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SOME CHARACTERISTICS OF MONOCARBOXYLIC ACID TRANSFER ACROSS THE CELL MEMBRANE OF EPITHELIAL CELLS FROM RAT SMALL INTESTINE

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SUMMARY

1. The translocation of monovalent organic anions (pyruvate, propionate, acetate and butyrate) across the cell membrane of isolated epithelial cells from rat small intestine was studied by measuring competitive inhibition kinetics, exchange diffusion and temperature dependence of the efflux rate. A possible function of a monocarboxylate carrier in intestine will be discussed.

2. Earlier studies on the inhibition of pyruvate transport by fatty acids were extended to propionate and found to show the same characteristics. The kinetics, however, appeared to be more complex by the contribution of several diffusion pathways for propionate.

3. The mechanism of countertransport was most compatible with an "accelerated exchange diffusion" and could be studied at both sides of the membrane. This exchange diffusion exhibited saturation kinetics. It is proposed that different monocarboxylate anions may have different affinities for a common carrier.

4. Temperature dependence of the efflux of pyruvate and propionate was studied. Arrhenius plots obtained were not found to be linear between 0 and 5 °C. Between 5 and 15 °C activation energies for pyruvate and propionate efflux rates were found to be 19.6 and 12.6 kcal/mol, respectively.

INTRODUCTION

In a previous report [1] evidence was presented showing the existence of a carrier-mediated diffusion of pyruvate anions across the cell membrane of isolated epithelial cells. Experiments on the properties of the transport process, demonstrating inhibition by fatty acids, countertransport and influences of pH alterations, were presented in that study. The competitive inhibition by short-chain fatty acids found indicated that this translocation system might have a function in transepithelial transport of short-chain fatty acids in rat small intestine. A specific transfer mechanism for the transport of volatile fatty acids in rat small intestine was first proposed by Smyth and Taylor [2]. This mechanism was dependent on metabolic activity.

Later, several investigators [3–8] presented similar processes. Generally, these short-chain fatty acids are quantitatively not important as metabolic fuel for the rat. In ruminants, however, they are a major product of cellulose fermentation in the rumen [9, 10]. In these species an apparent correlation between fatty acid uptake and the appearance of bicarbonate in the lumen occurs [10]. It may be related to our finding of exchange of pyruvate and bicarbonate in epithelial cells [1]. Jackson et al. [6] proposed an additional mechanism for mucosal to serosal flux of weak electrolytes as a three-compartment model, including an intermediate compartment of high pH (intercellular space). Inhibition of transport of a weak acid by another weak acid was explained by alteration of the pH by non-ionic diffusion of the inhibitor. The model suggested that the two barriers separating the three compartments had different permeabilities to the ionic form of the acid. Using isolated epithelial cell preparations [1], we concluded from studies on pyruvate transfer that the anionic form was the only species transported. Therefore, we extended our investigations with the study of the kinetics of the anionic transport of monocarboxylate acids to get more insight in the contribution of this process to the overall transfer.

Recently it was discovered that pyruvate and lactate transport across the erythrocyte membrane was specifically inhibited by α-cyanohydroxycinnamate [11]. No effect of this inhibitor was seen on acetate and butyrate influx [12], suggesting that pyruvate and short-chain fatty acids do not share a common transport system in this cell type. This then would be at variance with our findings in rat small intestinal epithelium. Deuticke [13, 14] studied the transport of organic anions, such as pyruvate, acetate and propionate, in bovine red cells. A number of environmental parameters that influence anion permeability in the red cell were varied, including the concentration, the temperature and the pH. Clear-cut differences were found [14] between acetate and pyruvate permeation. Therefore, a second purpose of the present study was to compare more thoroughly the transfer characteristics of short-chain fatty acids with those of pyruvate with respect to the result obtained earlier [1]. The kinetics of organic anion transfer are based solely on measurements at 0 °C, because of the extremely rapid rate of transmembrane flux in small intestinal epithelial cells, which is also reported for the red blood cell [15]. Moreover, at 0 °C the possible interference of metabolism of the anions studied will be minimized.

