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L-Lactate Transport in Ehrlich Ascites-Tumour Cells

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1. Ehrlich ascites-tumour cells were investigated with regard to their ability to transport L-lactate by measuring either the distribution of [^{14}C]lactate or concomitant H^+ ion movements. The movement of lactate was dependent on the pH difference across the cell membrane and was electroneutral, as evidenced by an observed 1:1 antiport for OH^- ions or 1:1 symport with H^+ ions. 2. Kinetic experiments showed that lactate transport was saturable, with an apparent K_m of approx. 4.68 mM and a $V_{max.}$ as high as 680 nmol/min per mg of protein at pH 6.2 and 37°C. 3. Lactate transport exhibited a high temperature dependence (activation energy = 139 kJ/mol). 4. Lactate transport was inhibited competitively by (*a*) a variety of other substituted monocarboxylic acids (e.g. pyruvate, K_i = 6.3 mM), which were themselves transported, (*b*) the non-transportable analogues α-cyano-4-hydroxycinnamate (K_i = 0.5 mM), α-cyano-3-hydroxycinnamate (K_i = 2 mM) and DL-*p*-hydroxyphenyl-lactate (K_i = 3.6 mM) and (*c*) the thiol-group reagent mersalyl (K_i = 125 μM). 5. Transport of simple monocarboxylic acids, including acetate and propionate, was insensitive to these inhibitors; they presumably cross the membrane by means of a different mechanism. 6. Experiments using saturating amounts of mersalyl as an 'inhibitor stop' allowed measurements of the initial rates of net influx and of net efflux of [^{14}C]lactate. Influx and efflux of lactate were judged to be symmetrical reactions in that they exhibited similar concentration dependence. 7. It is concluded that lactate transport in Ehrlich ascites-tumour cells is mediated by a carrier capable of transporting a number of other substituted monocarboxylic acids, but not unsubstituted short-chain aliphatic acids.

It has been widely believed that the movement of monocarboxylic acids across cell membranes proceeds by simple physical diffusion of the undissociated lipophilic free acid through the hydrophobic matrix of the membrane, without mediation by a transport system (Hogben *et al.*, 1959; Huckabee, 1961; Hohorst *et al.*, 1965; Weiner, 1973). However, a transport system for lactate has been found in a bacterium (Harold & Levin, 1974), and some fragmentary evidence has appeared that the transport of pyruvate and lactate in animal tissues may also be mediated by a carrier system (Watts & Randle, 1967; Henderson *et al.*, 1969; Halestrap & Denton, 1974; Lamers & Hülsmann, 1975). Since lactate is delivered into the blood by actively glycolysing normal cells such as erythrocytes, skeletal muscle and retina, and is in turn extracted from the blood and metabolized by the liver and heart, specific transport systems for lactate and for pyruvate would thus enable rapid translocation of these important central metabolites across the membranes of certain types of cells.

This paper reports the first comprehensive study of lactate transport across the plasma membrane of a eukaryotic cell. We have chosen for this purpose Ehrlich ascites-tumour cells, which, like most types of cancer cells, produce lactate at a high rate from glucose. Extensive data are available on the transport of glucose, amino acids and Na^+ by Ehrlich cells, which also offer considerable experimental convenience for transport studies. The kinetics of lactate transport, its pH-dependence, inhibition by analogues and sensitivity to thiol-group reagents were among the factors investigated. The relationship between lactate and H^+ transport was also studied. From these studies it is concluded that a transport system is present in the Ehrlich tumour-cell membrane which translocates lactate at a high rate in either direction, depending on the gradients of lactate and H^+. The carrier also transports certain other substituted aliphatic acids, such as pyruvate, but does not transport unsubstituted acids such as acetate or propionate.

Experimental

Materials

NAD^+, L-lactate dehydrogenase (EC 1.1.1.27), L-lactic acid, D-lactate (calcium salt), DL-3-hydroxybutyrate acid, pyruvic acid, propionic acid, butyric acid, DL-phenyl-lactic acid, DL-*p*-hydroxyphenyllactic acid and mersalyl acid {*o*-[(3-hydroxymercuri-

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2 - methoxypropyl)carbamoyl]phenoxyacetic acid} were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. α-Cyano-3-hydroxycinnamic acid, α-cyano-4-hydroxycinnamic acid, 4-hydroxybutyric acid (sodium salt), 3-hydroxypropionic acid, 2-methylpropionic acid and carbonyl cyanide *m*-chlorophenylhydrazone were supplied by Aldrich Chemical Co., Milwaukee, Wis., U.S.A. 2-Chloropropionic acid, glycollic acid and chloroacetic acid (sodium salt) were products of Eastman Kodak, Rochester, N.Y., U.S.A. Dichloroacetate was obtained from ICN Life Sciences Group, Plainview, N.Y., U.S.A. L-[U-^{14}C]Lactic acid, [U-^{14}C]sucrose, [^{3}H]inulin and $^{3}H_2O$ were obtained from Amersham/Searle Corp., Arlington, Ill., U.S.A., and 5,5'-dimethyl[2-^{14}C]oxazolidine-2,4-dione from New England Nuclear Corp., Boston, Mass., U.S.A.

