Biochem. J. (1976) **160**, 121–123
Printed in Great Britain

The Transport and Oxidation of Succinate by Ehrlich Ascites-Tumour Cells

By TERENCE L. SPENCER
Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, U.S.A.

(*Received* 2 *August* 1976)

The transport and oxidation of succinate by functionally intact Ehrlich ascites-tumour cells was investigated. On the basis of pH dependence and inhibitor sensitivity it was concluded that succinate may be transported across the cell membrane by the organic anion carrier system. Thus the ability of isolated Ehrlich cells to oxidize succinate is real, and is not necessarily a result of damage to cell integrity.

Increasing use has been made of isolated cell systems to study metabolic processes (Archakov *et al.*, 1974; Dubinsky & Cockrell, 1974, 1975; Kleineke & Stadtman, 1974; Otto & Ontko, 1974; Bhargava *et al.*, 1975; Stubbs & Krebs, 1975). Succinate has been used as an exogenous respiratory substrate in many of these studies despite the reported low permeability of intact systems to this dicarboxylic acid (Ross *et al.*, 1967; Hems *et al.*, 1968). Indeed, Mapes & Harris (1975) have concluded that oxidation of succinate observed in isolated rat hepatocytes may be attributed to a proportion of 'leaky' cells in the preparations. On the other hand Aubert & Motais (1975) and Babcock *et al.* (1975) have provided some evidence for a succinate 'permease' located in ox erythrocyte and bull spermatozoa plasma membranes respectively.

Experiments reported in the present paper have been designed to resolve the question: does succinate enter intact cells? Ehrlich ascites-tumour cells were chosen as the ideal test system for this investigation since they are not easily damaged and large numbers of viable cells may be obtained by mild procedures. Interference or spurious results due to the presence of damaged cells was therefore minimized.

Materials and Methods

Succinic acid and phenylsuccinic acid were from Sigma Chemical Co., St. Louis, MO, U.S.A. [2,3-^{14}C]Succinic acid was from Amersham/Searle Corp., Arlington Heights, IL, U.S.A. Other chemicals and materials were obtained as given in Spencer & Lehninger (1976). Detailed information on propagation and harvesting of the Ehrlich ascites-tumour cells, methodology involved in performing the transport experiments, preparation of samples for liquid-scintillation counting and assay procedures has been published previously (Spencer & Lehninger, 1976). O_2 consumption was measured as indicated in Landry & Lehninger (1976). All incubation media contained 150mM-NaCl, 5mM-KCl and either 10mM-Mes [2-(*N*-morpholino)ethanesulphonic acid], pH6.2, 10mM-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid], pH7.2, or 10mM-Tris, pH8.2, at a temperature of 37°C. Cells were added at a concentration of 5mg of protein/ml from a stock solution of 80mg/ml.

Results and Discussion

Fig. 1 shows the uptake of succinate, added at a concentration of 5mM, by respiration-inhibited Ehrlich ascites-tumour cells. The transport rate is highly dependent on the medium pH. Initial rates of entry are 9.0nmol/min per mg of protein at pH6.2 and 0.3nmol/min per mg of protein at pH7.2. The maximal extent of uptake of succinate at pH6.2 of 23nmol/mg of protein is equivalent to an intracellular concentration of 7.8mM or an internal/external concentration ratio of at least 1.6 (assuming an even distribution throughout the nucleus and other cytoplasmic organelles). No entry of succinate is observable at pH8.2, and indeed succinate which is taken up by the cells at pH6.2 may be discharged intact by increasing the external pH to 8.2, as shown in Fig. 1.

These results are reminiscent of properties shown by lactate, which is transported into and out of Ehrlich cells by a relatively non-specific monocarboxylic acid carrier (Spencer & Lehninger, 1976). Corroborating evidence for this suggestion is provided by the sensitivity of succinate transport to a variety of inhibitors, as shown in Table 1. All of these compounds, with the exception of the succinate analogue phenylsuccinate, which was not tested against lactate transport, are inhibitors of the lactate transport system at equivalent concentrations (Spencer & Lehninger, 1976).

