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Topical Review

Monocarboxylate Transport in Erythrocytes*

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Introduction

Transport of anions across the red blood cell membrane has been studied intensively in recent years under the impact of the successful identification and molecular characterization of the transport protein mediating chloride/bicarbonate exchange.

The features of this electrically silent exchange process which is mediated by a 95-K Dalton intrinsic membrane protein, termed band 3 according to its position on SDS-polyacrylamide gels, have recently been treated comprehensively [67]. The transport system is characterized by a broad acceptance of substrates encompassing "regular" mono- and divalent inorganic anions (halides, oxyanions) [67], but also more exotic passengers [24, 35, 74]. Numerous organic anions most likely also permeate the erythrocyte membrane in this way, e.g., dicarboxylates [25, 39, 76], aliphatic and aromatic sulfonates [17, 18, 33, 45, 64, 67, 76]; (B. Deuticke, unpublished results) as well as organic phosphates [9, 48] and even certain amino acids [108].

The question of parallel, alternative pathways of anion transport in the erythrocyte membrane has hitherto met less attention. Pathways independent of the anion exchange system and insensitive to its specific inhibitors may account for slow movements of Cl^- [63] and SCN^- [31]. Alternative routes have mainly been discussed for monocarboxylates. Being the anions of weak and often rather lipophilic acids, they have long been postulated [41, 54] to permeate by nonionic diffusion of the undissociated acid via the lipid domain of the membrane following Fick's law and Overton's principles of nonelectrolyte selectivity. Recent investigations have provided more detailed information on this type of transport [19, 20, 76]. It has, however, also emerged that aliphatic and aromatic monocarboxylates permeate to a certain extent by the classical anion exchange system [4, 18, 25, 76]. Most recently, convincing evidence has become available that in erythrocytes as in other types of cells aliphatic monocarboxylate transport involves a specialized "carrier" system [3, 28, 32, 46, 47]. In the following, the available information on the three parallel mechanisms of monocarboxylate transfer will be summarized, major emphasis being put on the specialized system. A prefatory section will deal with methodical aspects and general difficulties encountered in studying monocarboxylate transport; a concluding chapter is devoted to the physiological role of monocarboxylate movements across the erythrocyte membrane.

How to do it?

Radioactive tracers, in principle, permit unequivocal measurements of net and equilibrium fluxes of monocarboxylates. Problems may arise in case of anions which are subject to enzymatic modification inside the red cell, in particular, by lactate dehydrogenase (LDH). In view of the low specificity of this enzyme for aliphatic 2-oxo- or 2-hydroxy-monocarboxylates [34, 55], the labeled species of these anions may become transformed either by net reduction (formation of a 2-hydroxy- from a 2-oxo-anion) or by isotope exchange

$$RCOCOO^- + R^*CHOHCOO^- \underset{\text{LDH}}{\rightleftharpoons} RCHOHCOO^- + R^*COCOO^-$$

* Dedicated to Professor Dr. A. Fleckenstein, Freiburg i. Br., on the occasion of his 65th birthday.

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between a labeled hydroxy- or oxo-monocarboxylate and an endogenous redox partner (lactate, pyruvate) whose presence is difficult to avoid as long as intact cells are used. The tracer fluxes observed will be taken erroneously for those of the original anion. Difficulties may also result from decomposition of the labeled anion [91]. Adequate chromatographic [49] or other analytical techniques [95] have to be used to cope with these problems.

Among techniques avoiding these complications arising in tracer experiments, enzymatic determinations have been used for following the net movements of substrates of LDH (lactate, pyruvate) [32, 83, 84]. In a more sophisticated approach H_1NMR spin-echo techniques were applied to follow transmembrane net movements of lactate [7].

Additional problems arise in establishing substrate patterns of the specific moncarboxylate carrier system in view of its stereoselectivity. Physiologically relevant substrates, e.g., lactate, are commercially available as defined L- or D-enantiomers. Other substrates, however, can usually be obtained only in unspecified chirality, presumably in the racemic form. Techniques for the preparative separation and identification of enantiomers are available but tedious [5].

The investigation of the nonionic diffusion of monocarboxylic acids is mainly complicated by the high permeabilities of the more lipophilic acids. The qualitative question, whether a given monocarboxylate diffuses in the nonionized form to any major extent can be answered using criteria presented and discussed in detail elsewhere [4, 17, 18, 76]. Quantitative studies require measurements of rapid pH changes or of tracer fluxes at equilibrium [66, 99, 107]. Rate coefficients of tracer fluxes of a monocarboxylate (labeled anion plus labeled undissociated acid), obtained by conventional techniques at pH values far above the pK′ value of the acid, can be converted into rate coefficients and permeabilities for the undissociated acid, provided that only nonionic diffusion but no parallel shunt pathways for the ionized species have to be considered [25].

Pathways of Monocarboxylate Transfer

Easy Does It: Transport by Nonionic Diffusion

For unsubstituted aliphatic monocarboxylates there has never been much doubt that nonionic diffusion would mainly account for their penetration in erythrocytes. Qualitative studies bear out this expectation [4, 17, 18]. For acetate, numerous criteria to be expected in case of simple diffusion via a lipid phase were also established [18, 20]. Substituted aliphatic monocarboxylates of high lipid solubility (e.g., halogen- or cyano- derivatives) should in principle also permeate through the lipid phase. Their lower pK values will, however, induce a lower apparent permeability of the anion at a given pH. For this reason, nonionic diffusion could not be detected by the qualitative criteria of refs. 17 and 76[1] for chloro- and fluoro-substituted acetates (pK′ < 3.0) and 2-chloropropionate (pK′ 2.88), while 3-chloropropionate (pK′ 4.0) permeates by nonionic diffusion [46]. In case of physiologically relevant hydroxy- and oxo-substituted fatty acids (e.g., lactate, pyruvate, 2-hydroxybutyrate, acetoacetate, glycerate, glyoxylate), predictions are difficult. Oxo-compounds up to C_4 and α-hydroxy derivatives up to C_3 penetrate predominantly as anions [4, 17, 18]. Elongation of the chain or shifting of the substituents to positions more distant from the carboxyl group leads to an increased contribution of nonionic diffusion [76].

The qualitative criteria on which this classification was based are not sensitive enough to detect minor contributions of nonionic diffusion and cannot be used for quantitative studies except in combination with advanced techniques of rapid pH measurement [66, 99]. Tracer flux measurements in the presence of inhibitors of specific transport components can be used, however, to establish permeabilities of undissociated acids [25]. Lactic acid permeability (3.7×10^{-5} cm·sec^{-1} at 30 °C, activation enthalpy 24 kcal/mole) compares well to the lactic acid permeability of black lipid films [107]. Permeability to glycolic acid is at least one order of magnitude lower (B. Deuticke, *unpublished results*).