Methods

Cell growth and harvesting. Ehrlich ascites-tumour cells (Northwestern University strain, hyperdiploid), initially provided by Dr. E. Coe, Department of Biochemistry, Northwestern University Medical School, Chicago, Ill., U.S.A., were propagated by injection into the peritoneal cavity of Swiss albino mice (Buckberg Laboratories, Tomkins Cove, N.Y., U.S.A.) and harvested after 7–10 days. The cells were washed three times to remove the ascitic fluid and contaminating erythrocytes with a solution containing 150mM-NaCl, 5mM-KCl, 5mM-Tris/HCl, pH 8.2. Centrifugations were carried out at 60*g* for 5min in an International Clinical Centrifuge (Damon I.E.C. Division, Needham Heights, Mass., U.S.A.) equipped with a swinging-bucket head.

Incubations. Unless otherwise stated, incubations were performed in a buffered medium of the composition 150mM-NaCl, 5mM-KCl and 10mM-Mes [2-(*N*-morpholino)ethanesulphonic acid], pH 6.2, in water-jacketed thermostatically controlled vessels at a temperature of 37°C; mixing was accomplished with a magnetic flea. A 1min preincubation period was generally used to achieve temperature equilibration of the cells (5mg of protein/ml), and the reaction started by the addition of the compound under test from a 0.5M stock solution by using a glass microsyringe.

pH changes were followed under the above conditions by using a micro-pH electrode, pH-meter (Expandomatic SS-22; Beckman Instruments, Fullerton, Calif., U.S.A.) and recorder (model DS2G, Sargent–Welch Scientific Co., Skokig, Ill., U.S.A.) at a chart speed of 5in (12.5cm)/min, with 0.25 unit giving full-scale deflexion. The system was calibrated by back titration with standard 0.1M-HCl.

Experiments involving transport of radioactively labelled substrates (final specific radioactivity 20nCi/μmol) were performed as above, and reactions terminated at the time indicated by centrifugation of a portion of the cell suspension (usually 200μl) for 15s in an Eppendorf Microfuge (Brinkman Instruments, Westbury, N.Y., U.S.A.). The supernatant was removed by gentle aspiration and the sample treated as indicated below. [^{3}H]Inulin (0.2μCi/ml) was added to correct for extracellular water spaces.

Cell volumes. These were determined by using $^{3}H_2O$ (0.25μCi/ml) and [^{14}C]sucrose (50nCi/ml) to correct for extracellular water. The cells were separated from the medium as described above.

Intracellular pH. This was determined by measuring the distribution of the weak acid 5,5'-dimethyl[2-^{14}C]-oxazolidine-2,4-dione (50nCi/ml) by published methods (Poole, 1967; Addanki *et al.*, 1968). Again, [^{3}H]inulin was used to correct for the extracellular water space.

Inhibitor stop. Since it was found that the thiol-group reagent mersalyl was capable of inhibiting lactate transport (see Fig. 6), this property was utilized to obtain kinetic data by using the inhibitor-stop technique, which enabled the time-resolution to be increased by nearly an order of magnitude. Essentially the method for removing samples and quantifying the time of termination of the reaction was as indicated by Coty & Pedersen (1974). The stop mixture consisted of 0.8ml of standard incubation medium containing 10mM-mersalyl, to which 0.2ml samples of the incubating cells were added. Subsequent steps were identical with those used in the centrifugal stop method.

Scintillation counting. Radioactivity was determined by solubilizing the cell pellet in Soluene 350 (Packard Instrument Co., Downers Grove, Ill., U.S.A.) and dissolving the subsequent digest in 10ml of a mixture of 600ml of toluene, 400ml of methoxyethanol and 6g of 5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole. For measurements of total radioactivity in the system, a portion of the total cell suspension was solubilized. The samples were then counted for radioactivity in a Beckman LS150 scintillation spectrometer by using the automatic quench correction to discriminate between ^{3}H and ^{14}C.

Assays. Lactate was assayed enzymically by the method of Hohorst (1963). Protein was determined by a modified biuret method (Szarkowska & Klingenberg, 1963) by using KCN to correct for the turbidity caused by lipids, which accounted for 20–50% of the original absorbance. Small-column chromatography with Dowex 1 was performed as indicated by Busch (1953).

Results

Effect of the pH of the medium on entry of L-*lactate into Ehrlich ascites-tumour cells*

Since lactic acid has a p*K'* of 3.86 and is almost completely dissociated at physiological pH, it

appeared possible that the lactate ion may undergo co-transport (symport) with H^+ or exchange (antiport) with OH^- across the cell membrane (Mitchell, 1967). If this is the case then transport of lactate could be expected to be pH-dependent. Data in Fig. 1(*a*) show the effect of the suspending medium pH on the entry of [^{14}C]lactate, at an initial concentration of 5mM, as a function of time. The maximum extent of lactate uptake varies from approx. 52nmol/mg of total cell protein at pH6.2 to 21nmol/mg at pH8.2. On the basis of the measured water content of the cells of 3.0μl/mg, these values are equivalent to inside/outside lactate ratios of 3.5 and 1.4 respectively. Greater than 90% of the radioactive label which entered the cells was in the form of lactate at the end of the 15min incubation period, as indicated by small-column chromatography. Concomitant with the entry of lactate the internal pH decreases (Fig. 1*b*). This decrease is approx. 0.6 and 0.10pH unit at suspending buffer pH values of 6.2 and 7.2 respectively. At the highest external pH value tested there is no discernible change in intracellular pH, probably owing to the buffering capacity of the cytoplasm.

