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A Mitochondrial Monocarboxylate Transporter in Rat Liver and Heart and its Possible Function in Cell Control

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Several hydroxy- and keto-substituted monocarboxylates were found to undergo co- as well as counter-exchange across the mitochondrial membrane. The results argue against a simple Donnan system and may be explained by the existence of a transporter for monocarboxylates. In support of this explanation it was apparently possible to 'pump' pyruvate to the sucrose-inaccessible space by using the dicarboxylate transporter. Further, several aromatic and aliphatic analogues of pyruvate, but not of di- or tri-carboxylate transport inhibitors, have been shown to prevent pyruvate-exchange reactions. Palmitoylcarnitine was found to have a much stronger affinity for the carrier than either carnitine or pyruvate and the possible consequences of this for carnitine–palmitoylcarnitine exchange and on the control of the pyruvate dehydrogenase complex are explored. In view of the range of transport inhibitors and substrates it is suggested that the carrier has a fairly broad specificity. 'Inhibitor-stop' kinetic studies show that the speed of translocation of pyruvate at 1°C is of the same order as malate. The possible correlation between the role of a hydroxy–keto acid transporter in substrate exchange and some whole animal experiments is briefly discussed. It is proposed that for reasons of control the cell will require membrane monocarboxylate transporters no less than di- or tri-carboxylate carriers.

The finding that it is possible to inhibit the exchange diffusion of pyruvate between the medium and the sucrose-impermeable space of rat liver and heart mitochondria with certain 2-oxo acids (Mowbray, 1974) suggests that a pyruvate transporter may be present in the mitochondrial inner membrane. Halestrap & Denton (1974) have reported decreased pyruvate content in rat liver mitochondria and human erythrocytes when the medium also contains small concentrations of a substituted cinnamic acid. The fact that this cinnamate decreased loss of pre-loaded pyruvate during centrifugation of the mitochondria would imply that it may be an inhibitor of a pyruvate-transport system. It has also been found that certain 2-oxo acids which do not inhibit enzymes involved in pyruvate oxidation extracted from rat brain mitochondria (Land & Clark, 1973) are, nevertheless, strong inhibitors of pyruvate and 2-hydroxybutyrate oxidation in intact mitochondria (Clark & Land, 1974; Land & Clark, 1974). These authors suggest that it is access of the monocarboxylate to the mitochondrial matrix that is impaired. The present paper describes some of the properties of a system involved in the inhibitible exchange of monocarboxylates across the inner mitochondrial membrane. Mitochondria from both rat heart and liver have been used. There appears to be very little difference, if any, in monocarboxylate transport between the two tissues: for this reason and because of the necessity of sacrificing a relatively large number of animals for heart work, many of the observations first made on heart mitochondria were followed up in liver preparations.

Experimental

Methods

Livers and hearts from male white rats weighing about 230–250g were minced finely with scissors in 250mM-sucrose–25mM-triethanolamine–HCl–1mM-EDTA buffer, pH7.2, and homogenized with two passes in a Teflon–glass homogenizer. The mitochondrial pellet was washed twice and resuspended at a concentration of about 50mg of mitochondrial protein/ml in 0.25M-sucrose–10mM-KCl–70mM-Tris–HCl – 1.15mM-succinate – 21.5mM-oxamate, pH7.4. All experiments were carried out in the suspending buffer containing also 0.75mM-arsenite, 6.3μM-rotenone and 10μCi/ml of ^3H_2O, and the substrate content was measured after centrifuging the mitochondria through an oil layer as described by Mowbray (1974). Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin (Armour Pharmaceutical Co., Eastbourne, Sussex, U.K.), dried to constant weight, as standard. Scintillation counting was performed in a toluene-based cocktail containing 4g of BBOT [2,5-bis-(5-t-

butylbenzoxazol-2-yl)thiophen], 80g of naphthalene and 400ml of 2-methoxyethanol per litre of solution. Quench correction was by external standard in a Beckman LS-200 spectrometer. The kinetics of pyruvate penetration were measured by the 'inhibitor-stop' method. The mitochondria (approx. 10mg) were preincubated for 3min in 1.2ml of pH7.4 buffer containing rotenone and arsenite (Mowbray, 1974), and the experiment was started by the addition of ^{14}C-labelled substrate. Samples (100μl) were withdrawn at set times and mixed immediately with 110μl of 100mM-2-oxo-4-methylpentanoic acid in the same buffer. A 200μl sample of this mixture was then centrifuged through an oil layer as above. [^{14}C]Sucrose was used as the extramitochondrial marker.

