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Sulfate transport in human placenta: further evidence for a sodium-independent mechanism

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Sulfate transport in isolated placental brush-border membrane vesicles has properties consistent with an anion exchange process. To ascertain the relevance of this finding to sulfate accumulation by the fetus and placenta *in vivo*, we examined sulfate transport in human placental tissue slices, comparing sulfate uptake with that of a non-metabolizable amino acid marker, α-aminoisobutyrate (AIB). In contrast to AIB, which was actively concentrated from physiological media, sulfate uptake by the placenta slice was concentrative only in the absence of sodium and at low pH. Uptake of sulfate reached a steady state after 60 min. It was blocked by DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonate), a specific inhibitor of anion transport, but not by ouabain. We found no evidence for Na^+-dependent uptake of sulfate in incubated placental tissue. It seems unlikely that Na^+-dependent sulfate transport by the placenta can be responsible for net sulfate accumulation by the human fetus.

Introduction

The placenta is a specialized organ whose principal function is to provide an adequate environment for the embryo and fetus during intrauterine growth and development. Placental tissues separate maternal and fetal circulations, facilitating the transfer of nutrients from mother to fetus [1,2]. Inorganic sulfate is one such nutrient that is utilized by a variety of biosynthetic reactions essential for fetal growth and development [3–5]. In clinical studies of sulfate metabolism of pregnant women and their infants at delivery, we previously found evidence for a small concentrative gradient across the placenta [6]. However, subsequent experiments with human placental brush-border membrane vesicles (BBMV) revealed a transmembrane transport system with properties more indicative of anion exchange [2,7]. As yet, sulfate transport has not been examined in isolated intact placental tissue. We therefore examined sulfate uptake by human placental slices using α-aminoisobutyrate (AIB), a non-metabolizable amino acid, a marker solute whose Na^+-dependent uptake by placenta is well characterized [8–14].

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Methods

Placental slice preparation

Intact human placentas from uncomplicated term pregnancies were obtained from the Grace Maternity Hospital (Halifax, NS, Canada) shortly after delivery and brought to the laboratory on ice. Tissue slices averaging 1 mm thickness were then cut from the maternal surface using a Stadie-Riggs Microtome (Thomas Scientific, Swedesboro, NJ) according to the method of Smith and co-workers [8–10]. Slices were placed on ice, cut into squares weighing 10–50 mg, and placed in incubation medium within 45 min of placental delivery.

Chemicals

Reagent-grade chemicals and distilled, deionized water (dH_2O, > 10 megohm/cm resistance) were used for all experiments. Carrier-free $Na_2{}^{35}SO_4$ (> 10 mCi/mmol), α-amino[*methyl*-^{3}H]isobutyric acid (^{3}H-AIB, 10 mCi/mmol) and ^{3}H-polyethylene glycol (^{3}H-PEG, 4000 M_r, 0.7 mCi/g) were obtained from Dupont (NEN Canada), Montreal, PQ. Minimal essential medium (MEM; Cat No. 320–1090) from Gibco Co. (Toronto, ON) contained 0.80 mM sulfate as $MgSO_4 \cdot 7H_2O$. Tissue solubilizer (BTS-450) and liquid scintillation cocktail (MP) were from Beckman Scientific (Fullerton CA). Non-specific arylsulfatase enzyme (from *Helix pommatia*), Tris and Pipes buffers, 7-hydroxy-

coumarin, ouabain, SITS (4-isothiocyano-4′-acetamidostilbene-2,2′-disulfonate) and DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonate) were all obtained from Sigma Chemicals, St. Louis, MO.

Uptake experiments

Tissue slices were incubated as previously described [15], modified according to Smith et al. [8–10]. Three incubation media were used: (i) MEM; (ii) a balanced salt medium (BSM), consisting of NaCl (116 mM), KCl (5.4 mM), $CaCl_2$ (1.8 mM), $NaHCO_3$ (26.2 mM), NaH_2PO_4 (1 mM), $MgCl_2$ (0.8 mM); and (iii) a buffered sucrose medium, consisting of sucrose (280 mM) and Tris-Pipes buffer (pH 6.5; 10 mM). When bicarbonate-based MEM was used, slices were incubated in a Dubnoff shaker bath at 60 cycles per min thermostated to 37°C, under a 95% O_2/5% CO_2 atmosphere; otherwise 100% O_2 was used. Substitutions and other additions for specific experiments are indicated in the text.

