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INHIBITION OF PYRUVATE TRANSPORT BY FATTY ACIDS IN ISOLATED CELLS FROM RAT SMALL INTESTINE

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SUMMARY

1. A technique is described for the rapid separation of intestinal epithelial cells from the incubation medium by passage through a silicon-oil layer and collection in acid, in which their soluble constituents are released.

2. The inhibition by fatty acids of pyruvate oxidation is further studied. Measurement of pyruvate transport in epithelial cells at 0 °C showed that short- and medium-chain fatty acids as well as ricinoleate inhibit this transport. Propionate inhibits pyruvate transport by another mechanism than octanoate.

3. Differences between pyruvate, propionate and octanoate transport across the epithelial cell membrane were obtained in efflux studies. These studies revealed that acetate, propionate, butyrate and high concentrations of bicarbonate readily stimulate the efflux of pyruvate, probably by anionic counter-transport. No effects were seen with octanoate and hexanoate. The data obtained in these efflux studies suggest that lipophilicity and the pK_a values of the monocarboxylic acids determine the contribution of non-ionic diffusion to overall transport.

4. Saturation kinetics, competitive inhibition by short-chain fatty acids and counter-transport suggest a carrier-mediated transport of pyruvate.

INTRODUCTION

Earlier experiments with rat heart [1, 2], diaphragm [1], liver [3] or isolated kidney tubules [4] showed that during fatty acid oxidation, oxidative decarboxylation of pyruvate is inhibited. Determination of the state of the pyruvate dehydrogenase complex [5, 6] during fatty acid oxidation has established that enzyme phosphorylation plays an important role in the decrease of pyruvate oxidation in these tissues. Studies in rat intestine [7, 8] on the inhibition of fatty acids of pyruvate oxidation suggested that a small part of this decrease can be ascribed to phosphorylation of the enzyme complex too. The decrease in pyruvate dehydrogenase activity measured under the same conditions, however, was much lower than the depression of $^{14}CO_2$ production from [1-^{14}C]pyruvate. To a minor extent this discrepancy was also found by Wieland et al. [9] in studies with perfused rat heart. Inhibition of the pyruvate

dehydrogenase complex by increased levels of acetyl-CoA and NADH have been mentioned as additional metabolic mediators of fatty acid action [1, 8, 9]. Randle et al. [10] concluded from the effects of fatty acids on pyruvate oxidation in rat heart that there may be an effect of fatty acids on the permeability of cellular membranes for pyruvate. Moreover, we concluded from earlier results that fatty acids not only inhibit the oxidative decarboxylation of pyruvate in rat small intestine, but also its conversion to lactate, possibly by inhibition of pyruvate transport.

It has generally been assumed [11–13] that pyruvate freely permeates the cell membrane by passive diffusion so that changes of the intracellular level will be reflected by similar changes in the blood. There are only a few reports [12, 14, 15] of studies on the mechanism of pyruvate translocation across cellular membranes. Some evidence has been obtained for the involvement of a carrier system in the transport of pyruvate in heart [16] and more recently in erythrocytes [17]. In the latter case this is clearly demonstrated by the specific inhibition of the transport by α-cyano-4-hydroxycinnamate.

The access of pyruvate to its dehydrogenase in mitochondria is also of great interest in view of the central role of pyruvate oxidation in carbohydrate metabolism. Evidence has now become available [17–21] for the occurrence of a transport carrier for pyruvate in the mitochondrial membrane, although it has been argued by Klingenberg [22] that sufficient undissociated monocarboxylic acid exists intracellularly to render such a transport carrier redundant. Recently Bakker et al. [23] described the mechanism by which monocarboxylic acids, such as pyruvate and acetate move across artificial membrane vesicles (liposomes). Their results suggest that pyruvate crosses the membrane in the undissociated form and readily exchanges with other weak monocarboxylic acids. It is the purpose of the present study to investigate the mechanism of translocation of pyruvate across the epithelial membrane, to compare it with that of other monocarboxylic acids and to study their mutual influence.

MATERIALS AND METHODS

Reagents

All chemicals were of analytical purity. Sodium [1-^{14}C]pyruvate (13.1 Ci/mol), sodium [2-^{14}C]pyruvate (9.89 Ci/mol), [^{3}H]inulin (860 Ci/mol), [U-^{14}C]sorbitol (8.7 Ci/mol), [1-^{14}C]propionic acid (57.5 Ci/mol), [1-^{14}C]octanoic acid (31.8 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.). Prostaglandin E_1 was a generous gift of the Unilever Research Laboratories (Vlaardingen, the Netherlands).

