The Transport of Citrate and Other Tricarboxylic Acids in Two Species of *Pseudomonas*

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When cells of *Pseudomonas* are grown on citrate as the sole carbon source they oxidize citrate and isocitrate rapidly. Fluorocitrate inhibits the oxidation of citrate. Fluorocitrate-treated cells accumulate [6-¹⁴C]citrate, as shown by a rapid Millipore-filtration technique. In the absence of fluorocitrate most of the [6-¹⁴C]-citrate is lost in the form of ¹⁴CO₂. The isolation of a pseudomonad characterized by its ability to grow on tricarballoylate as a sole carbon source has facilitated the study of the tricarboxylate-carrier specificity. Cells grown on citrate will exchange radioactive citrate for unlabelled citrate or isocitrate but not for *cis*-aconitate, *trans*-aconitate or tricarballoylate. Cells grown on tricarballoylate will exchange radioactive citrate for unlabelled citrate, *cis*-aconitate or tricarballoylate, but not for isocitrate or *trans*-aconitate. The properties of the exchange system involved are compared with those of the related system in mitochondria.

The ability of intact cells of *Pseudomonas* to oxidize intermediates of the tricarboxylic acid cycle depends on the nature of the carbon source used for growth. Since cell-free extracts of such cells exhibit all the enzymic activities of that cycle, it has been inferred that the adaptive behaviour of the intact cells is due to the elaboration of specific inducible permeability factors (Kogut & Podoski, 1953; Barrett & Kallio, 1953; Clarke & Meadow, 1959). Villarreal-Moguel & Ruiz-Herrera (1969) have described the induction and properties of the citrate-transport system in *Aerobacter aerogenes*. Kay & Kornberg (1969) have described an inducible carrier system of *Escherichia coli* specifically involved in the uptake of the dicarboxylic acid intermediates of the tricarboxylic acid cycle. Direct measurement of the entry into bacterial cells of the tricarboxylic acid-cycle intermediates is difficult because of the rapidity with which the acids are metabolized (Davis, 1956). Using fluorocitrate as inhibitor, we have been able to decrease the ability of *Pseudomonas* to metabolize citrate such that measurable accumulation of radioactive citrate occurs in appropriately adapted cells. It is the purpose of this paper to report some of the characteristics of this accumulation process.

MATERIALS

All chemicals used were the purest possible (reagent grade). Propane-1,2,3-tricarboxylic acid (tricarballoylic acid) was obtained from Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A. [6-¹⁴C]Citrate (specific radio-

activity 5.14 mCi/mmol) was from New England Nuclear Corp., Boston, Mass., U.S.A. and [1,5-¹⁴C₂]citrate (specific radioactivity 22 mCi/mmol) was from Amersham/Searle, Don Mills, Ont., Canada. The fluorocitrate was obtained from Calbiochem, Los Angeles, Calif., U.S.A. as the barium salt of DL-fluorocitric acid. The barium salt was dissolved in 0.1 M-HCl and sufficient 2.5 M-H₂SO₄ was added until no further BaSO₄ precipitate was formed. The fluorocitric acid was neutralized with 6 M-KOH after centrifugation to remove the BaSO₄ precipitate. *trans*-Aconitic acid was obtained from Koch-Light Lab. Ltd., Colnbrook, Bucks., U.K. *threo*-D₃-Isocitrate, *cis*-aconitate, and L-malate were all from Sigma Chemical Co., St Louis, Mo., U.S.A. DL-Isocitrate was also purchased from Sigma Chemical Co. and was reported to be 43% D-isocitrate, and total isocitrate content was 96%. The potassium salts of all organic acids were used in all experiments.

METHODS

Growth of bacteria. *Pseudomonas fluorescens* (A.T.C.C. 13525) was purchased from the American Type Culture Collection and *Pseudomonas* sp. was isolated by using tricarballoylate as the carbon source in the selecting medium. The latter organism was identified as a strain of *Pseudomonas fluorescens* through the facilities of the Department of Bacteriology, Hospital for Sick Children, Toronto. This organism is currently being further characterized for subsequent deposition in the National Research Culture Collection, Ottawa, Ont., Canada. The isolation of fluorescent pseudomonads capable of growth on tricarballoylate has been previously reported by Gray & Brooke (1954) and Altekari & Rao (1963). The organisms were maintained on nutrient agar slants and for this study were grown in batch culture at room temperature in mineral salts Medium 63 (Sistrom, 1958) containing

