J. Membrane Biol. 71, 163–171 (1983)

The Journal of
Membrane Biology

The Mechanism of Na^+-L-Lactate Cotransport by Brush Border Membrane Vesicles from Horse Kidney: Analysis of Rapid Equilibrium Kinetics in Absence of Membrane Potential

Raymond Mengual and Pierre Sudaka

Laboratoire de Biochimie, Faculté de Médecine, Chemin de Vallombrose, F-06034 Nice Cedex, France

Summary. Membrane transport of lactate was studied using vesicles prepared from horse kidney brush border. It is shown that the carrier-mediated transport of L-lactate is Na dependent and the D-lactate Na dependence seems weaker than the L stereoisomer. Augmented transport rate is observed following imposition of an artificial chemical Na^+ gradient of electrical potential difference. The effect of Na^+ chemical gradient on the L-lactate uptake was analyzed using membrane vesicles incubated with 50 mM KCl and valinomycin in order to short circuit any contribution of transmembrane electrical potential to the transport. Kinetics results and principally the absence of linearity between $1/v$ (lactate) versus $1/Na^+$ show that the L-lactate transport mechanism fit the properties of an ordered process with two Na^+ ions cotransported with one L-lactate anion. The L-lactate and sodium affinities (K_m) determined under Na^+ chemical gradient were 1.05 and 48 mM for L-lactate and Na, respectively. The sodium activation was shown to be highly cooperative with a Hill number of 2 although no "sigmoidal" activation effect was observed.

Key words lactate · anion · Na mechanism · cotransport · renal brush border

Introduction

Membrane transport of L-lactate has been studied in erythrocytes [13, 16, 20], mitochondria [19] and Ehrlich ascite tumor cells [33]. In these cases lactate movement was found to be mediated by a carrier. Studies with isolated perfused rat kidneys [8, 9] showed a correlation between net sodium reabsorption by the kidney tubules and lactate oxidation. More recently, studies using vesicles from rat or rabbit renal cortex [4] or enterocytes [11, 22, 35] have shown that lactate absorption is sodium dependent. Lactate thus seems to follow the same sodium-dependent absorption and activation processes as sugars, amino acids [26, 37] and phosphate [6, 23].

In the present paper we re-examine the mechanism of L-lactate transport, in terms of the gradient hypothesis [12, 31], using membrane vesicles isolated from horse kidney. We first specified the general characteristics of this transport system: 1) specificity of Na activation; 2) stereospecificity; 3) role of Na chemical gradient and electical potential.

In a second part, we attempt to get more insight into the mechanism of coupling between Na ion and substrate by kinetic means. We have assimilated the transport entity to a rapid equilibrium multireactant system. Experimental conditions were chosen so that only chemical Na and lactate gradient – but not electrical gradient – were present.

It is important to stress that, on these conditions, isotopic movements of L-lactate corresponded to net movements. This approach is therefore different, although complementary, from the equilibrium (isotope) exchange's developed by Hopfer [24] for analysis of the Na-glucose cotransport studies.

Thus the mechanism of the sodium dependence by analogy with enzyme systems [32, pp. 320–329] is the subject of the present study and based on net flux data derived from initial rate measurements. Our study complements numerous kinetic studies conceived to explain the transport and coupling mechanism [1, 2, 10, 14, 15, 24, 26, 36]. In our next paper (*unpublished*) isotopic exchanges of Na and lactate were used in order to study the mechanism of Na-lactate translocation.

Materials and Methods

Vesicle Preparation

Brush border vesicles of the renal cortex of the horse were prepared as already described [29]. The vesicles were sus-

0022-2631/83/0071-0163 $01.80

pended at a concentration of 10 mg/ml in a 300-mM mannitol buffer and 10 mM Hepes (4(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid)-Tris, pH 7.4.

The transport system was characterized by one type of experiment in which external sodium and L-lactate concentrations formed a gradient with those of the intravesicular medium.

Lactate Uptake Experiments

The osmolarity of each incubation medium was adjusted to 300 mOsM by NaCl, KCl and/or choline chloride buffered with 10 mM Hepes-Tris, pH 7.4. The $U^{14}C$ lactate (8×10^5 to 1.5×10^6 dpm) was included in the incubation medium.

These transport experiments by filtration on Millipore filter are defined in each Figure. All conditions were repeated on 3 to 5 vesicle preparations, each point being done in triplicate. The radioactivity of filters was measured by the standard techniques with a liquid scintillation counter.

Measurement of Vesicular Volume

Vesicular volume was determined by centrifugation using double labeling with (^3H) H_2O and (methoxy^{14}C) inulin.

Material

D- and L-lactic acid (lithium salts and L-lactic acid in water solution were obtained from Serva (Heidelberg, Germany), D-mannitol and Hepes from Sigma Chemical Co. (St. Louis, Mo.), L-$U^{14}C$ lactic acid (90 mCi/mmol), and D-$U^{14}C$ lactic acid (45 mCi/mmol) were purchased from the Radiochemical Centre, (Amersham, England), L-2^3H lactic acid (90 mCi/mmol) and (^3H) H_2O by CEA (France) and (methoxy^{14}C) inulin by New England Nuclear (Boston, Mass.).

