is amplified morphologically in the form of a brush border. In a manner comparable to renal and intestinal epithelia the isolation of the brush border as membrane vesicles has enhanced our understanding of placental epithelial transport function by permitting the study of specific transport pathways present at the maternal surface of syncytiotrophoblasts [1]. The importance of membrane vesicle studies to defining the epithelial function of human placenta is significant because, unlike renal and intestinal epithelia, very limited information may be obtained from the alternative experimental models used to investigate placental transport. Indeed, the presence and nature of transport pathways in the basal or fetal-facing membrane remain largely unknown due to the inherent difficulties of basal membrane vesicle isolation from placental epithelia

Abbreviations: Mes, *N*-morpholinoethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TMA, tetramethylammonium; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid; DNDS, 4,4′-dinitrostilbene-2,2′-disulfonic acid; NADS, 4-amino-4′-nitrostilbene-2,2′-disulfonic acid; PAH, *p*-aminohippuric acid.

* Corresponding author. Fax: +1 (315) 4648014.

PII S0005-2736(96)00048-X

[2,3]. Recently, we have initiated membrane vesicle studies designed to identify and characterize molecular mechanisms of anion transport by the syncytiotrophoblast as a means to further define human placental epithelial transport function. Previously we described evidence for the presence of a placental brush-border membrane bicarbonate-coupled anion exchange mechanism for which chloride was a substrate and possibly involved in net transfer of fetal carbon dioxide as well as maintenance of normal fetal acid/base status [4]. We have extended our investigation of placental anion transport by assessing the possible presence of a bicarbonate-coupled anion exchange mechanism as a pathway for sulfate transport across the apical membrane of human placenta. Inorganic sulfate is an essential metabolite utilized by the developing fetus in the synthesis of sulfated mucopolysaccharides, proteins and steroids [5]. Previous evidence suggests sulfate transport across the placental brush-border membrane is mediated and occurs by a mechanism which is not coupled to sodium but may be coupled to protons via cotransport or to hydroxyl ions and/or bicarbonate ions via anion exchange [6–11]. We now wish to add our own observations on the nature of human placental sulfate transport by describing evidence for the presence of a placental brush-border membrane anion exchange mechanism coupling the electroneutral exchange of two bicarbonate ions or one carbonate ion for sulfate.

2. Materials and methods

Materials. Valinomycin, furosemide, bumetanide, probenicid, amiloride, harmaline, PAH, uric acid, salicylic acid, isethionic acid, formic acid, acetic acid, pyruvic acid, lactic acid, butyric acid, succinic acid, α-ketoglutaric acid, malonic acid, oxalic acid, molybdic acid, and sodium selenate were purchased from Sigma. Sodium tungstate and sodium chromate were purchased from Merck and Fluka, respectively. [^{35}S]Sulfate was obtained from DuPont-New England Nuclear. Valinomycin was dissolved in 95% ethanol and was added to the membrane suspension in a 1:100 dilution. Equivalent volumes of ethanol were added to control aliquots of membrane. All solutions were prepared with distilled-deionized water and passed through 0.22 μm Millipore filters.

Membrane preparations. Brush-border membrane vesicles were isolated from human term placenta by divalent cation aggregation and differential centrifugation as described previously [12,13]. Briefly, the villous tissue of placenta obtained within 15 min of elective caesarean section was quickly dissected and minced into small ($\approx$ 1 cm) fragments at 4°C. The tissue fragments were rinsed three times in 300 mM mannitol, 10 mM Hepes/TMA (OH), pH 7, and gently stirred for approximately 30 min using a motor-driven spatula. The tissue suspension was filtered through cotton gauze, and phenylmethylsulfonyl fluoride was added to a final concentration 0.2 mM. The filtrate was centrifuged at 8100 rpm for 15 min using an SS-34 rotor (Sorvall). The low speed pellet was discarded, and the supernatant was centrifuged at 19000 rpm for 40 min. The high-speed pellet was gently resuspended and $MgCl_2$ added to a final concentration of 12 mM. After incubating for 10 min the membrane suspension was centrifuged at 5000 rpm for 15 min to pellet the Mg^{2+}-induced membrane aggregates. The low-speed supernatant was centrifuged at 19000 rpm for 40 min and the resulting pellet (brush-border membrane vesicles) resuspended and washed twice in buffers designated for each experiment. Membranes were stored frozen (−70°C) and used within 2 weeks of preparation. The isolated membrane vesicle fraction was typically enriched 25.4 ± 1.3-fold (n = 7) in alkaline phosphatase activity [14] compared with homogenates of villous tissue. Typical membrane marker enzyme enrichments for the basal membrane (Na^+/K^+-ATPase), mitochondria (succinate dehydrogenase), and endoplasmic reticulum (NADH dehydrogenase) were 0.68 ± 0.05 (n = 7), 0.43 ± 0.02 (n = 7), and 0.34 ± 0.03 (n = 7), respectively [15–17]. Protein was determined by a sodium dodecyl sulfate-Lowry assay using bovine serum albumin as the standard [18].

Isotopic flux measurements. Frozen (−70°C) aliquots of membrane vesicles were thawed at room temperature, and isosmotic solutions of appropriate ionic composition were added to obtain the desired intravesicular solution described for each experiment in the figure and table legends. The membrane suspension was incubated for 120 min at room temperature to attain transmembrane equilibration of the added media. During the pre-equilibration period the membranes were gassed continuously with humidified 100% N_2 or the CO_2/N_2 gas mixes described in the figure legends. The extravesicular media were prepared similarly and the final composition for each experiment is given in the figure and table legends. Intravesicular [^{35}S]sulfate content was assayed at least in triplicate at 37°C by a rapid filtration technique described previously [19]. Briefly, a small volume of media (10–1500 μl) containing radiolabeled substrate was placed at the bottom of a glass test tube followed by positioning a 2.5–10 μl aliquot of membrane suspension (50–200 μl) on the test tube wall immediately above the puddle of isotope-containing media. Vesicle uptake of radiolabeled substrate was initiated by rapidly mixing the two aliquots using a vortex, and after a predetermined time interval the uptake reaction was quenched by rapid dilution with isosmotic potassium gluconate, 10 mM Hepes/TMA (OH−), pH 7, kept at 4°C. The diluted membrane suspension was passed through a 0.65 μm Millipore filter (DAWP) and washed with an additional 9 ml of quench buffer. The process of quenching, filtration, and washing occurred routinely within a 15-s period. The filters were dissolved in 3 ml of Ready-Solv HP (Beckman) and counted by scintillation spectroscopy. The timed uptake values obtained were corrected

by the nonspecific retention of isotope by the filters. Although absolute sulfate uptake values expressed per mg of protein varied from membrane preparation to membrane preparation, relative changes resulting from experimental manipulations in individual membrane preparations were highly reproducible.