the appropriate carbon source at 10 mM concentration. A wrist-action shaker was used to keep the culture aerobic. Growth was determined turbidometrically at 500 nm with a Gilford spectrophotometer and a cuvette of 10 mm light-path; 1.0 ml of culture at E_{500} 0.5 was equivalent to 0.1 mg of dry wt. and gave the same colour intensity as 75 μ g of bovine serum albumin as determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Oxygen consumption. Washed cells were suspended in 0.1 M-potassium phosphate buffer, pH 7. Oxygen utilization was measured polarographically at 22°C by using a Clark oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.).

Accumulation of [14 C]citrate. The cells were harvested by centrifugation during late exponential or early stationary phase and washed twice with 125 mM-KCl-20 mM-tris-HCl buffer, pH 7, and resuspended in the same buffer to E_{500} between 0.5 and 0.6. Unless otherwise indicated all uptake studies were done at 22°C (room temperature). Fluorocitrate was added to the cell suspension 3 min before the addition of the radioactive material. At zero time [14 C]citrate was added by syringe to the flask containing the cell suspension and 1.0 ml samples were removed at appropriate time-intervals thereafter. The cells were rapidly separated from the radioactive medium and collected on membrane filters (25 mm H.A.W.P., 0.45 μ m pore size; Millipore Corp., Montreal, P.Q., Canada). The membrane was washed with 2.0 ml of ice-cold buffer and was rapidly transferred to vials containing scintillation medium [0.4% (w/v) 2,5-diphenyloxazole; 0.01% (w/v) 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)-benzene; 25% (v/v) ethanol; 75% (v/v) sulphur-free toluene].

A standard channels ratio method was used to convert observed counting rates into disintegrations per min. A counting efficiency of 70% was consistently obtained using this scintillation medium to count the Millipore membranes.

Determination of $^{14}\text{CO}_2$. The procedure outlined by McElroy & Williams (1969) was followed. The washed

cell suspension (1.0 ml) was placed in the outer diffusion chamber of a Conway micro-diffusion dish. An insert containing 1.0 M-Hyamine hydroxide (10-X; Packard Instrument Co. Inc., Downers Grove, Ill., U.S.A.) was used to trap the $^{14}\text{CO}_2$ released from [14 C]citrate. The reaction was terminated by the addition of 0.2 ml of 3 M-HClO₄ by syringe through a small hole in the lid which was quickly resealed with plasticine. After a diffusion period of 3 h the glass inserts were removed and placed in counting vials containing 10 ml of scintillation solution [0.4% (w/v) 2,5-diphenyloxazole; 0.01% (w/v) 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in sulphur-free toluene]. The vials were shaken before counting to ensure complete solution of the centre well contents.

High-voltage electrophoresis and radioautography. Neutralized HClO₄ extracts of cells loaded with either [14 C]citrate or [$^{14}\text{C}_2$]citrate were spotted in 1 inch bands on Whatman 3MM chromatography paper and dried. Appropriate radioactive and unlabelled markers were also applied in separate bands. The dimensions of the sheets used were 57 cm long $\times$ 25.4 cm broad (accommodating five 2 in strips) and the origin was placed 13 cm from the cathode end. The buffer used for the electrophoresis was formic acid-acetic acid-water (1.7:1.7:100, by vol.) and the pH was adjusted to 3.1 with pyridine. Approx. 2000 V (170 mA) was applied for 2 $\times$ 1/2 h (J. L. Orr, personal communication). For radioautography, X-ray film (Eastman-Kodak Co.) was exposed to the dried chromatogram for 4 weeks. The marker strip was sprayed with Bromocresol Green and ninhydrin.

RESULTS

The ability of pseudomonads to oxidize tri-carboxylic acids is markedly dependent on the nature of the carbon source used for growth (Kogut & Podoski, 1953; Barrett & Kallio, 1953; Clarke & Meadow, 1959; Gray & Brooke, 1954; Altekar & Rao, 1963). Although the ability to oxidize L-

Fig. 1. Inhibition of citrate oxidation by DL-fluorocitrate. Washed cells of *P. fluorescens* A.T.C.C. 13525 (0.5 mg of protein) grown on citrate as sole carbon source were tested for respiratory inhibition in the presence of 400 μ M-DL-fluorocitrate (f-cit.) (final concentration). The cells were suspended in 1.5 ml of 0.1 M-potassium phosphate buffer, pH 7, and oxygen consumption was measured polarographically with a Clark oxygen electrode at 22°C. The final concentrations of added citrate (cit.), cis-aconitate (cis-ac.) and threo-D₅-isocitrate (threo-D₅-isocit.) were 1 mM.