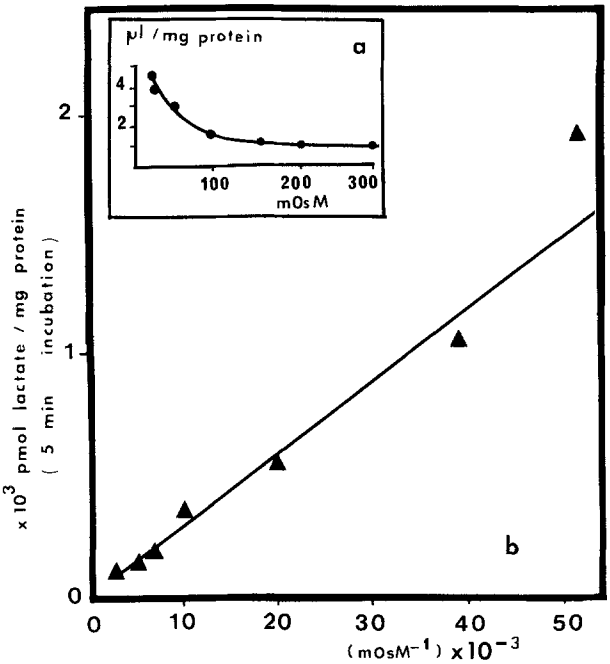


Fig. 1. Influence of increasing osmolarity on the intravesicular volume (*a*) and on the L-($U^{14}C$)-lactate (lithium salt) uptake (*b*). The increasing osmolarity was obtained in presence of 80 mM NaCl and increasing amounts of cellobiose in the incubation medium. Intravesicular volume was determined by double labeling and centrifugation: (^3H)$-H_2O$ for total volume and ($^{14}C-CH_3O$) methoxyinulin for extravesicular space. L-($U^{14}C$)-lactate uptake was determined after 5-min incubation in 80 mM NaCl, 40 mM mannitol, 10 mM Hepes-Tris, pH 7.45, and 1 mM L-($U^{14}C$)-lactate (lithium salt), medium. Each uptake value point in both *a* and *b* is the average of two assays

Results

Osmotic Sensitivity of Accumulated Lactate

It is important to verify that the radioactivity taken up by our vesicle preparations corresponds really to a penetration into the intravesicular space and not to a simple adsorption on the membrane surface. Figure 1 illustrates the effect of varying osmotic pressure on intravesicular volume and L-lactate transport.

Preliminary experiments showed that L-lactate uptake reached an equilibrium within 3 min: measurements were therefore taken after 5-min incubations. The impermeant solute cellobiose was used to modify the osmotic pressure of the incubation medium. Figure 1 (inset *a*) shows that the intravesicular volume (measured by double labeling) decreases when the osmotic pressure increases reaching a minimum at 0.9 µl/mg protein. Under the same conditions L-lactate absorption decreases proportionally to the increasing osmotic pressure (Fig. 1). This sensitivity to the osmotic gradient shows that the L-lactate is indeed accumulated in the intravesicular space and not adsorbed on membrane sites.

General Properties and Energetics of L-Lactate Accumulation

Figure 2 illustrates experiments indicating that Na specifically increases the transport of L-lactate. The maximum stimulation obtained with an electrochemical gradient of Na^+ ($[Na]_{out} > [Na]_{in}$) was about three times greater than that obtained with KCl at 25 sec (open symbols in Fig. 2). Although not shown, choline was observed to be ineffective to activate L-lactate accumulation.

In the absence of Na electrochemical gradient, i.e., when the vesicles were preincubated in the same NaCl concentration as that of the incubation medium, the overshoot no longer occurred (star symbols in Fig. 2); the rate of

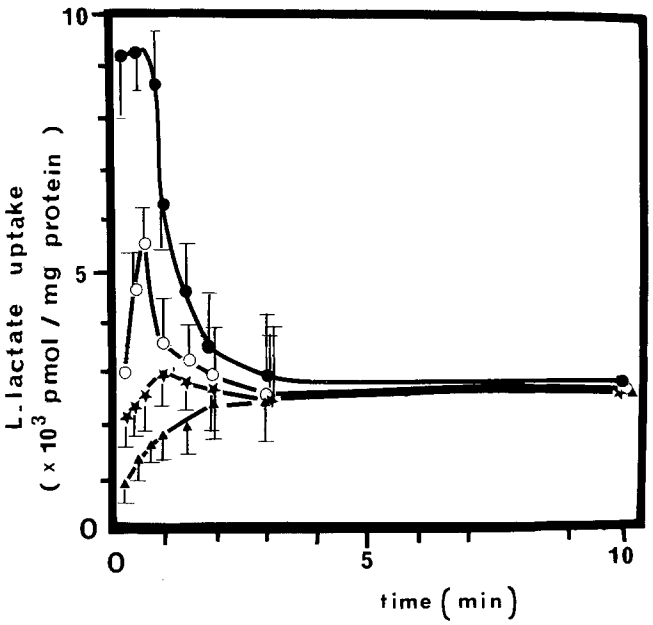


Fig. 2. Effect of various saline solutions on the time course of L-lactate uptake. Membrane vesicles were suspended either in buffered mannitol or preloaded in 80 mM NaCl, 140 mM mannitol. The uptake was initiated by dilution of vesicles (without NaCl) in incubation medium containing L-($U^{14}C$)-lactate (lithium salt) and various Na^+ gradients: NaSCN (●—●), NaCl (○—○), or K^+ gradient (KCl, ▲—▲); these salt concentrations were 80 mM with 140 mM mannitol buffered at pH 7.4 with Hepes-Tris 10 mM. NaCl preloaded vesicles were used with an incubation medium containing 80 mM NaCl (★—★) as in Fig. 1. L-lactate concentration was 1 mM in all experiments. Each membrane suspension aliquot had a volume of 20 µl (200 µg) and is added to 10 µl of incubation medium. The uptake was stopped by diluting these 120 µl with 1 ml of a 150 mM NaCl, 10 mM Hepes-Tris solution, pH 7.4 (4 °C), filtered on nitrocellulose membrane (0.45 µm size pore-Millipore) rinsed with 4 ml of the same buffer; each filtration assay lasted 15 to 20 sec for each incubation time indicated. Means and SE are indicated by points and bars, respectively, and obtained from two measurements in triplicate