Permeabilities to unsubstituted small fatty acids have also been measured by this technique (Table 1). Numbers for red cells are considerably lower than those for simple artificial lipid membranes [2, 102, 103]. Since unstirred layer effects

[1] The nonionic diffusion of a weak acid into erythrocytes can be distinguished from its ionic permeation by demonstrating sensitivity to inhibitors of carbonic anhydrase of the exit of chloride from cells equilibrating – in unbuffered solutions – with the anion of a weak acid. Carbonic anhydrase is required to keep going the Jacobs-Stewart cycle [57] that catalyzes the continuous dissipation of the transmembrane pH gradient established by the entry of the weak acid acting as a protonophore. Entry of an organic anion in direct exchange for cellular chloride, e.g., via the anion exchange system, does not create pH gradients and is therefore independent of the function of the Jacobs-Stewart cycle.

Table 1. Membrane permeability to small monocarboxylic acids

		Human erythrocytes ($cm \cdot sec^{-1} \cdot 10^4$)	Artificial lipid membranes ($cm \cdot sec^{-1} \cdot 10^4$)
4 °C	C_1	1.74[a]	
	C_2	0.34[a]; 0.5[b]	5[c]
	C_3	1.31[a]	
25 °C	C_1	35[d]	110[e]
	C_2	7[d]	66[e]
	C_3	27[d]	260[e]

[a] B. Deuticke, *unpublished results.*
[b] [99].
[c] [2].
[d] Calculated from the measured values at 4 °C, using an activation energy of 23 kcal/mole [20].
[e] [102, 103].

can be disregarded in both systems [44, 102], intrinsic differences in permeability, e.g., due to the presence of cholesterol, etc. [20], have to be invoked to explain the difference. In both systems, however, the permeability reaches a minimum for the C_2 compound.

Black Passenger: Transport via the Anion Exchange System

Monocarboxylate transport via the Cl^-/HCO_3^- exchange system is difficult to study. Contributions of the two other pathways cannot be excluded *a priori* and will even predominate in many cases. The problem has been assessed taking advantage of selective inhibitors of the Cl^--system. Sufficient inhibitory selectivity is only warranted by stilbene disulfonate compounds [11, 70]; most other inhibitors also affect the specific monocarboxylate carrier [25, 28]. The rate coefficient of DIDS-sensitive[2] lactate equilibrium exchange is about twice that of sulfate transfer, but 10^4 times lower than that for Cl^- (Table 2). A decrease of the chain length from C_3 (lactate) to C_2 (glycolate) enhances transport. Rate coefficients for the two monocarboxylates are comparable to those of the analogous dicarboxylates. Pyruvate self-exchange cannot be studied reliably in erythrocytes, due to its conversion into lactate. Data obtained for pyruvate net fluxes in ghosts indicate a considerable contribution of the Cl^- system [83, 84]. These data, however, are at variance with influx measurements in intact cells [46], indicating a low contribution of the Cl^- system.

Table 2. Comparison of rate coefficients of mono-and dicarboxylate self-exchange via the inorganic anion transport system (10 °C, chloride media, substrate concentration 5 mM, pH_e = 7.4)

	min^{-1}		min^{-1}
Glycolate[a]	0.0300	Oxalate	0.0750
Lactate[a]	0.0010	Malonate	0.0010
Chloride[b]	~20	Sulfate	0.0005

[a] Measured as the difference between PCMBS-insensitive and PCMBS + DIDS-insensitive flux.
[b] [6].

DIDS-sensitive monocarboxylate exchange is inhibited by inorganic anions; its activation energy (30 kcal/mole)agrees with data for other substrates of the Cl^--system [18]. The values are constant between 5 and 35 °C, in contrast to the situation for Cl^- and Br^- exchange [6], but in agreement with the data for divalent anions [13, 86] and for slowly penetrating monovalent anions [15, 31, 33].

Kinetic parameters for monocarboxylate movements via the Cl^--system are only available for the heteroexchange of lactate or pyruvate against Cl^-. In an evaluation of nonlinear Lineweaver-Burk plots, Halestrap [46] estimated a very low affinity (K_T about 300 mM) and a capacity (at 10 °C) of 50 μmol/ml cells/min, much lower than J_{max} of Cl^- self-exchange [6] or Cl^-/HCO_3^- heteroexchange [69] at this temperature, but 50 times higher than the J_{max} of lactate net transfer via the specific moncarboxylate carrier (*see* below).

The further analysis of moncarboxylate movements via the Cl^--system will be facilitated by using ruminant erythrocytes, in which the specific monocarboxylate carrier is absent [28]. Studies on these species have already shown the Cl^--system to lack stereoselectivity in monocarboxylate transport – in contrast to the specific monocarboxylate carrier [28]

A provisional pattern of monocarboxylates transported by the chloride system is given in Table 3. To what extent unsubstituted monocarboxylates move via the Cl^- system is unsettled. Transport of formate has been demonstrated unequivocally [4]. For acetate and propionate, inhibition of the Cl^--system by these anions was demonstrated [16, 43, 87], but interpreted controversially as competitive [87] or noncompetitive [43].

The structural properties that make monocarboxylates acceptable for the Cl^--system are not

[2] *Abbreviations and definitions:* DIDS – 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; DNDS – 4,4′-dinitro-2,2′-stilbenedisulfonic acid; DTNB – 5,5′-dithio-bis(2-nitrobenzoate); DTDP – 4,4′-dithiodipyridine; FDNB – 1-fluoro-2,4-dinitrobenzene; PCMB – *p*-chloromercuribenzoate; PCMBS – *p*-chloromercuriphenylsulfonic acid; TNBS – 2,4,6-trinitrobenzenesulfonate; *Kinetic terms: ee* = equilibrium exchange, *zt* = zero-*trans* (i.e., no substrate on *trans* side of membrane).

Table 3. Pattern of monocarboxylates transported by the inorganic-anion exchange system[a]

Formate > bicarbonate[b] (~12)
Chloroacetate[g] (0.0460)
Glyoxylate-hydrate > glycolate ≫ lactate > 2-hydroxybutyrate[g] (0.2620) (0.1200) (0.0040) (0.0025)
Pyruvate ≫ 2-oxobutyrate[g] (0.0650) (0.0080)
Oxamate, aceturate[c]
Glycine = serine > cysteine[d] (2.02) (1.81) (1.05)
Phenylglyoxylate, hippurate, *p*-aminohippurate[c]
α-Cyanocinnamates[e]
Phosphoenolpyruvate, 2-phospholactate, phosphoglycolate[f]

[a] Based on transport studies (chloride/monocarboxylate heteroexchange). Numbers in brackets are rate coefficients (min^{-1}), at 10 °C, pH 7.4), except for the amino acids.
[b] Calculated from data in [105]. [c] [76].
[d] Numbers refer to SITS-sensitive uptake into sheep erythrocytes in μmol/ml cell·hr [108].
[e] [46]. [f] [48].
[g] B. Deuticke, *unpublished results*.

well characterized. A "three point attachment" binding of the carboxyl group and an adjacent side group to the transfer site has been postulated [76]. Quantitative hetero-exchange experiments (*see* numbers in Table 3) indicate that polar (OH) and strongly electronegative (oxo- or chloro-) groups favor transport (pyruvate > chloracetate > glycolate ≫ acetate). Addition of a methyl group greatly reduces transport (α-OH-butyrate < lactate < glycolate, α-oxobutyrate < pyruvate); C_1-monocarboxylates are obviously transported much more rapidly than their C_2-congeners: formate ≫ acetate (which may not be transported at all), bicarbonate ≫ glycolate. From these observations one would tend to predict a considerable spatial restriction at the transfer site, a notion also borne out by the rapidly decreasing transport rates in the sequence of homologous dicarboxylate anions [19].