Fig. 2. Evolution of $^{14}\text{CO}_2$ from $[6\text{-}^{14}\text{C}]$ citrate by citrate-grown cells of *P. fluorescens* A.T.C.C. 13525 and *Pseudomonas* sp. The cells were washed and resuspended in 125 mM-KCl-20 mM-tris-HCl, pH 7.4 (both cell types were adjusted to the same E_{500} 0.57). Cells were pre-incubated with DL-fluorocitrate for 3 min before the addition of $[6\text{-}^{14}\text{C}]$ citrate (sp. radioactivity 5.14 mCi/mmol) to a final concentration of $10\text{ }\mu\text{M}$. The solid symbols represent results obtained with *Pseudomonas* sp. and the open symbols those with *P. fluorescens* A.T.C.C. 13525. The experiment was conducted at 22°C . $\bullet$, $\circ$, In the absence of DL-fluorocitrate; $\blacksquare$, $\square$, final concentration of $80\text{ }\mu\text{M}$ -DL-fluorocitrate added; $\blacktriangle$, $\triangle$, $400\text{ }\mu\text{M}$ -DL-fluorocitrate added. $\times$, Both strains were grown on L-malate as sole carbon source and the evolution of $^{14}\text{CO}_2$ was determined in the absence of DL-fluorocitrate.

malate is possessed by cells grown either on L-malate or citrate, the cells grown on L-malate are unable to oxidize citrate. Cells grown in L-malate brought about a significant oxidation rate when DL-isocitrate was added. This does not occur if *threo*-D₂-isocitrate is used. Since the rapid uptake of oxygen when DL-isocitrate is added is only transitory, we attribute the effect to impurities in the DL-isocitrate preparation. This is in contradistinction to cells grown on a tricarboxylic acid source. The polarographic measurement of oxygen consumption is of considerable utility in obtaining information before the direct measurement of the uptake of radioactively labelled citrate into the cells. For example, Fig. 1 shows that the added fluorocitrate is effective in blocking the metabolism of citrate and *cis*-aconitate, but not that of isocitrate. The effectiveness of fluorocitrate as an inhibitor of aconitase is also shown in Fig. 2 where the evolution of $^{14}\text{CO}_2$ from

Fig. 3. Accumulation of $[6\text{-}^{14}\text{C}]$ citrate by washed cells of *P. fluorescens* A.T.C.C. 13525 grown on citrate. Stationary phase cells were washed and resuspended in 125 mM-KCl-20 mM-tris-HCl, pH 7.4, and adjusted to E_{500} 0.60. The final concentration of $[6\text{-}^{14}\text{C}]$ citrate (sp. radioactivity 5.14 mCi/mmol) added at zero time was $10\text{ }\mu\text{M}$. Final concentrations of DL-fluorocitrate were as follows: Δ , no DL-fluorocitrate added; $\square$, $40\text{ }\mu\text{M}$; $\blacktriangle$, $100\text{ }\mu\text{M}$; $\bullet$, $400\text{ }\mu\text{M}$; $\circ$, 2 mM . $\blacksquare$, Represents cells of the same strain grown on L-malate as a sole carbon source; uptake was measured in the presence of $400\text{ }\mu\text{M}$ -DL-fluorocitrate; the washed cells were adjusted to the same E_{500} as those grown on citrate.

$[6\text{-}^{14}\text{C}]$ citrate is measured. As would be expected, cells grown on L-malate as sole carbon source do not produce $^{14}\text{CO}_2$ from such labelled citrate. The effectiveness of fluorocitrate also appears to be strain-dependent, as shown in Fig. 2. Pertinent to this finding is a report by Kirk & Goldman (1970) of their isolation of a pseudomonad capable of growth on fluorocitrate. This organism will selectively degrade the inhibitory isomer of fluorocitrate. We have noted that cell-free extracts of our strains will reduce NADP when fluorocitrate is added. Differences in the fluorocitrate sensitivity may therefore reside in the differential capacity to metabolize this 'inhibitor'.