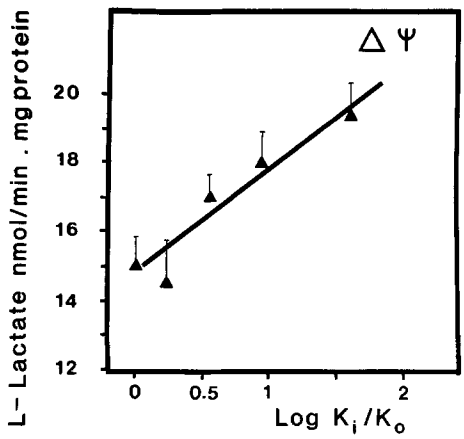


Fig. 3. Influence of membrane potential ($\Delta\psi$) in absence of Na^+ chemical potential ($\Delta\bar{\mu}_{Na^+}$). Membrane vesicles are preloaded in 100 mM NaCl, 100 mM KCl, 10 mM Hepes-Tris, pH 7.4. Various KCl concentrations in incubation medium were tested always in presence of 100 mM NaCl, 2 mM L-lactate. Choline chloride was used to compensate variation of KCl concentrations. Preloading and incubation mediums contained 10 µM valinomycin. Initial rates of uptake were taken after 5 sec incubation time. Means and SE from four experiments in triplicate are indicated by points and bars, respectively

uptake of lactate, however, was higher than that recorded in a KCl gradient (filled triangles in Fig. 2). This experiment shows that the transient Na chemical gradient provides the energy for the transient accumulation of L-lactate.

Two types of evidence suggest that the Na-L-lactate cotransport is a voltage-dependent mechanism. First when Cl^- was replaced by the more permeant anion SCN^-, the amplitude of the overshoot increased (filled circles in Fig. 2); this potentiation is probably the result of an increase in the rate of the L-lactate uptake under the influence of the negative electrical potential generated by the diffusion SCN^- into the intravesicular space [18]. The second evidence of the $\Delta\Psi$ effect is given in Fig. 3. The experiments illustrated in Fig. 3 show the membrane potential effect alone on the L-lactate uptake. As was found out with the D-glucose uptake [30], the L-lactate uptake was observed to be a direct function of the membrane potential. In absence of chemical gradient of Na^+ (100 mM NaCl equilibrated) and in presence of 10 µM valinomycin (optimal concentration tested) with various $[K^+]_{in}/[K^+]_{out}$ ratios, uptake of L-lactate was found to be linearly related to the log $[K^+]_{in}/[K^+]_{out}$.

Vesicles incubated in the presence of D- or L-lactate have different uptake time courses depending upon which isomer is present (Fig. 4). In the presence of 100 mM NaCl gradient, over the membrane, the lactate uptake shows the overshoot typical of a transport activated by sodium; D-lactate transport, however, was scarcely activated by 100 mM sodium. After 40 to 50 sec the uptake of the two isomers tends towards the same equilibrium value.

Initial Rate of L-Lactate Transport as a Function of External Na and Lactate Concentration

The aim of the following experiments is to analyze the variations in initial rates of L-lactate transport as a function of L-lactate concen-

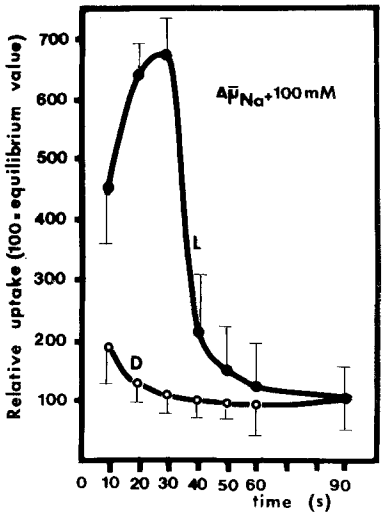


Fig. 4. Influence of a Na^+ electrochemical gradient ($\Delta\bar{\mu}_{Na^+}$) on the time course of L- and D-lactate uptake by brush border membrane vesicles. Incubation medium-containing 100 mM NaCl (100 mM mannitol, 10 mM Hepes-Tris, pH 7.4) and ($U^{14}C$)-D-lactate (2.5 mM) (o—o) or ($U^{14}C$)-L-lactate, (lithium salt 2.5 mM) (●—●), is added to membrane vesicles at differents times indicated in the Figure. All filtration conditions are the same as in Fig. 2. Means and SE from four experiments in triplicate are indicated by points and bars, respectively

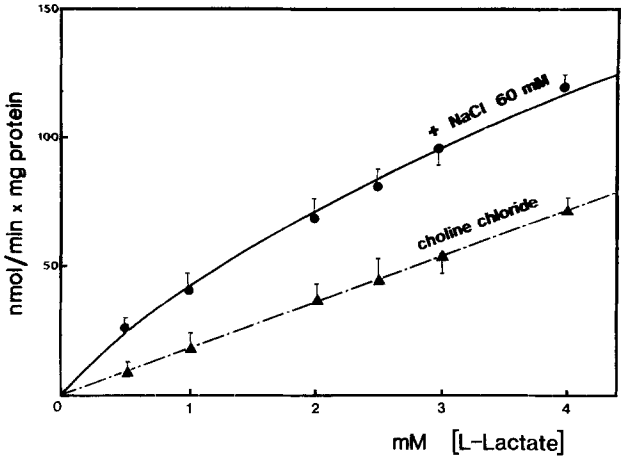


Fig. 5. Influence of L-lactate concentration in the incubation medium on lactate uptake by renal brush border vesicles, in absence of membrane potential (with 10 μM valinomycin). L-lactate used for this experimentation is L-lactic acid buffered by 1 mM Hepes-Tris, pH 7.4 (and not a lithium salt). The incubation medium contains 60 mM NaCl, 50 mM KCl, 60 mM choline chloride, 10 μM valinomycin, (●—●), or 50 mM KCl, 120 mM choline chloride, 10 μM valinomycin (▲—▲). Concentrations of L-lactate are indicated in the Figure. The uptake is initiated by addition of membranes suspended in 50 mM KCl, 100 mM choline chloride, 10 μM valinomycin. The initial rate of uptake is obtained after 5 sec of incubation. Each point represents 12–15 experiments with their SE