3. Results and discussion

3.1. HCO_3^- gradient-driven SO_4^{2-} influx

The presence of a SO_4^{2-}/HCO_3^- exchange mechanism in human placental brush-border membrane would be suggested by the ability of a bicarbonate concentration gradient to serve as a driving force for concentrative accumulation of intravesicular sulfate. The time course of intravesicular sulfate accumulation ($[SO_4^{2-}]_o = 10$ μM) is illustrated in Fig. 1 as a function of an imposed transmembrane pH and bicarbonate gradient. In the absence of a bicarbonate and pH gradient (pH_o 6/pH_i 6) sulfate uptake was low and slowly approached an equilibrium value measured at 2 h. The imposition of an outwardly directed bicarbonate gradient (pH_o 6/pH_i 7.5 + CO_2/HCO_3^-) resulted in a marked stimulation of sulfate uptake achieving levels approximately 4-fold greater than observed at equilibrium. The imposition of an inside-alkaline pH gradient alone in the nominal absence of CO_2/HCO_3^- (pH_o 6/pH_i 7.5 − CO_2/HCO_3^-) also induced an increased uptake of sulfate to levels at or slightly above equilibrium but substantially below those observed in the presence of a bicarbonate gradient. The concentrative accumulation of sulfate noted in the presence of an outwardly directed bicarbonate gradient suggests a possible coupling of bicarbonate efflux to sulfate influx consistent with the presence of a SO_4^{2-}/HCO_3^- exchange mechanism. However, the nature of sulfate and bicarbonate flux coupling does not necessarily require a direct interaction between sulfate and bicarbonate with a common transport mechanism. Rather, the apparent flux coupling may have arisen from an indirect interaction between sulfate and bicarbonate secondary to the generation of an inside-positive bicarbonate gradient-induced diffusion potential.

The nature of sulfate and bicarbonate flux coupling was assessed by determining the effect of maneuvers designed to shunt the possible formation of ion-gradient (H^+, OH^-, HCO_3^-)-induced diffusion potentials. To the extent that sulfate uptake was conductive and electrostatically coupled to an inside-positive voltage difference, a reduced level of sulfate uptake would be expected in the presence of charge

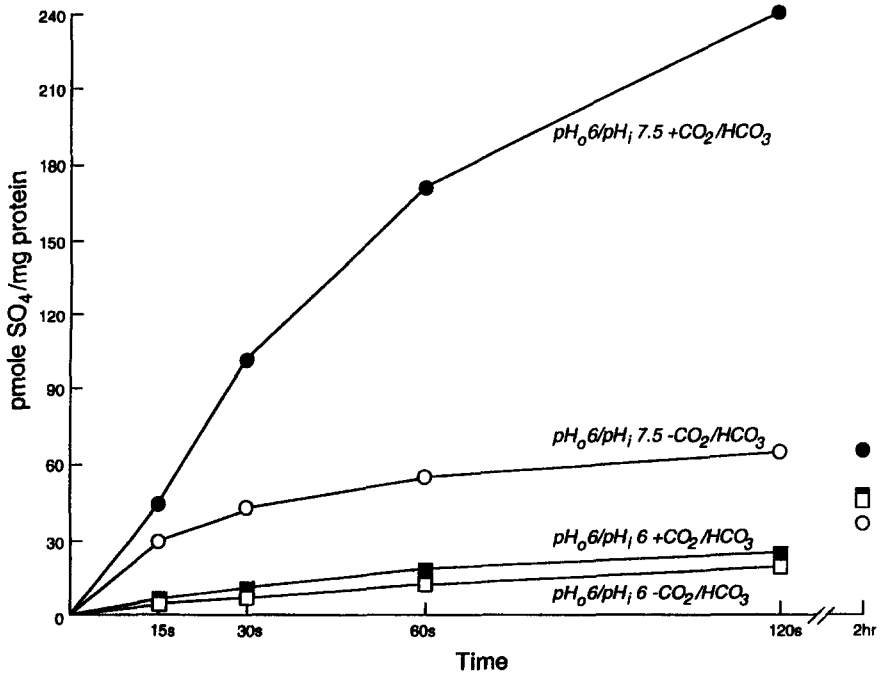

Fig. 1. Effect of bicarbonate gradient on sulfate influx. Brush-border membrane vesicles were pre-equilibrated under 10% CO_2/90% N_2 with: (pH_o 6/pH_i 6 + CO_2/HCO_3^-), 53 mM TMA gluconate, 57 mM potassium gluconate, 52 mM Mes, 45 mM Hepes, 25 mM TMA (OH^-), 2 mM bicarbonate; (pH_o 6/pH_i 7.5 + CO_2/HCO_3^-), 53 mM TMA gluconate, 57 mM potassium bicarbonate, 52 mM mannitol, 45 mM Hepes, 25 mM TMA (OH^-) or under 100% N_2 with similar solutions substituting gluconate for bicarbonate. Uptake of [^{35}S]SO_4 (10 μM) occurred from an extravesicular solution pre-equilibrated under 10% CO_2/90% N_2 containing: (pH_o 6/pH_i 6 + CO_2/HCO_3^-), 51 mM TMA gluconate, 57 mM potassium gluconate, 52 mM Mes, 44 mM Hepes, 25 mM TMA (OH^-), 2 mM bicarbonate; (pH_o 6/pH_i 7.5 + CO_2/HCO_3^-), 51 mM TMA gluconate, 59 mM K^+, 61 mM gluconate, 47 mM Mes, 13 mM gluconate, 29 mM mannitol, 25 mM TMA (OH^-), 2 mM bicarbonate or from an extravesicular solution pre-equilibrated under 100% N_2 containing: (pH_o 6/pH_i 6 − CO_2/HCO_3^-), 51 mM TMA gluconate, 57 mM potassium gluconate, 52 mM Mes, 44 mM Hepes, 25 mM TMA (OH^-); (pH_o 6/pH_i 7.5 + CO_2/HCO_3^-), 51 mM TMA gluconate, 59 mM K^+, 61 mM gluconate, 47 mM Mes, 42 mM mannitol, 25 mM TMA (OH^-). A representative experiment of three independent observations is illustrated.