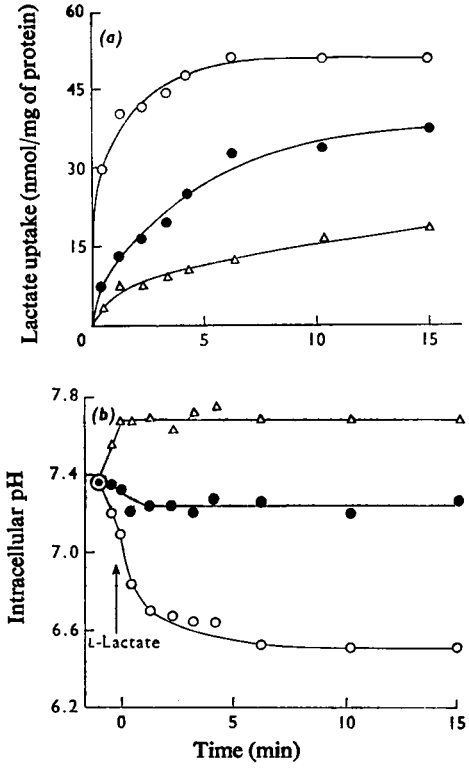

Fig. 1. *Effect of pH on the uptake of* L-*lactate*

Incubations were carried out and lactate uptake and intracellular pH changes determined as described under 'Methods' by using the centrifugal-stop technique. Suspending buffers contained 150mM-NaCl, 5mM-KCl and either 10mM-Tris/HCl (pH8.2), 10mM-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N*'-yl)ethanesulphonic acid]/NaOH (pH7.2) or 10mM-Mes/NaOH (pH6.2). Lactate was present at a concentration of 5mM. ○, pH6.2; ●, pH7.2; △, pH8.2. (*a*) Time-course of lactate entry; (*b*) time-course of intracellular pH change.

The pH-dependence of the lactate distribution across the Ehrlich cell membrane is further illustrated in Fig. 2(*a*), where the lactate content of the cells is increased by lowering the external pH to 6.2, and subsequently decreased by increasing the pH to 8.2, in a medium containing 5mM extracellular lactate. These experiments also show that the lactate content of the cells does not simply reflect the external pH, but is actually determined by the pH gradient existing across the cell membrane, a conclusion that follows from comparison of the data at points A and B in Fig. 2. Fig. 2(*b*) shows that at A the inside/outside lactate ratio is 3.0 whereas at B it is 5.5, although the extracellular pH is approximately equal at these points. Fig. 2(*c*) shows that the intracellular pH, immediately before initiation of lactate uptake, is 0.25 unit higher for the latter (cf. 7.8 and 8.05 at points A' and B' respectively). Thus the calculated inward-directed pH gradient driving the uptake of lactate is 1.5pH units for A (7.8 − 6.3) and 1.75pH units for B (8.05 − 6.3). Observed pH gradients across the cell membrane, during the course of the experiment, vary from a low of −0.35pH to a high of approx. +1.1pH (Fig. 2*d*).

Effect of ionophores on lactate movement

The data presented in Table 1 show the effects of nigericin, carbonyl cyanide *m*-chlorophenylhydrazone and valinomycin on the distribution of lactate, added at a concentration of 5mM, between cells and medium. Nigericin, which is known to induce an electroneutral K^+/H^+ exchange, lowers by more than 60% the amount of lactate present in the cells, whether the inhibitor is added to the medium before lactate or after a steady-state lactate concentration is attained. Nigericin appears to induce an equilibrium in which the lactate concentration is identical on both sides of the cell membrane. Producing a similar effect is the combination of carbonyl cyanide *m*-chlorophenylhydrazone and valinomycin, which together also induce an electroneutral K^+/H^+ exchange. Carbonyl cyanide *m*-chlorophenylhydrazone or valinomycin alone yields only about half the decrease in lactate concentration given by nigericin. Measurements of intracellular pH showed that there is a direct correlation between the ability of these compounds to decrease cell lactate and their ability to dissipate the pH gradient across the cell membrane. The mitochondrial inhibitors antimycin A, rotenone and oligomycin had no effect on lactate distribution.