tration as well as of external Na concentration. In order to avoid any complicating contribution of electrical parameters on the transport activity, vesicles were treated with 10 μM valinomycin and pre-equilibrated with 50 mM KCl. If such vesicles are diluted in a medium containing 60 mM NaCl (final concentration) and varying concentrations of L-lactate, [^{14}C] L-lactate uptake rate curve as a function of external L-lactate has a strongly activated saturation profile (Fig. 5) (lactate absorption being linear for the first 10 sec for 100 mM Na or 100 mM choline, measurement of the initial lactate absorption rates were therefore made after 5 sec). In contrast, when diluted in choline chloride the uptake is a simple diffusion process. These experiments suggest that the total uptake of L-lactate measured in presence of Na is made of two components: one carrier-mediated component requiring presence of external Na ions and saturation as a function of L-lactate concentrations; a second component which appears to be a simple diffusion process independent of the presence of Na ions and probably not carrier mediated.

On this basis, the rate of L-lactate uptake by the transport system, in all the conditions described below will be corrected for the diffusion component. Figure 6 shows the influence of Na^+ and L-lactate on initial rates of lactate transport. Figure 6*a* gives a reciprocal plot of the relationship between the initial rates of uptake and lactate concentration of the extravesicular medium at 5 sodium concentrations. As can already be seen in Fig. 5, absorption obeys saturable kinetics. One can also see that sodium acts as an activator: more sodium in the extravesicular medium raises the initial rate and increases the apparent affinity of the transport system for lactate. Finally, it can be seen that activation is of a competitive type; in other words, at any sodium concentration the maximum rate is constant.

The plot of $1/v$ (uptake rate) versus $1/[Na^+]$ (sodium concentration obtained) was curved as for allosteric enzyme and approached a horizontal line which intersects the $1/v$ axis at various levels; nevertheless the reciprocal plot of v versus the square of Na concentration ($1/v$ versus $1/[Na]^2$) has linear shapes which also indicate a mixed type of activation. The sodium apparent affinity ($K_{m_{app}Na^+}$) for the transport sys-

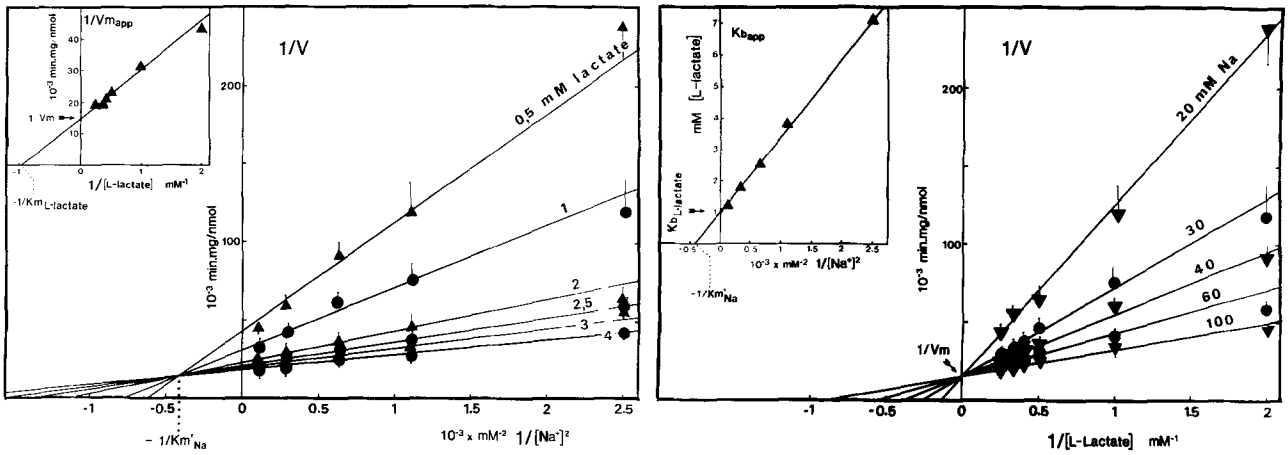
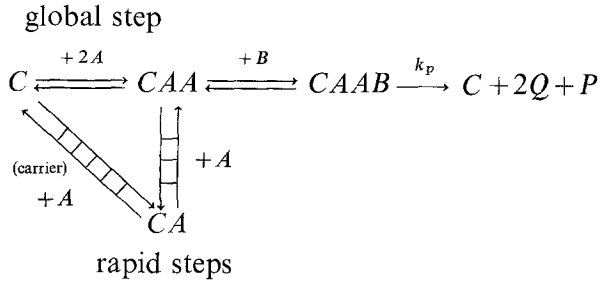