compensating movements of potassium across valinomycin-treated membranes. Although, as shown in Fig. 2, the stimulation of sulfate uptake measured in the presence of an outwardly directed bicarbonate gradient was reduced in valinomycin-treated membranes in the presumed absence of voltage difference, the concentrative accumulation of sulfate persisted. This finding suggests a direct chemical coupling of bicarbonate efflux to sulfate influx consistent with the presence of a SO_4^{2-}/HCO_3^- exchange mechanism in placental brush-border membrane. Also notable in Fig. 2 in the nominal absence of bicarbonate is the reduced but sustained stimulation of sulfate uptake in valinomycin-treated membranes resulting from imposition of an inside-alkaline pH gradient. This observation suggests hydroxyl ions may serve as a substrate for exchange with sulfate through the SO_4^{2-}/HCO_3^- exchanger or an as yet unidentified sulfate transport pathway.

The reduced level of sulfate uptake resulting from maneuvers designed to minimize a membrane potential difference suggests some fraction of bicarbonate gradient-induced sulfate accumulation may have occurred via a conductive uptake pathway for sulfate present in parallel with a SO_4^{2-}/HCO_3^- exchange mechanism. However, the relative impermeability of biological membranes in general to highly charged molecules such as sulfate make consideration of this possibility less likely and indeed, an inside-positive voltage difference was observed to have no effect on the level of placental brush-border membrane vesicle sulfate uptake [8]. We therefore entertained an alternate possibility attributing the reduced level of bicarbonate gradient-induced sulfate uptake to an effect of short-circuiting membrane potential on promoting a more rapid dissipation of the imposed bicarbonate gradient. We tested this possibility by examining the effect of valinomycin on bicarbonate gradient sulfate uptake at early time points as is shown in Fig. 3. Sulfate uptake was essentially identical in the presence and absence of valinomycin at 5 s but thereafter was progressively reduced in short-circuited membranes. This observation indicates that after only 5 s the imposed bicarbonate gradient had not dissipated sufficiently to where differences in sulfate uptake may be distinguished between membranes treated with and without valinomycin. The similarity of 5-s sulfate uptake values in the presence and absence of valinomycin would not be expected if the effect of short-circuiting membrane potential was due to inhibition of conductive sulfate uptake. We include this observation as further evidence for the presence of a placental brush-border SO_4^{2-}/HCO_3^- exchange mechanism by suggesting the decreased sulfate uptake measured in short-circuited membrane vesicles resulted from a more rapid decay of the driving force for exchange. The data shown in Fig. 2 suggest gradients of bicarbonate and hydroxyl ions may serve independently as a driving force for accumulation of intravesicular sulfate by the same or different transport mechanisms. We attempted to distinguish between these two possibilities by assessing the inhibitor sensitivity of sulfate uptake in the presence of the same pH gradient but in the presence and absence of

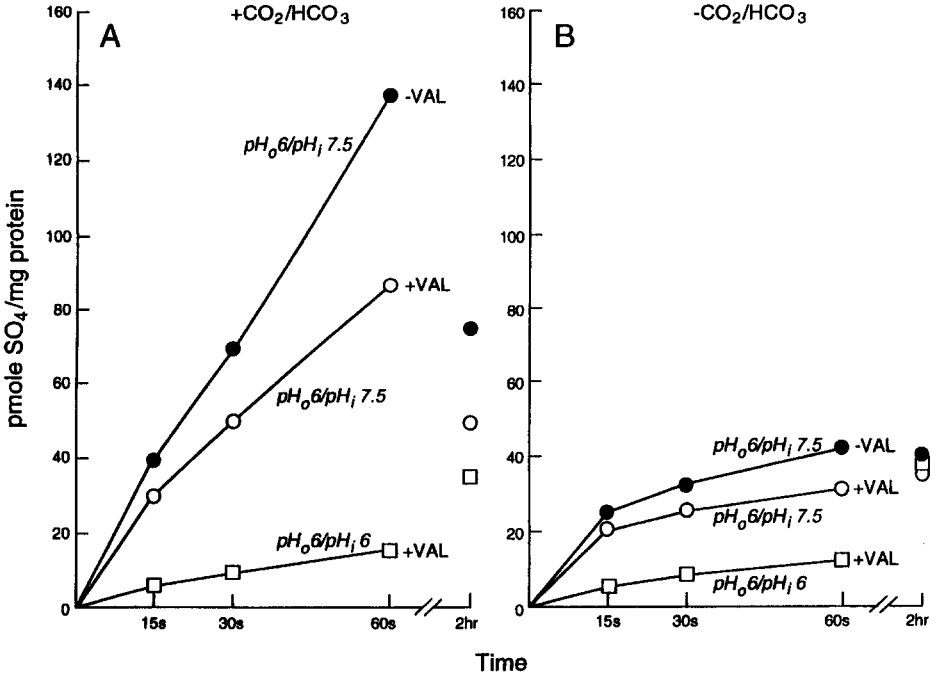

Fig. 2. Effect of valinomycin (VAL) on bicarbonate and hydroxyl gradient-driven sulfate influx. Brush-border membrane vesicles were pre-equilibrated as described in the legend to Fig. 1. Uptake of [^{35}S]sulfate (10 μM) occurred from extravesicular solutions described in the legend to Fig. 1. Where indicated (+VAL) membrane vesicles were preincubated with valinomycin (0.25 mg/ml) for a minimum of 30 min. A representative experiment of three independent observations is shown.