Control experiments in which the pyruvate content of liver mitochondria (4.7–5.1mg of protein per sample) was measured enzymically (Bucher *et al.*, 1963) and by using [^{14}C]pyruvate after a 5min incubation in 1mM-pyruvate, showed that there was no difference in the content measured by the two methods. This suggests that there was no significant metabolism of pyruvate under the conditions used. It has, however, to be borne in mind that the sucrose space (4.20±0.16μl/mg of protein) was large compared with the internal space (0.66±0.06μl/mg) and so a small conversion of pyruvate would not necessarily be detected easily. The identity of radioactivity and pyruvate is strongly supported by the exchange experiments in which after incubation of mitochondria with a saturating amount of pyruvate, the decrease in radioactivity on adding unlabelled pyruvate agrees closely with the predicted dilution in specific radioactivity (see below; Mowbray, 1974): thus the ^{14}C label behaves identically with chemical pyruvate.

Materials

[U-^{14}C]Pyruvate, DL-[3-^{14}C]hydroxybutyrate, L-[U-^{14}C]lactate and L-[U-^{14}C]malate were used as sodium salts at a specific radioactivity of 135mCi/mol in 0.25M-sucrose – 10mM-KCl – 20mM-Tris – HCl, pH7.4. These radiochemicals together with [U-^{14}C]-sucrose and tritiated water were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. 2-Oxopentanoic acid and 2-oxo-4-methylpentanoic acid were obtained from Fluka A.-G., Buchs, Switzerland: 2-oxo-3-phenylpropionic (phenylpyruvic) acid, benzene-1,2,3-tricarboxylic acid and L-carnitine hydrochloride were obtained from Koch–Light, Colnbrook, Bucks., U.K.: DL-palmitoylcarnitine, L-malic acid, L-lactic acid and rotenone were from Sigma Chemical Co., St. Louis, Mo., U.S.A.: sodium DL-3-hydroxybutyrate and sodium pyruvate were obtained from the Boehringer Corp., London W.5, U.K., and α-cyanocinnamic acid was bought from Ralph Emanuel Ltd., Wembley, Middx., U.K. Lactic acid solutions were boiled for 10min to depolymerize lactides before neutralization, and solutions of lactate and pyruvate were standardized enzymically. All other materials were of the highest quality available commercially.

Results and Discussion

Substrate exchange

A concentrated mitochondrial suspension was added to about 2ml of incubation mixture at 25°C containing a ^{14}C-labelled substrate so that the final mitochondrial concentration was about 5mg of mitochondrial protein per ml. After 5min of incubation, 200μl samples of the suspension were centrifuged above an oil layer. A small volume (5 or 10μl) of competing unlabelled substrate was now added to the incubation and the mixture sampled after a further 2min. Three further additions of competing substrate allowed sampling over a range of competing substrate up to 10 times the concentration of ^{14}C-labelled substrate. The ^{14}C-labelled substrate contents in heart mitochondria at two concentrations of competitor are given in Table 1. Similar additions of

Table 1. *Percentage of initial ^{14}C-labelled substrate remaining in the presence of added unlabelled competing substrate*

The data are means from three separate heart mitochondrial preparations (10 hearts per preparation). The mitochondrial substrate content varied between 2.1 and 2.5μmol/g of protein. The intramitochondrial space was 0.95±0.07μl/mg of protein. The concentration of the ^{14}C-labelled substrate was 1mM.