Fig. 6. Effects of Na^+ and L-lactate concentrations on L-lactate uptake. All values of initial velocities from Na^+ gradient conditions are corrected by subtracting from them the corresponding values in choline chloride gradient. These initial velocities are taken at 5 sec of incubation. (*a*) Reciprocal plots of initial velocities of L-lactate uptake as a function of lactate concentration for various sodium gradients, as indicated on the Figure. (*Inset*): linear plotting of kinetic parameters: $K_{b_{app}}$ (lactate) versus $1/(Na^+)^2\,mM^{-2}$. (*b*) Reciprocal plots of initial velocities of L-lactate uptake as a function of Na^+ gradient concentrations for various lactate concentrations, as indicated on the Figure. (*Inset*): replot from (*b*) or $1/V_{m_{app}}$ as a function of 1/(L-lactate) mM^{-1}. (All these experiments were conducted in 10 µM valinomycin and at the equilibrium 50 mM KCl). Each point represents 15 experiments with their SE

tem diminishes as the lactate concentration increases. The data seem to indicate that more than one Na^+ ion interacts with the L-lactate transport system. The influence of sodium on the apparent affinity constant of lactate indicates that sodium is an essential activator which directs the transporter towards the form which combines with lactate.

Since both sodium and lactate combine with the transport system, this could be brought about in a random or an ordered form.

Our experimental results can be interpreted by an activation mechanism correlated to a well-defined velocity equation. The only mechanism corresponding to an ordered system of two sequenced interactions is in accordance with our experimental results and velocity equation derived from rapid equilibrium assumptions [32, pp. 320–329]. The agreement was obtained when two Na^+ ions (A) interact in the schematic process:



The uptake velocity is proportional to the decomposition of the hypothetic quaternary complex:

$$v = k_p[CAAB]. \tag{1}$$

The conservation equation applied to the equilibrium is:

$$[C]_T = [C] + [CAA] + [CAAB]. \tag{2}$$

k_p: constant velocity of lactate transport, $[C]_T$: total carrier concentration, $[C]$: free carrier concentration, $[CAA]$, $[CAAB]$, are the sodium-activated carrier concentrations, the latter being combined with lactate. $[A]$, $[B]$ = extracellular concentrations, $[P]$ = absorbed lactate, $[Q]$ = absorbed sodium[1].

$$K'_a = \frac{[A]^2[C]}{[CAA]}; \tag{3}$$

$$K_b = \frac{[B][CAA]}{[CAAB]}. \tag{4}$$

The velocity is expressed:

$$\frac{v}{[C]_T} = \frac{k_p[CAAB]}{[C] + [CAA] + [CAAB]} \tag{5}$$

[1] In the case of (A) and (B) substrates there is an order consideration contrary to the (P) and (Q) products for the debinding.

or as function $[C]$ and dissociation constants:

$$\frac{v}{k_p[C]_T}=\frac{\dfrac{[B][A]^2[C]}{K_bK'_b}}{[C]+\dfrac{[A]^2[C]}{K'_a}+\dfrac{[B][A]^2[C]}{K_bK'_a}} \tag{6}$$

if $V_m=k_p[C]_T$.

The velocity equation becomes a function of $[B]$:

$$\frac{v}{V_m}=\frac{[B]}{\dfrac{K_bK'_a}{[A]^2}+K_b+[B]} \tag{7}$$

or

$$\frac{1}{v}=\frac{1}{[B]}\left[\frac{K_b}{V_m}\left(1+\frac{K'_a}{[A]^2}\right)\right]+\frac{1}{V_m}. \tag{8}$$

In function of $[A]^2$ the velocity equation corresponds to:

$$\frac{v}{V_m}=\frac{[A]^2}{\dfrac{K_bK'_a}{[B]}+[A]^2\left(\dfrac{K_b}{[B]}+1\right)} \tag{9}$$

or

$$\frac{1}{v}=\frac{1}{[A]^2}\left(\frac{K_bK'_a}{[B]V_m}\right)+\frac{1}{V_m}\left(1+\frac{K_b}{[B]}\right). \tag{10}$$

We can observe from experimental data that $[A]$ corresponds to sodium and $[B]$ to L-lactate concentrations. Both sodium ions interact in first, L-lactate being the second component $[B]$. The binary complex $[CA]$ cannot be used in this mechanism applied on our results. Thus K'_a and K_b correspond to the dissociation constants of two sodium ions and L-lactate, respectively. (These dissociation constants are taken to be equivalent to the affinity constant K_m.) $K'_{a_{app}}$ and $K_{b_{app}}$ are the apparent dissociation constants. Thus Eqs. (8) and (10) correspond to Figs. 6*a* and 6*b*, respectively. In this case from Fig. 6*a* we observe that the $K_{b_{app(lactate)}}$ varies with the Na^+ concentration; the linearization of $K_{b_{app}}$ versus $1/[Na^+]^2$ is shown in the inset and from Eq. (8) to:

$$K_{b_{app}}=K'_aK_b\frac{1}{[A]^2}+K_b \tag{11}$$

which confirms the K_b value or the K_m L-lactate of 1.05 mM. The intercept plot with the $1/[A]^2$ axis gives $1/K'_a$ or $K'_a=2304\,mM^2$, i.e. for one sodium ion $\sqrt{2304}\,mM^2$, $K_{a_{Na^+}}=48$ mM.

On Fig. 6*b* and from Eq. (10), the intercept point of the family of curves is common and corresponds to $1/K'_a$, $0.44\times10^{-3}\,mM^{-2}$ or $2272\,mM^2$, i.e. a value of 47 mM for the dissociation constant of one Na^+ ion. The different intercept plots on $1/v$ axis corresponding to $1/V_{m_{app}}$ ($V_{m_{app}}$ = apparent maximal velocity) can be expressed from Eq. (10):

$$\frac{1}{V_{m_{app}}}=\frac{1}{[B]}\cdot\frac{K_b}{V_m}+\frac{1}{V_m} \tag{12}$$

which confirms the value of $1/V_m$ 15×10^{-3} min mg $nmol^{-1}$, i.e. 66.6 $nmol\cdot min^{-1}\,mg^{-1}$ (inset of Fig. 6*b*).