Preparation and extraction of epithelial cell suspensions

Normal fed male Wistar rats, weighing approx. 200 g, were maintained on a normal laboratory diet and H_2O ad libitum. The rats were narcotized with ether and killed by removal of the heart. The small intestine was removed and the lumen flushed with 100 ml ice-cold saline. Villous cells, originating from the jejenum, were harvested

according to the high frequency vibration technique of Harrison and Webster [24, 25], as described earlier [8]. The cell pellet (20–30 mg protein) was washed two times with incubation buffer, Krebs/Henseleit/bicarbonate [26] or Krebs/phosphate [27], both containing 1.4 mM $CaCl_2$, 0.6 mM $MgSO_4$ and 0.1 % fatty acid-poor Pentex serum albumin from Fluka (Buchs, Switzerland). The cells were resuspended, by passage through a plastic pipette, in 2–3 ml buffer and directly used for transport measurements. Care was taken that the cell suspension was standing not longer than 5 min before the start of the transport studies, since strong aggregation may occur after 5 min and this was found to interfere with the reproducibility of the transport rates. This aggregation was greatly reduced when the cells were standing as dilute suspensions (1 mg protein/ml). Metabolism of [1-^{14}C]pyruvate was measured as described in the previous report [8].

Silicon oil filtration technique

Eppendorf plastic microtest tubes (No. 3810, 1.5 ml volume) were filled with 0.1 ml 0.5 M $HClO_4$ and 0.4 ml silicon oil (TEGILOXAN® A. V. 100, Goldschmidt Ltd., Essen, G.F.R.) and 0.5 ml incubation buffer, with labelled substrate present and [U-^{14}C]sorbitol or [^{3}H]inulin as marker for extracellular space (1 mM and 0.4 mM final concentrations, respectively). After layering the tubes, they were briefly centrifuged in an Eppendorf 3200 centrifuge to separate the silicon oil from both aqueous layers. They were stood for a minimum of 15 min in an ice-bath to insure that the incubation layer was at 0 °C. When uptake of substrate was to be determined, 50 μl of a concentrated, disaggregated, cell suspension was added to the incubation layer and during incubation the tubes were gently shaken by hand (about twice/min). At the end of the incubation the cells were sedimented at 10 000 $\times g$ for 4 min in the Eppendorf centrifuge. This centrifuge reaches maximal g values after 5 s and the cells were separated from the medium by the silicon oil layer within 15 s. The cellular content was released when the cells came into contact with the bottom perchloric acid layer. Parallel tests were done with incubation buffer (sometimes with the inhibitors present) containing ^3H_2O and [U-^{14}C]sorbitol to find out the intracellular space of the cells and the total water space that was mixed with the acid underphase. After samples had been taken from the cell-free upperphase for radioactivity measurement, it was washed away with the help of acetone/water (50 : 50, v/v). A sample of 70 μl of the clear acid underphase was then taken with a Hamilton syringe for the analysis of radioactivity. When substrates or inhibitors (e.g. fatty acids) had to be added in a higher concentration than 1 mM they were added as 123 mM sodium salt solutions in NaCl-free Krebs/phosphate or Krebs/Henseleit/bicarbonate, for maintenance of isotonicity. The description of the method of studying efflux of substrates from cells is given in the legend to Fig. 6.

Calculation of results

Cellular ^{14}C-labelled substrate content was calculated by correcting the ^{14}C-labelled substrate content of the pellet for substrate in the extracellular space, as determined from the distribution of [^{3}H]inulin. Calculation of the intracellular water in a typical experiment (example 0.77 mg cell protein) proceeds as follows. Specific activity of ^3H_2O in the upperphase was 1149 cpm/μl and ^3H_2O measured in underphase (70 μl) was 7780 cmp; the total water space then is

$$\frac{100}{70 - \frac{7780}{1149}} \times \frac{7780}{1149} = 10.71\ \mu l.$$

Furthermore the specific activity of [U-^{14}C]sorbitol in upperphase was 285 cpm/μl and [U-^{14}C]sorbitol measured in underphase (70 μl) yielded 1372 cpm. The total adherent space then equals

$$\frac{100+10.71}{70} \times \frac{1372}{285} \text{ or } 7.61\ \mu l.$$

The intracellular water in this experiment then amounted to $(10.71-7.61) = 3.10\ \mu l$.

Counting of radioactivity

The counting vials contained 10 ml Instagel (Packard) and ^{3}H and ^{14}C activities were measured in a liquid scintillation counter (Packard, model 3380) with automatic standardization.

RESULTS AND DISCUSSION

Effects of fatty acids and the fatty acid derivatives ricinoleic acid and prostaglandin E, on oxidative decarboxylation of pyruvate at 30 °C

In earlier experiments from our laboratory [8] it was observed that short-chain and medium-chain fatty acids decrease the rate of $^{14}CO_2$ production from [1-^{14}C]pyruvate in intestinal epithelial cells. Interconversion of the pyruvate dehydrogenase complex determines to a minor extent the inhibition of pyruvate oxidation by fatty acids. Therefore we proposed two possible mechanisms for this discrepancy. Firstly the inhibition of the pyruvate dehydrogenase complex may be caused by high acetyl CoA/CoASH– and NADH/NAD$^+$ ratios as the result of fatty acid oxidation. Indeed in later experiments we found a clearcut increase in acetyl-CoA/CoASH ratio after incubation of cells with octanoate or albumin-bound oleate (results not shown). Secondly, fatty acids may interfere with pyruvate transport across the membrane of the epithelial cells.