The Textbook Way: Transport via a Monocarboxylate-Specific Carrier System

Halestrap and Denton [47] first suggested a pyruvate and lactate carrier in the erythrocyte membrane on the basis of transport inhibition by an aromatic analogue of these monocarboxylates, α-cyano-4-hydroxycinnamate. While the specificity of this inhibition is less pronounced than first claimed, the presence of the carrier has been firmly established [3, 21, 22, 25, 28, 32, 81, 82]. The independent nature of this carrier is demonstrated by its inhibitor sensitivity pattern, substrate specificity, pH dependence and the type of flux coupling. These features are presented subsequently. Moreover, this section will deal with kinetic models and the possible molecular identity of the system.

Inhibitor-Sensitivity

Among the inhibitors of anion exchange via the Cl^--system, several stilbenedisulfonates are most effective [11]. At the levels completely blocking the Cl^--system, stilbenedisulfonates produce a saturating but incomplete inhibition of monocarboxylate movements, varying from anion to anion [3, 25, 28]. SH-group modifying mercurials (PCMBS, PCMB, Hg^{++}) and dithiol compounds (DTNB, DTDP and several analogues), on the other hand, block that component of monocarboxylate transfer that is *in*sensitive to stilbenedisulfonates, while not affecting – except for DTDP – the Cl^--system [25, 28]. This pattern can be used to discriminate the two pathways of mediated monocarboxylate transport [25].

Amphiphilic organic compounds of a very divergent structure inhibit the Cl^--system [11, 20, 67]. With two exceptions, all compounds studied as yet also inhibit specific monocarboxylate transport, even at identical or very similar concentrations. This parallelism raises numerous questions as to the common molecular basis of the inhibition, although it has to be realized that the kinetics of inhibition of the two pathways may differ [46]. One of the exceptions are stilbenedisulfonates. Their lack of affinity for the monocarboxylate system may result from a particular charge distribution at the monocarboxylate binding site, since other aromatic mono- and disulfonates inhibit this system as well as the chloride system [25]; (B. Deuticke, *unpublished*). As a further exception, methylxanthines (methylisobutylxanthine, pentoxiphylline) inhibit monocarboxylate movements at I_{50} values between 0.3 and 1.5 mM ([3]; B. Deuticke, *unpublished results*) without affecting anion movements via the Cl^--system (B. Deuticke, *unpublished results*). Cyano-cinnamates are sometimes regarded as specific inhibitors of monocarboxylate transport. While this may be true for mitochondria [47], it is certainly not true for the erythrocyte, where these anions are unselective inhibitors of both anion transport systems ([3, 46]; B. Deuticke, *unpublished results*).

Table 4. Kinetic standard parameters of monocarboxylate transport via the specific system[a]

			J_{max} (pmol/cm^2·sec)	J_{max} (μmol/ml cells·min)	K_T (mM)	$\frac{J_{max}}{K_T}$
Equilibrium exchange at Donnan equilibrium, $pH_e = 7.4$	L-Lactate[b]	20 °C	4.3	3.9	46.3_e	0.092
		10 °C	2.1	1.9	72.8_e	0.029
	3-Hydroxybutyrate[c]	0 °C			72	
	Chloride[d]	10 °C		1,900	35_e	54.3
	Sulfate[e]	10 °C		0.1	22_e	0.0045
Net flux at Donnan equilibrium of H^+, $pH_e = 7.4$		*Zero-trans-efflux*[b]				
	L-Lactate	20 °C	0.97	0.87	10.5_i	0.092
		10 °C	0.10	0.09	3.0_i	0.033
		5 °C	0.02	0.02	1.7_i	0.012
		Zero-trans-influx[f]				
		25 °C	3.0	3.3	13.4_e	0.2
		Trans-acceleration (from outside)[b]				
		5 °C			4.0_e[g]	
		Zero-trans-influx[h]				
	3-Hydroxybutyrate	10 °C	1.3	1.43	16_e	0.081

[a] Index $_e$ refers to extracellular, $_i$ to intracellular. Data for the inorganic anion exchange system (chloride, sulfate) is given for comparison.
[b] Fluxes were measured in presence of stilbene disulfonate inhibitors of the inorganic-anion exchange system (B. Deuticke, *unpublished results*).
[c] [82]. [d] [6].
[e] Calculated from Table 2 in Ref. [87], using a factor of 1.3 between 10 and 25 °C for converting K_T values, and the activation energy of sulfate self-exchange [86] for converting J_{max}.
[f] [32]. [g] K_T for transacceleration equals K_T for zero-*trans*-influx [29]. [h] [81].

Kinetics, Flux Coupling and pH-Dependency: on the Way to a Transport Model

PCMBS-sensitive, DIDS-insensitive fluxes of monocarboxylates follow Michaelis-Menten-type kinetics under equilibrium exchange and net flux conditions ([28, 32, 81, 82]; B. Deuticke, *unpublished results*). Self-inhibition at high substrate concentrations, so characteristic for the chloride system [14], has not been observed. Unidirectional net efflux exhibits the phenomenon of transacceleration by external substrates [21, 22], indicative of transport by a mobile carrier (*see* below) moving faster in the substrate-loaded than in the free form [50]. The half saturation constants K_T for equilibrium exchange (Table 4), which correspond to the dissociation constant of the substrate-carrier complex (at a given pH) are of the same order of magnitude as those for the Cl^--system [cf. 6, 14, 67]; the maximal capacity J_{max} (also under equilibrium exchange conditions) is $10^3 \times$ lower than the chloride exchange capacity of the inorganic anion transfer system [6], but about $20 \times$ higher than the sulfate exchange capacity of that system [87]. Net fluxes under zero-*trans* (*zt*) conditions have a much lower J_{max} and a lower K_T than equilibrium exchange. The ratios J_{max}/K_T coincide for equilibrium exchange and zero-*trans* efflux (at $pH_i \approx pH_e$), indicating that the numbers are consistent with each other [52, 71]. The half saturation constant for *trans*-acceleration (K_T^{ta}) of unidirectional lactate efflux by external lactate (at low internal lactate) which is a measure of K_T^{zt} influx [29], is higher than K_T^{zt} (efflux). In terms of a mobile carrier model, this difference indicates an asymmetric system [50, 71]. Moreover, in terms of such a model the considerable difference between J_{max}^{ee} and J_{max}^{net} suggests the free carrier to move more slowly than the substrate-loaded one. The low half-saturation constants of net fluxes result from the combined influence of carrier affinity and mobility on these half saturation constants (*see* below and [50, 71, 88]).