MATERIALS AND METHODS

Reagents. All chemicals were of analytical purity. Sodium [1-^{14}C]pyruvate (13.1 Ci/mol), [1-^{14}C]propionic acid (57.5 Ci/mol), [^{3}H]inulin (860 Ci/mol), poly[^{14}C]ethyleneglycol (average molecular weight 6000; 75 Ci/mol), $^{3}H_2O$ (90.9 Ci/mol) were supplied by the Radiochemical Centre (Amersham, England). Because of instability of aqueous solutions of pyruvate, in every experiment a part of solid [^{14}C]pyruvate was dissolved in a freshly prepared non-radioactive sodium pyruvate solution. Ricinoleic acid was purchased from Sigma (St. Louis, U.S.A.).

Measurement of uptake of monocarboxylic acids. The procedures of isolation, of epithelial cells from rat jejunum by the high frequency vibration technique of Harrison and Webster [16, 17] as well as the centrifugal filtration of the cells through silicon oil were described in detail previously [1]. The minor modifications of this procedure employed will be indicated in the legends to the figures and tables.

Measurement of efflux. Epithelial cells (4–8 mg protein/ml) were first preincubated at 0 °C with 1 mM labelled substrate. After 20 min, 50 μl of this preincubated cell suspension was added to 700 μl Krebs-phosphate buffer (pH 7.4) (in some experiments 300 μl, as indicated), which was layered on silicon oil. The buffer sometimes contained countersubstrate. After the time indicated, the cells were rapidly centrifuged (10 000 × *g*) through the oil layer in an Eppendorf 3200 centrifuge. The substrate content at zero time was determined by the filtration of 200 μl undiluted cell suspension, to which [^{3}H]inulin had been added. In the studies on temperature dependence of efflux rates, [1-^{14}C]pyruvate or [1-^{14}C]propionate efflux was followed for a short time (30 s). At this stage of the investigations it was found that the initial ^{14}C-labelled substrate content at 0 °C was sometimes lower than the values after 30 s efflux at 0 °C. It was supposed that the ^{14}C-labelled substrate content estimated in efflux studies was more reliable since the adherent ^{14}C-labelled substrate transported with the cells through the silicon oil amounted to only 5–10% from the labelled cellular content. In order to explore further this curious finding, another marker for the extracellular space, ^{14}C-labelled polyethyleneglycol, was tested. A comparison of the values in these experiments showed that ^3H_2O, [^{3}H]inulin and poly[^{14}C]ethyleneglycol yielded 11.35±0.58, 6.83±0.37 and 5.85±0.45 μl/mg protein ($n = 5$), respectively. This overestimation of the volume of adherent fluid when [^{3}H]inulin was used as a marker became considerably important when the efflux was small. Sallee et al. [18] also noted a slightly larger adherent mucosal fluid in everted sacs of intestine, when measured with [^{3}H]inulin. It was ascribed to the rapid exchange of tritium with tissue protons. Calculations of results on temperature dependence were corrected by using the poly[^{14}C]ethyleneglycol space instead of [^{3}H]inulin in parallel incubations. The adherent spaces were found to vary between different cell suspensions, which makes corrections in earlier experiments impossible.

RESULTS AND DISCUSSION

Inhibition of propionate transport by monocarboxylic acids

Table I shows the inhibition of propionate (1 mM) uptake at 30 °C by pyruvate octanoate or ricinoleate in epithelial cells from rat jejunum. It can be seen that pyruvate (10 mM) inhibition at 0 and 30 °C was largely abolished at higher propionate concentrations (4–5 mM), suggesting a competitive nature of the inhibition. On the other hand, the increase of the propionate concentration was without effect on the relative inhibition by octanoate or ricinoleate. These data agree with our earlier studies on pyruvate transport [1] and probably reflect the presence of a common translocation system for pyruvate and propionate. The differences between pyruvate and propionate transport across the epithelial cell membrane obtained in earlier efflux studies [1] were also confirmed with respect to the kinetics of the uptake at different concentrations of propionate (Table I). An important contribution of non-ionic diffusion to the overall transfer of propionate made it an ill-suited model for a kinetic study of anionic translocation, the more so as only an approximation of the initial rate could be determined. Lineweaver-Burk plots (not shown) of the values given in Table I showed non-linear relationships. The rate of propionate transport was obviously not proportional with the concentration used. The data of Table I,

TABLE I

EFFECT OF MONOCARBOXYLIC ACIDS ON THE UPTAKE OF PROPIONATE IN RAT SMALL INTESTINAL EPITHELIUM

The incubations were carried out at 0 and 30 °C in Krebs-phosphate buffer (pH 7.4) containing different concentrations of [1-^{14}C]propionate, 0.4 mM [^{3}H]inulin and 0.1 % albumin. After 5 min the cells were rapidly centrifuged (10000 × *g*, 2 min) through a silicon oil layer in the $HClO_4$ phase. Further treatment of the samples is described under Materials and Methods. Each value represents the mean of four separate experiments (±S.E.).