Experiments to measure distribution and net uptake of solute were performed as previously described [15] with minor modifications, as follows: At the end of incubation, tissue slices were weighed and placed in 7 ml vials for liquid scintillation counting, then digested with 0.5 ml of BTS-450 tissue solubilizer overnight at 50°C. The digested tissue was suspended in 5 ml of scintillation cocktail containing 7 ml/l glacial acetic acid and counted using automated quench correction.

In keeping with previous studies [15], water spaces were estimated for each new experiment. For this purpose, some of the tissue slices were weighed using a Cahn electrobalance (Ventron Instruments, Paramount, CA) before and after drying overnight at 110°C. The difference was taken to be the total tissue water (TTW). The extracellular water (ECW) compartment was determined using labelled 4000 M_r poly(ethylene glycol) (PEG) [16] and intracellular water (ICW) volume calculated by difference.

Isotopic distribution ratios ($C_i/C_o \equiv$ dpm per ml ICW/dpm per ml medium) for the two solutes, sulfate and AIB, and saturation kinetics were examined as described previously [15].

Sulfoesterification experiments

The possible contribution of sulfoesterification to sulfate uptake was assayed using 7-hydroxycoumarin as substrate in a method modified from Chen et al. [17]. Slices totalling 500 mg wet weight were incubated in 30 ml of sucrose medium containing 0.5 mM Na_2SO_4 for 15 min at 37°C, then transferred to medium containing 100 μM 7-hydroxycoumarin and incubated for a further 60 min. Slices were then blotted, weighed and homogenized with a Elvehejm pestle in 3 ml of homogenate. Unreacted 7-hydroxycoumarin in the incubation mixture was extracted by mixing 1 volume of the homogenate with an equal volume of 1.5% (v/v) isoamyl alcohol/diethyl ether. A 0.2 ml aliquot of the aqueous phase containing the hydroxycoumarin sulfoester formed during the incubation was then hydrolyzed with 0.2 ml arylsulfatase enzyme in 0.6 ml citrate buffer (pH 4.8; 250 mM) at 37°C, for 16 h in a Dubnoff shaking water bath. The reaction was terminated by addition of 0.125 ml 15% TCA and the non-polar products were extracted with 2 ml $CHCl_3$. After thorough mixing, a 1 ml aliquot of the $CHCl_3$ phase was removed and added to 1.5 ml of 1 M NaCl in 0.01 M NaOH. The amount of 7-hydroxycoumarin released by arylsulfatase action on the synthesized coumarin 7-sulfate was determined spectrofluorometrically using excitation and emission wavelengths of 368 nm and 456 nm, respectively.

TABLE I

Water spaces for human placental slices in different media

Slices were incubated in the media for 60 min with ^{3}H-PEG (final activity = $3 \cdot 10^6$ dpm/ml). The values are means ± S.E. (n = experiments, each with three replicates) for water spaces expressed as a percent of the total wet weight.

Medium	TTW (%)	ECW (%)	ICW (%)
MEM ($n = 3$)	82.2 ± 1.6	35.4 ± 3.3	46.8 ± 1.3
BSM ($n = 5$)	83.3 ± 1.5	30.2 ± 2.1	52.7 ± 2.1
Sucrose ($n = 10$)	78.7 ± 1.4	38.6 ± 1.3	40.0 ± 2.4

Statistical

Uptakes are expressed means ± S.E. with 3 replicates at each point, unless otherwise stated. Differences between means were tested by the Student's t-test.

Results

Water spaces and distribution of label in placental slices

Total tissue water (TTW) was a relatively constant fraction of slice wet weight but distribution between intra- and extracellular spaces was dependent on composition of the medium (Table I), as noted by others [18]. The time required for the PEG marker to reach steady state was less than 60 min in all three media (data not shown); therefore, water spaces for other media were measured at 60 min in all subsequent experiments.

