APPENDIX

Labelling of liver glutamate by [1-¹⁴C]- and [2-¹⁴C]-pyruvate as a measure of oxaloacetate–fumarate disequilibrium in the mitochondria

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Symbols are defined as follows (see also abbreviations in the main paper).

Z , the C3/C2 ratio of label in glutamate from C2-pyruvate during the first passage of label round the citrate cycle. It measures oxaloacetate–fumarate disequilibrium in the mitochondria.

M , the measured ratio of glutamate label: (by C1-pyruvate)/(by C2-pyruvate).

$F1$, the measured fraction of glutamate label from C2-pyruvate that was in the C1 position.

$F5$, the measured fraction in the C5 position.

x , the fraction of label transferred via fumarase from C2 to C3 or C1 to C4 in oxaloacetate.

Scheme A1 shows the distribution of glutamate label during the first two turns of the citrate cycle when 1 unit of label is introduced into oxaloacetate via C1- or C2-pyruvate, and y units of label enter C5-glutamate via the pyruvate dehydrogenase reaction. (No label enters by the latter route from C1-pyruvate.) The factor f is the fraction of C3-label in glutamate that has returned to the same position after one turn of the cycle by the time the liver specimen was taken.

Inspection of Scheme A1 shows that:

$$Z = (1 - x)/x \quad (1)$$

$$M = x/(\text{total label}) \quad (2)$$

$$F1 = f(x + y)/(\text{total label}) \quad (3)$$

$$F5 = y/(\text{total label}) \quad (4)$$

$$\text{Total label from C2-pyruvate} = 1 + y + f(2 - x + y) \quad (5)$$

Substitution for x and y from eqns. (2) and (4) in (3) gives:

$$f = F1/(M + F5) \quad (6)$$

whence f can be calculated from experimental quantities. Then substitution of x and y from eqns. (2) and (4) into eqn. (5) and rearrangement give:

$$\text{Total label} = (1 + 2f)/[1 + f \cdot M - (1 + f) \cdot F5] \quad (7)$$

whence x can be calculated by eqn. (2) and Z by eqn. (1).

In the experiments reported in the main text, $f = 0.025$, corresponding in all to the recycling of 0.05 of the C3-label. The kinetics of recycling are such that the fraction recycled more than once would have been less than 0.05², a negligible amount.

The treatment above implies infinitely rapid turn-over of mitochondrial malate and fumarate. The effects of finite rates can be calculated (Heath & Phillips, 1972). Their computations have been extended to estimate the dependence of the effects on the fractions of the cellular malate that is mitochondrial and of cellular glutamate that becomes labelled (to allow for effects of possible compartmentation), and the effects of biphasic uptake of pyruvate by the liver, very fast from the arterial blood, slower and delayed from the portal blood (Okajima *et al.*, 1981). In summary, it was found that, if the liver specimen was taken 1.5 min after injection of pyruvate, the value of the ratio would differ by less than 3% from that if malate and fumarate turnover was infinitely fast, provided

Scheme A1. Distribution of label from C1- and C2-pyruvate in glutamate during the first and second turns of the citrate cycle from the entry of label into oxaloacetate

Abbreviations are as in Scheme 2 of the main paper. Symbols are as defined in this Appendix.

that no more than half the malate was mitochondrial, at least 25% of the glutamate became labelled and uptake from the arterial supply was in the range 20–80% of the total uptake.

When the rate of malate–oxaloacetate exchange, ROM (Scheme 1 of the main paper), was calculated, it was assumed that there was a loss of malate label by transport to the cytosol at rate $RFO \times (S.R. \text{ malate})$, but that negligible label returned within 1.5 min of injection, since label once

transported to cytosolic malate would be nearly all either incorporated into glucose or held up in transit through cytosolic aspartate and fumarate.

References

- Heath, D. F. & Phillips, J. C. (1972) *Biochem. J.* **127**, 453–470
Okajima, F., Chenoweth, M., Rognstad, R., Dunn, A. & Katz, J. (1981) *Biochem. J.* **194**, 525–540