epithelial cells and found in the mucosal solution, mucosal tissue and serosal solution; 3–5% appeared as CO₂. The overall recovery was 94–99% of the glucose absorbed. Half of the CO₂, all of the lactate and all mucosal tissue glucose originated in glucose absorbed from the mucosal solution. The apparent specific radioactivity of glucose entering the serosal solution was 1.37-fold greater than that predicted; a model is proposed to account for these results (Porteous & Pritchard, 1972b).

The minimum free energy required to sustain observed transintestinal glucose and lactate concentration gradients is not more than 1% of the energy available from concomitant glucose catabolism. No obligatory coupling between this energy source and these solute concentration gradients is implied.

The lactate dehydrogenase activity of mucosa is 300-fold greater than the pyruvate dehydrogenase activity. Mucosal lactate dehydrogenase exhibits an isoenzyme pattern like that of skeletal muscle, unlike that of heart. We postulate that the preponderance of lactate dehydrogenase over pyruvate dehydrogenase, and the regulatory responses of a 'muscle-type' lactate dehydrogenase, together ensure the high rate of lactate production by intestine, despite the high O₂ concentration required to sustain concomitant glucose transport.

Iemhoff, W. G. J., van den Bergh, J. W. O., Pijper, A. M. & Hülsmann, W. C. (1970) *Biochim. Biophys. Acta* **215**, 229

Kimmich, G. A. (1970) *Biochemistry* **9**, 3659

McDougal, D. B., Little, Katherine D. & Crane, R. K. (1960) *Biochim. Biophys. Acta* **45**, 483

Porteous, J. W. & Pritchard, P. (1972a). *Biochem. J.* **127**, 61P

Porteous, J. W. & Pritchard, P. (1972b) *Biochem. J.* **127**, 80P

Carrier-Mediated Transport across the Erythrocyte Membrane: a Rigorous Test for the Simple Carrier Model

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The study of transport of non-electrolytes across the erythrocyte membrane shows that, in general, the substrate flux is related to the substrate concentration by a simple Michaelis-Menten equation. Values for V and K_m can be determined from several different types of kinetic experiments. These values of K_m present a serious problem of interpretation, since the same substrate can give different values of K_m from each type of experiment. The present communication presents an analysis, based on a simple carrier model, that predicts a definite relationship between the parameters from four different types of experiment.

In entry experiments the inward substrate flux into substrate-free cells is measured, and in exit experiments the outward flux from preloaded cells into substrate-free solution. Exchange experiments involve the measurement of inward fluxes with intracellular and extracellular substrate concentrations equal, and Sen-Widdas experiments (Sen & Widdas, 1962) measure the effect of extracellular substrate on the outward flux from a high and saturating intracellular substrate concentration.

Differences between K_m (entry) and K_m (exchange) can be explained by a simple carrier model with different reorientation rates for loaded and empty carriers (Levine *et al.*, 1965), and similar differences between K_m (entry) and K_m (exit) can arise from differences between carrier concentration or substrate affinity between the two membrane surfaces (Hoare, 1972). However, differences between K_m (Sen-Widdas) and other K_m values have been taken as evidence for a carrier molecule with two types of substrate-binding site with different affinities (Lieb & Stein, 1971).

In the present communication it is shown that the parameters from these four types of experiment are related by eqn. (1):

$$K_m \text{ (Sen-Widdas)} = \frac{K_m \text{ (exchange)}}{1 + \frac{V(\text{exit})}{V(\text{entry})} + \frac{V(\text{exit})}{V(\text{exchange})}} \quad (1)$$

provided that transport is mediated by a simple carrier. There may therefore be no need to postulate a carrier with different types of binding sites to account for K_m (Sen-Widdas) values. Experimental verification of eqn. (1) under a variety of conditions would provide strong support for the simple carrier model.

Hoare, D. G. (1972) *J. Physiol. (London)* in the press
Levine, M., Oxender, D. L. & Stein, W. D. (1965) *Biochim. Biophys. Acta* **109**, 151

Lieb, W. R. & Stein, W. D. (1971) *Nature New Biol. (London)* **230**, 108

Sen, A. K. & Widdas, W. F. (1962) *J. Physiol. (London)* **160**, 392

Malate Permeation as the Pacemaker for Exogenous Malate Metabolism in *Escherichia coli*

By I. D. HAMILTON and W. H. HOLMS (*Department of Biochemistry, University of Glasgow, Glasgow W.2, U.K.*)

When *Escherichia coli* strain ML308 is trained to glucose, glycerol or acetate as sole source of carbon and energy in simple defined media it will sub-

sequently grow on malate only after a period of adaptation. Glucose-trained cells have difficulty in adapting to malate, and on inoculation into glucose-malate media display diauxic growth in which malate is used after glucose. Glycerol-trained cells do not show diauxie in glycerol-malate media, but the degree of malate adaptation depends on the period of growth before glycerol is exhausted. Cells subcultured from glycerol into media containing an excess of malate and various limited amounts of glycerol grow on malate, after glycerol exhaustion, with different rates of growth, O_2 uptake and malate utilization. Whatever the rate of growth on malate, it is stimulated by addition of acetate.