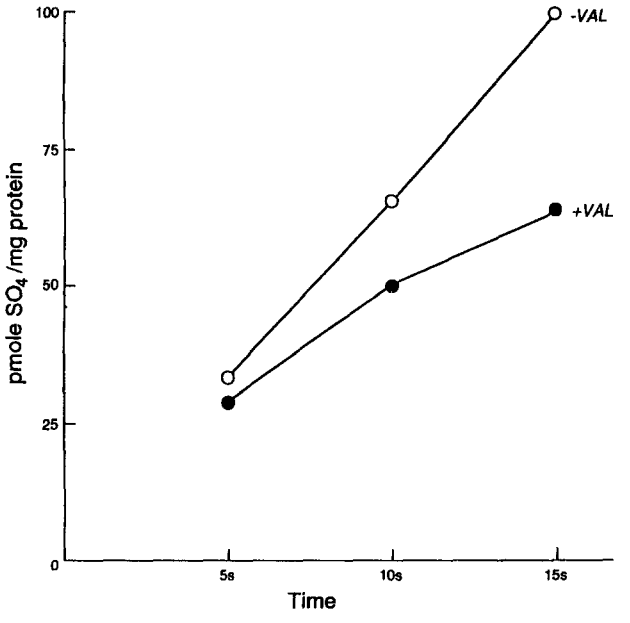

Fig. 3. Effect of valinomycin (VAL) on bicarbonate gradient-driven sulfate influx at early time points. Brush-border membrane vesicles were pre-equilibrated under 10% CO_2/90% N_2 as described in the legend to Fig. 1 for pH_o 6/pH_i 7.5+CO_2/HCO_3^-. Uptake of [^{35}S]sulfate (10 μM) occurred from an extravesicular solution described in the legend to Fig. 1 for pH_o 6/pH_i 7.5+CO_2/HCO_3^-. A representative experiment of three independent observations is illustrated.

CO_2/HCO_3^-. As shown in Fig. 4 the concentration-dependent inhibition of sulfate uptake by the well-known anion transport inhibitor DIDS was essentially indistinguishable in the presence and absence of CO_2/HCO_3^-. This finding suggests bicarbonate and hydroxyl gradient-induced sulfate uptake occurs by an inhibitor-sensitive, mediated transport pathway in both instances and on the strength of the observed similarity in inhibitor sensitivity represents functional modes of the same anion exchange mechanism.

3.2. Stoichiometric coupling of SO_4^{2-}/HCO_3^- exchange

The stoichiometric coupling of bicarbonate and sulfate fluxes through the anion exchange mechanism was estimated thermodynamically by attempting to identify the magnitude of opposing driving forces which bring the mechanism of exchange to equilibrium [20,21]. The mechanism mediating sulfate for bicarbonate exchange will be at equilibrium and mediate no net flux when equal and opposite electrochemical potential differences for sulfate and bicarbonate exist across the vesicle membrane. This relation may be expressed in terms of relevant intra- and extravesicular ion concentrations and membrane potential by:

$$\frac{[SO_4^{2-}]_i}{[SO_4^{2-}]_o} = \left(\frac{[HCO_3^-]_i}{[HCO_3^-]_o}\right)^n \left(\frac{[K^+]_i}{[K^+]_o}\right)^{n-2} \quad (1)$$

where n is the number of bicarbonate ions exchanged per sulfate ion and, in the presence of the potassium ionophore valinomycin, the membrane potential approaches the Nernst potential for potassium. Thus, as shown in Fig. 5, n was calculated when $[K^+]_o = [K^+]_i$ by identifying the magni-

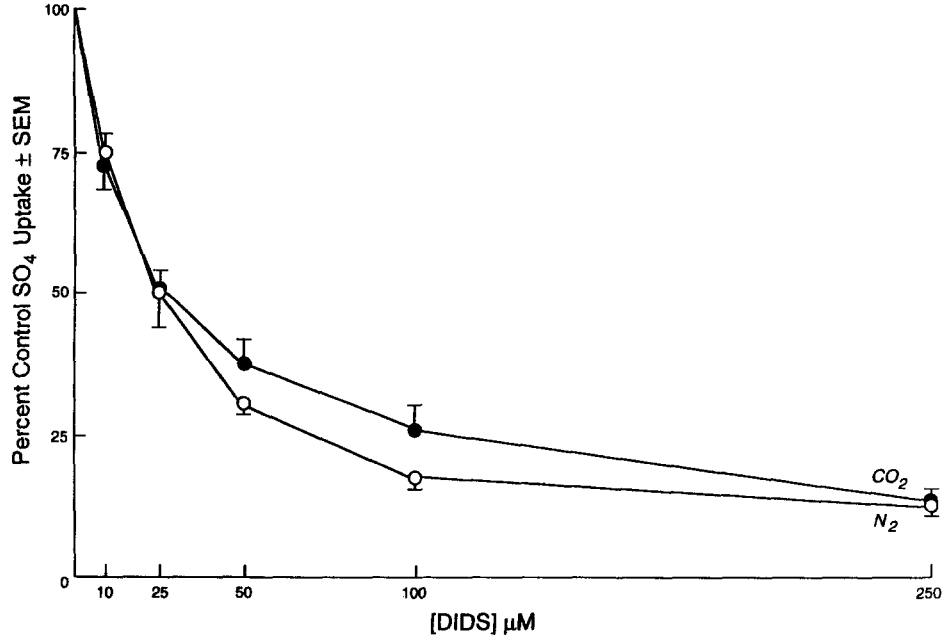

Fig. 4. Effect of DIDS on bicarbonate and hydroxyl gradient-driven sulfate influx. Brush-border membrane vesicles were pre-equilibrated at pH 7.5 under 10% CO_2/90% N_2 or 100% N_2 as described in the legend to Fig. 1. The 10-s uptake of [^{35}S]sulfate (10 μM) was measured from extravesicular solutions pre-equilibrated with 10% CO_2/90% N_2 or 100% N_2 as described in the legend to Fig. 1 and containing DIDS at the concentrations shown. Membrane vesicles were preincubated with valinomycin (0.25 mg/ml) for a minimum of 30 min. The data are expressed as % of control sulfate uptake determined in the absence of DIDS: +CO_2/HCO_3^-, 27.3 ± 2.5 pmol/mg and −CO_2/HCO_3^-, 23 ± 0.4 pmol/mg. The mean ± S.E. of four experiments each using a different membrane preparation is shown.