Lactate net fluxes are of a considerable magnitude. Unless they are electrogenic, electroneutrality has to be maintained by a molecular coupling of ion fluxes. Electrogenicity is unlikely since lactate net fluxes are insensitive to changes of membrane potential (B. Deuticke, *unpublished*). Electroneutrality is not preserved by a coupled exchange of lactate against inorganic anions, since such anions are no substrates of the monocarboxylate system

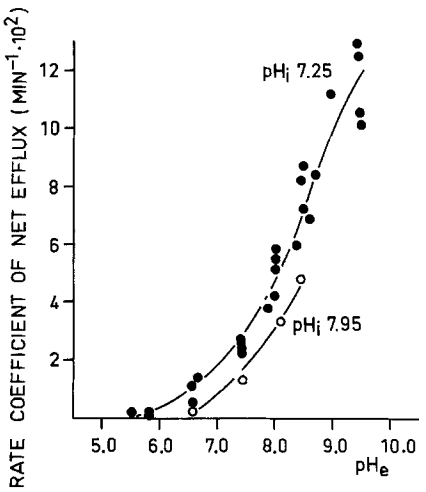

Fig. 1. Dependence of lactate net efflux on extracellular pH at two values of intracellular pH. pH gradients were maintained during flux measurements by blockage of pH equilibration via the inorganic anion exchange system and by extracellular buffering (from ref. 25)

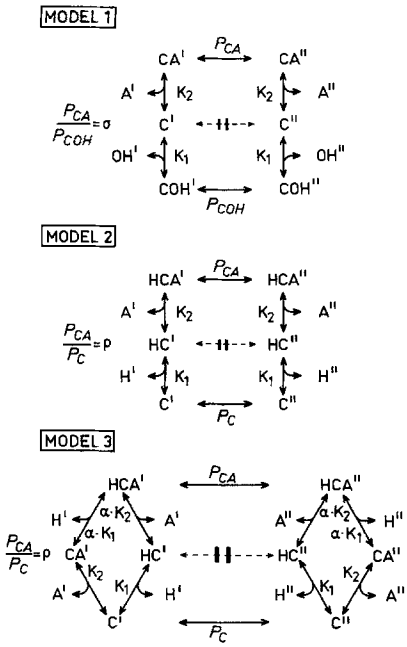

Fig. 2. Kinetic models of the monocarboxylate transport system in the erythrocyte membrane. The model is illustrated by the classical ferry boat symbolism but assumed to represent the alternating association and dissociation of substrate, at two sides of the membrane, to and from conformational isomers of an intrinsic protein spanning the barrier domain of the membrane. For specification of the simplifying assumptions, *see* text

[25]. As an alternative, a coupled exchange against OH^- or its phenomenological equivalent, a monocarboxylate-H^+-cotransport have been postulated [25, 32, 81, 82] in analogy to a similar mechanism in ascites tumor cells [92]. Evidence for this concept comes from results indicative of a 1:1 coupled movement of lactate and H^+ [32] and from marked effects of pH changes at one or both sides of the membrane on monocarboxylate movements [25, 32, 81, 82]. These effects are as follows:

1) Net (zero-*trans*) flux at fixed pH on the *cis* side of the membrane increases with increasing pH on the *trans* side at pH values > 6 [25, 32, 81] (Fig. 1).

2) Net (zero-*trans*) flux at fixed *trans*-pH decreases with increasing *cis*-pH [25, 81].

3) Equilibrium-exchange flux increases with decreasing pH at both sides of the membrane (pH equilibrium) [28, 82].

These data can be rationalized in terms of one of the kinetic carrier models compiled in Fig. 2.

In *model 1*, OH^- and monocarboxylate anions compete for a common binding site, the free carrier C is unable to reorient, COH and CA are mobile. *Trans*-alkalinization stimulates by increasing the concentration of a substrate of the carrier (OH^-) required for its reorientation to the *cis*-side under net flux conditions. Inhibition by *cis*- or bilateral alkalinization is due to competition. *Models 2 and 3* are based on a successive binding, in ordered or random sequence, of H^+ and monocarboxylate, to the "carrier" which reorients only in its free form or in combination with both substrates. In these cotransport models *trans*-alkalinization stimulates net flux by favoring the formation of the deprotonated, mobile form of the carrier at the *trans* side. *Cis*- or bilateral alkalinization diminishes the fraction of protonated carrier able to bind substrates.

These models are very simple, inasmuch as only "direct" pH effects (formation or disappearance of transport sites) are considered. pH effects on carrier mobility have not been included, since they cannot be the object of predictive models. The experimental finding of opposite effects of *cis*- and *trans*-pH on net fluxes rules out a dominating contribution of such indirect effects.

We shall now examine the kinetic differences between the models. General treatments of cotransport models are available [50, 56, 100]. For considerations mainly concerning pH effects, a number of simplifications seems justified, namely the assumptions of

1) rapid equilibria at the interfaces, supported by the ratio of J^{ee}_{max} and J^{net}_{max} [52];

2) symmetry of the carrier-substrate dissociation constants and the carrier-reorientation rate constants. At least one of these two assumptions may not be fulfilled since the carrier is asymmetric with

Table 5. Standard parameters of H^+/monocarboxylate cotransport, for an ordered and a random bi-bi mechanism of transport, in terms of the molecular constants introduced in Fig. 2[a]

	J_{max}		K_T	
	Model 2	Model 3	Model 2	Model 3
Situation I Simple equilibrium $H'=H''$ $Lac'=Lac''$	$\rho \cdot P_c \cdot \frac{C_T}{2}$	$\rho \cdot P_c \cdot \frac{C_T}{2\left(1+\alpha \cdot \frac{K_1}{H}\right)}$	$K_2 \cdot \left(1+\frac{K_1}{H}\right)$	$\alpha \cdot K_2 \frac{1+\frac{K_1}{H}}{1+\alpha \cdot \frac{K_1}{H}}$
Situation II Donnan equilibrium $\frac{Lac'}{Lac''}=\frac{H''}{H'}$	$\rho \cdot P_c \cdot \frac{C_T}{2}$	$\rho \cdot P_c \cdot \frac{C_T}{2+\alpha\left(\frac{K_1}{H'}+\frac{K_1}{H''}\right)}$	$K_2 \frac{1+2\frac{K_1}{H'}+\frac{H''}{H'}}{2}$	$\alpha \cdot K_2 \frac{1+2\frac{K_1}{H'}+\frac{H''}{H'}}{1+\alpha\left(\frac{K_1}{H'}+\frac{K_1}{H''}\right)}$
Situation III Partial disequilibrium $Lac'=Lac''$ $H' \neq H''$ Flux $'\rightarrow''$	$\rho \cdot P_c \cdot \frac{C_T}{1+N \cdot \frac{H''}{H'}}$	$\rho \cdot P_c \frac{C_T}{1+Z\left(\frac{K_1}{H'}+\frac{H''}{H'}\right)+\alpha\frac{K_1}{H'}}$	$K_2 \cdot \frac{1+\frac{K_1}{H'}+N\left(\frac{K_1}{H'}+\frac{H''}{H'}\right)}{1+N\frac{H''}{H'}}$	$\alpha \cdot K_2 \frac{1+\frac{K_1}{H'}+Z\left(\frac{K_1}{H'}+\frac{H''}{H'}\right)}{1+\alpha\frac{K_1}{H'}+Z\left(\frac{K_1}{H'}+\frac{H''}{H'}\right)}$
Situation IV Zero trans-flux $'\rightarrow''$ $H' \neq H''$ $Lac''=0$	$\rho \cdot P_c \cdot \frac{C_T}{1+\rho\left(1+\frac{H''}{K_1}\right)}$	$\rho \cdot P_c \frac{C_T}{1+\rho\left(1+\frac{H''}{K_1}\right)+\alpha\frac{K_1}{H'}}$	$K_2 \cdot \frac{1+2\frac{K_1}{H'}+\frac{H''}{H'}}{1+\rho \cdot \left(1+\frac{H''}{K_1}\right)}$	$\alpha \cdot K_2 \frac{1+2\frac{K_1}{H'}+\frac{H''}{H'}}{1+\rho\left(1+\frac{H''}{K_1}\right)+\alpha \cdot \frac{K_1}{H'}}$