In cells, otherwise physiologically competent, the possible pacemakers in malate utilization are: (1) permeation of malate into the cells; (2) manufacture of biosynthetic precursors derived from phosphoenolpyruvate; (3) production of energy or withdrawal of intermediates from the tricarboxylic acid cycle, which would depend on the production of acetyl-CoA from malate.

As acetate cannot be converted into phosphoenolpyruvate by these cells (Kornberg, 1966), it is unlikely that the conversion of malate into phosphoenolpyruvate limits growth on malate. The provision from malate of C_2 fragments for the tricarboxylic acid cycle depends on 'malic' enzyme to yield pyruvate and thence on pyruvate dehydrogenase to give acetyl-CoA. Measurements of these enzyme systems during adaptation to malate shows that they are not limiting malate metabolism.

It follows that the intracellular supply of malate limits growth and that adaptation to malate depends on synthesis of the mechanism for malate permeation (Kay & Kornberg, 1971). Even maximal malate utilization is limited by permeation, and acetate stimulation reflects a greater total flux of carbon fragments into the cell.

Kay, W. W. & Kornberg, H. L. (1971) *Eur. J. Biochem.* **18**, 274

Kornberg, H. L. (1966) *Essays Biochem.* **2**, 1

Preliminary Separation of Enzymes Extracted from *Bacillus stearothermophilus*

By A. ATKINSON, B. W. PHILLIPS, D. S. CALLOW, W. R. STONES and P. A. BRADFORD (*Microbiological Research Establishment, Porton, nr. Salisbury, Wilts., U.K.*)

A method has been developed for large-scale enzyme preparation from *Bacillus stearothermophilus*, giving extracts suitable for the eventual isolation of hundred-milligram quantities of several pure enzymes.

A 5 kg batch of *B. stearothermophilus* cell paste

(Sargeant *et al.*, 1971) was suspended in 5 litres of 0.1M-imidazole buffer, pH 8.0, and disrupted by a single pass at 40 litres/h through a Manton-Gaulin homogenizer at 550 kg/cm² (J. Melling, A. Atkinson, B. W. Phillips & W. R. Stones, unpublished work). Then 20 mg of deoxyribonuclease (300 units/mg; BDH Chemicals Ltd., Poole, Dorset, U.K.) was added and the suspension was left at 4°C for 30 min. Cell debris was removed by centrifugation at 23 000 g for 45 min and washed with 2 litres of 0.02M-imidazole buffer, pH 7.0.

The combined supernatants (conductivity 3.5 mmho; pH 7.0 ± 0.2) were then added to 5 kg of DEAE-cellulose (conductivity 8 mmho; pH 7.0) and left for 30 min at 4°C after mixing. Unbound protein was removed and DEAE-cellulose collected by centrifugation in a spin-drier. Batch elution of enzymes was accomplished with stepwise increases in NaCl concentration, with two washes with 6 litres of buffer at each stage, by using a spin-drier to remove the ion-exchanger. The 0.1M-, 0.5M- and 1.0M-NaCl fractions were obtained. The last was treated with ethanol (2.0 vol.) at 4°C to precipitate tRNA, which was further purified by reprecipitation with ethanol. The 66%-(v/v)-ethanol supernatant contained the enzyme triose phosphate isomerase (Fahey *et al.*, 1971), which was adsorbed on hydroxyapatite.

The unbound protein fraction and the 0.1M-NaCl fraction together contained 40% of the total phosphofructokinase activity, free from alcohol dehydrogenase. The 0.5M-NaCl fraction contained glyceraldehyde 3-phosphate dehydrogenase, alcohol dehydrogenase, aminoacyl-tRNA synthetases, trans-formylase, fructose diphosphate aldolase and 60% of the total phosphofructokinase activity. With the exception of the last the recovery of each enzyme in this fraction was 90% of the combined supernatant activity with a purification factor of 2.5, and 70% of the broken-cell activity. The 66%-(v/v)-ethanol supernatant from tRNA precipitation contained 60% of the triose phosphate isomerase activity of the combined supernatant with a purification factor of 40. The tRNA (total yield 7g), which contained little protein or DNA, had a methionyl acceptor activity of 50 pmol/ E_{260} unit.

Further purification of these enzymes and of tRNA from these extracts has been carried out in the Medical Research Council Laboratory of Molecular Biology, Cambridge, U.K., by Dr. C. J. Bruton, Dr. B. F. C. Clark, Dr. R. C. Fahey, Dr. J. I. Harris, Dr. R. Jack, Dr. E. Kolb and Dr. G. Koch.

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Fahey, R. C., Kolb, E. & Harris, J. I. (1971) *Biochem. J.* **124**, 77 P