Propionate concentration (mM)	Temperature (°C)	Control	+pyruvate (10 mM)	+octanoate (10 mM)	+ricinoleate (0.5 mM)
		(nmol/mg protein per 5 min)			
0.5	30	2.91±0.21	2.32±0.13	1.69±0.18	1.96±0.17
1.0	30	5.33±0.50	4.15±0.04	2.79±0.35	3.04±0.16
2.0	30	9.62±0.69	9.63±0.14	5.34±0.67	6.95±0.92
5.0	30	21.9 ±1.6	19.9 ±1.3	12.5 ±1.2	12.2
10.0	30	37.5 ±3.0	35.6 ±1.6	22.6 ±0.9	26.5 ±0.8
0.2	0	1.06±0.13	0.63±0.08	—	—
0.4	0	1.72±0.26	1.06±0.22	—	—
1.0	0	4.18±0.35	2.73±0.50	—	—
2.0	0	7.16±1.11	5.80±0.82	—	—
4.0	0	11.9 ±1.7	9.87±0.48	—	—

suggesting affinity of both anions for a common (hypothetical) carrier were used to approximate the (apparent) affinity values of both anions for this carrier.

Mutual competitive inhibition between pyruvate and propionate

The K_i of propionate inhibition on pyruvate uptake was calculated from double reciprocal plots of the rate of pyruvate transport versus the pyruvate concentration used [1]. The K_i (21 mM) differed markedly from the apparent K_m (2 mM) of pyruvate. These affinities might be overestimated by the interference of other anionic diffusion pathways for pyruvate. For that reason we attempted to measure the affinity or half saturation constant K_m by varying the concentration of the inhibitor at a fixed pyruvate concentration. If a carrier mediated transport is involved in monocarboxylate anion transport, this process might be described by the equation:

$$\frac{v_i}{v - v_i} = \frac{K_i}{[I]}\left(\frac{[S]}{K_m} + 1\right)$$

which is derived from Michaelis-Menten equations:

$$v = \frac{V}{1 + \frac{K_m}{[S]}} \quad \text{and} \quad v_i = \frac{V}{1 + \frac{K_m}{[S]}\left(1 + \frac{[I]}{K_i}\right)}$$

in which v is the rate with substrate alone and v_i the rate in the presence of an inhibitor of concentration $[I]$. K_i and K_m are the proposed affinity constants of inhibitor and substrate, respectively. Experiments on the effects of different concentra-

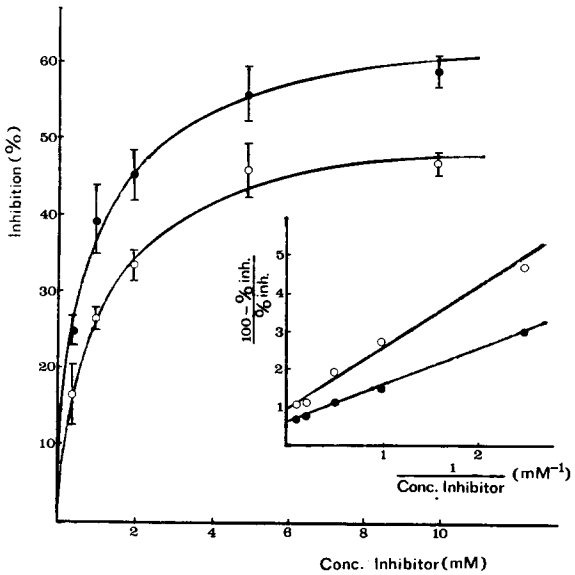

Fig. 1. Mutual competitive inhibition between pyruvate and propionate. The incubations were carried out at 0 °C (5 min) in Krebs-phosphate buffer (pH 7.4) containing 0.4 mM [^{3}H]inulin and 0.1 % albumin. ●—●, 0.4 mM [1-^{14}C]pyruvate in the presence of different concentrations propionate; ○—○, 0.4 mM [1-^{14}C]propionate in the presence of different concentrations pyruvate. The theoretical description of the 100−%inhibition/%inhibition versus 1/[I] plots was given in the text. Each point represents the mean of four experiments.