Time-dependence of sulfate uptake

The time courses for uptake of AIB and sulfate in three different media are shown in Fig. 1. The concentrative uptake of AIB was much less in minimal essential medium (MEM) than in the balanced salt medium, presumably because of competition for Na^+-dependent transport by other MEM constituents, particularly the neutral amino acids. Therefore, balanced salt medium was used in subsequent experiments. In contrast to AIB, uptake of sulfate was not concentrative

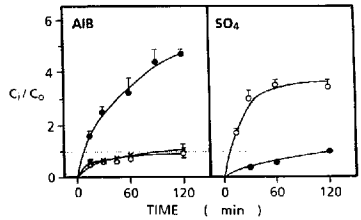

Fig. 1. Uptake of 0.1 mM AIB (^{3}H-AIB: $2 \cdot 10^6$ dpm/ml) (left) and 0.1 mM SO_4 ($^{35}SO_4$: $2 \cdot 10^5$ dpm/ml) (right) in balanced salt medium (BSM, ●), minimal essential medium (MEM, ×) and sucrose medium (○). Data points and bars represent means ± S.E. for three slices. The interrupted line indicates the equilibrium ratio (C_i/C_o) of unity.

in Na^+-based medium but increased with time in the sucrose medium, achieving a 3-fold concentration gradient by 30 min, but there was no increase after 60 min. This medium-dependent uptake effect was reproducible for all preparations, but at 60 min the magnitude of the gradient for AIB in the physiological salt medium and sulfate in the sucrose medium was variable, depending on the placental preparation (Table II).

Absence of detectable sulfoesterification

We were unable to detect any sulfoester formation in the sucrose medium at 60 min (data not shown). The sensitivity of the assay was such that utilization of 2% of the 500 μM sulfate present in the sucrose medium during the original incubation would have been readily detectable as greater than 10% esterification of added 7-hydroxycoumarin. Thus, sulfoesterification cannot account for the concentrative uptake observed in the sucrose medium.

Inhibition by anion-exchange transport inhibitors

Uptake of sulfate from sucrose medium was reduced markedly by DIDS (Table III). In most experiments, we observed significant residual uptake, but it was never concentrative. Residual (DIDS-insensitive) uptake varied significantly between preparations. In one placental preparation, SITS was substituted for DIDS at the same concentration (0.5 mM). The degree of inhibition observed was not significantly different (data not shown). Inhibition of sulfate uptake by DIDS was concentration-dependent (Fig. 2), with an apparent inhibition constant (K_i) for DIDS of about 10^{-4} M. No inhibition of AIB uptake was observed.

TABLE II

Uptake of SO_4 and AIB by human placenta slices

Slices were incubated for 1 h in BSM or sucrose medium and then incubated for 1 h in the same medium containing either 0.1 mM AIB ($2 \cdot 10^6$ dpm ^{3}H-AIB/ml) or 0.1 mM Na_2SO_4 ($2 \cdot 10^6$ dpm $^{35}SO_4$/ml).

Solute	C_i/C_o	
	BSM	sucrose medium
^{3}H-AIB	3.03 ± 0.60 ($n = 4$)	0.68 ± 0.08 ($n = 4$)
$^{35}SO_4$	0.63 ± 0.18 ($n = 3$)	3.14 ± 0.22 ($n = 11$)

TABLE III

Inhibition of sulfate uptake by DIDS

Slices were incubated for 60 min in sucrose medium with (+ DIDS) or without (− DIDS) 0.5 mM inhibitor. Slices were then transferred to identical fresh medium containing 0.1 mM Na_2SO_4 with added label ($2 \cdot 10^5$ $^{35}SO_4$) for 60 min and then removed for counting. Residual activity is defined as uptake in the presence of DIDS, expressed as a percentage of that without inhibitor (− DIDS).

Placental preparation	Uptake ratio (C_i/C_o)		
	− DIDS	+ DIDS	residual activity (%)
1	5.49 ± 0.71	0.50 ± 0.08	9 ± 2 *
2	3.36 ± 0.45	0.05 ± 0.03	1 ± 1
3	2.31 ± 0.16	0.18 ± 0.09	8 ± 4 *
4	1.79 ± 0.10	0.22 ± 0.11	12 ± 6 *
5	3.65 ± 0.20	0.38 ± 0.05	10 ± 1 *
Mean [a]	3.32 ± 0.64	0.27 ± 0.08	8 ± 2 *

[a] Data are means ± S.E. for five experiments.
* Significantly different ($P < 0.05$) from 0.