[a] Derived by procedures as described, e.g., in Ref. 50. Simplifying assumptions are discussed in the text.

$\rho=\frac{P_{CA}}{P_C}$; $N=\frac{K_1 \cdot K_2+\rho \cdot H' \cdot Lac}{K_1 \cdot K_2+\rho \cdot H'' \cdot Lac}$. $Z=\frac{\alpha \cdot K_1 \cdot K_2+\rho \cdot H' \cdot Lac}{\alpha \cdot K_1 \cdot K_2+\rho \cdot H'' \cdot Lac}$. C_T = Sum of all forms of the carrier.

respect to the half-saturation constants of the net fluxes (Table 4). However, this does not affect the issue.

Models 1 and 2 cannot be distinguished on a kinetic basis. They describe in essence identical situations, except that the molecular constants have a somewhat different meaning in the two models. Model 3 differs from model 2 in characteristic features, as becomes evident from different relationships between the standard kinetic parameters and the molecular coefficients (Table 5).

The following situations are considered:

I $Lac' = Lac''$, $H' = H''$

II $\frac{Lac'}{Lac''}=\frac{H''}{H'}$ (i.e., Donnan equilibrium)

III $Lac' = Lac''$, $H' \neq H''$

IV $Lac''=O$ $H' \neq H''$.

In situation I, J_{max} is pH-independent for model 2, but strongly pH-dependent for model 3. K_T is strongly pH-dependent for model 2, but only pH-dependent for model 3 when α is significantly different from 1.

Situation II, which describes the normal situation of a red cell in which anions and protons are distributed according to a Donnan equilibrium, corresponds to situation I except that the proton gradient across the membrane, i.e. the Donnan ratio $r_H = H''/H'$, influences the standard parameters.

In situation III ($Lac' = Lac''$, $H' \neq H''$), which essentially describes an asymmetric situation, the differences between the two models are not readily predictable. In both models J_{max} as well as K_T are related to pH′ and pH″ by functions involving the products and sums of proton concentrations and dissociation constants. At fixed pH′, pH″ will have a more pronounced effect on J_{max} than on K_T.

In situation IV ($Lac''=O$, $H' \neq H''$) J_{max} only depends on pH″ in model 2, but on pH′ and pH″ for model 3. K_T is dependent on pH′, pH″ and the pH difference in all three models. Fluxes in opposite direction exhibit asymmetry governed by the proton concentration ratio and described by

$$\frac{J'^{\rightarrow''}_{max}}{K'_T}=\frac{H'}{H''} \cdot \frac{J''^{\rightarrow'}_{max}}{K''_T}.$$

The pH effects predicted by the models can be compared with the pH dependences of standard parameters for monocarboxylate transfer experimentally obtained. For equilibrium exchange (situ-

Table 6. pH-dependence of standard parameters of monocarboxylate transfer by the specific system[a]

	pH_e		K_{T_e} (mM)	J_{max} (relat. units)
Equilibrium exchange[b]	6.3		125	6
3-Hydroxybutyrate, rat RBC, 10 °C	8.3		37–40	1
Zero-*trans-efflux*[c]	pH_i	pH_e	K_T	J_{max}
L-Lactate, human RBC, 10 °C	(=*cis*)	(=*trans*)	(mM)	(pmol/cm²·sec)
	7.25	7.35	3.0	0.08
		8.0	5.3	0.29
	6.5	7.4	1.2	0.09
	8.0		4.9	0.07
Zero-*trans-influx*	pH_i	pH_e		(μmol/ml cells·min)
	(=*trans*)	(=*cis*)		
L-Lactate, human RBC, 25 °C[d]	7.3	6.5	4.5	3.3
		7.5	13.4	3.3
3-Hydroxybutyrate, rat RBC, 10 °C[e]	7.5	6.6	10	1.38
		8.0	29	1.52

[a] Indices $_i$ and $_e$ refer to intra- and extracellular compartments.
[b] [82]. [c] (B. Deuticke, *unpublished results*).
[d] [32]. [e] [81].

ation I, β-hydroxybutyrate in rat erythrocytes [82]) the increase of J_{max} at low pH (Table 6) favors model 3; the concomitant increase of K_T is compatible with this model if $\alpha > 1$, but also compatible with model 2. For net fluxes ([23, 32, 82]; B. Deuticke, *unpublished*) (situation IV) the J_{max} values increase at decreasing H″ (*trans*) but are not affected by H′ (*cis*) (Table 6). This is compatible with model 3 if $\alpha K_1/H'$ is very small relative to $1+\rho\cdot(1+H''/K_1)$. This latter assumption may indeed be true since ρ is certainly much larger than 1 in view of the *trans*-acceleration exhibited by lactate transfer. Model 2, however, is also reconcilable with the data. The increase of K_T with decreasing H′ and H″ is predicted by both models. The available data thus slightly favor model 3 but do not yet prove its validity.

Substrate Affinity: Portrait of a Binding Site

According to direct transport studies the monocarboxylate carrier transports not only "physiological" substrates, such as pyruvate and L-lactate, but also the related anions D-lactate, glycolate [28] and β-hydroxybutyrate [3, 81, 82]. In rabbit erythrocytes acetate could be directly shown to be a substrate (B. Deuticke, *unpublished results*). More extensive transport studies are hampered by the lack of labeled substrates or adequate methods of chemical analysis. Competitive inhibition, and *trans*-acceleration or *trans*-inhibition [29] by substrate analogues, however, can be used to establish substrate patterns indirectly.