tions of propionate on the influx of pyruvate (0.4 mM) and the reversed process are summarized in Fig. 1. The inset clearly shows a straight line in a plot of the ratio uninhibited influx to inhibited influx ($v_i/(v-v_i)$ or 100−% inhibition/% inhibition) versus the inverse inhibitor concentration (1/[I]). Affinity constants of 1.7 and 1.2 mM could be calculated from the slopes of the two plots for pyruvate and propionate, respectively. Likewise it was concluded from Fig. 1 that the maximal inhibition differed from 100%, indicating that the monocarboxylate entry process was composed of several contributions. Consequently an attempt was made to find a method that could distinguish between the carrier-mediated anionic diffusion and other processes. Therefore the exchange diffusion between pyruvate and short-chain fatty acids was used to confirm results obtained from the influx studies.

Exchange diffusion of monocarboxylate anions

Earlier studies [1] on the efflux of propionate and pyruvate showed that the movement of the substrate across the membrane could be accelerated by the presence of the substrate or its homologue on the opposite side of the membrane. The non-competitive inhibitor of influx, octanoate, had no effect on this exchange diffusion, indicating an asymmetrical behaviour of the monocarboxylate carrier. On the other hand, if binding of anions to the membrane constituents occurred, efflux stimulation could be explained by inhibition of the re-entry of the effluxed anion due to the blockade of binding sites by "counter" anions. In view of this possibility, we performed experiments measuring the effect of preloading cells for 5 min with unlabelled

TABLE II

EFFECT OF PRELOADING OF CELLS WITH UNLABELLED SUBSTRATE ON THE INFLUX RATE OF [1-^{14}C]PYRUVATE OR [1-^{14}C]PROPIONATE AT 0 °C

Cells preloaded at 0 °C for 5 min with 1 or 2 mM unlabelled substrate (50 μl) were added to 700 μl Krebs-phosphate buffer (pH 7.4) containing 1 mM labelled substrate, 0.4 mM [^{3}H]inulin and 0.1 % albumin. After 5 min the cells were rapidly sedimented through silicon oil, as described under Materials and Methods. The results are means of four experiments (±S.E.). The significance of each comparison with the control was determined using Student's *t*-test for paired data.

Preincubation	Influx rate of pyruvate (nmol/mg protein per 5 min)	Influx rate of propionate (nmol/mg protein per 5 min)
None	3.18±0.27	4.64±0.19
1 mM propionate	3.82±0.26 ($P < 0.05$)	—
2 mM propionate	4.51±0.50 ($P < 0.05$)	—
1 mM pyruvate	—	5.25±0.13 ($P < 0.05$)
2 mM pyruvate	—	5.63±0.17 ($P < 0.01$)

substrate on the influx of another labelled substrate. The results are shown in Table II. It can be seen that cells preloaded with unlabelled propionate (2 mM) show an increase of the rate of influx of [1-^{14}C]pyruvate of 42 %. The degree of stimulation was dependent on the concentration of propionate used. These findings are compatible with a reversible membrane transport model in which the organic anions penetrate the membrane in the form of a complex with some unknown carrier.

Countertransport is a term that has been used for two different processes at the cellular level, which have been designated by Stein [19] as "competitive exchange

TABLE III

THE INFLUENCE OF THE EXTERNAL VOLUME ON THE STIMULATION OF EFFLUX BY A COUNTERANION

Epithelial cells, preincubated for 15 min at 0 °C with 1 mM [1-^{14}C]propionate (50 μl), were added to 0.7 or 0.3 ml buffer, layered on silicon oil. The buffer contained countersubstrate and [^{3}H]inulin. After 30 s the cells were rapidly centrifuged through silicon oil as described under Materials and Methods. The results are the mean of four experiments (±S.E.).