Concentration dependence of sulfate uptake

Saturation analysis was conducted on the DIDS-inhibitable component in the presence of 1 mM inhibitor with sulfate concentrations in the physiological range (Fig. 3). Saturation was not observed and Eadie-Augustinsson transformation (Inset Fig. 3) did not indicate a single affinity uptake mechanism. The variability be-

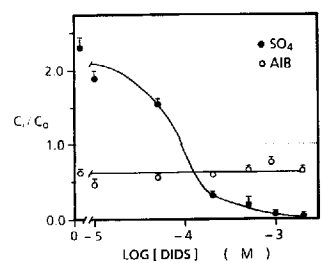

Fig. 2. DIDS inhibition of uptake. Slice were preincubated for 60 min with DIDS in either BSM (○) or sucrose medium (●). Slices were then transferred to identical media containing either ^{3}H-AIB or Na_2 $^{35}SO_4$, respectively (both at 0.1 mM concentration and $2 \cdot 10^6$ dpm label/ml of medium.

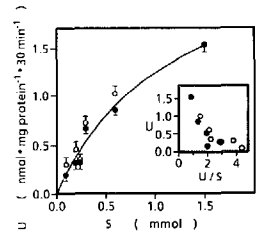

Fig. 3. Concentration dependence of sulfate uptake. Shown are uptakes from two different experiments (closed and open circles). Inset: Eadie-Angustinsson transformation.

tween placental preparations was sufficient to preclude calculation of specific affinity constant(s).

Effects of cations (H^+, Na^+, K^+)

In the sucrose medium, concentrative uptake of sulfate but not AIB is strongly stimulated by hydrogen ion (Fig. 4). Sulfate showed a modest dependence on the buffer used but the uptake was most marked in citrate-phosphate buffer at pH 4.2. At lower pH values, sulfate uptake declined, but marked increases in apparent ECW volumes suggested that tissue viability was compromised (data not shown).

The presence of chloride salts in the sucrose medium inhibited sulfate uptake significantly (Table IV). At physiological concentrations of K^+ (5 mmol/l), the inhibitory effect was less than 10%. Similar concentrations of NaCl well below the physiological range of

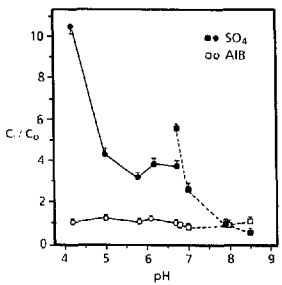

Fig. 4. Effect of pH on SO_4 (■, ●) and AIB (□, ○) uptake in placental slices incubated using 10 mM Tris-Pipes buffer (squares, pH 6.5 to 8) or citrate-phosphate buffer (circles, pH 4 to 6.5) with $Na_2{}^{35}SO_4$ (0.1 mM; $2 \cdot 10^6$ dpm/ml) was added to the medium. Isotopic distribution ratios (C_i/C_o) are plotted against the pH measured in each vial before addition of slices.

TABLE IV

Effect of chloride salts on sulfate uptake in sucrose medium

Slices were incubated in sucrose medium with NaCl or KCl replacing sucrose to maintain isomolarity. Uptake was measured with 0.1 mM Na_2SO_4 containing $^{35}SO_4$ ($4 \cdot 10^5$ dpm/ml). The data are expressed as means ± S.E. for three slices, the uptake ratio (C_i/C_o) with no salt added was 3.03 ± 0.29.

Final salt Conc. (mM)	Uptake of % of control		
	NaCl	KCl	NaCl+KCl
2.5	79 ± 6	97 ± 3	–
5.0	85 ± 3	90 ± 3	76 ± 5
10	49 ± 10	80 ± 3	82 ± 6
20	45 ± 2	62 ± 2	55 ± 1
40	42 ± 3	54 ± 4	–

[#] Added in equimolar amounts (e.g. 2.5 mM NaCl + 2.5 mM KCl for final concentration of 5 mM, etc).

120–180 mmol/l were more inhibitory. Mixing experiments (Table IV) did not indicate synergistic inhibition by cation. To examine chloride anion as an inhibitor, uptake was compared in separate experiments (titrated to pH 6.5 with KOH or NaOH) with Pipes anions replacing chloride. With 10 mM K^+ present, the inhibition amounted to 35% (Ratios of 4.98 ± 1.25 and 3.22 ± 6.41 for K^+-Pipes and KCl, respectively), while for Na^+-Pipes, the inhibition was 75% (Ratios of 2.25 ± 0.20 and 0.56 ± 0.01, respectively).