		% of initial ^{14}C-labelled substrate remaining							
	Competing substrate ...	Pyruvate		DL-3-Hydroxybutyrate		L-Lactate		L-Malate	
^{14}C-labelled substrate	Concn. (mM) ...	1	6	1	6	1	6	1	6
Pyruvate		—	—	53	13	61	35	58	35
DL-3-Hydroxybutyrate		25	3	—	—	41	19	33	10
L-Lactate		56	27	58	39	—	—	70	57
L-Malate		32	9	129	110	120	81	—	—

substrate had no effect on intramitochondrial space, and comparable data were obtained with liver mitochondria. There appears to be considerable variation between the substrates in their ability to compete with one another: pyruvate is shown to be a particularly effective competitor for 3-hydroxybutyrate and for malate.

Under certain conditions addition of 'competitors' increased the ^{14}C-labelled substrate content. Examples of this are seen in Table 1 where both lactate and DL-3-hydroxybutyrate have enhanced the malate content. At low ^{14}C-labelled substrate concentrations (about 0.3mM) this phenomenon was more widespread and pyruvate addition has been observed to lead to an increase in the [^{14}C]malate content, and conversely malate increased the pyruvate and lactate contents. This relationship between substrate concentration and mitochondrial content was further investigated in liver mitochondria by measuring the mitochondrial content over a range of ^{14}C-labelled substrate concentration. Fig. 1(*a*) shows that under the conditions used pyruvate content has essentially reached a maximum at about 2mM-pyruvate in the suspending medium. Increasing the ^{14}C-labelled substrate concentration up to 6mM did not further increase the mitochondrial content. Prior addition of 0.4mM-malate increased markedly the pyruvate content (Fig. 1*a*) without, however, changing the concentration at which saturation is reached. This suggests that although malate does not influence the affinity of the mitochondrion for pyruvate it increases access of pyruvate to the matrix space or to mitochondrial binding sites.

This co-transporting phenomenon is not compatible with the suggestion that pyruvate and malate are distributed across the mitochondrial membrane in the manner predicted for a simple Donnan system (Harris & Berent, 1970). The data are more easily explained on the basis that anion accumulation is achieved by a series of exchanges between different species (Chappell & Crofts, 1966; Klingenberg, 1970). Thus 'competitor' initially enters in exchange for other unspecified mitochondrial substrate and then exchanges inwards additional ^{14}C-labelled substrate. This model requires that the exchange is selective and specific: that is, malate can displace another mitochondrial substrate which pyruvate cannot. Since it is difficult to explain how pyruvate may then exchange in a non-specific manner for malate but not for this unspecified substrate, this co- and then counter-transport implies that pyruvate, like malate, is specifically transported. Since mitochondria can accumulate phosphate and subsequently exchange it stoicheiometrically for malate (Johnson & Chappell, 1973), phosphate addition has the predicted effect of increasing the maximum pyruvate content (Fig. 1*a*). Although entering molecules displace others selectively this need not imply that only a single carrier is involved. This appears to be the simplest hypothesis, but selective connexions between several carriers or uncon-