The stronger inhibition of pyruvate oxidation by 5 mM octanoate than by 5 mM hexanoate (Table I) might suggest chainlength dependency. Therefore the long-chain fatty acids palmitate and myristate were also tested. However, they were not more effective than octanoate when tested at 80 μM.

Recently Ammon and Phillips described the influence of fatty acids on the absorption of water and electrolytes in canine Thiry-Vella fistulas of ileum [28] and human jejunum [29]. Long-chain fatty acids, particularly the hydroxy fatty acids ricinoleate (the active principle of castor oil) and 10 (9)-hydroxystearic acid (major fatty acid of fatty stools [30]), strongly impaired electrolyte- and water absorptions in vivo; some inhibition was seen with octanoate and hexanoate [28]. Ricinoleate at a lower concentration (0.12 mM; see Table I) was as effective as 80 μM octanoate, but at 0.5 mM it inhibited pyruvate oxidation almost completely. Perfusion of human jejunum in vivo with ricinoleic acid or oleic acid evokes water secretion [31], the latter has been described also for prostaglandin E_1 in vivo [32, 33]. Prostaglandin E_1 at 0.1 mM (Table I) resulted in a 30 % decrease of pyruvate oxidation, but no effect

TABLE I

EFFECT OF FATTY ACIDS, RICINOLEIC ACID AND PROSTAGLANDIN E, ON OXIDATIVE DECARBOXYLATION OF PYRUVATE IN RAT SMALL INTESTINE

Pyruvate oxidation ([1-^{14}C]pyruvate → $^{14}CO_2$) was measured in Krebs/Henseleit/bicarbonate buffer (pH 7.4) fortified with 1 mM sodium [1-^{14}C]pyruvate (spec. act. varied from 5 to 30 μCi/mol) at 30 C, as described in detail in the previous report [8]. The values are derived from individual experiments, in which in each case the rate of CO_2 production was set at 100 % when no additions were made.

Addition to the incubation medium	Concentration (mM)	n	$^{14}CO_2$ Production (percentage of control)
—	—	8	100
Hexanoate	5	3	67.2±2.1
Octanoate	0.08	3	81.8±3.1
Octanoate	5	4	44.4±2.0
Myristate	0.08	3	82.3±4.0
Palmitate	0.08	4	84.3±5.5
Ricinoleate	0.12	5	81.7±7.5
Ricinoleate	0.5	5	14.0±2.1
Prostaglandin E_1	0.1	4	69.5±5.6
Prostaglandin E_1	0.02	1	98.8

was observed at 0.02 mM, the concentration needed for maximal activation of adenylcyclase [32]. The latter result might suggest a fatty acid-like effect of prostaglandin E_1 on pyruvate utilization. Moreover, octanoate and ricinoleate are not able to increase cyclic AMP levels in intestinal villous cells under the same conditions as described here (H. R. de Jonge, unpublished).

Effect of octanoate on pyruvate transport in epithelial cells at 0 °C

In studies on the uptake of substrates that readily cross the membrane it is important to separate the cells from the incubation medium rapidly; secondly, it is essential that the solute composition of the cells should not change during the separation. Techniques for the separation of intestinal cells have been described, in particular in studies on aminoacid- and glucose uptake [35–37]. Reiser and Christiansen [35] followed millipore filtration of intestinal cells microscopically and observed extensive morphological damage, probably attributed to the presence of mucus. Also, collection of the cells by centrifugation reduced the viability of the cells, although in this case amino acid uptake against a concentration gradient could be measured [35]. Therefore our attention was directed to the centrifugal filtration method, successfully used in many mitochondrial uptake studies [38, 39]. Recently this method has also been applied to cell separations (thymocytes [40], kidney tubules [4] and fat cells [41]). The viability of the cells after passage through the oil appeared to be unchanged [40, 41], as judged by their hormone sensitivity, oxygen consumption and sodium content.

The rate of utilization of pyruvate in intestinal epithelial cells at physiological temperature has been found to be very rapid [8], as judged by CO_2– and lactate production rates. For that reason we decided to study transport at 0 °C, since at that temperature its metabolism is relatively sluggish. Experiments not shown indicated