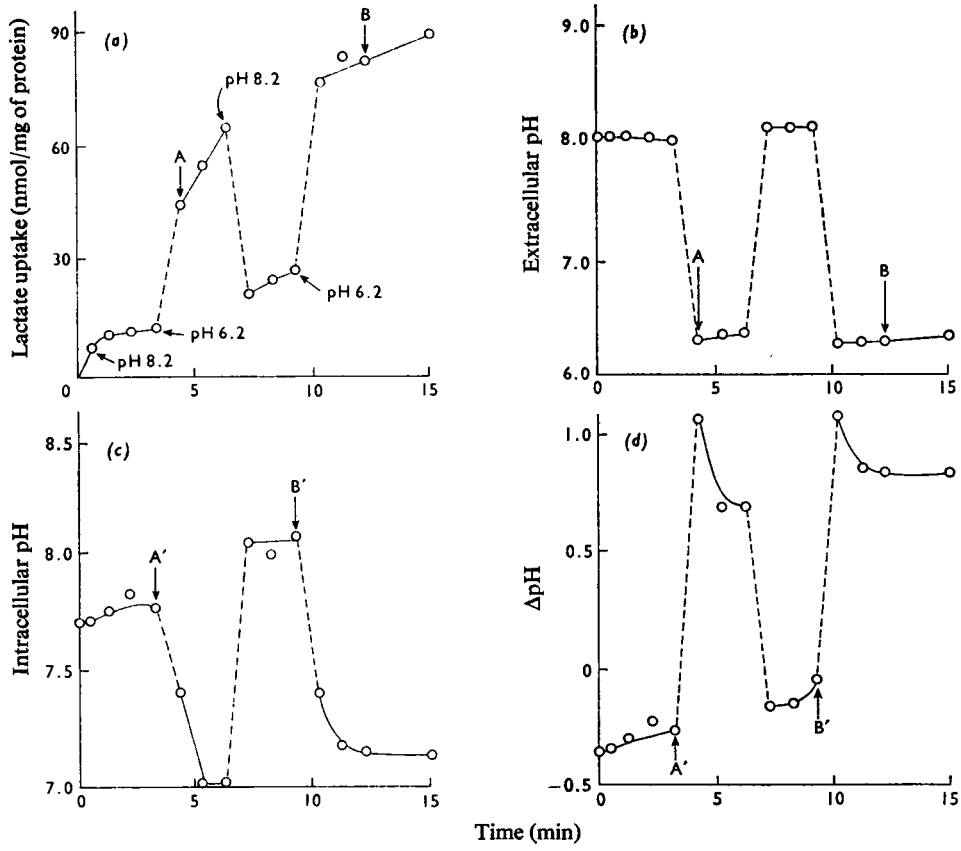

Fig. 2. *Changes in lactate content and intracellular pH of ascites cells induced by varying extracellular pH*

Experiments were performed as described in the legend to Fig. 1 at an initial pH of 8.2. The pH was lowered to 6.2 by the addition of a small amount of 1 M-Mes and increased to 8.2 by using 2.5 M-Tris base. (*a*) Lactate accumulation; (*b*) extracellular pH; (*c*) intracellular pH; (*d*) pH differential across the cell membrane (intracellular pH minus extracellular pH). A, A', B and B' are referred to in the text.

Table 1. *Effect of ionophores on lactate uptake*

Lactate uptake was determined as in Fig. 1 at pH 6.2 and with 5 mM-lactate. Other additions were: 10 μM-nigericin, 10 μM-valinomycin and 2 μM-carbonyl cyanide *m*-chlorophenylhydrazone. The compounds to be tested were preincubated with the cells for 1 min before the addition of the lactate in (*a*) and added 2 min after lactate in (*b*). Cells were separated from the medium and the [^{14}C]lactate uptake was determined after a further 2 min incubation period.

	L-Lactate uptake (*a*)		L-Lactate uptake (*b*)	
Inhibitor added	(nmol/min per mg of protein)	(% of control)	(nmol/min per mg of protein)	(% of control)
None	43	100	41	100
Nigericin	16	37	14	34
Valinomycin + carbonyl cyanide *m*-chlorophenylhydrazone	19.5	45	20	49
Valinomycin	34	79	37	90
Carbonyl cyanide *m*-chlorophenylhydrazone	33	77	36	88

Kinetics of lactate transport

Data in Fig. 3(*a*) show the time-course of the change in pH of the medium initiated by the uptake of lactate. The initial pH of the medium is 6.20, which increases to approx. 6.25 on addition of a portion of cells, owing to a combination of the buffering capacity of the cell medium (fast component of the change) and partial equilibration of the pH of the cell and the medium (slow component). The latter is probably due, in part, to outward movement of endogenous lactate and other permeant acids. On the addition of 5mM-L-lactate to the medium there is a rapid increase in the pH of the medium due to the uptake of H^+ ions (or exit of OH^- ions) concomitant with entry of lactate. This process is complete after approx. 45s; it has a half-time of less than 10s. These data, after correction for the slow baseline change in the absence of lactate, were recalculated and expressed in the form of a first-order plot (Fig. 3*b*). The first-order rate constant derived from the linear representation is $2.58\,min^{-1}$, which is equivalent to an initial rate of 252nmol of lactate transported/min per mg of protein.

Fig. 4 shows the stoicheiometry of the movement of H^+ ions and [^{14}C]lactate taken up by Ehrlich cells in the presence of 5mM-lactate. Over the time-course studied the $H^+/^{14}C$ transport ratio shows a median value of approx. 1.05. Other experiments not shown indicated that a similar equimolar relationship holds for a variety of different lactate concentrations at different pH values and temperatures, and also for other monocarboxylic acids, including pyruvate, 3-hydroxybutyrate and acetate. The measurement of the lactate transport rate, as illustrated in Fig. 3, afforded a simple and convenient method for investigating the kinetics of this process and has been utilized in some of the following experiments.

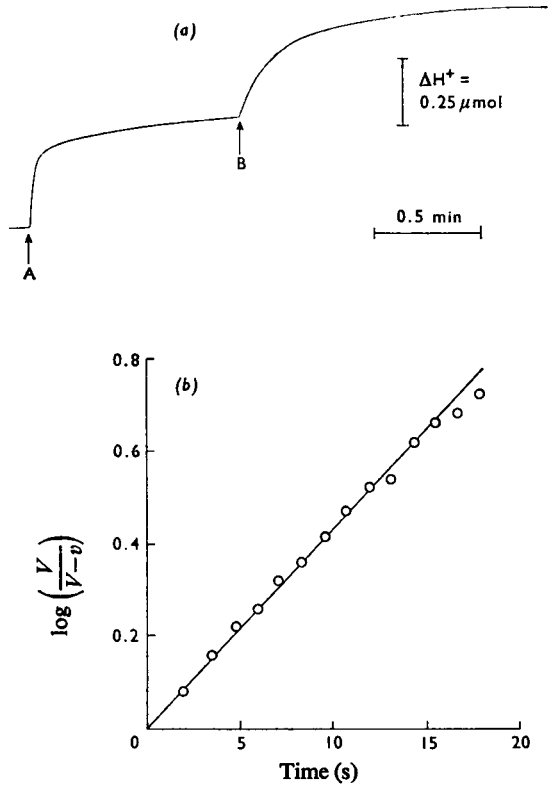

Fig. 3. *Lactate-induced proton uptake*

pH changes in the suspending buffer were followed as indicated under 'Methods'. Ehrlich cells were added at A, and 5mM-lactate was added at B. (*a*) Time-course; (*b*) first-order plot of the data from (*a*) after the addition of lactate. *V* refers to the maximal extent of uptake and *v* extent of uptake at time *t*, expressed as nmol/g.