tude of an outwardly directed sulfate gradient which would just balance an outwardly directed bicarbonate gradient so as to bring net flux of sulfate through the exchange mechanism to zero. Membrane vesicles were preloaded with [^{35}S]sulfate and bicarbonate and progressively diluted into increasing volumes of extravesicular buffer maintaining a constant 10:1 outward bicarbonate gradient and the intra- to extravesicular sulfate concentration ratios shown. The possible effect of ion gradient-induced diffusion potentials on the flux measurement were minimized by preforming the experiment in the presence of valinomycin where intra- and extravesicular potassium was equal and constant at each dilution. The net change in intravesicular sulfate content was assayed 5 s after imposition of the ion gradients shown and is assumed to represent only net flux through the anion exchange mechanism. For the exchange mechanism to be at equilibrium when $n = 1$ or a 1:1 bicarbonate to sulfate coupling ratio no net flux of sulfate would be expected in the presence of outward bicarbonate and sulfate gradients of equal magnitude. However, as shown in Fig. 5, a 10:1 outward bicarbonate gradient induces a net influx of sulfate against a 10:1 outward sulfate gradient which indicates the exchange mechanism is not at equilibrium and, therefore, $n = 1$ underestimates the bicarbonate to sulfate coupling ratio. In contrast, an increasingly larger net efflux of sulfate was observed to result from the imposition of outwardly directed sulfate gradients of 300:1 and 1000:1, respectively, which indicates n values of 2.5 and 3 overestimate the bicarbonate to sulfate coupling ratio. These results suggest the value for n is greater than 1 but less than 2.5 and indeed net sulfate flux most closely approximated zero in the presence of a 100:1 outward sulfate gradient where n is 2. Thus static head determinations of zero net sulfate flux estimate the bicarbonate to sulfate coupling ratio to be 2:1 or its thermodynamic equivalent of one carbonate exchanged per sulfate. Interestingly, a similar 2:1 bicarbonate to sulfate coupling ratio has been determined for a SO_4^{2-}/HCO_3^- exchange mechanism present in basolateral membrane of renal proximal tubule cells [21] which further suggests the anion exchange in both kidney and placenta occurs by an electroneutral process neither contributing to nor being driven by membrane potential.

An attempt was made to distinguish between bicarbonate and carbonate as the buffer ion species preferred for exchange with sulfate through the transport mechanism. Adjusting intravesicular pH under the appropriate pCO_2 according to the Henderson–Hasselbalch equation the effect of increasing intravesicular carbonate on sulfate uptake was determined at constant intravesicular bicarbonate

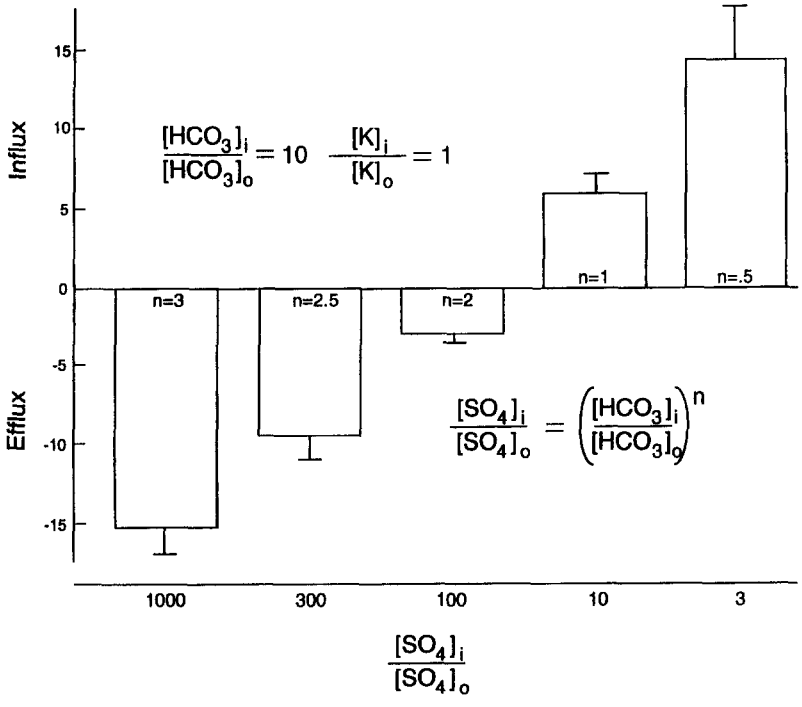

Fig. 5. Effect of bicarbonate and sulfate concentration gradients on intravesicular sulfate content. Brush-border membrane vesicles were pre-equilibrated under 5% CO_2/95% N_2 with: 70 mM potassium gluconate 30 mM potassium bicarbonate, 70 μM potassium carbonate, 60 mM mannitol, 60 mM Hepes, 30 mM TMA, 20 μM [^{35}S]sulfate, pH 7.5. Intravesicular sulfate content was assayed before and 5 s after 1:3, 1:10, 1:100, 1:300 and 1:1000 dilution and incubation under 5% CO_2/95% N_2 at pH 6.5 in 97 mM potassium gluconate, 3 mM $KHCO_3$ and $n = 5$: 26 mM mannitol, 20 mM Hepes, 60 mM Mes, 41 mM TMA; $n = 1$: 6 mM mannitol, 6 mM Hepes, 81 mM Mes, 52 mM TMA; $n = 2$: 0.6 mM Hepes, 89 mM Mes, 58 mM TMA; $n = 2.5$ and 3: 0.2 mM Hepes, 90 mM Mes, 57 mM TMA. Membrane vesicles were preincubated with valinomycin (0.25 mg/ml) for a minimum of 30 min. Net influx and efflux of sulfate are expressed as percent of initial intravesicular sulfate content and represent the mean ± S.E. for three separate experiments each performed with a different membrane preparation.