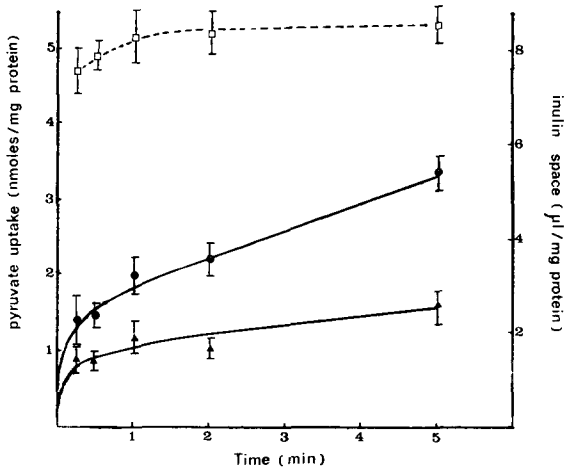

Fig. 1. Effect of 5 mM octanoate on transport of 1 mM pyruvate in epithelial cells at 0 °C. The incubation was carried out at 0 °C in Krebs/Henseleit bicarbonate buffer (pH 7.4), containing 1 mM [2-^{14}C]pyruvate, 0.4 mM [^{3}H]inulin and 0.1 % albumin. After the time indicated, the cells were rapidly centrifuged (10 000 × *g*) through a silicon oil layer, as described under Methods: ● — ●, control; ▲ — ▲, 5 mM octanoate present; □- - -□, adherent water, calculated from [^{3}H]inulin transported with the cells. Intracellular water volumes, calculated from the distribution of $^{3}H_2O$ and [U-^{14}C]sorbitol were 4.18 ± 0.21 (n = 16) μl/mg protein, determined after 5 min incubation. Each point represents the mean of six experiments (± S.E.).

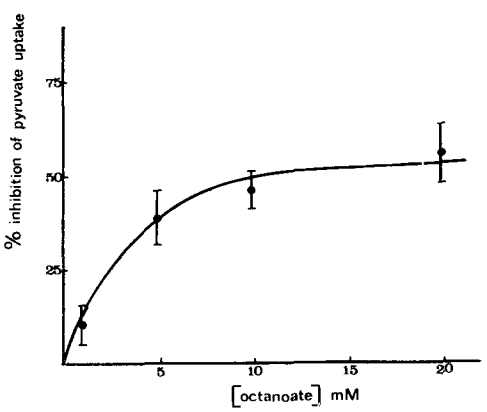

Fig. 2. Inhibition of pyruvate transport at 0 °C as a function of the concentration of octanoate. The incubations are carried out at 0 °C (5 min) in Krebs/phosphate buffer (pH 7.4), containing 1 mM labeled substrate, 0.4 mM [^{3}H]inulin and 0.1 % albumin. For details of the transport assay see Methods. Each point represents the mean of four experiments (± S.E.).

that at 0 °C the rate of conversion of [1-^{14}C]pyruvate to $^{14}CO_2$ and lactate amounted only to 0.1 nmol/min/mg protein, whereas the data of Fig. 1 indicate that the initial rate of pyruvate transport exceeds 5 nmol/min/mg protein. In agreement with the discrepancy between the rates of metabolism and transport is the observation that the radioactivity within the cells is largely due to the presence of pyruvate as measured by double-beam spectrophotometry (data not shown). Fig. 1 demonstrates further-

more that 5 mM octanoate clearly inhibited the translocation of pyruvate across the membrane. The study of the effects of different concentrations of octanoate on pyruvate transport (5 min) revealed that at 10 mM octanoate the inhibition of pyruvate transport was nearly maximal (Fig. 2). In Fig. 1 it is also demonstrated that the adherent space, calculated from [^{3}H]inulin transported with the cells, appeared not to be constant during the first min of incubation. This might be due to the relatively long time required for the equilibration of inulin with the adherent space of the cells, as pointed out in another report [42]. Therefore, the uptake values for [2-^{14}C]pyruvate at times shorter than 30 s could be somewhat overestimated. It seems more probable to us that at short incubation times there is an increase of adherent water with time.

Comparison of the effects of fatty acids on the rate of pyruvate transport

In order to compare the effects of fatty acids other than octanoate on pyruvate transport we decided to measure uptake rates at 5 min. In the first experiments we used Krebs/Henseleit/bicarbonate buffer (Table II). From the relative inhibitions of octanoate, hexanoate and D,L-3-hydroxybutyrate we concluded that there was the same chainlength dependency as observed in the effect of fatty acids on pyruvate oxidation at 30 °C (compare ref. 8 and the previous section). In later studies we measured transport in Krebs/phosphate buffer owing to its stronger buffering capacity. Surprisingly, in this buffer a significantly higher rate of pyruvate uptake was measured, but the effect of octanoate was relatively the same. Ricinoleate and prostaglandin E_1 were also inhibitory to pyruvate transport.

TABLE II

INFLUENCE OF FATTY ACIDS ON PYRUVATE TRANSPORT IN INTESTINAL EPITHELIAL CELLS AT 0 °C

The incubations were carried out for 5 min at 0 °C. The conditions are described in the legend to Fig. 1 and under Materials and Methods. The number of experiments is indicated in parentheses.