The transfer system seems to be specific for small aliphatic monocarboxylates, but rather unspecific within that group. Inorganic anions, dicarboxylates (e.g., oxalate) as well as aliphatic monosulfonates are not transported ([22]; B. Deuticke, *unpublished results*). From Table 7 the following specificity pattern emerges.

1) The system accepts unsubstituted monocarboxylates, but prefers substituted derivatives with chain lengths between two and four members. Five-membered chains (e.g., 2-hydroxyvalerate) act inhibitory, i.e., are presumably bound to the system without being transported.

2) Affinity for the carrier is enhanced by substituents in the order $(=0)>(-CN, -N_3, -F, -Cl, -Br, -J)>(-(OH)$ at position 2. Substitution at position 3 is less efficient but follows the same order.

3) Increasing the number of substituent −OH groups abolishes affinity (glyoxylate-hydrate vs. glycolate, glycerate vs. lactate). In case of halogenated analogues, mono- and disubstituted derivatives are well accepted, tri-substituted ones are much less readily transported (trifluoroacetate) or even inhibit (trichloroacetate, tribromoacetate).

4) Replacement of oxygen in a side group by a sulfur or a nitrogen atom (i.e., mercapto, amino-, or carboxamido substitution) lowers or abolishes affinity and transport (thioglycolate or oxamate vs. glycolate; thiolactate or alanin vs. lactate). Carbon atoms in the backbone, however, can be replaced by other atoms: methoxyacetate (and

Table 7. Substrate pattern of the monocarboxylate carrier

	Accepted substrates				Unaccepted monocarboxylates
	Accelerating ($P_{CA} > P_C$)		Inhibitory ($P_{CA} < P_C$)		
C_1					Formate Bicarbonate
C_2	Acetate	+	Cl_3-acetate	(−)	Glyoxylate
	Glycolate	+	Br_3-acetate	− −	Glycine
	X-acetates	+ +	Phenyl-acetate	− −	Oxamate
	X_2-acetates	+ +	Cyclohexyl-acetate	−	Oxalate
	F_3-acetate	+	Phenylglyoxylate	− − −[b]	
	Thioglycolate	+	*X*-Cinnamates	− − −	
		(X = CN-,F-,Cl-,Br-,J-)			
C_3	L-lactate	+ +	3-Br-propionate	(−)	Glycerate
	D-lactate	+	Phenylpyruvate[b]	− −	3-Mercapto-propionate
	Thiolactate	(+)			Alanin
	3-Cl-lactate	+			Malonate
	3-OH-propionate	(+)			
	2-(N_3-,Cl-,Br-,Cl_2)-propionate	+ +			
	3-(N_3-,Cl-)-propionate	+			
	2,3-Cl_2-propionate	+ +			
	2,2′-Cl_2-propionate	+			
	Pyruvate	+ + +			
	3-Br-pyruvate	+ + +			
C_4	2-OH-butyrate	+ +	Butyrate	(−)	Isobutyrate
	3-OH-butyrate	(+)	Vinylacetate (Δ 3,4)	(−)	2-OH-isobutyrate
	2-Cl-butyrate	+	Crotonate (Δ 2,3)	(−)	N-methylglycine
	2-oxo-butyrate	+ + +	Cyclopropancarboxylate	(−)	N-formylglycine
	3-oxo-butyrate (=acetoacetate)	+			
	Methoxyacetate	+ +			
	Methylthioacetate	+ +			
C_5	2-OH-valerate	(+)	Valerate	(−)	Isovalerate
C_6			Hexanate	−	4-oxo-valerate
			2-OH-hexanate	−	2-oxo-isovalerate
					Ethoxyacetate
					N-acetylglycine

[a] Based on *trans*-acceleration, *trans*-inhibition and competition studies. Substrates are classified according to an index + + + to − − −, based on values for J_{max}/K_T derived according to [29]. *P* values as defined in Fig. 2.

methylthioacetate) are better substrates than butyrate, and accepted as well as acetoacetate.

5) Unsaturated analogues (vinylacetate, crotonate) inhibit slightly, i.e., are bound but not well transported, while branched isomers (2-hydroxy-isobutyrate, 2-oxoisovalerate) are not accepted.

6) Aromatic and alicyclic derivatives act inhibitory, some of them very powerfully.

The acidity of the carboxyl group is probably involved in these patterns, since acids with lower pK′ values are usually the better substrates. Unambiguous statements concerning the relevance of acidity, however, are presently impossible since substitutions or replacements which change the acidity usually also change the monocarboxylates' capacity for interacting with its binding site on the carrier by either weak polar, or hydrophobic interactions.

The pattern is based, in case of optically active compounds, on work with analogues of unknown chirality, which limits the evaluation. Nevertheless, the substrate pattern tentatively suggests some structural elements of the substrate binding site (Fig. 3):

1) A single positively charged group, binding carboxyl groups of sufficient acidity (dicarboxylates not accepted, simple moncarboxylates not well accepted).

2) A single H-acceptor group (e.g., C=0) enforcing the binding of substrates that carry a single H-donor group (i.e., −OH). A second OH-group cannot be accommodated.

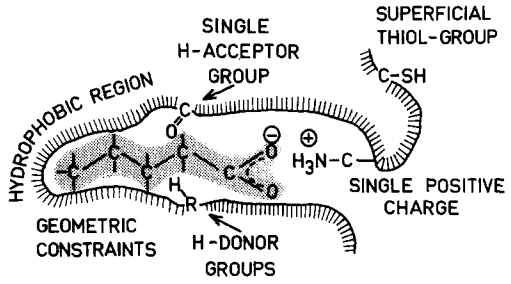

Fig. 3. Schematic view of the substrate binding site of the monocarboxylate/H^+-cotransport system based on the pattern outlined in Table 7

3) One or several H-donor groups interacting with electronegative substituents of the substrate (=0, $-CN$, $-N_3$, $-F$, etc.). Both donor and acceptor group on the binding site must be very near to the positive charge interacting with the carboxylate group since substituents in position 3 are much less favorable than in position 2.

4) No adequate negatively charged binding site for a positively charged substituent (amino and N-substituted amino acids not accepted).

5) Geometric constraints, which restrict binding and transport (branched isomers, analogues $>C_5$ not accepted).

6) A hydrophobic region favoring substrate binding by interaction with an apolar substrate domain (*compare* formate with acetate, glycolate with lactate) but probably also of critical importance for the conformation change involved in substrate translocation: very hydrophobic analogues (phenyl and cycloalkyl derivatives) and aliphatic analogues (e.g., 2-OH-valerate) are probably bound, but prevent carrier reorientation).

Further properties to be incorporated into this portrait encompass stereo-selectivity of the system [3, 28] and the pH-dependence of transport. The site of protonation is undefined. From the titration curve in Fig. 1 an apparent pK of about 8.5 can be deduced for the outward directed form of the free carrier, indicative of an ε-amino group. The slope of this titration curve is lower than that expected for the protonation of a single dissociable group. Although other explanations cannot be discarded [22], the low slope may merely reflect the simultaneous change of J_{max} and K_T, predicted by the kinetic models.