Although the block of citrate metabolism by externally added fluorocitrate is not complete, it is sufficiently effective that on addition of $[6\text{-}^{14}\text{C}]$ citrate 3 min after the inhibitor (see the Methods section) label accumulates in the cells (Fig. 3). The label is found solely as citrate (and isocitrate, which is not separated from citrate by high-voltage electrophoresis). The activity of isocitratase (EC 4.1.3.1) in this organism, grown on

citrate, must be sufficiently low that negligible amounts of $[6-^{14}\text{C}]$ citrate are converted into $[^{14}\text{C}]$ -succinate, etc. When $[1,5-^{14}\text{C}_2]$ citrate is supplied to the organism an additional spot appears on the radioautograph; this spot has the same mobility as the glutamate marker. The presence of significant amounts of label in the cells in forms other than citrate (and isocitrate) would make interpretations in terms of transport hazardous. All the experiments reported in this paper were therefore performed with $[6-^{14}\text{C}]$ citrate. When no fluorocitrate is administered to the cells only a small amount of label remains on the Millipore membrane after washing. For this reason we conclude that any $^{14}\text{CO}_2$ produced by the cells during uptake experiments does not remain associated with the filter. Fig. 3 further demonstrates that cells grown on L-malate as sole carbon source do not accumulate radioactive citrate and also that washing the Millipore filter effectively removes the extracellular radioactive citrate.

The retention of radioactive citrate does not occur in the presence of potassium cyanide (5 mM), 2,4-dinitrophenol (0.5 mM), or carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (100 μM).

The initial rate of accumulation of $[6-^{14}\text{C}]$ citrate has been studied as a function of citrate concentration. The Michaelis constant was calculated to be 33 μM (Fig. 4). This agrees well with the K_m value

Fig. 4. Double-reciprocal plot of citrate concentration and initial rate of uptake of citrate by *P. fluorescens* A.T.C.C. 13525 at 22°C in the presence of 400 μM -DL-fluorocitrate (final concentration). Details are given in the text.

(approx. 50 μM) observed in polarographic studies where the initial rate of oxygen consumption was measured as a function of citrate concentration.

If unlabelled citrate or certain other related compounds are added to cells that have accumulated $[6-^{14}\text{C}]$ citrate in this way, the radioactivity retained on the Millipore filters rapidly decreases. This is shown for A.T.C.C. 13525 cells in Fig. 5. The effect exhibits marked specificity; for instance, neither *cis*-aconitate nor tricarballylate would bring about this effect with these cells.

The strain of *Pseudomonas* sp. isolated on tricarballylate, however, possesses a different range of specificity for this phenomenon. When the organism is grown on citrate it is able to accumulate $[6-^{14}\text{C}]$ -citrate and the radioactivity retained falls rapidly on addition of unlabelled citrate. The process resembles that shown in Fig. 5 and, as in that case, tricarballylate is ineffective in decreasing the amount of retained $[6-^{14}\text{C}]$ citrate. If the cells are grown on tricarballylate they will not usually

Fig. 5. Citrate exchange from $[6-^{14}\text{C}]$ citrate-loaded cells of *P. fluorescens* A.T.C.C. 13525. Washed cells (E_{500} 0.60) were allowed to accumulate $[6-^{14}\text{C}]$ citrate (final concentrations of 10 μM - $[6-^{14}\text{C}]$ citrate (sp. radioactivity 5.14 mCi/mmol) and 400 μM -DL-fluorocitrate). Arrow indicates addition of unlabelled tricarboxylic acids. ▲, Control; ○, either *trans*-aconitate or tricarballylate; □, *cis*-aconitate; ●, citrate; Δ, *threo*-D₃-isocitrate. Final concentration of each tricarboxylic acid added was 1 mM.