Influence of the pH of the medium on the apparent affinity for lactate

The effect of incubation media of different pH values on the influx of lactate, measured as a function of lactate concentration, is shown in Fig. 5(*a*). As may be predicted from the data in Fig. 1 the rate of entry of lactate at a given concentration increases with decreasing pH of the medium, i.e. with a decreasing cell–medium gradient of H^+. These data yield linear double-reciprocal plots (Fig. 5*b*) at all pH values tested; the plots show a common intercept equivalent to a $V_{max.}$ of 580nmol/min per mg of protein. However, the apparent affinity constant (K_m) for lactate is pH-dependent, possessing values of 4.6, 20 and 100 mM at pH values of 6.2, 7.2 and 8.2, respectively.

Influence of thiol-group reagents on lactate transport

As shown by data in Fig. 6, increasing concentrations of mersalyl produce increasing degrees of inhibition of the initial rate of uptake of 5mM-lactate. The reaction is virtually 100% inhibited at a concentration of 2.0mM, with a half-maximal effect observable at about 0.25mM. Other experiments, not shown here, indicate that complete inhibition of transport may be obtained at lower mersalyl concentrations by means of longer preincubation times. At 10mM-mersalyl the inhibition of lactate transport appears to be instantaneous. Mersalyl has accordingly been utilized, in some of the experiments described below, as an 'inhibitor stop', to measure initial transport velocities under conditions where the pH method of Fig. 3 is inadequate, for example, in the presence of other permeant acids.

Data summarized in the double-reciprocal plot of Fig. 7 show that the inhibitory action of mersalyl is competitive in nature, at least under the conditions investigated. In the presence of 0.5mM inhibitor the

$V_{max.}$ remains constant at approx. 680nmol/min per mg of protein, whereas the apparent K_m for lactate increases fivefold from 4.4 to 22mM. By using standard relationships the calculated K_i is 125μM for mersalyl.

Comparison of the influx and efflux of lactate

Results presented in Fig. 8(*a*) show the time-course of entry and exit of lactate by using the inhibitor-stop technique. Influx of lactate, initially added at 5mM, was induced by a lowering of the external pH and efflux by an increase in external pH, so that the pH gradient across the membrane was the same but oppositely directed. Consequently, the H^+ 'pressure' imposed on lactate to move across the membrane is the same in both directions. When these data are plotted (Fig. 8*b*) two essentially straight lines are obtained, which yield first-order rate constants of 3.35min^{-1} for the influx and 7.6min^{-1} for the efflux of lactate, equivalent to transport rates of 359 and 795nmol/min per mg of protein respectively. These rates are approximately what one would expect after taking into account the calculated internal lactate concentration, before initiation of efflux, of 17.5mM, compared with the extracellular concentration of 5.0mM (see Fig. 5).

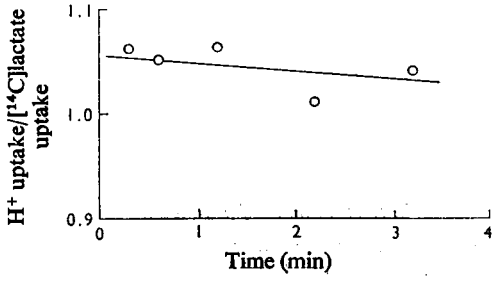

Fig. 4. *Stoicheiometry of proton and lactate entry*

H^+ uptake was measured as in Fig. 3 and lactate uptake as in Fig. 1.

Inhibition of lactate transport by α-cyanohydroxycinnamate

α-Cyanohydroxycinnamate is a specific inhibitor of pyruvate and lactate transport in both mitochondria and erythrocytes (Halestrap & Denton, 1974; Halestrap, 1975); inhibition of pyruvate transport in mitochondria has been confirmed by Paradies & Papa (1975). Data in Fig. 9 show that 2.5mM-α-cyano-4-hydroxycinnamate and α-cyano-3-hydroxycinnamate inhibit lactate transport in Ehrlich ascites cells. As indicated by the convergence of the linear double-reciprocal plots, both compounds are competitive inhibitors of the transport process. The derived inhibitor constants (K_i) are 0.5mM for the 4-hydroxy and 2mM for the 3-hydroxy isomer. These values are much higher than those obtained for pyruvate transport in rat liver mitochondria (Halestrap, 1975; Paradies & Papa, 1975). The distribution of α-cyanohydroxycinnamate between cells and supernatant fluid was also determined, by utilizing its large extinction coefficient in alkaline solution. These experiments indicated that the Ehrlich cell membrane is impermeable to this compound, results that are at variance with those reported by Halestrap & Denton (1975) using epididymal fat-pads, kidney-cortex slices and erythrocytes, and Mendes-Mourao *et al.* (1975) using rat and mouse liver cell preparations. This observation may reflect an intrinsic