as shown in Fig. 6. Bicarbonate buffer-dependent sulfate uptake was determined at each intravesicular pH shown by correcting total sulfate uptake with sulfate uptake measured in the absence of CO_2 and buffer ions. As shown in Fig. 6, at a constant intravesicular bicarbonate concentration of approximately 28 mM sulfate uptake was observed to increase with increasing intravesicular carbonate concentration and may suggest carbonate as a substrate for the sulfate exchange mechanism. The possibility of carbonate as a substrate for anion exchange was investigated further by examining the effect of increasing intravesicular bicarbonate on sulfate uptake while maintaining intravesicular carbonate constant. Using a similar approach described for the preceding experiment shown in Fig. 6, adjusting intravesicular pH under the appropriate pCO_2 permitted an equilibrium between a constant intravesicular carbonate concentration of 35 μM and the three different bicarbonate concentrations shown in Fig. 7 according to the Henderson–Hasselbalch equation. However, in contrast to the preceding experiment in which sulfate uptake was observed to increase with increasing intravesicular carbonate Fig. 7 shows essentially no effect of increasing intravesicular bicarbonate on sulfate uptake when intravesicular carbonate was constant. Thus, the results shown in Figs. 6 and 7 indicate the level of sulfate uptake varies more as a function of intravesicular carbonate than bicarbonate across the range of concentrations examined and therefore may suggest carbonate rather than bicarbonate as the preferred anion exchanged for sulfate through the transport mechanism. However, the constraints imposed by the Henderson–Hasselbalch relation require intravesicular pH to also vary when determining the effect of increasing intravesicular bicarbonate or carbonate on sulfate uptake and therefore the possible down-regulation of SO_4^{2-}/HCO_3^- exchange by titration must also be considered. The precedent for allosteric regulation of transport by protons has been established for the Na^+/H^+ exchange mechanism [22] and the Cl^-/HCO_3^- exchange mechanism [23].

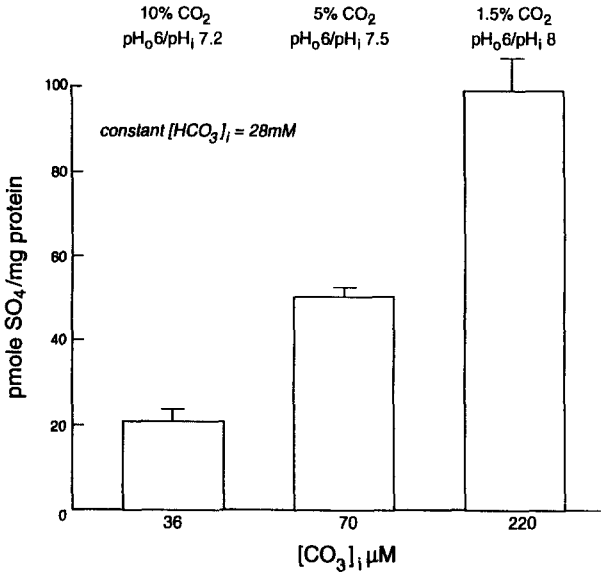

Fig. 6. Effect of increasing intravesicular carbonate on sulfate influx. Brush-border membrane vesicles were pre-equilibrated with 70 mM potassium gluconate, 30 mM potassium bicarbonate, 60 mM Hepes/20 mM TMA (OH^-) and at pH 7.2 under 10% CO_2 /90% N_2 with 26 mM mannitol and 36 μM potassium carbonate; at pH 7.5 under 5% CO_2 /95% N_2 with 10 mM TMA (OH^-), 16 mM mannitol and 70 μM potassium carbonate; at pH 8 under 1.5% CO_2 /98.5% N_2 with 26 mM TMA (OH^-), 220 μM potassium carbonate. A parallel set of membranes were prepared similarly in the absence of bicarbonate and carbonate under 100% N_2 by substituting gluconate for bicarbonate. The 10-s uptake of [^{35}S]sulfate (10 μM) was assayed at pH 6 following 1:200 dilution in 100 mM potassium gluconate, 75 mM Mes, 20 mM TMA (OH^-), 3 mM mannitol pre-equilibrated with respective CO_2 /N_2 gasses or 100% N_2. Bicarbonate-dependent sulfate uptake is shown and was determined from total sulfate uptake minus sulfate uptake measured under 100% N_2. The data represent the mean ± S.E. for five experiments using five different membrane vesicle preparations.

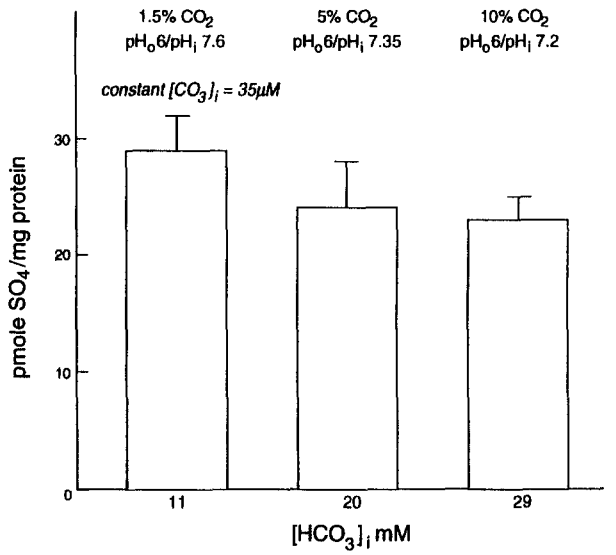

Fig. 7. Effect of increasing intravesicular bicarbonate on sulfate influx. Brush-border membrane vesicles were pre-equilibrated with 70 mM potassium gluconate, 60 mM Hepes, 20 mM TMA (OH^-), 36 μM potassium carbonate and at pH 7.2 under 10% CO_2 /90% N_2 with 30 mM potassium bicarbonate, 26 mM mannitol; at pH 7.35 under 5% CO_2 /90% N_2 with 20 mM potassium bicarbonate, 10 mM potassium gluconate, 4 mM TMA (OH^-), 22 mM mannitol; at pH 7.6 under 1.5% CO_2 /98.5% N_2 with 11 mM potassium bicarbonate, 19 mM potassium gluconate, 12 mM TMA (OH^-), 13 mM mannitol. A parallel set of membranes were prepared similarly in the absence of bicarbonate and carbonate under 100% N_2 by substituting gluconate for bicarbonate. The 10-s uptake of [^{35}S]sulfate (10 μM) was assayed at pH 6 following 1:200 dilution in 100 mM potassium gluconate, 75 mM MES, 28 mM TMA (OH−), 3 mM mannitol pre-equilibrated with respective CO_2 /N_2 gasses or 100% N_2. Bicarbonate-dependent sulfate uptake is shown and was determined from total sulfate uptake minus sulfate uptake measured under 100% N_2. The data represent the mean ± S.E. of five experiments performed with five different membrane vesicle preparations.