Molecular Features: in Search of Structural Equivalent

Information on the molecular nature of the carrier and its environment in the membrane comes from

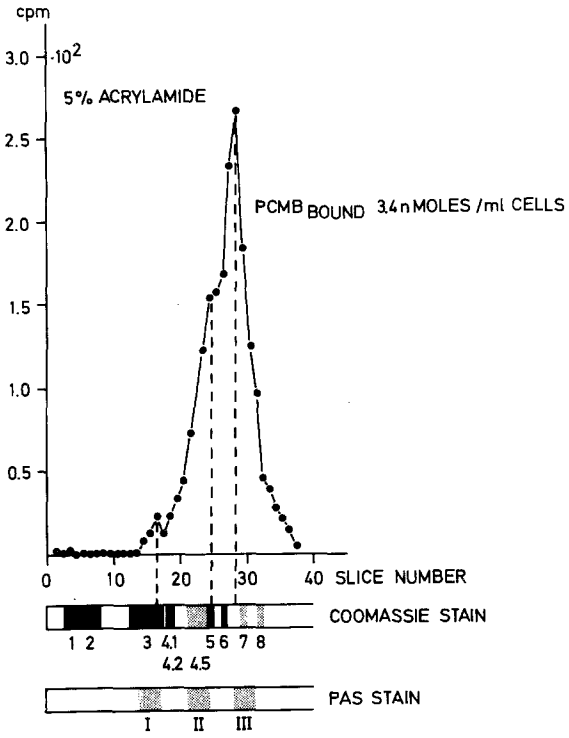

Fig. 4. Distribution of ^{14}C-PCMB over the peptide fractions of the human erythrocyte membrane, after brief exposure (< 1 min) of native cells to the reagent. Isolation and solubilization of membranes under adequate precautions against migration of labeled to unreacted SH-groups. Numbering of bands according to Steck [94]. Localization of PCMB to bands 3, 4.5, and 7 was further substantiated by extracting loosely bound peptides (band 1, 2, 4.1, 4.2, 5, 6) without loss of label (*see also* ref. 21)

studies with covalent inhibitors and lipid modulation. The major questions are:
a) in which peptide fraction does the carrier reside, and
b) how does the operation of the carrier depend on the surrounding lipids?

a) Identifying the Peptide Involved. Inhibition of lactate transport by FDNB, TNBS and pyridoxal phosphate [28, 46] and a lactic anhydride derivative [59] suggest the involvement of amino groups. In view of their inhibitory influence on inorganic anion exchange [28, 59, 67] and of the rather uncharacteristic binding pattern of such reagents [93, 109], they will probably not help to identify the transport protein. Mercurials are more promising tools in view of their high affinity for the monocarboxylate carrier [28]. Complete inhibition is obtained by exposing cells to 40 nmol PCMBS/ml cells for less than 10 sec at 0 °C [28]. During this exposure time PCMBS binds to about 1.5×10^5 membrane SH groups/cell [21] on the exofacial

membrane surface. Since the total number of exofacial SH-groups amounts to 9×10^5 [1, 12, 96, 97], a maximum of 20% of these groups is possibly involved in monocarboxylate transport.

This high affinity binding of PCMB(S), which is only reversible by prolonged exposure to thiols [28], has been used to look for the carrier among the membrane peptide fractions. The pattern of gel-electrophoretic bands labeled by PCMB during very brief exposure revealed three possible candidates [21]: band 3, which binds very little (5–15%) of the total mercurial, the band 4.5 region, and band 7, which bind the major part (*cf.* Fig. 4).

PCMB-labeling of the band 3 region is unlikely to involve primary binding to the inorganic anion exchange system (95K-dalton peptide), which has no exofacial SH group in its native state [79, 80]. The band 4.5 region, which represents approximately 10% of the membrane protein [94], binds about 1–1.5 nmol PCMB/ml cells, equivalent to $6–9 \times 10^4$ sites per cell. This heterogeneous fraction contains at least six peptide fractions (about 10^5 copies per cell of each) between 45K and 55K daltons [61]. At least some of them span the membrane [1, 21, 94]. Carrier functions of the erythrocyte membrane previously assigned to peptides migrating in this region comprise nucleoside [58] and, particularly, monosaccharide transport [62].

Band 7, which contributes 3% of the membrane protein mass (4×10^5 copies/cell) [94], also binds about 1–1.5 nmol PCMB/ml cells at complete inhibition of monocarboxylate transport. This 30K-dalton peptide seems to span the erythrocyte membrane [21, 94] and behaves as a very tightly bound intrinsic protein.

Band 7, a subfraction of band 4.5, and even a minor (<1%) fraction of the band 3 region can all be regarded as candidates for the monocarboxylate carrier function when turnover numbers are considered. From the J_{max} value of lactate equilibrium exchange a maximal exchange rate of lactate (at 20 °C) can be computed, equivalent to a turnover number (TON) per cell, which may be compared to similar numbers for other facilitated transport processes (Table 8).

The TON for lactate compares well to values for systems transporting other organic substrates, but also to sulfate exchange, while chloride and glucose have much higher values. TON's per transport site, computed for those processes, for which the number of carriers has been (tentatively) established, differ by four orders of magnitude. Taking the minimal and maximal TON as limits, the number of monocarboxylate transport sites could vary between 4×10^2 and 4×10^6 sites per cell at pH 7.4, 20 °C. PCMB-labeling data restrict the range to a maximum of 10^5 sites per cell, all compatible with the three peptides.

Table 8. Turnover numbers for various transport systems in the human erythrocyte membrane[a]

	[Molecules/cell/sec] $\cdot 10^{-6}$	No. of transport sites/cell	TON (molecules/site/sec)
L-lactate	4	$<10^5$	$>10^2$(?)
Chloride[b]	8,300	$\sim 10^6$[g]	$\sim 10^4$
Sulfate[c]	0.4	$\sim 10^6$[g]	~ 1
Leucine[d]	0.4	?	–
Uridine[e]	2	$\sim 10^4$[h]	$\sim 2 \times 10^2$
Glucose[f]	270	$\sim 10^5$[i]	$\sim 2.7 \times 10^3$

[a] Data computed from J_{max} values for equilibrium exchange at 20 °C, using published Q_{10} values, wherever possible, and an estimated Q_{10} of 4 where no data were available.
[b] [6]. [c] [87]. [d] [53]. [e] [10].
[f] [104]. [g] [67]. [h] [98]. [i] [62].

b) Lipid Dependence. A membrane-spanning protein mediating monocarboxylate transport is likely to sense its lipid environment in analogy to other membrane enzyme and transport systems. Experimental alteration of membrane lipids, but also investigations of the temperature dependence of transport may be used to establish such interactions (for reviews *see* 27, 38, 78, 85).