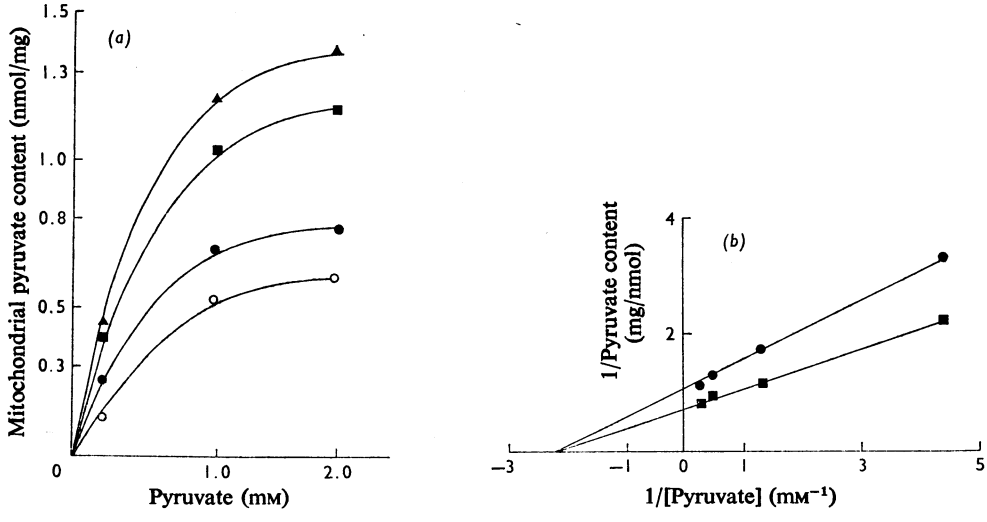

Fig. 1. *Effect of pyruvate concentration on mitochondrial pyruvate content*

(*a*) The intramitochondrial pyruvate content was measured after incubation of liver mitochondria (7.7mg of protein/ml) for 3min at 25°C in the presence of the shown concentrations of pyruvate (●), and containing in addition 0.4mM-malate (■) or 0.4mM-malate+0.4mM-phosphate (▲) or 5.0mM-phenylpyruvate (○). The intramitochondrial space was 0.73μl/mg of protein. (*b*) As for (*a*) with 5.0mg of mitochondria/ml and a space of 1.06μl/mg of protein.

nected transporters allowing selective competition for intramitochondrial cations or cationic sites are among the alternative possibilities.

3-Hydroxybutyrate, alone of the monocarboxylates tested, is strongly exchanged by malate (Table 1). This may possibly be related to the fact that L-3-hydroxybutyrate, the unnatural isomer, is structurally very similar to L-malate.

In a dynamic situation such as *in vivo*, the actual substrates counter-exchanging across the mitochondrial membrane will depend on the free concentrations and the relative affinities of substrates able to compete for a carrier. The ability of alternative mitochondrial substrates such as pyruvate and 3-hydroxybutyrate to undergo obligatory exchange via a transporter would provide a reasonable control for use of oxidizable substrate. In addition to showing relatively strong mutual exchange with 3-hydroxybutyrate, pyruvate also appears able to counter-transport L-malate rather effectively (Table 1). This may operate as a self-limiting process in pyruvate production since the increased cytoplasmic malate will slow the reoxidation of reduced coenzyme by malate dehydrogenase and the higher reduced coenzyme pool may restrict glyceraldehyde 3-phosphate oxidation and lead, in addition, to pyruvate reduction to lactate.

Exchange transport of monocarboxylates across the mitochondrial membrane may also offer an explanation for the anomalous (cf. Williamson, 1964) effect of a constant lactate pool (production of lactate is balanced exactly by its utilization) in the perfusate on pyruvate oxidation in perfused rat heart. Under these conditions and in the presence of glucose and insulin, pyruvate oxidation is suppressed (Mowbray & Ottaway, 1973*a*). It may be that the presence of lactate (2.2mM in perfusate; 1.7μmol/g in tissue) decreases the mitochondrial pool of pyruvate by exchange. The addition of pyruvate to the perfusate reverses this inhibition (Mowbray & Ottaway, 1973*a*). In support of this explanation it has been observed that in rat heart perfused under aerobic conditions with glucose, lactate production is negligible but increases appreciably on addition of lactate itself to the perfusate (Mowbray, 1969). Thus it may be proposed that a decreased availability of pyruvate to the mitochondrion consequently increases the competitiveness of lactate dehydrogenase for pyruvate.

Measurement of affinity constants for pyruvate exchange

From saturation experiments of the type described above, it is possible to evaluate the Langmuir absorption constant, α (corresponds to the Michaelis constant), for pyruvate. Reciprocal plots were linear (Fig. 1*b*) over a range of pyruvate concentrations up to 6mM and α was estimated to be 0.447 ± 0.007mM (six experiments). The corresponding inhibition constant (K_i) for competing mitochondrial substrate-exchange reactions is easily determined (see Harris & Manger, 1968), and the K_i values for pyruvate 'inhibition' of substrate retention in liver mitochondria were 0.5 ± 0.2mM for malate, 0.5 ± 0.2mM for lactate and 0.4 ± 0.2mM for 3-hydroxybutyrate. These data do not necessarily imply the existence of a specific membrane carrier system; merely that anion exchangers are involved. It is worth noting, however, that an apparently identical binding of pyruvate is effective in displacing substrates for two of which specific and different enzyme sites exist within the mitochondrion, suggesting that the data may relate to a transporter. Also, although malate can be transferred by the di- or tri-carboxylate carrier, neither benzene-1,2,3-tricarboxylate (50mM) nor 2-*n*-butylmalonate (15mM) was found to prevent pyruvate counter-transport.