Volume efflux medium (ml)	Counteranion	Propionate intracellularly after 30 s incubation (nmol/mg protein)	Efflux stimulation by counteranion (nmol/mg protein)
0.70	—	3.70±0.16*	—
0.70	2 mM pyruvate	2.82±0.13	0.88±0.06**
0.30	—	4.15±0.21*	—
0.30	2 mM pyruvate	3.00±0.19	1.15±0.14**

Between * marked values significance of difference was $P < 0.1$.
Between ** marked values significance of difference was $P < 0.01$.

diffusion" and "accelerative exchange diffusion". In a recent paper, Robinson [20] described a method for distinguishing between these two models for transport of amino acids in rat small intestine. The volume of the external medium was changed and it was investigated whether the stimulation of efflux was affected. A change of the external medium in the present efflux experiments (Table III) from 0.7 to 0.3 ml might be expected to approximately double the extracellular concentration of [1-^{14}C]-propionate so that the re-entry may be stimulated and the shielding of the external binding sites for the labelled propionate by the unlabelled pyruvate might become less efficient. In the "accelerative model" the stimulation of efflux should be unaffected. It can be seen from Table III that the intracellular concentration of [1-^{14}C]-propionate was higher after 30 s when 0.3 ml external medium was used, indicating increased backflux of labelled substrate. The stimulation of efflux, however, was significantly increased under this condition. This disagrees with the concept that the presence of external unlabelled pyruvate decreases the backflux of [1-^{14}C]propionate. Analogous results were obtained with the stimulated efflux of [1-^{14}C]pyruvate by external propionate (results not shown). Stein [19] clearly formulated the basis for this phenomenon, that some component of the membrane moves through the membrane at a faster rate when combined with substrate than when free: "accelerative exchange diffusion". The possibility remains, however, that the pyruvate anion could induce the exchange for propionate of equivalent electrical charge. Therefore, the specificity of the stimulation of efflux was studied by examining the ability of four substrates, pyruvate, acetate, propionate and butyrate, to accelerate exchange. In this substrate sequence the contribution of ionic diffusion to overall transport would be expected to decrease because of the increase in lipophilicity of the undissociated acid [1]. Previous results [1] clearly showed that pyruvate in contrast to its rapid rate of influx moves slowly out of the cells. The initial phase (1 min) of pyruvate flux could be stimulated by counteranions (Fig. 6 of ref. 1). For convenience of measurement, an approximation of concentration-dependent efflux-stimulation by different

TABLE IV

DEPENDENCE OF THE INTRACELLULAR PYRUVATE CONCENTRATION ON THE PRESENCE OF COUNTERSUBSTRATES

Epithelial cells preincubated for 15 min at 0 °C with 1 mM [1-^{14}C]pyruvate (50 μl) were added to 0.7 ml buffer present on top of silicon oil. The buffer contained countersubstrates at the concentration indicated as well as [^{3}H]inulin. After 5 min incubation the cells were rapidly centrifuged through silicon oil as described under Materials and Methods. The results are the mean of four experiments (±S.E.).

Counteranion concentration (mM)	Pyruvate intracellularly after 5 min incubation (nmol/mg protein)			
	Pyruvate*	Acetate*	Propionate*	Butyrate*
0	4.04±0.13	4.06±0.14	3.76±0.27	4.28±0.30
0.4	3.34±0.25	3.85±0.18	3.43±0.27	3.65±0.32
0.8	2.90±0.12	3.68±0.20	3.26±0.24	3.55±0.30
2.0	2.59±0.15	3.46±0.20	2.98±0.25	3.26±0.27
4.0	2.11±0.06	3.19±0.15	2.78±0.29	3.22±0.30

* i.e. this anion was added to the external medium at the indicated concentration.

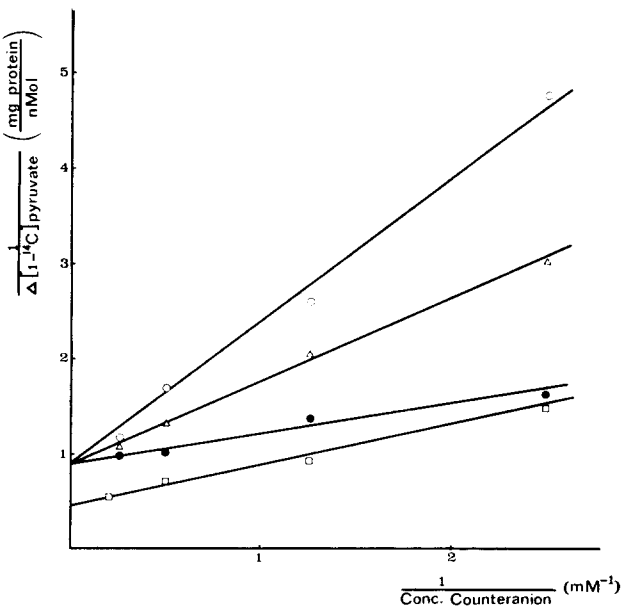

Fig. 2. Lineweaver-Burk plots of the stimulation of efflux as a function of the concentration of counteranion. The values represent the difference in efflux in the presence and absence of counteranion calculated from the data in Table IV. Different counteranions used were: ●—●, butyrate; △—△, propionate; ○—○, acetate; □—□, pyruvate.