Addition to the influx medium	Concentration (mM)	Buffer used for influx measurements	
		Krebs/Henseleit/bicarbonate	Krebs/phosphate
		Influx rates of pyruvate (nmol/mg protein/5 min)	
—	—	3.25±0.35 (5)	4.69±0.13 (9)
Acetate	10	—	3.34±0.08 (4)
Propionate	10	—	3.52±0.12 (4)
Butyrate	10	—	3.14±0.12 (4)
Octanoate	5	2.00±0.20 (5)	2.82±0.15 (5)
Hexanoate	5	2.55±0.24 (5)	—
D,L-3-hydroxybutyrate	5	2.97±0.21 (4)	—
Prostaglandin E_1	0.1	2.82±0.27 (4)	3.85±0.15 (3)
Ricinoleate	0.5	1.99±0.22 (4)	2.78±0.19 (5)

The short-chain fatty acids, acetate, propionate and butyrate at 10 mM concentration were found to inhibit pyruvate transport (Table II). To elucidate the character of the inhibition of the fatty acids tested, we studied the effect of fatty acids on the rate of transport at different pyruvate concentrations (Fig. 3). In the first experiments we tried to investigate the kinetics during very short incubation periods (30 s, results

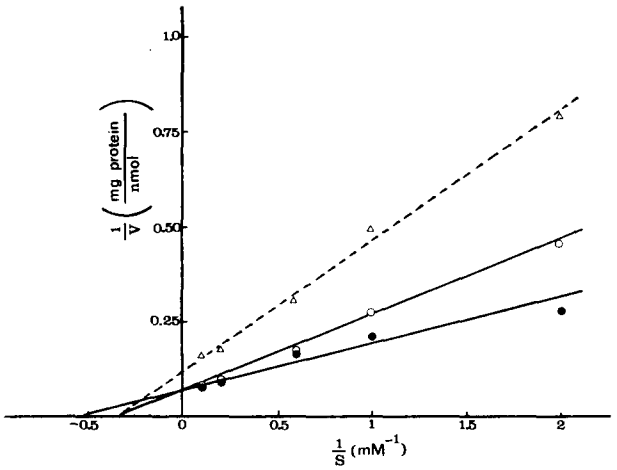

Fig. 3. Lineweaver-Burk plots of the rates of pyruvate transport as a function of the concentration of pyruvate. Effects of 10 mM octanoate and 10 mM propionate. For details of transport assay see Methods. The incubations were carried out at 0 °C (5 min) in Krebs/phosphate buffer (pH 7.4), containing 0.4 mM [^{3}H]inulin and 0.1 % albumin. ● — ●, pyruvate alone; ○ — ○, pyruvate in the presence of 10 mM propionate; △- - -△, pyruvate in the presence of 10 mM octanoate. Each point represents the mean of four experiments.

not shown). It was found that the amount of adherent substrate accounted for a large percentage of the total substrate found in the sample. This introduced a large error in the uptake rate measurements at 0 °C and made the results unreliable. Therefore, in the experiments shown (Fig. 3) uptake was measured over 5 min, after it had been shown in Fig. 1 that at 5 min the uptake of pyruvate was not maximal. Lineweaver-Burk plots of the results suggested that uptake of pyruvate at 0 °C was a saturable process. This argues for the presence of a carrier for pyruvate translocation in epithelial cells, as already described for the cell membranes of heart [16] and erythrocytes [17]. This proposal was further supported by the competitive nature of the propionate inhibition (Fig. 3), suggesting the presence of a translocator for monocarboxylic acids in intestinal epithelial cell membranes. The same experiments were done in the presence of octanoate, the inhibition of which appeared to be of non-competitive nature. This led us to study the nature of diffusion of pyruvate, propionate and octanoate across the epithelial cell membrane.

Effect of pH on the influx of pyruvate, propionate and octanoate at 0 °C

The mechanism involved in the transport of weak electrolytes by small intestine has been the subject of several previous investigations [42–48]. The transport of short-chain fatty acids may be a combination of carrier-facilitated or active transport [43–45] and non-ionic diffusion [42, 46–48]. In these studies net mucosal to serosal fluxes were measured. Our studies, however, involve isolated epithelial cell sheets in which both brush borders and basolateral plasma membranes are exposed to the incubation fluid simultaneously, not allowing definitive conclusions to be made about the localization of the translocation sites. The contribution of diffusion of the non-ionized form of short-chain fatty acids to overall transport should be influenced by varying the pH of the incubation medium. While changing the pH of the Krebs/