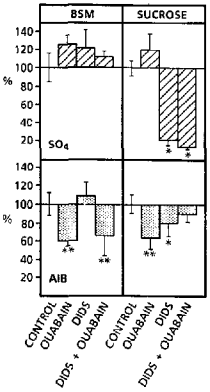

Fig. 5. Effect of ouabain and DIDS on SO_4 and AIB uptake in balanced salt medium (BSM, left) and sucrose medium (right). Slices were incubated for 1 h in medium containing either 0.5 mM DIDS or 0.1 mM ouabain or both. They were then transferred to identical medium with added 0.1 mM Na_2SO_4 (hatched bars; $2 \cdot 10^6$ dpm/ml) or 0.1 mM AIB (shaded bars; $5 \cdot 10^6$ dpm/ml) (* $P < 0.05$; ** $P < 0.01$).

Effect of ouabain

At 1 mM concentration, ouabain significantly inhibited concentrative uptake of AIB from balanced salt medium to 61% of control and inhibited equilibrium uptake to the same degree (Fig. 5, lower panel). This inhibition was largely independent of DIDS. However, ouabain had no inhibitory effect on concentrative uptake of sulfate from the sucrose medium or equilibrium uptake from the salt medium, and did not affect the inhibition of sulfate uptake by DIDS.

Discussion

In mammals, inorganic sulfate is non-uniformly distributed throughout extra- and intracellular spaces. Excess sulfate formed from sulfur amino oxidation is excreted in the urine but the extracellular pool is maintained by renal reabsorption [3,19]. Net uptake by the tubular epithelium depends on Na^+-dependent active transport in the luminal membrane, with the accumulated sulfate diffusing passively from the intracellular to the peritubular space via an anion-exchange system in the contraluminal membrane. This system and the one found in gut are functionally related to the asymmetric localization of transport systems in absorptive epithelial plasma membranes [20].

The placental syncytiotrophoblast also serves as an absorptive epithelium, transporting essential nutrients from mother to fetus, including sulfate. Animal studies suggest that maternal pools supply sulfate for the fetus. However, concentrations are about 7% higher in human cord blood than in maternal samples [6]. This led us to consider that sulfate transport across the placenta might be driven by a transport system akin to the Na^+-dependent systems in gut and kidney. However, sulfate transport in properties brush-border membrane vesicle (BBMV) preparations isolated from human placenta are in keeping with an anion exchange system. These properties include: lack of stimulation by sodium, transstimulation by bicarbonate and other anions, and inhibition by anion-transport inhibitors, such as SITS and DIDS [2,7,23–25].

Extrapolation of these findings to the human fetal-maternal unit depends on several assumptions, including negligibie metabolic conversion of sulfate in intact tissue under physiological conditions and lack of participation by other cell types – notably the cytotrophoblast and trophoblast mesenchyme – in the transport process. We have used incubated placental slices to assess sulfate transport in intact placental tissue. As others have reported for both renal and placental slices [8–10,15,26], uptake of the non-metabolizable amino acid marker, α-aminoisobutyrate (AIB), is concentrative and inhibited by ouabain. These results are consistent with the presence of a specific Na^+-dependent amino acid co-transporter in the placental brush-border [12,14] and rule out major contributions by any contaminating erythrocytes, which do not concentrate AIB, to the observed uptake [27].

In placental slices, we find that sulfate uptake is concentrative only in non-physiological media, displaying characteristics more consistent with a H^+-dependent, Na^+-independent transport mechanism, inhibitable by compounds specific for anion exchange pathways. There is no evidence for sulfoesterification, making metabolic conversion an unlikely mechanism of uptake under these conditions.

In isolated BBMV, sulfate transport occurs by anion-exchange at physiologically relevant concentrations (0.1–0.5 mmol/l sulfate) [2,7,23–25]. Saturation kinetics are compatible with a single tranporter [23], but residual uptake has been observed even in the presence of excess amounts of anion transport inhibitor [23,25], a finding we have also noted in the intact slice. Whether this represents an alternative protein-mediated pathway of low affinity such as that seen in fibroblasts [28] or a passive diffusion component has not been determined. Our slice data also indicate that the DIDS-sensitive component of uptake is not kinetically homogeneous over the physiological range of sulfate concentrations, which differs from our observations in renal slices [15]. Other investigators have found that the renal contraluminal membrane recognizes three different substrate specificities for anion transport [20,21]. All three systems catalyze sulfate transport to differing degrees and all are inhibited to some extent by the distilbene sulfonates. Saturation kinetics in other intact-cell preparations, including fibroblasts, transformed astroglial cells and hepatocytes [28–30], indicate similar heterogeneity, compatible with at least two kinetically distinguishable transport systems. Our data would extend this kinetic heterogeneity to intact placental tissue, a conclusion supported by immunochemical evidence for different placental isoforms of band 3 anion-transport protein at the brush-border and the basal-lateral surfaces of human placental syncytiotrophoblast [31].