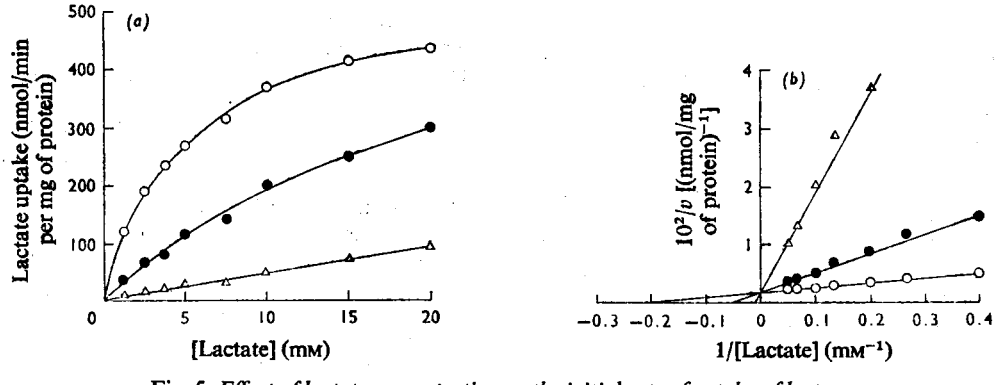

Fig. 5. *Effect of lactate concentration on the initial rate of uptake of lactate*

Cells were incubated, and lactate entry followed, as described in Fig. 3; various concentrations of lactate were present as shown: ○, pH6.2; ●, pH7.2; △, pH8.2. (*a*) Michaelis–Menten plot; (*b*) double-reciprocal plot. Rates of entry of lactate were determined from the initial rate of H^+ ion uptake by using a lactate/H^+ ion ratio of 1.

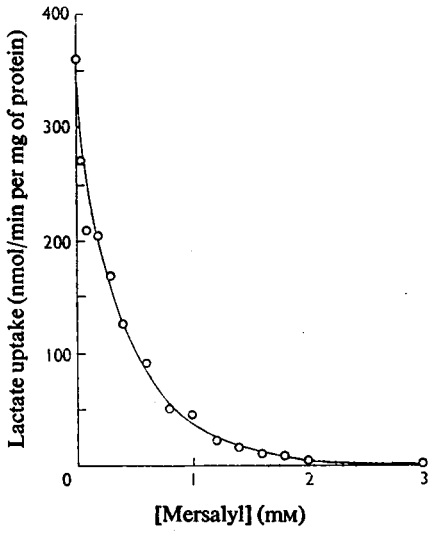

Fig. 6. *Effect of mersalyl on the uptake of lactate*

Lactate (5 mM) entry was followed by using the pH method (Fig. 3) in cells that had been preincubated for 1 min with various concentrations of mersalyl. Initial-velocity kinetics were determined as in Fig. 5.

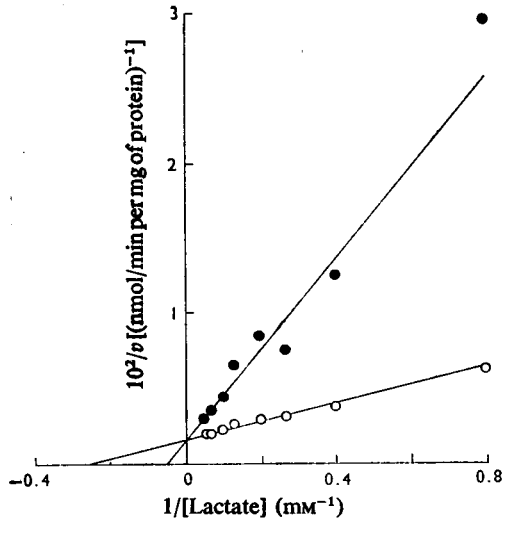

Fig. 7. *Effect of mersalyl on the apparent affinity for lactate*

Lactate entry was followed as indicated in the legend to Fig. 5 in the presence of various concentrations of substrate as shown. ○, Control; ●, 0.5 mM-mersalyl present.

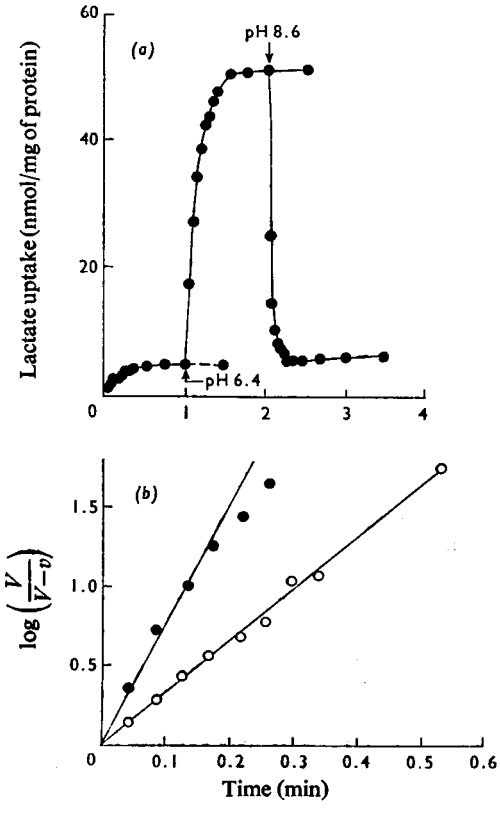

Fig. 8. *Rates of entry and exit of* [^{14}C]*lactate*

Lactate influx and efflux was stopped at timed intervals by the action of 10 mM-mersalyl as described under 'Methods'. Lactate was present at 5 mM and the initial pH was buffered to 8.4 by using 10 mM-Tris/HCl. External pH was changed as indicated in Fig. 2. (*a*) Time-course, (*b*) first-order plots; ○, lactate influx; ●, lactate efflux. V and v have the same meanings and units as indicated in the legend to Fig. 3.

permeability difference between these normal cell types and the Ehrlich ascites carcinoma or a difference in the intactness of the membranes of the cell preparations (Mapes & Harris, 1975).