Since succinate has been shown to enter Ehrlich ascites-tumour cells readily at pH6.2 (see Fig. 1) I decided to investigate cell respiration at this pH

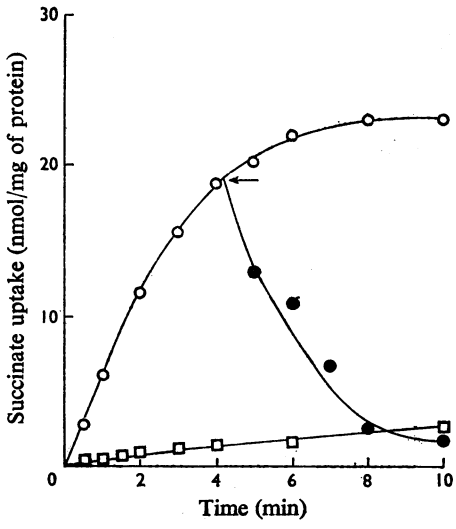

Fig. 1. *Time-course of uptake of succinate by Ehrlich ascites-tumour cells*

Cells were incubated and transport experiments performed as described in the Materials and Methods section in the presence of 5mM-[^{14}C]succinate. 2μM-Antimycin A was added to inhibit oxidation of the added substrate. ○, Uptake at pH6.2; □, uptake at pH7.2; ●, release at pH8.2 initiated by the addition of 2M-Tris base to a cell suspension previously preloaded at pH6.2 (arrowed).

and compare it with that observed at pH7.2 and 8.2. As shown in Fig. 2, addition of 3μM-rotenone, a potent inhibitor of the oxidation of NADH-linked substrates, decreases the respiration rate by approx. 96% in all cases. The further addition of 10mM-succinate at pH6.2 produces a ninefold increase in the rate of O_2 consumption, equivalent to 38% of that seen in the non-treated control cells. However, when similar amounts of succinate are added to cells incubated at pH7.2 and 8.2 the activations are

Table 1. *Inhibitors of succinate transport*

Cells were incubated as indicated in the legend to Fig. 1 at pH6.2 in the presence of 2μM-antimycin A, 5mM-[^{14}C]succinate and other additions as shown. Transport rates were determined after a 1min incubation.

Inhibitor	Transport rate (nmol/min per mg of protein)	Inhibitory effect (%)
None	9.3	—
2mM-Mersalyl	0.2	98
2.5mM-α-Cyano-4-hydroxycinnamate	2.2	76
10mM-Phenylsuccinate	5.0	46
20mM-4-Hydroxyphenyl-lactate	4.6	51
5μM-Nigericin	0.3	97

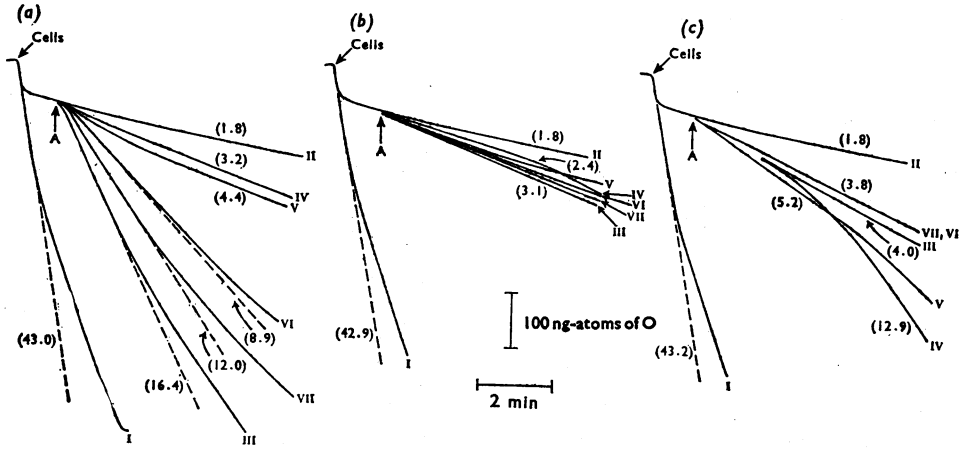

Fig. 2. *Effect of pH and inhibitors on the oxidation of succinate by Ehrlich ascites-tumour cells*

O_2 consumption by the cell suspension was measured as described in the Materials and Methods section in a total volume of 1.65ml and additions were made as indicated. (*a*) pH6.2; (*b*) pH7.2; (*c*) pH8.2. Rotenone was present at a concentration of 3μM in II–VII and 10mM-succinate was added to III–VII at point A. I–III, No further additions; IV, 1mM-mersalyl present; V, 5mM-α-cyano-4-hydroxycinnamate present; VI, 20mM-4-hydroxyphenyl-lactate present; VII, 10mM-phenylsuccinate present. All inhibitors were present from time zero. Numbers in parentheses represent calculated respiration rates in ng-atoms of O/min per mg of protein.