Although the plotting in direct coordinates does not show some cooperative effect of the Na^+ ion on the lactate initial rate uptake, the cooperativity and number from the Hill plot were analyzed. The expression of v/V_m versus $[Na^+]$ for each lactate concentration provides that the $S\,0.9/S\,0.1$ (sodium ion concentrations which correspond, respectively, to 0.9 and 0.1 value of V_m apparent) is always 20, indicating a high cooperativity of the Na^+ ion with the lactate carrier system. The Hill plot of V/V_{m-v} versus Na^+ ion, in log scale, showed a Hill number of 2 providing the stoichiometry previously expressed (*not shown*). The lactate has revealed no cooperative effect on uptake itself.

Thus from an analysis of the experimental data one can obtain the respective affinities of lactate and sodium during lactate transport, using equations derived from rapid equilibrium transport system assumptions [32, pp. 320–329]; the interactions of sodium and lactate with the transport system are therefore coordinated, sodium being the first to react.

Discussion

The main purpose of this study was to determine the characteristics of lactate translocation across renal brush border membranes. The present experiments support the concept that transmembrane lactate reabsorption depends on a cotransport with sodium, and yield further insight on the functional mechanism of the lactate transporter.

Our results with the membrane vesicles suggest the following conclusions: (1) The energy necessary for the active (secondary) transport of L-lactate is furnished by the electrochemical gradient of extravesicular Na^+; sodium does

not activate D- and L-lactate transport to the same degree, i.e. the transport systems studied seem specific. (2) L-lactate absorption is brought about by an ordered mechanism with two substrates (sodium preceding lactate); $2\,Na^+$ must interact with the transport system indicating a stoichiometry of 2:1, Na^+ versus lactate.

Characteristics and Energy Relations of Lactate Transport

At equal concentrations D- and L-lactate are not equally sensitive to an electrochemical gradient of Na^+. Sodium gradient-dependent lactate transport would therefore appear to be more specific to the L isomer than to the D, but not as strongly as in the case for phenylalanine [27] and for D-glucose as compared with its L isomer [29]. Furthermore, as to the specificity of the lactate carrier, it is possible that the D and L isomers interact with the same carrier, the L isomer having the greater affinity owing to its molecular structure: this interpretation is supported by the fact that the D-lactate changes the overshoot of the time course of L-lactate absorption [4].

The L-lactate transport system described here shares numerous properties of different Na-dependent cotransport systems for sugar, amino acids and anions in other brush border membranes. In these systems the Na gradient is the driving force which provides energy for the accumulation by the two components of the electrochemical potential ($\Delta\mu$ and $\Delta\psi$) or of the chemical potential ($\Delta\mu_{Na}$) alone. From the data obtained by several authors and those presented in this paper, the electrogenic character varies in function of the membrane origin (enterocytes or kidney proximal tubule cells). It is interesting to compare first the electrogenic properties of L-lactate uptake with other systems, and second the influence of chemical potential on the L-lactate uptake kinetics. Indeed the time course of L-lactate absorption shows an overshoot dependent of the sodium gradient, as observed in brush border vesicles of enterocytes of rabbit [22], rat [11, 35], and rat renal cortex [4]. This uptake which can be activated even in vesicles preloaded in NaCl, indicated that the lactate is coupled with sodium in a cotransport process; since a KCl gradient has no effect, sodium is specific for this process as it has been reported for glucose [3] and amino acids [17, 28].

In the presence of a NaSCN gradient, replacing Cl^- by SCN^- has the effect of increasing the membrane potential ($\Delta\psi$), and an increase of maximum lactate absorption occurs at the level of the overshoot. When the intravesicular medium is made more negative, lactate is therefore absorbed more rapidly, as has already been shown to occur for glucose [5], proline [30] and phenylalanine [17] where the sodium cotransports are electrogenic contrary to the phosphate uptake demonstrated to be electroneutral [23].

Moreover, we can note that according to the nature of the membrane in question, the electrochemical Na^+ gradient is completely or partially used as the energy source for lactate transport: for enterocytes [22], Na-lactate cotransport is insensitive to any variation of membrane potential, whereas in the kidney, chemical and electrical potentials act on lactate absorption.

These properties concerning this membrane potential dependence [4] corroborate with the gradient hypothesis [12, 13] which postulates that NaCl ionic or H^+ gradient provide the driving force for the cotransports systems.

Ordered Cotransport of Na^+ and L-Lactate under Na Chemical Potential Conditions

The saturable L-lactate transport is a function of the chemical potential of sodium and is closely regulated by the electrochemical potential (Figs. 2 and 3). The chemical potential of the lactate itself does not give sufficient energy for facilitating and activating L-lactate transport.

Several general analyses of cotransport have been made [2, 10, 31, 34, 36]. According to Heintz's terminology [21], our system is of an "affinity" type: sodium modifies the affinity of lactate for its transport system. The analysis of the kinetics of the cotransport system in rapid equilibrium agrees with the experimental lactate influx results. This model where the sodium acts as an essential cofactor in transport mechanism, advances the presence of a binary complex (carrier-sodium) and a ternary one (carrier-sodium-lactate). Thus we can observe for the range of concentrations studied, that first the sodium interacts with the membrane system, then the lactate, the uptake of two Na ions being coupled to one lactate anion. Since the system is coordinated, the sites of lactate and sodium interaction are distinct but functionally interdependent. This sequential system is almost a kinetic fact but does not correspond necessarily to an existing entity, especially with regard to the ternary complex ($C-AA-B$) as

such; the sodium (two ions), while being carried, could have an activating effect on a functional lipoprotein component of the membrane lactate carrier, like an energization process.