Specificity of the lactate-transport system

Table 2 shows data from experiments in which a variety of monocarboxylic acids were tested for their ability to act as substrates for the lactate-transport system. All of the compounds exhibit apparent saturation kinetics. There are large differences both in the apparent affinity (cf. extremes of 4.6 mM for L-lactate and 63 mM for phenyl-lactate) and in the $V_{max.}$ (cf. 2000 and 240 nmol/min per mg of protein for acetate and trichloroacetate respectively). However, not all the acids tested appear to be transported

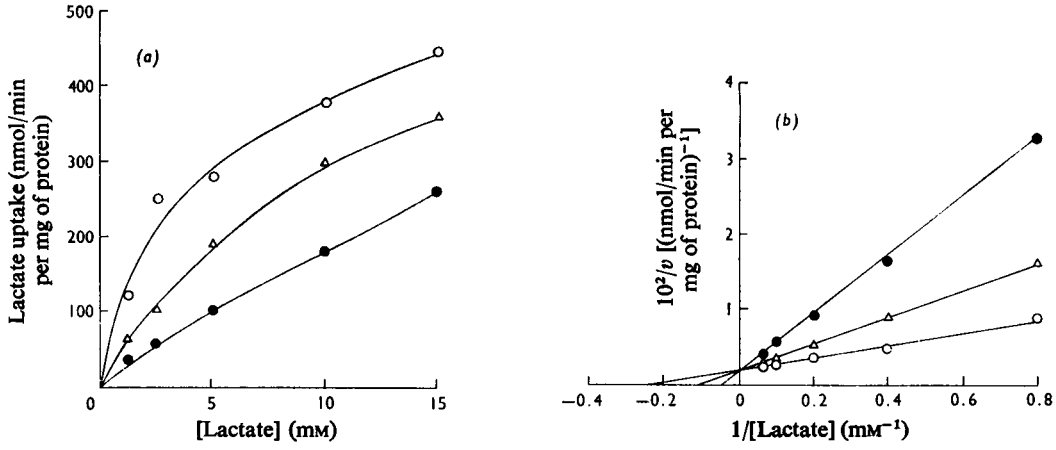

Fig. 9. *Effect of α-cyanohydroxycinnamate on lactate uptake*

Rates of lactate transport were determined as in Fig. 5 at various substrate concentrations and Lineweaver–Burk plots made of the data. ○, Control; ●, 2.5mM-α-cyano-4-hydroxycinnamate present; △, 2.5mM-α-cyano-3-hydroxycinnamate present.

Table 2. *Specificity of the lactate-transport system*

Cells were incubated and $V_{max.}$ and K_m values as well as sensitivity to 2.5mM-α-cyano-4-hydroxycinnamate and 0.5mM-mersalyl determined as indicated in the legend to Fig. 5 at a pH of 6.2. K_i values were determined by following the kinetics of [^{14}C]lactate entry by using the inhibitor-stop technique as described under 'Methods', in the absence and presence of competing anions. pK' values were obtained from data listed by Kortüm *et al.* (1961). C refers to competitive and N to non-competitive inhibition. +, Noticeable inhibition; – negligible effect; ND, not determined.

	a					*b*	
				Sensitivity to			
Substrate	$V_{max.}$ (nmol/min per mg of protein)	Apparent K_m (mM)	pK_a	α-Cyano-4-hydroxycinnamate	Mersalyl	Inhibition of lactate transport	K_i (mM)
L-Lactate	660	4.7	3.86	+	+	ND	ND
D-Lactate	630	5.1	3.86	+	+	ND	ND
Pyruvate	470	8.5	2.49	+	+	C	6.3
Glycollate	410	6.5	3.83	+	+	ND	ND
DL-3-Hydroxybutyrate	600	10	4.39	+	+	C	13.5
DL-4-Hydroxybutyrate	610	5.5	4.31	+	+	ND	ND
2-Chloropropionate	790	5.6	2.88	+	+	ND	ND
DL-3-Hydroxypropionate	460	8	3.73	+	+	ND	ND
Chloroacetate	640	5.4	2.87	+	+	C	7.0
Dichloroacetate	600	5.9	1.79	+	+	ND	ND
Trichloroacetate	240	5.8	0.66	+	+	ND	ND
DL-Phenyl-lactate	760	63	3.36	+	+	ND	ND
DL-*p*-Hydroxyphenyl-lactate	Not transported		3.22	ND	ND	C	3.6
Formate	640	33	3.75	–	–	N	>100
Acetate	2000	12.5	4.76	–	–	N	22.5
Propionate	2200	8.5	4.87	–	–	ND	ND
Isobutyrate	2450	7.9	4.86	–	–	ND	ND

by the same process as lactate. The substituted monocarboxylic acids tested, such as pyruvate and chloroacetate, are apparently transported by the lactate system, as indicated by the inhibition of their movement by both mersalyl and α-cyano-4-hydroxycinnamate. On the other hand the simple unsubstituted aliphatic acids, such as formate and acetate, appear not to be transported by the lactate-carrier