Effect of inhibitors

Mowbray (1974) has shown that it is possible to inhibit pyruvate exchange across the mitochondrial membrane by using 2-oxo-4-methylpentanoate. This compound which accumulates in tissues in maple-syrup-urine disease has been reported to inhibit pyruvate and 3-hydroxybutyrate oxidation in intact brain mitochondria (Land & Clark, 1974) and has been found to inhibit pyruvate and 3-hydroxybutyrate transfer across the mitochondrial membrane in that tissue (J. Mowbray, J. B. Clark & J. M. Land, unpublished results). 2-Oxo-3-phenylpropionate (phenylpyruvate) which has also been observed to inhibit pyruvate oxidation in preparations of brain (Bowden & McArthur, 1972; Clark & Land, 1974; Land & Clark, 1974) and of liver (Arinze & Patel, 1973) which contain whole mitochondria, is relatively ineffective at preventing pyruvate entry to liver mitochondria when used at a concentration of 5mM (Fig. 1*a*). The maximum pyruvate content (tested up to 6mM-[^{14}C]pyruvate) was, however, decreased over the control value, presumably reflecting competitive binding. This observation emphasizes the advisability of investigating not only substrate accumulation (including binding) but also exchange in the presence of inhibitor. At concentrations of 2-oxo-3-phenylpropionate between 20 and 50mM it does, however, function as an inhibitor of pyruvate exchange from the sucrose-impermeable space. Fig. 2 demonstrates the effect of adding inhibitor, after prior incubation of liver mitochondria in pyruvate, on the ability of 3-hydroxybutyrate to displace the accumulated pyruvate. The addition of inhibitor decreases the pyruvate content by 40% but permits no significant exchange with the added competitor. Essentially identical results were obtained with lactate as competing substrate. Halestrap & Denton (1974) have suggested that a substituted cyanocinnamic acid is an

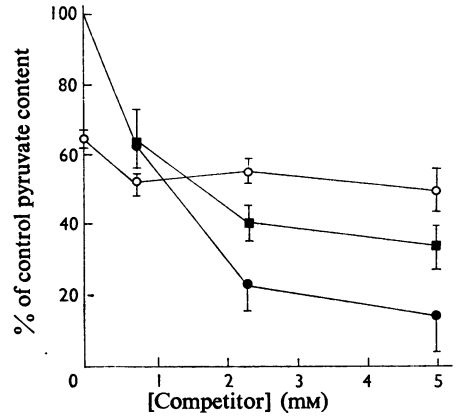

Fig. 2. *Effect of lactate and 3-hydroxybutyrate concentration on mitochondrial pyruvate content*

3-Hydroxybutyrate (●), lactate (■) and 23mM-phenylpyruvate followed by 3-hydroxybutyrate (○) were added to liver mitochondria incubated at 25°C in 1mM-[^{14}C]-pyruvate. The initial pyruvate content was 1.04nmol/mg of protein and the data show the means±S.E.M. of four different preparations. The mean intramitochondrial volume was 0.71μl/mg.

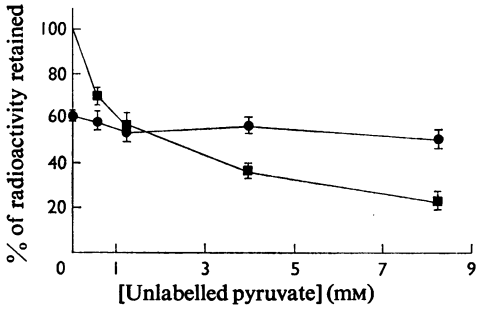

Fig. 3. *Inhibition of pyruvate–pyruvate exchange by α-cyanocinnamic acid*

Liver mitochondria suspended in 2.2mM-[^{14}C]pyruvate were incubated at 25°C after addition of no inhibitor (■) or 2mM-α-cyanocinnamic acid (●) followed by unlabelled pyruvate to give the concentration shown. The initial pyruvate content was 2.1nmol/mg, the mitochondrial space was 0.55μl/mg and the data are means±S.E.M. of four experiments. The cyanocinnamic acid was added in ethanol solution and an equal volume of ethanol was added to the control incubation.