only 1.7- and 2.2-fold respectively. Mersalyl, α-cyano-4-hydroxycinnamate and 4-hydroxyphenyl-lactate inhibit the stimulatory effects of succinate at pH6.2 and 7.2, but not at pH8.2; the observed stimulatory effects of succinate at pH8.2 may be ascribed to a non-specific increase in membrane permeability under these conditions. This conclusion is based on the ineffectiveness of all inhibitors at this pH except phenylsuccinate, which is capable of inhibiting the mitochondrial dicarboxylate carrier.

It may be argued that the entry and oxidation of succinate added to Ehrlich ascites-tumour cell suspensions is due to a breakdown of the membrane structure and/or the presence of damaged cells. Numerous lines of evidence indicate that this is not true. The first is that the 'viability' of the Ehrlich cells as used in this study was found to be over 95% and usually over 98% as determined by using a dye-exclusion method. Secondly, the proportion of succinate-induced respiration that is insensitive to mersalyl inhibition, which could be taken as a reflection of the number of cells that are non-specifically permeable to succinate, is very small. These values are 3.4% $\{[(3.2-1.8)/(43.0-1.8)]\times 100\}$ and 1.4% $\{[(2.4-1.8)/(42.9-1.8)]\times 100\}$ at pH6.2 and 7.2 respectively (taken from data contained in Figs. 2*a* and 2*b*) (average = 2.4%). Thirdly, the transport and oxidation of succinate is inhibited by a variety of compounds of which only phenylsuccinate is an effective inhibitor of mitochondrial succinate oxidation. Fourthly, the highest rate of transport and oxidation is observed at an acidic pH, pH6.2, where the viability of cells (Penttila & Trump, 1975) and the stability of cell membranes (Dodge *et al.*, 1962) has been shown to be optimal.

In conclusion, the experiments reported herein show that succinate may indeed be transported into and oxidized by functionally intact cells, in this case Ehrlich ascites-tumour cells, under certain conditions, specifically the presence of a transmembrane H^+ gradient (alkaline inside). The entry of the dicarboxylic acid succinate is facilitated by the organic anion transport system which also transports substituted monocarboxylates (Spencer & Lehninger, 1976).

This work was supported by a contract (NO1-CP-45610) from the National Cancer Institute. I thank Dr. Albert L. Lehninger for advice in the preparation of this manuscript.

References

Archakov, A. I., Karyakin, A. V. & Skulachev, V. P. (1974) *FEBS Lett.* **39**, 239–242
Aubert, L. & Motais, R. (1975) *J. Physiol. (London)* **246**, 159–179
Babcock, D. F., First, N. L. & Lardy, H. A. (1975) *J. Biol. Chem.* **250**, 6488–6495
Bhargava, P. M., Siddiqui, M. A., Kranti Kumar, G. & Prasad, K. S. N. (1975) *J. Membr. Biol.* **22**, 357–368
Dodge, J. T., Mitchell, C. & Honahan, D. (1962) *Arch. Biochem. Biophys.* **100**, 119–130
Dubinsky, W. P. & Cockrell, R. S. (1974) *Biochem. Biophys. Res. Commun.* **56**, 415–422
Dubinsky, W. P. & Cockrell, R. S. (1975) *FEBS Lett.* **59**, 39–43
Hems, R., Stubbs, M. & Krebs, H. A. (1968) *Biochem. J.* **107**, 807–815
Kleineke, J. & Stratman, F. W. (1974) *FEBS Lett.* **43**, 75–80
Landry, Y. & Lehninger, A. L. (1976) *Biochem. J.* **158**, 427–438
Mapes, J. P. & Harris, R. A. (1975) *FEBS Lett.* **51**, 80–83
Otto, D. A. & Ontko, J. A. (1974) *Biochem. Biophys. Res. Commun.* **61**, 693–700
Penttila, A. & Trump, B. F. (1975) *Virchows Arch. B* **18**, 1–16
Ross, B. D., Hems, R. & Krebs, H. A. (1967) *Biochem. J.* **102**, 942–951
Spencer, T. L. & Lehninger, A. L. (1976) *Biochem. J.* **154**, 405–414
Stubbs, M. & Krebs, H. A. (1975) *Biochem. J.* **150**, 41–45