counteranions could still be obtained after 5 min incubation, since only the initial phase showed large differences. It can be seen from Table IV that indeed butyrate is more efficient than propionate or acetate in removing pyruvate from the cells. The reciprocal efflux stimulation is plotted versus the reciprocal of counteranion concentration in Fig. 2. These plots reveal that the stimulation evoked by acetate, propionate or butyrate gave the same V. However, the affinity constants (1.8, 1.0 and 0.4 mM, respectively) were considerably different. The affinity constant for propionate obtained here correlated with the value calculated from the results of Fig. 1 (1.2 mM) rather well. It can be seen (Fig. 2) that the V with pyruvate as counteranion is clearly higher than the V obtained with short-chain fatty acid anions. As a possible explanation for this discrepancy it is suggested that pyruvate induces not only movement of [1-^{14}C]pyruvate over the cellular membrane, but also the exchange over the mitochondrial membrane. In a recent report [21], evidence was presented showing the existence in rat liver mitochondria of a translocator, which mediates an exchange between mitochondrial [^{14}C]pyruvate and external pyruvate, but no exchange could be demonstrated with external acetate. Indeed, another set of experiments (results not shown) revealed that stimulation of [1-^{14}C]propionate efflux by pyruvate resulted in a V close to the one found with propionate as the counteranion.

Temperature dependence of monocarboxylic acid efflux

Temperature dependence of anion transfer has puzzled many investigators. Reported Q_{10} values range from 1.2 to 1.5 [22], as would be expected in the case of

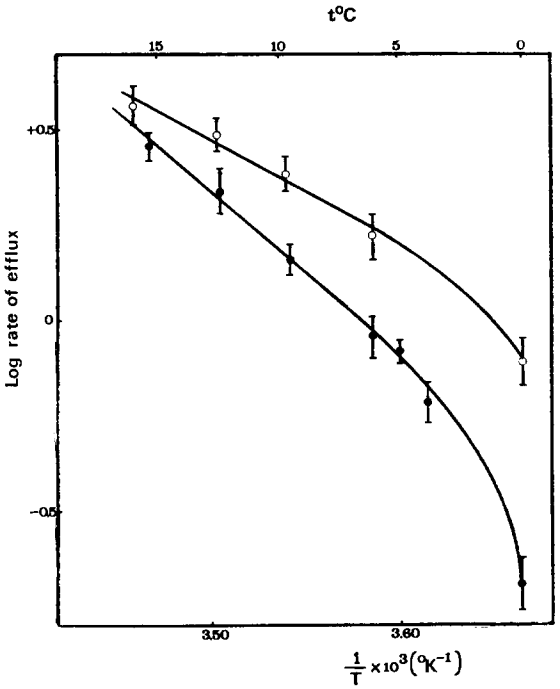

Fig. 3. Temperature dependence of efflux of propionate and pyruvate from isolated epithelial cells. Cells, preincubated for 20 min at 0 °C with 1 mM labelled substrate (50 μl) were added to 0.7 ml Krebs-phosphate buffer layered on silicon oil. After 30 s the cells were rapidly centrifuged through silicon oil. The initial ^{14}C-labelled substrate content was determined as described under Materials and Methods. The rate of efflux was calculated as nmol/mg protein. ●—●, [1-^{14}C]pyruvate efflux; ○—○, [1-^{14}C]propionate efflux. Each value represents the mean of four measurements (±S.E.).

a diffusion process. In contrast, Q_{10} values for phosphate, sulphate, chloride, pyruvate, lactate and bicarbonate [13, 15, 23, 24] were found to be 5–8, corresponding to an activation energy of 30–40 kcal/mol). There appeared to be no simple correlation between the absolute rates of anion permeation and the magnitude of the activation energy. Fig. 3 demonstrates that in the Arrhenius diagrams of initial rates (30 s) of pyruvate and propionate efflux a deviation from linearity in the temperature interval 0–5 °C occurs. Firstly, this may confirm some peculiar high temperature dependence in the range 0–5 °C, in accordance with the values found between 0 and 10 °C for other anion transfer systems [23, 24], but secondly such a change in activation energy may also indicate a transition in the membrane structure at the deflection point of the diagram. The lower activation energy of propionate efflux (12.6 kcal/mol) found between 5 and 15 °C, when compared with pyruvate (19.6 kcal/mol) may have its origin in a partial contribution of non-ionic diffusion to propionate permeation (compare ref. 1). Unpublished observations showed that the temperature dependence of propionate-[1-^{14}C]propionate exchange between 6 and 15 °C yielded the same activation energy as propionate efflux. Measurements at temperatures higher than 15 °C were of limited significance by the inability to estimate linear rates of transport.