inhibitor of pyruvate accumulation in rat liver mitochondria and in human erythrocytes. Fig. 3 presents results which show that the addition of 2mM-α-cyanocinnamic acid to mitochondria suspended in a saturating (Fig. 1*a*) concentration of [^{14}C]pyruvate, prevents the dilution of mitochondrial specific radioactivity by added unlabelled pyruvate. The effectiveness of this inhibitor at relatively low concentrations may be partly due to its extreme insolubility in aqueous media but it may be significant that it is an enol analogue of pyruvate, especially since the transporter appears capable of exchanging other hydroxy acids. It does appear, however, that the 2-hydroxy acid, lactate, is less capable as an exchanger than 3-hydroxy acids (Table 1; Fig. 2). Relevant to the question of whether the carrier recognizes the keto or enol form of pyruvate is the observation that although the 3-hydroxy acid L-carnitine exchanges pyruvate as well as 3-hydroxybutyrate, the palmitoyl ester of carnitine is an order of magnitude more effective as a pyruvate counter-transporter (see below). On the assumption that a single carrier is involved, molecular models suggest that the keto group of the palmitoyl and the carboxyl group of the carnitine moiety would exist in juxtaposition, closely resembling the structure of (keto) pyruvate. Thus it seems likely that the transporter specificity is not exclusive for either the keto or hydroxy forms of monocarboxylates.

Kinetic experiments

The rate of penetration of pyruvate to the sucrose-inaccessible space at 1°C was studied by the 'inhibitor-stop' technique with 50mM-2-oxo-4-methylpentanoate as inhibitor. Fig. 4 demonstrates that pyruvate uptake appears to follow a first-order type of reaction similar to that shown for adenine nucleotide (Pfaff *et al.*, 1969), succinate (Quagliariello *et al.*, 1969) and citrate (Palmieri *et al.*, 1972) transfer across the mitochondrial membrane. At 1°C the first-order constant was estimated to be 1.66min^{-1} and the half-time of penetration 25s; this is about 25% faster than the translocation of the dicarboxylate succinate (Quagliariello *et al.*, 1969) and very considerably faster (estimated to be about four times at this temperature) than the tricarboxylate, citrate (Palmieri *et al.*, 1972).

Fatty acid–pyruvate exchange

If the monocarboxylate transporter transfers 3-hydroxybutyrate, it might be expected also to accept the 3-hydroxy acid carnitine as a substrate. Fig. 5(*a*) demonstrates that pyruvate does indeed exchange readily with carnitine, which, although not proof, would be consistent with this hypothesis. However, when palmitoylcarnitine was similarly tested it proved to be a very powerful exchanger of pyruvate, capable of displacing all the mitochondrial pyruvate at concentrations of palmitoylcarnitine an order of magnitude lower than other substrates, or than pyruvate itself, require (Fig. 5*b*). This displacement of pyruvate by palmitoylcarnitine can be prevented by 2-oxo-4-methylpentanoate (Fig. 5*b*) and appears therefore to

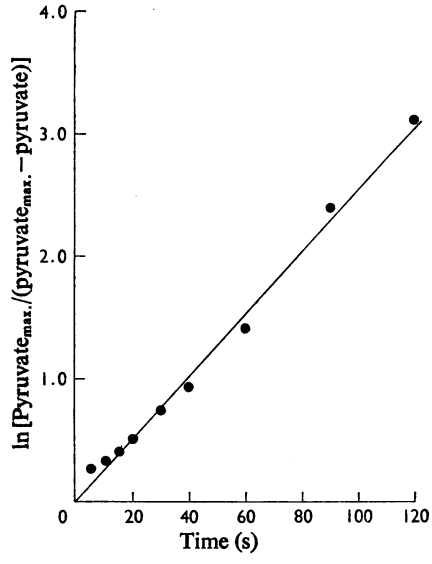

Fig. 4. *Semilogarithmic plot of pyruvate penetration with time into liver mitochondria demonstrating first-order-type kinetics*