Taken in concert with *in vitro* studies of placental BBMV [2,7], our findings in intact placental slices indicate that concentrative sulfate uptake by the fetus, as it occurs *in vivo*, utilizes mechanisms other than the transmembrane sodium gradient that allows concentrative uptake of other nutrients, such as glucose and amino acids.

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References

1 Trueman, P. and Ford, H.C. (1984) Biochim. Biophys. Acta 779, 139–160.
2 Shennan, D.B. and Boyd, C.A.R. (1987) Biochim. Biophys. Acta 906, 437–457.
3 Mulder, G.J., Caldwell, J., Van Kempen, G.M.J. and Vonk, R.J. (eds) (1982) Sulfate metabolism and sulfate conjugation, Taylor and Francis, London.
4 Halstead, P.K. and Roe, D.A. (1981) Drug Nutr. Interact. 1, 75–86.
5 Cole, D.E.C., Evans, J.R., Hamilton, D.A. and Raad, M. (1990) Biol. Neonat. 57, 292–299.
6 Cole, D.E.C., Baldwin, L.S. and Stirk, L.J. (1984) Am. J. Obstet. Gynecol. 148, 596–599.
7 Cole D.E.C. (1984) Biochem. Biophys. Res. Commun. 123, 223–229.
8 Smith, C.H., Adcock, E.W., Teasdale, F., Meschia, G. and Battaglia, F.C. (1973) Am. J. Physiol. 224, 559–564.
9 Smith, C.H. and Depper, R. (1974) Pediatr. Res. 8, 697–703.
10 Enders, R.H., Judd, Donohue, T.M. and Smith, C.H. (1976) Am. J. Physiol. 230, 706–710.
11 Ruzycki, S.M., Kelley, L.K. and Smith, C.H. (1978) Am. J. Physiol. 3, C27–C35.
12 Yudilevich, B.L. and Sweiry, J.H. (1985) Biochim. Biophys. Acta 822, 169–201.
13 Karl, P.I., Chang, B. and Fisher, S.E. (1988) Pediatr. Res. 23, 9–13.
14 Johnson, L.W. and Smith, C.H. (1988) Am. J. Physiol. 254, C773–C780.
15 Cole, D.E.C., Koltay, M. and Scriver, C.R. (1984) Biochim. Biophys. Acta 776, 113–121.
16 Chesney, R.W., Jax, D.K., Mohyuddin F. and Scriver, C.R. (1978) Renal Physiol. 1, 166–170.
17 Chen, Q.Y. and Schwarz, L.R. (1985) Biochem. Pharmacol. 34, 1363–1366.
18 McIver, D.J.L. and Raine, A.E.G. (1972) J. Physiol. 225, 555–564.
19 Tallgren, L.G. (1980) Acta Med. Scand. Suppl. 640, 1–100.
20 Ullrich, K.J. and Rumrich, G. (1988) Am. J. Physiol. 254, F453–F462.
21 Aronson, P.C. (1989) Annu. Rev. Physiol. 51, 419–41.
22 Ullrich, K.J. and Murer, H. (1982) Phil. Trans. R. Soc. Lond. B299, 549–558.
23 Boyd, C.A.R. and Shennan, D.B. (1986) J. Physiol. 377, 15–24.
24 Boyd, C.A.R. and Shennan, D.B. (1986) J. Physiol. 379, 367–376.
25 Deves, R. and Boyd, C.A.R. (1989) Proc. R. Soc. Lond. B237, 85–97.
26 Scriver, C.R. and Mohyuddin, F. (1968) J. Biol Chem 243, 3207–3213.
27 Young, J.D. and Ellory, J.C. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds.), pp. 301–325, Academic Press, New York.
28 Elgavish, A., Smith, J.B., Pillion, D.J. and Meezan, E. (1985) J. Cell Physiol. 125, 243–250.
29 Wolpaw, E.W. and Martin, D.L. (1986) Biochim. Biophys. Acta 855, 302–311.
30 Von Dippe, P. and Levy, D. (1982) J. Biol. Chem. 257, 4381–4385.
31 Vanderpuye, O.A., Kelley, L.K., Morrison, M.M. and Smith, C.H. (1988) Biochim. Biophys. Acta 943, 277–287.