The stoichiometry observed during the lactate transport, in presence of chemical potential is consistent with the electrogenic properties of the lactate uptake and explains that the global charge of Na-lactate is not zero.[2] The absence of a "sigmoidal" effect of the Na ion on the lactate uptake, but the high cooperativity and the apparent Hill number of 2 observed, can indicate the weak representation of the carrier complex (*CA*), with one Na ion. The apparent process seems to result from the single interaction of 2 Na ions; these data were observed from the correspondence between the velocity equations and experimental curves of the lactate transport. This conclusion was also suggested when the proton gradient, across the brush border membrane, was found without stimulation on the sodium-dependence of lactate [25].

The experimental data also show that the salt sodium lactate is not the true substrate of the transporter; in fact if this were the case the reciprocal plot, $1/V$ versus 1/lactate would be straight and parallel lines at low lactate concentrations and would form a series of plateaus at the level of the $1/V$ axis at high lactate concentrations [32, pp. 245–250]. Our results thus agree with the presence of distinct sites for lactate and sodium.

We can again observe that this L-lactate uptake seems to present some analogy with the phenylalanine uptake [17] of which the data were obtained without annulling the membrane potential and which is characterized by an electrogenic transfer and a coupling factor of two, 2 Na ions for one phenylalanine molecule. If we compare the lactate system to that of inorganic anions, a study of the kinetics of absorption and binding (using specific probes) let to the concept of a sequential exchange mechanism [7]. In fact these studies showed that, in addition to an anion-absorption site, a second site may exist: the "modifier site". A series of interactions would thus be necessary for activating the inorganic anion absorption mechanism.

Finally, a comment should be made on the nature of the information obtained using either our "rapid equilibrium" approach or that resulting from an "isotopic exchange" technique as developed by Hopfer [24]. It is clear that our approach enables us to be precise only about the first step of the transport reaction, i.e. the sequence of binding of the cotransported species (ordered process). Hopfer's approach, one the other hand, indicates whether the process is ordered and also shows that the first solute which interacts with the membrane carrier is the first one which is released. The "rapid equilibrium" technique appears therefore to bring complementary information on the mechanism of Na-solute cotransport.

Thus to exclude some limit of this proposed mechanism, namely, about the debinding step of the solute from the membrane carrier system, a second paper using lactate and sodium isotopic exchange will confirm more insight into Na-lactate transport as an isoordered bibi-type mechanism corresponding to an *A.S.* glide model [36] (a system where the binding and unbinding steps of rectants are symmetrical, i.e., the activator (*A*) interacts before the solute (*S*) for the binding on the carrier, and also this activator is released first in the intravesicular medium corresponding to a glide model).

Addendum

The time course of the (^{14}C) L-lactate (5 mM) and ^{22}Na (120 mM) uptake, measured under isotopic exchange conditions has shown a coupling factor of 1.67 (R. Mengual, *unpublished results*).

Prof. R. Motais, and Drs. J.L. Cousin and G. LeBlanc are thanked for their helpful advice and critical reading of the manuscript. We also thank Professor Dr. K.J. Ullrich and Dr. H. Murer for their interest and stimulating discussion on the present work. Also we thank Mrs. M. Lanteri and Mrs. G. Levanti for their excellent technical assistance, and R. Strazzanti for secretarial assistance.

References

1. Alvarado, F., Mahmood, A. 1974. Cotransport of organic solutes and sodium ions in the small intestine: A general model. Amino acid transport. *Biochemistry* **13**:2882–2890
2. Alvarado, F., Robinson, J.W.L. 1979. A kinetic study of the interactions between amino acids and monosaccharides at the intestinal brush-border membrane. *J. Physiol. (London)* **295**:457–475

[2] A same stoichiometry of Na-coupled lactate absorption (2 Na versus one lactate anion) is suggested by analysis of the cell potential response (depolarization) under lactate perfusion conditions (I. Samarzya, V. Molnar and E. Fromter, 1980.) Advances in Physiological Science Vol. 11, Kidney and Body Fluids. L. Tabács editor. pp. 419–423. 28th International Congress of Physiological Sciences (Budapest). Pergamon, Akademiai Kiadó, Budapest, 1981.