$\ln[\text{Pyruvate}_{max.}/(\text{pyruvate}_{max.}-\text{pyruvate})] = kt$ where pyruvate$_{max.}$ refers to the intramitochondrial pyruvate content at equilibrium. The data are means from four mitochondrial preparations and the suspending [^{14}C]-pyruvate concentration was varied between 0.1 and 1.65mM. The pyruvate$_{max.}$ values correspondingly varied between 0.2 and 2.4nmol/mg of protein and the mean intramitochondrial volume was 0.96μl/mg.

involve the monocarboxylate-transport system. The physiological implications of this exchange are that it may provide an explanation for the effective preference which the liver and muscle cell shows for fatty acid over pyruvate as oxidizable substrate. Long-chain fatty acyl-CoA supplied to carnitine palmitoyltransferase I situated on the outside of the inner mitochondrial membrane (Brosnan *et al.*, 1973) will find there carnitine displaced by, for instance, pyruvate. The subsequent strong counter-transfer of pyruvate by the palmitoylcarnitine will result in lowered pyruvate oxidation. The carnitine released by the intramitochondrial carnitine palmitoyltransferase II will presumably be counter-transported for incoming palmitoylcarnitine, ensuring an external carnitine supply. It has been observed that there is a remarkable quantitative agreement between the entry of pyruvate and its expulsion by palmitoylcarnitine and the effects of these two mitochondrial substrates on the activation–inactivation cycle of the pyruvate dehydrogenase complex in liver mitochondria incubated *in vitro* (Mowbray, 1974). Thus the expulsion of pyruvate may have the further effect of allowing the control of the pyruvate dehydrogenase complex to reside solely in the ATP/ADP ratio (Wieland & Portenhauser, 1974) since control seems to reside in the pyruvate dehydrogenase kinase alone (Walajtys *et al.*, 1974).

Need for monocarboxylate transporters

The argument has been made (Klingenberg, 1970) that there may be no need of membrane transporters for monocarboxylates since they exist in sufficient

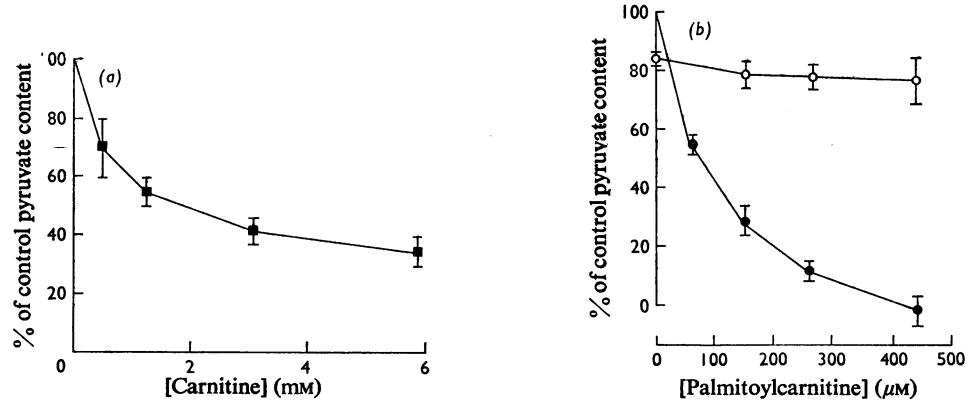

Fig. 5. *Effect of* (*a*) L-*carnitine* (■) *and* (*b*) DL-*palmitoylcarnitine* (●) *in the presence of* 2.2mM [^{14}C]*pyruvate on the intramitochondrial pyruvate content*

The mean initial pyruvate content was 1.43nmol/mg and the intramitochondrial space was 1.04μl/mg of protein. The result of adding 50mM-2-oxo-4-methylpentanoate before palmitoylcarnitine is shown (○).