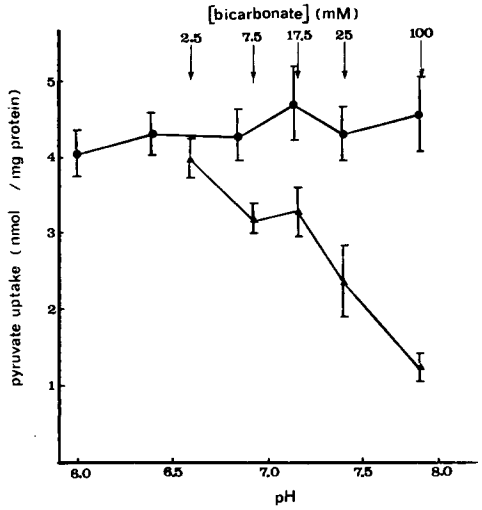

Fig. 4. Influence of pH on transport of 1 mM pyruvate into epithelial cells at 0 °C, measured in two isotonic buffers. The incubations were carried out at 0 °C (5 min) in buffer containing 1 mM [2-^{14}C]-pyruvate, 0.4 mM [^{3}H]inulin and 0.1 % albumin. The pH of the Krebs/Henseleit/bicarbonate buffer was varied by adjustment of the concentrations of HCO_3^- and Cl^-, while maintaining the other ion concentrations constant. For details of the transport assay see Methods. ● — ●, pH varied in Krebs-phosphate buffer; ▲ — ▲, pH varied in Krebs/bicarbonate buffer. Each point represents the mean of four experiments (±S.E.).

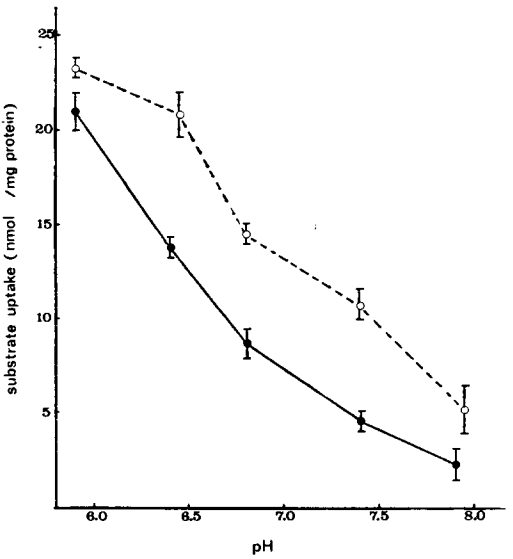

Fig. 5. Influence of pH on the transport of 1 mM propionate and 1 mM octanoate in epithelial cells at 0 °C, measured in Krebs/phosphate buffer. For details of transport assay see Methods. ● — ●, 1 mM [1-^{14}C]propionate; ○- - -○, 1 mM [1-^{14}C]octanoate. The measurement of transport of octanoate by silicon oil filtration was complicated by the precipitation of the fatty acid in the acid underphase, and the strong influence of the pH on the distribution of octanoate between adherent water and silicon oil during passage of the cells. Therefore in these experiments the silicon oil layer, the acid extract and the pellet were all tested for radioactivity. Each point represents the mean of three experiments (±S.E.). The incubation time was 5 min.

Henseleit bicarbonate buffer (by varying the bicarbonate concentration from 0–100 mM), as used by Barry and Smyth [44] in studies on propionate transport in everted sacs, we found a dependence of pyruvate transport on the pH (Fig. 4). But repeating the same experiments in Krebs/phosphate, we found no significant variation between pH 5.9 to pH 7.9 (Fig. 4). The effect found in Krebs/bicarbonate can be ascribed to inhibition of pyruvate transport by high concentrations of bicarbonate (compare also Table II). By contrast, propionate or octanoate influx, measured in Krebs/phosphate, were both strongly dependent on pH (Fig. 5). Assuming a homogeneous partition of carboxylic acid in the cell water (intracellular water of cells amounted to 4.18 ± 0.21 ($n = 16$) μl/mg protein), the calculated concentrations of pyruvate, propionate and octanoate at pH 7.4 were 1.03, 1.10 and 2.50 mM respectively. This leads to the conclusion that a concentration gradient of octanoate across the membrane is probably present at 0 °C at physiological pH. Octanoate transport in intestine has been studied by Gallagher and Playoust [49] and by Bloch et al. [45]. The latter authors showed an accumulation of octanoate against a concentration gradient in vitro at physiological pH. Taking into account our results obtained at 0 °C (Fig. 5), a part of the fatty acid concentration gradient may be explained by non-ionic diffusion [46]. The fatty acids used in the present study have pK_a values close to 5. Pyruvate on the other hand has a pK_a value of 2.5, so that the absence of an effect of pH lowering on the influx rate tends to support the conclusion that the ionic form is the only species transported. From the data of Fig. 5 it can be concluded that at pH 7.4 the amount of propionate taken up after 5 min amounts to 4.6 nmol/mg protein. When it is considered that the initial rate is many times larger (as concluded from unpublished experiments carried out for 30 s when a rate of 8 nmol/min/mg protein was found) and assuming a Q_{10} of 3.5 (as was suggested by experiments on propionate efflux carried out at 0 °C and at 10 °C; not shown) propionate transport is very fast when compared with propionate metabolism [43]. Since the presence of substrates at 5 min incubation is not solely due to influx, but a result of influx accompanied by efflux, efflux studies were undertaken.