Fig. 6. Citrate exchange from $[6-^{14}\text{C}]$ citrate-loaded cells of *Pseudomonas* sp. grown on tricarballoylate as sole carbon source. Washed cells (E_{500} 0.60) were allowed to accumulate $[6-^{14}\text{C}]$ citrate under conditions as described for Fig. 5. (Note the difference in scale of the ordinate when comparing this figure with Figs. 5 and 7). ▲, Control; ●, *threo*-D-isocitrate; □, *trans*-aconitate; ×, tricarballoylate; ■, *cis*-aconitate; ○, citrate. Final concentration of each tricarboxylic acid added was 1 mM.

accumulate $[6-^{14}\text{C}]$ citrate. Occasionally they will do so (e.g. Fig. 6), but will always do so if a low concentration of citrate (1 mM) is added to the growth medium (Fig. 7). Whether the ability to take up $[6-^{14}\text{C}]$ citrate is acquired with or without added citrate, such cells grown on tricarballoylate will now lose their radioactivity if tricarballoylate is added (Figs. 6 & 7) in marked contradistinction to cells grown solely on citrate.

For reasons mentioned in the Discussion section it was important to prove that the decrease in counts retained on the filter brought about by the addition of unlabelled tricarboxylic acids was in fact due to the egress of the counts in the form of citrate rather than conversion into $^{14}\text{CO}_2$. That the decrease is caused by the loss of $[6-^{14}\text{C}]$ citrate to the medium was demonstrated by taking cells that had been exposed to fluorocitrate for 3 min followed by $[6-^{14}\text{C}]$ citrate for 8 min. The cells were collected by centrifugation at 0°C and resuspended at 20°C in buffer containing fluorocitrate. Unlabelled tricarboxylic acid was added and at given time-

Fig. 7. Uptake and exchange of $[6-^{14}\text{C}]$ citrate from variously induced cells of *Pseudomonas* sp. All cultures were washed and resuspended to E_{500} 0.60. Cells were grown either on 10 mM-citrate (●) or on 10 mM-tricarballoylate (△) as sole carbon sources, or on 10 mM-citrate to which 1 mM-tricarballoylate was added (○), or on 10 mM-tricarballoylate to which 1 mM-citrate was added (▲). At 8 min after the addition of radioactive citrate, tricarballoylate (T) (1 mM final concentration) was added to each suspension, and, for the cells grown solely on citrate, citrate (C) (0.5 mM final concentration) was added after a further 4 min.

intervals cells and medium were separated as described in the Methods section. It was important that the resuspension was done in the presence of fluorocitrate; if the inhibitor were not present the counts retained decreased rapidly. This breakdown of fluorocitrate inhibition might be due to the loss of inhibitor from the cells or its metabolic conversion. Such experiments showed that a large proportion of the counts no longer retained in the cells could be accounted for by an increase in the radioactivity of the filtrate (Fig. 8).

DISCUSSION

The accumulation of citrate by *Pseudomonas* possesses two very marked characteristics. It is entirely dependent on the growth of the cells on appropriate substrates, and the ability of the cells to accumulate $[6-^{14}\text{C}]$ citrate in the presence of added fluorocitrate is induced by the same growth conditions that also permit, in the absence of fluorocitrate, the uptake of oxygen on the addition of citrate and production of $^{14}\text{CO}_2$ from $[6-^{14}\text{C}]$ citrate. The conclusions of Barrett & Kallio (1953), Clarke & Meadow (1959) and Kogut & Podoski (1953) that growth of *Pseudomonas* on citrate induces an ability to transport citrate receives direct confirmation from these results. The second characteristic of the process is its sensitivity to

Fig. 8. Citrate-grown cells of *P. fluorescens* A.T.C.C. 13525 were allowed to accumulate $[6-^{14}\text{C}]$ citrate under conditions described in Methods section. The cells were removed from the radioactive medium by centrifugation at 0°C and were quickly resuspended in 125 mM-KCl-20 mM-tris-HCl buffer, pH 7.4 at 20°C (final concentration of DL-fluorocitrate added to resuspension medium was $150\ \mu\text{M}$). Samples (1.0 ml) were separated by Millipore filtration at time-intervals indicated. The Millipore membranes were washed with 0.5 ml of ice-cold buffer and placed into the ethanolic scintillation medium described in the Methods section. The filtrates were collected into tubes containing 0.5 ml of 1.6 M- HClO_4 ; the filtrates were evacuated over KOH in a vacuum desiccator for 10 min to remove labelled carbon dioxide and then neutralized with 3 M- K_2CO_3 before adding a portion (500 μl) to a scintillator for counting. Arrow indicates the addition of tricarboxylic acid (1 mM final concentration). Open symbols on the dashed line represent radioactivity found in filtrates, solid symbols on the solid line represent radioactivity found on the membrane. $\blacktriangle$, $\triangle$, Citrate; $\bullet$, $\circ$, *threo*- D_3 -isocitrate; $\blacksquare$, $\square$, no tricarboxylic acid added. Note that loaded cells would retain the label if kept at 0°C after resuspension in the absence of fluorocitrate.