proportion in the undissociated form at neutral pH. As such they are able to dissolve in membrane lipid and thus penetrate the non-aqueous barrier. This does not mean, however, that the effective free concentration of a given monocarboxylate is sufficient to provide an adequate transport rate by this mechanism. By using the total tissue concentrations in rat heart (Kraupp *et al.*, 1967), it would appear that more malate [for which a transporter is considered necessary; see Klingenberg (1970)] could exist in the undissociated form than pyruvate at pH 7. To ensure controlled membrane penetration, especially in an environment with competing binding sites, transporters may be required even for weakly dissociated monocarboxylates. Further, there seems some reason to believe that monocarboxylate transporters are to be found also in the plasma membranes of cells. In the perfused rat heart the penetration of pyruvate (Watts & Randle, 1967; Mowbray & Ottaway, 1973*b*) and the distribution of lactate between tissue and perfusate (Opie & Mansford, 1971; Mowbray & Ottaway, 1973*a*) has been found to be incompatible with free diffusion. Also competition to cross the blood–brain barrier has been shown between pyruvate and butyrate, propionate and L(+)-lactate (Oldendorf, 1973). Finally, Halestrap & Denton (1974) have observed that hydroxycyanocinnamate, a mitochondrial monocarboxylate-transport inhibitor, decreases the pyruvate and lactate content of human erythrocytes incubated in the presence of these substrates.

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References

Arinze, I. J. & Patel, M. S. (1973) *Biochemistry* **12**, 4473–4478

Bowden, J. A. & McArthur, C. L. (1972) *Nature (London)* **235**, 230

Brosnan, J. T., Kopec, B. & Fritz, I. B. (1973) *J. Biol. Chem.* **248**, 4075–4082

Bucher, Th., Czok, R., Lamprecht, W. & Latzko, E. (1963) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 253–259, Academic Press, New York and London

Chappell, J. B. & Crofts, A. (1966) in *Regulation of Metabolic Processes in Mitochondria* (Tager, J., Papa, S., Quagliariello, E. & Slater, E. C., eds.), pp. 293–314, Elsevier, Amsterdam

Clark, J. B. & Land, J. M. (1974) *Biochem. J.* **140**, 25–29

Halestrap, A. P. & Denton, R. M. (1974) *Biochem. J.* **138**, 313–316

Harris, E. J. & Berent, C. (1970) *FEBS Lett.* **10**, 6–12

Harris, E. J. & Manger, J. R. (1968) *Biochem. J.* **109**, 239–246

Johnson, R. N. & Chappell, J. B. (1973) *Biochem. J.* **134**, 769–774

Klingenberg, M. (1970) *Essays Biochem.* **6**, 119–159

Kraupp, O., Adler-Kastner, L., Niessner, H. & Plank, B. (1967) *Eur. J. Biochem.* **2**, 197–214

Land, J. M. & Clark, J. B. (1973) *Biochem. J.* **134**, 539–544

Land, J. M. & Clark, J. B. (1974) *FEBS Lett.* **44**, 348–351

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mowbray, J. (1969) Ph.D. Thesis, University of Edinburgh

Mowbray, J. (1974) *FEBS Lett.* **44**, 344–347

Mowbray, J. & Ottaway, J. H. (1973*a*) *Eur. J. Biochem.* **36**, 362–368

Mowbray, J. & Ottaway, J. H. (1973*b*) *Eur. J. Biochem.* **36**, 369–379

Oldendorf, W. H. (1973) *Amer. J. Physiol.* **224**, 1450–1453

Opie, L. H. & Mansford, K. R. L. (1971) *Eur. J. Clin. Invest.* **1**, 295–306

Palmieri, F., Stipani, I., Quagliariello, E. & Klingenberg, M. (1972) *Eur. J. Biochem.* **26**, 587–594

Pfaff, E., Heldt, H. W. & Klingenberg, M. (1969) *Eur. J. Biochem.* **10**, 484–493

Quagliariello, E., Palmieri, F., Prezioso, G. & Klingenberg, M. (1969) *FEBS Lett.* **4**, 251–254

Walajtys, E. I., Gottesman, D. P. & Williamson, J. R. (1974) *J. Biol. Chem.* **249**, 1857–1865

Watts, D. J. & Randle, P. J. (1967) *Biochem. J.* **104**, 51P

Wieland, O. H. & Portenhauser, R. (1974) *Eur. J. Biochem.* **45**, 577–588

Williamson, J. R. (1964) *Biochem. J.* **93**, 97–106