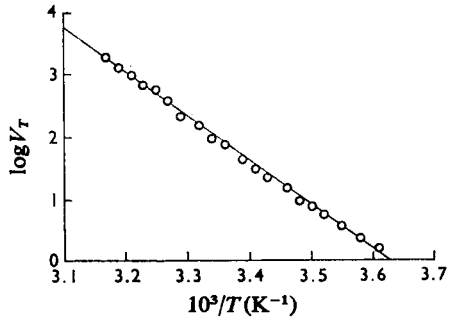

Fig. 10. *Effect of temperature on lactate transport*

Initial rates of lactate entry into ascites cells were determined as described in the legend to Fig. 5 by using a saturating concentration of substrate (20mM). V_T is expressed as nmol/min per mg of protein.

system, since their influx is not inhibited by these agents. The data in Table 2(*b*) show that unsubstituted aliphatic acids are non-competitive inhibitors of lactate transport, unlike pyruvate and 3-hydroxybutyrate, for example, which show competitive inhibition of lactate transport. It is noteworthy that the D and L isomers of lactate are transported at nearly equal rates.

Effect of temperature on lactate transport

The Arrhenius plot of Fig. 10 shows that the rate of entry of lactate into the ascites cells increases from 13 to 1900nmol/min per mg of protein between 4° and 42°C, equivalent to an activation energy of 139kJ/mol. Over this temperature range no significant break is discernible in the linearity of the plot.

Discussion

The results presented in this paper show that the Ehrlich ascites-cell membrane contains a carrier system capable of transporting lactate and certain other substituted acids, both into and out of the cytoplasmic space. This transport process is passive, but is dependent on the pH gradient existing across the plasma membrane, as shown by the induction of uptake (Figs. 1, 2, 8) or of release (Figs. 2, 8) of lactate through manipulation of the extracellular pH. The transport of lactate across the cell membrane is coupled in an obligatory equimolar manner to either the counter-transport (antiport) of a OH^- ion or the co-transport (symport) of an H^+ ion. Experimental limitations do not permit a distinction between these alternatives at present. Lactate transport in *Streptococcus faecalis* (Harold & Levin, 1974), and pyruvate transport in erythrocytes (Halestrap & Denton, 1974) and rat small-intestine cells (Lamers & Hülsmann, 1975) appear to be promoted by systems similar to that described here.

That a carrier is involved in the transport of lactate comes from six interrelated pieces of evidence. (1) The initial rate of lactate transport follows typical saturation kinetics, and exhibits an apparent K_m, under conditions of neutral pH (Fig. 5), of the same magnitude as the lactate concentrations found *in vivo* in the tumour cells, i.e. approx. 20mM (T. L. Spencer, unpublished work). (2) Mersalyl, a thiol-group reagent, is a strong inhibitor of lactate transport. (3) The compounds α-cyanohydroxycinnamate and hydroxyphenyl-lactate are specific competitive inhibitors of lactate transport. (4) Ehrlich ascites cells transport compounds structurally related to lactate by the same mechanism as lactate. (5) The transport process has a relatively high temperature dependence. (6) The carrier has distinct specificity in that simple unsubstituted aliphatic acids such as propionic acid are apparently not transported. It is significant that substitution of propionic acid by either a 2-hydroxyl group (lactate), 3-hydroxyl group (3-hydroxypropionate) or a 2-chlorine atom (2-chloropropionate) yields compounds transported by the carrier, but substitution with a methyl group (isobutyrate) does not. Since α-cyano-4-hydroxycinnamate and hydroxyphenyl-lactate are not transported, whereas phenyllactate and cyanocinnamate (results not shown) are, it appears possible that the presence of a phenolic hydroxyl group prevents transport of aromatic acids.

Little direct correlation is evident between the rate of transport (Table 2) and either the pK' values (cf. pyruvate and 3-hydroxybutyrate) or the position of the substituent on the carbon backbone (cf. 3- and 4-hydroxybutyrate). The rate of transport may also be determined by other factors, such as steric effects induced by the nature of the substitution (for example, compare chloroacetate with trichloroacetate and lactate with phenyl-lactate). No evidence apart from saturation kinetics, which by itself does not suffice, was obtained for carrier-mediated transport of the simple aliphatic monocarboxylic acids such as formate, acetate and propionate. It is most probable that these compounds cross the membrane by simple passive diffusion, as suggested by work in other systems (Hogben *et al.*, 1959; Hoek *et al.*, 1971; Weiner, 1973).

Technical difficulties concerned with establishing pH gradients of known magnitude across the cell membrane precluded a more thorough investigation of the kinetics and other properties of lactate transport in the more physiologically important outward direction. However, under physiological conditions (i.e. pH approx. 7.2 and lactate approx. 20mM) the rate of efflux, as extrapolated from data in Fig. 5, could be expected to be in the neighbourhood of 200nmol/min per mg of protein compared with observed maximal rates of glycolysis of 60nmol/min

per mg under similar conditions (Lee *et al.*, 1967). Lactate transport thus appears not to be the rate-limiting step in aerobic lactate production by the Ehrlich tumour cells. However, it is known that glycolysis is inhibited by low extracellular pH (Halperin *et al.*, 1969; Wilhelm *et al.*, 1971), conditions under which lactate efflux from the cell is also inhibited. This may argue for a regulatory role for the lactate-transport system; however, several glycolytic enzymes are known to have sharp pH-dependence (Wilhelm *et al.*, 1971). The lactate-transport system of Ehrlich cells can increase its apparent affinity for lactate and thus effectively increase the rate of transport when the outward-directed H^+ gradient is increased. The formation of H^+ ions together with the lactate anion from the neutral molecule glucose during glycolysis thus helps to furnish the driving force for lactate transport from the cell against a concentration gradient.

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