3.3. Inhibitor and substrate specificity of SO_4^{2-}/HCO_3^- exchange

The functional properties of the mechanism mediating placental brush-border membrane SO_4^{2-}/HCO_3^- exchange

were further characterized by assessing possible interaction with a number of anion transport inhibitors. A profile of the inhibitor sensitivity of the anion exchange mechanism as it functions in native placental brush-border membrane serves to distinguish the mechanism under study from other similar transport pathways as well as to facilitate the functional identification of the isolated, reconstituted transport protein. The stilbene derivatives are a well-known family of anion transport inhibitors and as shown in Table 1 the level of bicarbonate gradient-induced sulfate uptake was increasingly reduced in the presence of NADS, DNDS, SITS and DIDS, respectively. The observed inhibition of bicarbonate gradient-induced sulfate uptake by stilbenes is consistent with previous studies of the mechanism mediating placental sulfate transport using isolated membrane vesicles [8] and tissue slices [10]. Notably the rank order of inhibition by stilbene derivatives contrasts that of the red blood cell anion exchanger which is more sensitive to DNDS and less sensitive to SITS [24]. Similar to the previously observed inhibition of placental brush-border membrane SO_4^{2-}/SO_4^{2-} exchange [8], bicarbonate gradient-induced sulfate uptake was also reduced in the presence of the loop diuretics furosemide and bumetanide as well as the organic anion transport inhibitor probenecid. Furthermore, a comparable ranking of anion transport inhibitor sensitivity has also been determined for the SO_4^{2-}/HCO_3^- exchanger present in other epithelia such as the basolateral membrane of rat and rabbit proximal tubule cells [21,25] and rabbit ileum [26] as well as the canalicular membrane of rat liver [27]. To further elucidate the substrate specificity of placental brush-border membrane SO_4^{2-}/HCO_3^- exchange bicarbonate gradient-induced sulfate uptake was measured in the presence of various monovalent and divalent organic and inorganic anions as shown in Table 2. Among the monovalent organic and inorganic anions examined only salicylate, acetate and butyrate were noted to significantly reduce the level of bicarbonate gradient-induced sulfate accumulation. While this result may suggest a possible direct interaction of these compounds with the anion exchange mechanism, the reduced level of sulfate uptake may have also resulted from an indirect effect of these compounds, via nonionic diffusion of the protonated acid, to induce a more rapid collapse of the imposed pH and bicarbonate gradients. However, a direct interaction of salicylate with the mechanism mediating placental brush-border membrane sulfate transport is further suggested by the inhibition of SO_4^{2-}/SO_4^{2-} exchange observed in the absence of a transmembrane pH gradient [8]. Interestingly, where examined in other epithelia, salicylate was observed to have no effect on the SO_4^{2-}/HCO_3^- exchange mechanism present in the basolateral membrane of rat proximal tubule cells [25]. Furthermore, to the extent that the functional properties of the anion exchange mechanism are similar in different epithelia then the inability of acetate and butyrate to

Table 1
Effect of transport inhibitors on bicarbonate gradient-driven sulfate influx

Transport inhibitor (100 mM)	% Control
DIDS	35 ± 3.6
SITS	59 ± 2.3
DNDS	75 ± 4.5
NADS	86 ± 6.2
Furosemide	82 ± 3.5
Bumetanide	45 ± 6.8
Probenicid	40 ± 7.9
Amiloride	103 ± 4.9
Harmaline	97 ± 7.7

Brush-border membrane vesicles were pre-equilibrated under 10% CO_2/90% N_2 as described in the legend to Fig. 1 for pH_o 6/pH_i 7.5 + CO_2/HCO_3^-. The 10-s uptake of [^{35}S]sulfate (10 μM) occurred from an extravesicular solution pre-equilibrated under 10% CO_2/90% N_2 as described in the legend to Fig. 1 for pH_o 6/pH_i 7.5 + CO_2/HCO_3^- and containing 100 μM of the compounds shown. The data are expressed as percent of control sulfate uptake (17.1 ± 4 pmol/mg) determined in the absence of test compounds. The mean ± S.E. of four experiments each using a different membrane preparation is shown.

Table 2
Effect of monovalent and divalent anions on bicarbonate gradient-driven sulfate influx

	Anion (1 mM)	% Control
A. Monovalent	chloride	121 ± 4.0
	iodide	110 ± 5.6
	nitrate	112 ± 6.6
	thiocyanate	100 ± 4.6
	PAH	93 ± 3.0
	urate	96 ± 1.4
	salicylate	17 ± 0.6
	isethionate	77 ± 6.0
	formate	82 ± 2.6
	acetate	68 ± 1.8
	pyruvate	86 ± 8.0
	lactate	76 ± 5.6
	butyrate	54 ± 1.5
B. Divalent	sulfate	11 ± 0.0
	thiosulfate	13 ± 1.2
	sulfite	14 ± 1.2
	phosphate	103 ± 5.8
	molybdate	86 ± 6.6
	selenate	14 ± 0.6
	chromate	12 ± 1.0
	arsenate	101 ± 2.4
	tungstate	106 ± 8.0
	succinate	89 ± 4.0
	α-ketoglutarate	93 ± 0.7
	malonate	87 ± 9.0
	oxalate	21 ± 2.6

Brush-border membrane vesicles were pre-equilibrated under 10% CO_2/90% N_2 as described in the legend to Fig. 1 for pH_o 6/pH_i 7.5 + CO_2/HCO_3^-. The 10-s uptake of [^{35}S]sulfate (10 μM) occurred from an extravesicular solution pre-equilibrated under 10% CO_2/90% N_2 as described in the legend to Fig. 1 for pH_o 6/pH_i 7.5 + CO_2/HCO_3^- and containing 1 mM of anion. The data are expressed as percent of control sulfate uptake (32.8 ± 3.2 pmol/mg) determined in the absence of test anions. The mean ± S.E. of three experiments each using a different membrane preparation is shown.