Efflux of pyruvate, propionate and octanoate at 0 °C from epithelial cells and the effects of monocarboxylic acids and pH on these processes

Efflux studies approximate unidirectional fluxes better than the 5 min uptake studies as presented above. They were performed after preloading the cells for 15 min with labeled pyruvate or fatty acid at 0 °C. A small aliquot of these suspensions was added to a medium with or without exchangeable substrate. The concentration of labeled extracellular substrate then rapidly decreased to approx. 6 % of its original value in the preloading period. Fig. 6 clearly shows that pyruvate moves slowly out of the cells. This process was not found to be pH-dependent (compare Fig. 7). The initial phase (1 min) of pyruvate efflux could be stimulated by propionate, probably by countertransport. However, this propionate-pyruvate exchange was greatly inhibited when the pH was lowered to pH 5.9 (Fig. 7), probably due to very rapid equilibration of propionate between the various compartments at that pH. Fig. 5 indeed shows a strong stimulation of propionate influx by lowering the pH.

Propionate moves out much faster than pyruvate (Fig. 6). This rate was clearly depressed at pH 5.9 (Fig. 7). This, together with the results on influx, indicates that the pH gradient across the membrane influences the rate of propionate movement.

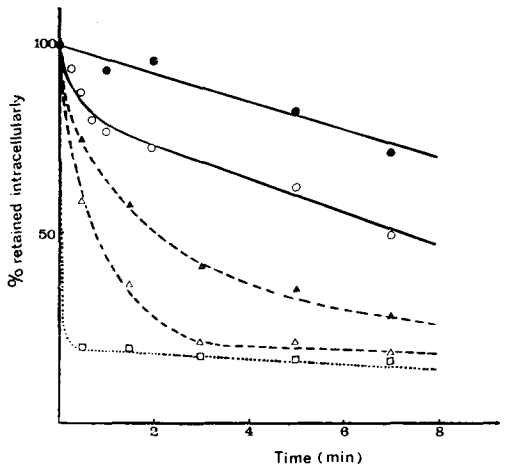

Fig. 6. Efflux of [2-^{14}C]pyruvate, [1-^{14}C]propionate and [1-^{14}C]octanoate from epithelial cells in Krebs/phosphate buffer (pH 7.4); effect of counter ions. Epithelial cells were preincubated at 0 °C with 1 mM labeled substrate. After 15 min 50 μl of these preincubated cell suspensions were added to 0.7 ml buffer layered on silicon oil. The buffer contained counter-substrate and [^{3}H]inulin. After the time indicated the cells were rapidly centrifuged (10 000 × g) through the oil layer as described in Methods. The substrate content at "zero time" was determined by filtration of 0.5 ml of undiluted cell suspension, to which [^{3}H]inulin was added, through a silicon oil layer. This initial ^{14}C-labelled substrate content of the cells amounted for pyruvate, propionate and octanoate 5.16, 5.05 and 12.50 nmol/mg protein respectively. ● — ●, [2-^{14}C]pyruvate efflux; ○ — ○, [2-^{14}C]pyruvate efflux in the presence of 2 mM propionate; ▲ — ▲, [1-^{14}C]propionate efflux; △- - -△, [1-^{14}C]propionate efflux in the presence of 2 mM pyruvate; □. . . .□, [1-^{14}C]octanoate efflux.

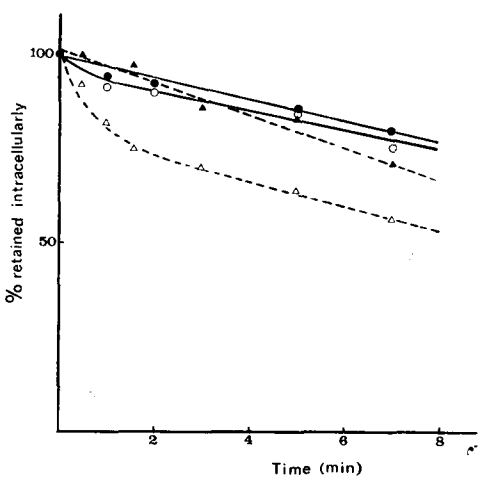

Fig. 7. Efflux of [2-^{14}C]pyruvate and [1-^{14}C]propionate from epithelial cells in Krebs/phosphate buffer (pH 5.9). Effect of counter-ions. Details are described in the legend to Fig. 6. ● — ●, [2-^{14}C]-pyruvate efflux; ○ — ○, [2-^{14}C]pyruvate efflux in the presence of 2 mM propionate; ▲- - -▲, [1-^{14}C]propionate efflux; △- - -△, [1-^{14}C]propionate efflux in the presence of 2 mM pyruvate.