potassium cyanide and to classical uncouplers of oxidative phosphorylation. Effects of these inhibitors in bacterial systems are always difficult to interpret because of the uncertainties surrounding bacterial respiration and associated energy conservation, but it is probably safe to suggest that the bacterial citrate-transport system shows the same energy dependence that has been demonstrated for other bacterial permeases (Cohen & Monod, 1957).

In the experiments reported in this paper the inhibition of citrate metabolism by fluorocitrate

enables the measurement of the accumulation of radioactivity present solely as citrate, provided the radioisotope is confined to C-6. It should be recognized, however, that this approach contains two hazards, which must be borne in mind in interpreting the results. First, it is likely that fluorocitrate is an inhibitor or competitive substrate of the putative tricarboxylate-transport system. This is suggested by Fig. 3; in the presence of 2 mM-fluorocitrate the rate of uptake of citrate is significantly retarded. Secondly, the inhibition of citrate

metabolism is incomplete (Fig. 2) and the radioactivity found is therefore a measure of a steady-state concentration of citrate, not an equilibrium value. A decrease in the [6-¹⁴C]citrate found may therefore represent either the exit of citrate from the cells or an inhibition of entry. The experiments in Fig. 8 suggest that most, if not all, of the decrease in radioactivity is due to movement out of the cells and we have for this reason interpreted the results of Figs. 5 & 6 in terms of an outward flow of [6-¹⁴C]citrate. These experiments, however, have all been performed with cells having their walls intact. Until such time as the experiments are repeated with protoplasts it cannot be ruled out that some of the label is retained by the cells in the small compartment between the plasma membrane and the cell wall postulated by Winkler & Wilson (1966).

The outward movement of citrate in response to added tricarboxylate ions gives a clear indication of the specificity of the exchange system for these ions. In this respect particular interest attaches to the transport properties of the *Pseudomonas* sp. isolated in this laboratory and characterized by the ability to grow on tricarballoylate. When grown on citrate this organism will not exchange [6-¹⁴C]citrate for tricarballoylate and when grown on the latter compound as sole carbon source it will no longer consistently accumulate [6-¹⁴C]citrate. The ability of cells grown on tricarballoylate plus low concentrations of citrate to accumulate [6-¹⁴C]citrate and then to exchange it for tricarballoylate is a most significant finding. The fact that exchange occurs at all rules out the existence of a mixed population of cells some possessing a tricarballoylate 'carrier' and some a 'carrier' specific for citrate. On the other hand, the fact that cells grown on tricarballoylate alone do not usually accumulate [6-¹⁴C]citrate demonstrates that the tricarballoylate 'carrier' cannot transport citrate. In the doubly induced cells the exchange must take place at two distinct carriers and not by the type of 'revolving door' mechanism usually implied for antiport. The nature of the forces bringing about counter-movement under these circumstances is unclear but may concern either the necessity to preserve electroneutrality or displacement from internal binding sites.

A number of laboratories have carried out detailed studies of the carboxylate-anion-exchange carriers of the inner membrane of mitochondria. The system studied here appears to differ from the

mitochondrial system in two important respects. The exchange of [6-¹⁴C]citrate for other tricarboxylate anions is insensitive both to 2-ethylcitrate and benzene-1,2,3-tricarboxylate, which inhibit the tricarboxylate carrier of rat liver mitochondria (Robinson, Williams, Halperin & Leznoff, 1971). We have been unable to find any evidence for malate-citrate antiport, although this is a marked feature of mitochondrial tricarboxylate transport. Despite these differences it may well be that there are important analogies at the molecular level. If such analogies may be made, our results on the exchange of tricarballoylate and [6-¹⁴C]citrate would favour the models put forward by Harris & Manger (1968) over the strict antiport-exchange carrier model presently favoured for mitochondria (Robinson *et al.* 1971).

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