Lactate equilibrium exchange is markedly inhibited by cholesterol depletion; an increase of membrane cholesterol produces only a slight acceleration [42]. A similar pattern was observed for the monosaccharide carrier [42], while the transport systems for inorganic anions [42] and leucine (M. Piontek and B. Deuticke, *unpublished*) respond in the opposite direction. The cholesterol dependence of lactate exchange results, at least partly, from a continuous decrease of K_T^{ee} with increasing cholesterol [42]. Possible mechanisms of such effects of cholesterol range from direct interactions between the carrier protein and the sterol, as suggested for band 3 protein [65], to cholesterol-induced changes of the physical state of the lipid domain (*see* [27] for refs.). Membrane microviscosity, an indicator of order and mobility [51] of the lipids, as well as membrane polarity, characterized, e.g., by dielectric constants [75], may influence the orientation and conformation of membrane proteins and the rates of their molecular motion [37, 89], thus altering the substrate affinity and the rates of reorientation of the carrier. Dipole potentials and the network of hydrogen bonds in the headgroup region of the lipid domain are also sensitive to cholesterol [8, 98].

Phospholipid cleavage [26, 106] or depletion have provided information on a possible role of lecithin in monocarboxylate transport. The carrier system is inhibited noncompetitively (M. Piontek and B. Deuticke, *unpublished*) by cleavage of lecithin (in the exofacial layer of the membrane [101]) due to the formation of long-chain fatty acids [26]. Removal of lecithin also produces partial inhibition [26], indicating some sort of lecithin requirement, based on a direct interaction with the carrier protein or a fluidizing effect of lecithin in a membrane phase rich in sphingomyelin such as the erythrocyte membrane outer layer [90]. The sphingomyelin/lecithin ratio increases from 1:1 to 2:1 in the lecithin-depleted membrane. On the other hand, cleavage of sphingomyelin into ceramide and soluble phosphorylcholine also inhibits monocarboxylate transport as well as numerous other mediated transport processes [106].

c) Temperature Dependence. Temperature dependence is a further facet of monocarboxylate transport possibly reflecting lipid-protein interactions. Arrhenius diagrams for lactate transport (at J_{max}) exhibit breaks at 18–20 °C, going along with a decrease of the activation energy E_a for equilibrium exchange [28] (36 → 15 kcal/mole) and for net influx (38 → 22 kcal/mole) (B. Deuticke, *unpublished results*). For pyruvate [46] a break was observed at about 10 °C (35 → 18 kcal/mole).

Sudden changes of activation energies at a critical temperature have been reported for the self- or heteroexchange of rapidly penetrating monovalent anions via the inorganic anion transport system [6, 77], while slowly penetrating divalent anions have a constant high activation energy [13, 86]. Other transport processes in the erythrocyte membrane also exhibit breaks in their Arrhenius diagrams [40, 53, 60]. Attempts to attribute such changes of E_a to well-defined steps in the translocation process require detailed kinetic information [40, 52, 53]. In the present case the reorientation rates of both the substrate-loaded and the free carrier are probably affected in view of the change of activation energies for both, J^{ee}_{max} and J^{zt}_{max}. For an H^+/anion cotransport system such as the monocarboxylate carrier the interpretation of the temperature dependence of transport has also to consider the temperature-dependence of the H^+-carrier dissociation constants (K_1).

In molecular terms a change of the activation enthalpy of transport may result from a conformational transition of the carrier protein itself. As an alternative, thermotropic phase transitions or phase separations in the bulk lipid domain of the membrane or the immediate lipid environment of the carrier may influence the carrier function [72, 78, 85]. In the native erythrocyte membrane transitions of the bulk lipid phase do not occur [30, 68], mainly due to its high cholesterol content. Local transitions or lateral phase separations of lipids have been claimed on the basis of temperature studies involving a variety of physical probes, but are still a matter of controversy (*see* [36, 78] for refs.). Such local changes might occur in the vicinity of a membrane-spanning domain of the transport protein and thus influence its conformation, orientation, or ligand-induced molecular motions, either by changes of specific lipid-protein interactions or by altering the solvent properties of the lipid such as dielectric constants or local viscosity.

What Do We Do with This Carrier?

The presence, in the erythrocyte membrane, of a specific mechanism to accelerate monocarboxylate equilibration between plasma and red cells shall finally also be considered under its physiological aspects. Glycolytic metabolism in human red cells produces lactic acid, i.e., lactate + H^+, at a rate of 1.5–2 μmol/ml cells/hr. Both metabolites have to leave the cells. One might ask, however, whether the capacity of the monocarboxylate carrier is needed for this purpose. Conceptually, lactate/H^+-cotransport, i.e., a joint removal of the two waste products, would seem a particularly adequate way of nature to handle this problem.

A theoretical treatment of the issue requires the kinetic parameters of lactate transport at 37 °C, which are not available. A very simple experimental approach, however, can also solve this problem: When erythrocytes are incubated in vitro, lactate accumulation in the extracellular medium is not retarded – as compared to accumulation in the whole suspension – by blockage of the monocarboxylate carrier, of the inorganic anion exchange system, or even of both systems (Fig. 5). Obviously, nonionic diffusion alone can manage the amounts of lactate produced in red cell metabolism, making the carrier dispensible for this purpose.

Alternatively, the carrier might be needed to accelerate the equilibration – between red cell and plasma – of lactate entering the blood during its passage through tissues producing lactate, e.g., the working muscle. The carrier would thus increase the solvent space for lactate and help to maintain gradients between interstitial fluid and plasma.

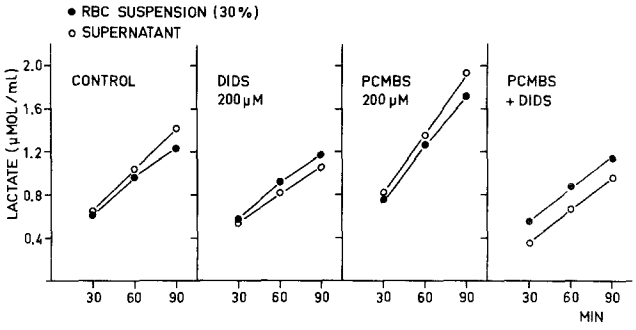

Fig. 5. Endogenous lactate equilibrates between cells and medium during incubation of erythrocytes (37 °C, pH 7.4) irrespective of the blockage of its two mediated transport systems. Increase of lactate production in the presence of PCMBS is probably due to formation of cation leaks [97] and a consecutive stimulation of energy-consuming ion pumps

Finally, the rapid, coupled cotransport of monocarboxylates and H^+ has been claimed [73] to be an important subsidiary mechanism of H^+ translocation across the erythrocyte membrane, necessary for improving the availability of the high buffer capacity of the red cells to H^+ ions entering the blood. In this sense the monocarboxylate carrier would act in parallel [23] to the well-established Jacobs-Stewart cycle [57] which requires the inorganic-anion exchange system.

The actual relevance of these two possible functions of the monocarboxylate carrier remains to be proven. Both would implicate an interesting contribution of the monocarboxylate carrier to the acid-base and electrolyte homeostasis, making the monocarboxylate carrier a promising object of the future attention not only of biophysicists and biochemists, but also of physiologists.

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