3. Aronson, P.S., Sacktor, B. 1975. The Na^+ gradient-dependent transport of D-glucose in renal brush border membranes. *J. Biol. Chem.* **250**:6032–6039
4. Barac-Nieto, M., Murer, H., Kinne, R. 1980. Lactate-sodium cotransport in rat renal brush border membranes. *Am. J. Physiol.* **239**:F496–F506
5. Beck, J.C., Sacktor, B. 1978. The sodium electrochemical potential-mediated uphill transport of D-glucose in renal brush border membrane vesicles. *J. Biol. Chem.* **253**:5531–5535
6. Berner, W., Kinne, R., Murer, H. 1976. Phosphate transport into brush-border membrane vesicles isolated from rat small intestine. *Biochem. J.* **160**:467–474
7. Cabantchick, Z.I., Knauf, P.A., Rothstein, A. 1978. The anion transport system of the red blood cell. The role of membrane protein evaluated by the use of probes. *Biochim. Biophys. Acta* **515**:239–302
8. Cohen, J.J., Barac-Nieto, M. 1973. Renal metabolism of substrates in relation to renal function. *In:* Handbook of Physiology. Section 8: Renal Physiology. J. Orloff and R.W. Berliner, editors. pp. 909–1001. American Physiological Society, Washington, D.C.
9. Cohen, J.J., Little, J.R. 1976. Lactate metabolism in the isolated perfused rat kidney; relations to renal function and gluconeogenesis. *J. Physiol. (London)* **255**:399–414
10. Cohen, S.R. 1980. The complete rate equation, including the explicit dependence on Na^+ ions, for the influx of α-aminoisobutyric acid into mouse brain slices. *J. Membrane Biol.* **52**:95–105
11. Corcelli, A., Storelli-Joss, C., Lippe, C., Storelli, C. 1979. Uptake di L-(+)lattato in membrane plasmatiche (luminali e contraluminali) isolate da enterociti di intestino tenue di ratto. *Boll. Soc. Ital. Biol. Sper.* Vol. **LV**:460–466
12. Crane, R.K. 1962. Hypothesis of mechanism of intestinal active transport of sugars. *Fed. Proc.* **21**:891–895
13. Deuticke, B., Rickert, I., Beyer, E. 1978. Stereoselective, SH-dependent transfer of lactate in mammalian erythrocytes. *Biochim. Biophys. Acta* **507**:137–155
14. Deves, R., Krupta, R.M. 1979. A simple experimental approach to the determination of carrier transport parameters for unlabeled substrate analogs. *Biochim. Biophys. Acta* **556**:524–532
15. Deves, R., Krupta, R.M. 1979. A general kinetic analysis of transport. Tests of the carrier model based on predicted relations among experimental parameters. *Biochim. Biophys. Acta* **556**:533–547
16. Dubinsky, W.P., Racker, E. 1978. The mechanism of lactate transport in human erythrocytes. *J. Membrane Biol.* **44**:25–36
17. Evers, J., Murer, H., Kinne, R. 1976. Phenylalanine uptake in isolated renal brush border vesicles. *Biochim. Biophys. Acta* **426**:598–615
18. Frömter, E., Rumrich, G., Ullrich, K.J. 1973. Phenomenologic description of Na^+, Cl^- and HCO_3 absorption from proximal tubules of the rat kidney. *Pfluegers Arch.* **343**:189–220
19. Halestrap, A.P. 1975. The mitochondrial pyruvate carrier. Kinetics and specificity for substrates and inhibitors. *Biochem. J.* **148**:85–96
20. Halestrap, A.P. 1976. Transport of pyruvate and lactate into human erythrocytes. *Biochem. J.* **156**:193–207
21. Heintz, E., Geck, P. 1978. Membrane Transport Processes. J.F. Hoffman, editor. Vol. 1, pp. 13–20. Raven Press, New York
22. Hildman, B., Storelli, C., Haase, W., Barac-Nieto, M., Murer, H. 1980. Sodium ion/L-lactate co-transport in rabbit small-intestinal brush-border-membrane vesicles. *Biochem. J.* **186**:169–176
23. Hoffman, N., Thees, M., Kinne, R. 1976. Phosphate transport by isolated renal brush border vesicles. *Pfluegers Arch.* **362**:147–156
24. Hopfer, U., Groseclose, R. 1980. The mechanism of Na^+-dependent D-glucose transport. *J. Biol. Chem.* **255**:4453–4462
25. Kinne, R., Barac, M., Murer, H. 1980. Sodium cotransport systems in the proximal tubule: Current developments. *In:* Current Topics in Membranes and Transport. Volume 13: Cellular mechanisms of renal tubular ion transport. F. Bronner and A. Kleinzeller, editors. pp. 303–313. Academic Press, New York
26. Kinne, R., Murer, H. 1978. Recent advances in the understanding of renal amino acid and sugar transport. *In:* Comptes rendus, VIIe congrès international de Néphrologie. S. Karger, editor. pp. 601–608. Les Presses de l'Université de Montréal
27. Murer, H., Hopfer, U., Kinne, R. 1976. Sodium/proton antiport in brush-border-membrane vesicles isolated from rat small intestine and kidney. *Biochem. J.* **154**:597–604
28. Murer, H., Sigrist-Nelson, K., Hopfer, U. 1975. On the mechanism of sugar and amino acid interaction in intestinal transport. *J. Biol. Chem.* **250**:7392–7396
29. Poirée, J.C., Vannier, C., Sudaka, P., Fehlmann, M. 1978. Glucose transport by horse kidney brush borders. I. Transport properties of brush border membrane closed vesicles. *Biochimie* **60**:645–651
30. Sacktor, B. 1980. Electrogenic and electroneutral Na^+ gradient-dependent transport systems in the renal brush border membrane vesicle. *In:* Current Topics in Membranes and Transport. Volume 13: Cellular mechanisms of renal tubular ion transport. F. Bronner and A. Kleinzeller, editors. pp. 291–300. Academic Press, New York
31. Schultz, S.G., Curran, P.F. 1970. Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**:637–718
32. Segel, I.H. 1975. Enzyme Kinetics. J. Wiley and Sons, New York
33. Spencer, T.L., Lehninger, A.L. 1976. L-lactate transport in Ehrlich ascites-tumor cells. *Biochem. J.* **154**:405–414
34. Stein, W.D. 1967. The Movement of Molecules Across Cell Membranes. pp. 192–206. Academic Press, New York, San Francisco, London
35. Storelli, C., Corcelli, A., Cassan, G., Hildman, R., Murer, H., Lippe, C. 1980. Polar distribution of sodium-dependent and sodium-independent transport system for L-lactate in the plasma membrane of rat enterocytes. *Pfluegers Arch.* **338**:11–16
36. Turner, R.J. 1981. Kinetic analysis of a family of cotransport models. *Biochim. Biophys. Acta* **649**:269–280
37. Ullrich, K.J. 1979. Sugar amino acid, and Na^+ cotransport in the proximal tubule. *Annu. Rev. Physiol.* **41**:181–195

Received 15 March 1982; revised 9 July 1982