CONCLUDING REMARKS

The present experiments strongly point to the involvement of a carrier in the movement of monocarboxylate anions across the cell membrane of the intestinal epithelial cell. Transfer shows competitive phenomena (compare also ref. 1), saturable exchange diffusion and a high temperature coefficient. Most experiments were carried out at 0 °C indicative of facilitated translocation, independent of metabolic activity. Furthermore, evidence from literature [11, 12, 25–28] makes it very likely that monocarboxylate transporters are to be found in the plasma membrane of the cell. Halestrap and Denton [11, 29] now obtained conclusive evidence that a carrier for pyruvate is present in erythrocytes that is sensitive to inhibition by α-cyano-4-hydroxycinnamate, an analogue of the enol form of pyruvate. The failure to demonstrate any inhibition of this compound on acetate transport [11, 29] is probably related to the observation of Deuticke [14] that acetate mainly penetrates by non-ionic diffusion in erythrocytes. They also failed to show inhibition of pyruvate or lactate transport across the heart cell membrane by α-cyanocinnamate analogues. The latter finding indicates that the proposed pyruvate carrier [25, 26] in this organ may have no affinity for these inhibitors. Oldendorf [27] demonstrated a saturable carrier transport system for the uptake of pyruvate, propionate and acetate in brain in vivo. In competition experiments it was found that the carrier was half saturated at approx. 1 mM pyruvate and 2.5 mM acetate, affinity constants approaching the values found in the present study on isolated epithelial cells from rat small intestine at 0 °C (Fig. 2). However, comparison of the values may not be permitted because of the large temperature differences involved. Because of both rapid metabolism and uptake of monocarboxylic acids, we were unable to measure these parameters at higher temperatures. Monocarboxylic acid transport across the erythrocyte membrane was studied extensively with respect to the ionic contribution to the transfer [13, 14]. Temperature, ion and pH dependencies suggested a specific anion transport system in this cell type. Also experimental evidence was provided for the contribution of non-ionic diffusion to the transfer, obscuring the kinetics of ionic transfer. The present paper demonstrates an easy means to discriminate between a specific anion and the non-ionic transport by studying the exchange diffusion. Apparent Michaelis constants determined in the exchange diffusion (Fig. 2) approached the values found in the net influx studies (Fig. 1).

In the earlier report [1] we speculated about the involvement of the monocarboxylate carrier in short-chain fatty acid transport and a possible localization in brush border membrane. Indeed, several investigations [2–4, 7] were indicative of a specific transport system for short-chain fatty acids in rat small intestine. There are, however, contradictory results about the mutual competition between these fatty acids and the existence of saturation kinetics [7, 8, 30]. Secondly, Jackson et al. [6] demonstrated that the transport of weak acids can be described in terms of a model system of three serial compartments, in which the pH of the intermediate compartment is higher than that of the bulk phases. On this basis he was able to explain the mutual inhibitory interactions between weak acids and the net mucosal to serosal fluxes found in experiments with stripped mucosa. Recently, it was clearly shown by the same group [31] that the development of a compartment of high pH within the intestinal wall may be associated with the serosally directed movement of metabolic

anions [32, 33] through lateral intercellular spaces of epithelium. In order to account for the observed mucosal to serosal flux of weak acids, it was necessary to suggest that the brush border membrane, in contrast to the serosal barrier, was impermeable to the anionic form of the acids. The anionic exchange diffusion described in the present paper may be a property of the basolateral membrane of the epithelial cell, accelerating the anionic transfer at this site with exchangeable anions at the serosal side (for example HCO_3^-). Localization of a monocarboxylate carrier at the blood side of the epithelial cell is more compatible with the carriers proposed in heart, brain or erythrocyte cell membranes [25–29, 11, 12].

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