TABLE III

INFLUENCE OF FATTY ACIDS AND BICARBONATE ON THE EFFLUX OF PYRUVATE OR PROPIONATE FROM INTESTINAL EPITHELIAL CELLS AT 0 °C

Epithelial cells were preincubated for 15 min at 0 °C in Krebs/phosphate buffer (pH 7.4) with 1 mM labelled substrate. Then 50 μl cell suspension were added to 0.7 ml buffer layered on a silicon-oil layer and containing counter-substrate or other compounds, as indicated as well as [^{3}H]-inulin. After 2 or 5 min, as indicated, the cells were rapidly centrifuged (10 000 · g) through the oil layer, as described under Methods.

Addition to the efflux medium	Concentration (mM)	n	[2-^{14}C]pyruvate-loaded cells		n	[1-^{14}C]propionate loaded cells	
			Efflux in nmol/mg protein/5 min	% Control*		Efflux in nmol/mg protein/2 min	% Control*
–		5	0.716	–	4	2.012	–
Acetate	2	3	0.924	166±11	4	2.258	111±5
Pyruvate	2		–	–	4	3.011	148±6
Propionate	2	5	1.744	256±30	4	–	–
Butyrate	2	5	1.683	253±26	4	2.699	134±4
Hexanoate	2	3	0.614	117±32	4	2.681	132±7
Octanoate	2	5	0.758	104±20	4	2.820	139±7
Propionate+ Octanoate	2	3	1.898	230±24	–	–	–
Butyrate+ Octanoate	2	3	1.950	230±30	–	–	
Pyruvate	1	3	1.500	199±18	–	–	–
Octanoate	5	5	0.755	99±18	–	–	–
Ricinoleate	0.5	5	0.718	89±18			–
Bicarbonate	25	4	1.280	176±20	–		
Bicarbonate	100	4	1.628	227±22	–	–	–

* In each experiment the efflux rate was set at 100% when no addition to efflux medium was made. The mean (±S.E.) values are given in this column.

Furthermore, propionate efflux could be stimulated by pyruvate (Fig. 7). Lowering the pH to 5.9 did not influence this exchange process, which is in agreement with the finding (Fig. 4) that pyruvate translocation across the membrane was not pH-dependent. The efflux of octanoate at pH 7.4 was too rapid to follow (Fig. 6). Comparison then of these data may indicate that lipophilicity of the undissociated form as well as the pK_a value of the carboxylic acid determines the rate of non-ionic diffusion. That for pyruvate or propionate the efflux rate is lower than the influx rate, reflects either the absence of a counterion or a low affinity of these substrates for the carrier at the inside of the membrane.

Efflux of pyruvate and propionate from epithelial cells at 0 °C; effects of the presence of fatty acids in the efflux medium

In a previous section it was shown that fatty acid could inhibit the net influx of pyruvate. It was also suggested that the nature of the inhibitory effect of e.g. propionate compared with octanoate on pyruvate influx was different (Fig. 3). Table III demonstrates the strong stimulations of 2 mM acetate, propionate or butyrate and 25 mM bicarbonate on pyruvate efflux, measured over 5 min. This suggests the presence of a monocarboxylate carrier with a high substrate affinity compared with that for bicarbonate. Hexanoate and octanoate in 2 mM concentrations, however, had no effect on the exit of pyruvate. This might be due to an inhibitory effect of these fatty acids on both in- and outward directed pyruvate fluxes, for instance by perturbation of the membrane lipoproteins or non-competitive binding to the monocarboxylate carrier. These possibilities were excluded by the observation that 2 mM hexanoate or 2 mM octanoate had no effect on the pyruvate efflux stimulated by 2 mM propionate or butyrate (Table III). This supports the hypothesis that octanoate and hexanoate are diffusing exclusively in the undissociated or perhaps in the micellar form across the membrane. Even higher concentrations (5 mM) of octanoate and 0.5 mM ricinoleate were not found to be inhibitory to the efflux of pyruvate. This was unexpected since 5 mM octanoate or 0.5 mM ricinoleate strongly inhibited the influx of pyruvate (Table II). This again would suggest an asymmetrical behaviour of the monocarboxylate carrier.

Propionate efflux may be stimulated by fatty acids and pyruvate in 2 mM concentrations. Comparable stimulations of pyruvate, hexanoate and octanoate on propionate efflux were obtained (Table III), which contrasts their dissimilar effects on pyruvate efflux. From the previous section it was concluded that propionate was moving out in the undissociated form at pH 7.4. Therefore stimulation of propionate exit by octanoate or hexanoate may be due to fatty acid exchange. The possible mechanism of this exchange might depend on the provision of protons at the inside of the membrane by influx of undissociated fatty acid, as described by Bakker and Van Dam [23] for liposomes.

CONCLUDING REMARKS

The transport of pyruvate in intestinal epithelial cells at 0 °C is characterized by saturation kinetics, competitive inhibition by short-chain fatty acids and counter-transport. These results could be explained by carrier-facilitated diffusion of monocarboxylic anions across the cell membrane. Since the outward flux rates of the

substrates are lower than the inward flux rates, a localization of the carrier within the brush border part of the cellular envelope, would explain the net mucosa to serosa fluxes of these monocarboxylic acids.

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