trans-stimulate or cis-inhibit the SO_4^{2-}/HCO_3^- exchange present in rabbit ileum may be considered evidence against acetate and butyrate as possible substrates for the placental anion exchanger [26]. Among the divalent anions tested for possible interaction with the placental brush-border membrane SO_4^{2-}/HCO_3^- exchanger the inorganic anions thiosulfate, sulfite, selenate and chromate were observed to significantly reduce the level of bicarbonate gradient-induced sulfate uptake. A similar inhibitory effect of thiosulfate, selenate and chromate has been reported for the mechanism mediating SO_4^{2-}/SO_4^{2-} exchange in placental brush-border membrane vesicles [7,9] as well as for the SO_4^{2-}/HCO_3^- exchange mechanism present in the basolateral membrane of rat proximal tubules [25]. These observations suggest the presence of anionic binding sites within the protein mediating renal and placental SO_4^{2-}/HCO_3^- exchange which recognizes and interacts with divalent oxyanions assuming a tetrahedral configuration. The observed inhibitory effect of selenate and chromate on sulfate uptake suggests these oxyanions as possible substrates for transport by the anion exchange mechanism which, in turn, may be an important pathway for the fetal accumulation of selenium and chromium as essential trace elements necessary for normal fetal development [28]. In addition, we further report an inhibition of the placental SO_4^{2-}/HCO_3^- exchanger by sulfite, a divalent oxyanion possessing a low trigonal or pyramidal geometry more closely resembling a planer carbonate ion than the previously described tetrahedral oxyanions [29]. The precedent for sulfite as a transportable substitute substrate for carbonate has been established from studies of the ionic dependence of the Na-bicarbonate-carbonate cotransport mechanism present in renal basolateral membrane vesicles [30]. Thus, consistent with the previously described effects of carbonate on sulfate uptake the inhibition of placental SO_4^{2-}/HCO_3^- exchange by sulfite may be considered additional evidence supporting carbonate as a substrate for the anion exchange mechanism. In addition to the inorganic divalent oxyanions mentioned, the dicarboxylic acid oxalate was the only organic divalent anion observed to reduce the level of placental brush-border membrane bicarbonate gradient-induced sulfate uptake. A similar inhibitory effect of oxalate has also been determined for the SO_4^{2-}/HCO_3^- exchanger present in other epithelia such as the basolateral membrane of rat and rabbit proximal tubule cells [21,25], rabbit ileum [26] as well as the canalicular membrane of rat liver [27]. Furthermore, direct evidence for oxalate as a transportable substrate of the SO_4^{2-}/HCO_3^- exchange mechanism has been obtained from membrane vesicle studies of the anion exchanger present in rabbit ileum [26] and renal proximal tubule cells [21]. The possibility of oxalate as a transportable substrate for the mechanism mediating SO_4^{2-}/HCO_3^- exchange in placental brush-border membrane is presently under study. In conclusion, sufficient evidence has been presented to suggest the presence of a bicarbonate-coupled anion exchange mechanism as an electroneutral pathway for sulfate transport across the maternal-facing brush-border membrane of human placenta.

Acknowledgements

The excellent secretarial assistance of Pattie Pisarek and technical assistance of Michelle Spaar is gratefully acknowledged. This work was supported by NIH HD29940.

References

[1] Smith, C.H., Moe, A.J. and Ganapathy, V. (1992) Annu. Rev. Nutr. 12, 183–206.
[2] Kelly, L.K., Smith, C.H. and King, B.F. (1987) Am. J. Physiol. 252, C38–C46.
[3] Illsley, N.P., Wang, Z., Gray, A., Sellers, M.C. and Jacobs, M.M. (1990) Biocesultshim. Biophys. Acta 1029, 218–226.
[4] Grassl, S.M. (1989) J. Biol. Chem. 264, 11103–11106.
[5] Cole, D.E.C., Baldwin, L.S. and Stirk, L.J. (1984) Am. J. Obstet. Gynecol. 148, 596–599.
[6] Cole, D.E.C. (1984) Biochem. Biophys. Res. Commun. 123, 223–229.
[7] Boyd, C.A.R. and Shennan, D.B. (1986) J. Physiol. 379, 367–376.
[8] Boyd, C.A.R. and Shennan, D.B. (1986) J. Physiol. 377, 15–24.
[9] Deves, R. and Boyd, C.A.R. (1989) Proc. R. Soc. Lond. B 237, 85–97.
[10] Cole, D.E.C. and Rastogil, N. (1991) Biochim. Biophys. Acta 1064, 287–292.
[11] Shennan, D.B. (1992) Placenta 13, 489–499.
[12] Ogin, C. and Grassl, S.M. (1989) Biochim. Biophys. Acta 980, 248–254.
[13] Grassl, S.M. (1992) J. Biol. Chem. 267, 22902–22906.
[14] Bowers, G.N. and McComb, R.B. (1966) Clin. Chem. 12, 70–89.
[15] Jørgensen, J.L. (1968) Biochim. Biophys. Acta 151, 212–224.
[16] Earl, D.C.N. and Korner, A. (1965) Biochem. J. 94, 721–734.
[17] Wallach, D.F.H. and Kamat, V.B. (1966) Methods Enzymol. 8, 165–166.
[18] Peterson, G.L. (1983) Methods Enzymol. 19, 95–115.
[19] Grassl, S. and Aronson, P.S. (1986) J. Biol. Chem. 261, 8778–8783.
[20] Aronson, P.S. (1981) Am. J. Physiol. 240, F1–F11.
[21] Kuo, S.-M. and Aronson, P.S. (1988) J. Biol. Chem. 263, 9710–9717.
[22] Aronson, P.S., Nee, J. and Suhm, M.A. (1982) Nature 299, 161–163.
[23] Olsnes, S., Tonnessen, T.I. and Sandvig, K. (1986) J. Cell Biol. 102, 967–971.
[24] Cabantchik, Z.I. and Greger, R. (1992) Am. J. Physiol. 262, C803–C827.
[25] David, C. and Ullrich, K. (1992) Pflugers Arch. 421, 455–465.
[26] Knickelbein, R.G. and Dobbins, J.W. (1990) Am. J. Physiol. 259, G807–G813.
[27] Meier, P.J., Valantinas, J., Hugentobler, G. and Rahm, I. (1987) Am. J. Physiol. 253, G461–G468.
[28] Underwood, E.J. (1977) Trace Elements in Human and Animal Nutrition, pp. 258–268 and pp. 302–340, Academic Press, London.
[29] Lister, M.W. (1965) Oxyacids, pp. 15–45, Elsevier, New York.
[30] Soleimani, M. and Aronson, P.S. (1989) J. Biol. Chem. 264, 18302–18308.