- Plaut, B. and Knowles, J. R. (1972) *Biochem. J.* **129**, 311–320
- Segel, I. H. and Martin, R. L. (1988) *J. Theor. Biol.* **135**, 445–453
- Selwyn, M. J. (1993) *Biochem. J.* **295**, 897–898
- Topham, C. M. and Brocklehurst, K. (1992) *Biochem. J.* **282**, 261–265
- Varón, R., García-Moreno, M., Garrido, C. and García-Canovas, F. (1992) *Biochem. J.* **288**, 1072–1073

Received 22 April 1993

Note: Varón and colleagues were given the opportunity to respond to the above Letters, but no acceptable reply has been received.

Differences in 2-oxoglutarate dehydrogenase regulation in liver and kidney

A paper with the above title by Smith et al. (1992) recently appeared in the *Biochemical Journal*. Its purpose was to seek an explanation for the different responses to acidosis of the liver, where gluconeogenesis and ammonia production for urea synthesis are decreased, compared with the kidney, where ammonia production and gluconeogenesis from glutamine are increased (see Smith et al., 1992). Its major conclusion was that this could be due to the differential pH-sensitivity, within these tissues, of what may be the key rate-controlling enzyme, the 2-oxoglutarate dehydrogenase complex (2-OGDH). This itself, on the basis of the data presented, was proposed to be a consequence of the different matrix environments of liver compared with kidney mitochondria. Thus it was proposed that the inherent H⁺-sensitivity of 2-OGDH (see below) in liver mitochondria is masked by the pH-sensitivity of glutamate-oxaloacetate transaminase (GOT), which is more active in liver than in kidney, and also the glutamate-aspartate carrier. In contrast, in kidney mitochondria the inherent pH-sensitivity of the enzyme is able to be expressed (Smith et al., 1992).

Although this is an attractive proposition, there are, however, a number of earlier reports (see below) which offer evidence that may be contradictory to these findings, or which offer alternative approaches with which different results may have been obtained. Indeed, it is surprising that these earlier reports, which clearly contain material very relevant to this work, and often contain precedent findings, were not quoted in Smith et al. (1992).

To begin with, Smith et al. (1992) eliminated the possibility that the extracted 2-OGDH activities from the two different tissues have different pH- or Ca²⁺-regulatory properties. (Ca²⁺ regulation is also thought to be important in mediating hormonal effects on ammonia production and gluconeogenesis in both tissues; increases in either [Ca²⁺] or [H⁺] within their physiological ranges each activate the enzyme by decreasing its *K_m* for 2-oxoglutarate.) However, this information could have been obtained from the literature, where extensive studies of the regulatory properties of both liver (McCormack, 1985) and kidney (McCormack et al., 1988) 2-OGDH have been described, and in which they were shown to be essentially similar to those found with the initially characterized pig heart enzyme (McCormack and Denton, 1979).

The second major finding of Smith et al. (1992), and on which their hypothesis is largely based, is that, whereas in intact kidney mitochondria increases in H⁺ concentration still stimulated the enzyme, in their studies with intact liver mitochondria they were unable to demonstrate any activation of the enzyme by increasing H⁺ concentration, and in fact reported some inhibition. Their third approach was then to measure transport and GOT activities, and hence, together with the apparent inability of H⁺ to regulate

2-OGDH within the intact liver mitochondrial matrix, to arrive at the conclusions outlined above.

However, a first criticism to be raised is that Smith et al. (1992) used a rather indirect method of calculating 2-OGDH flux (see their paper). In contrast, using a direct assay in which ¹⁴CO₂ production from 2-oxo[1-¹⁴C]glutarate was monitored, McCormack (1985) demonstrated that the enzyme did show pH-sensitivity when located within intact liver mitochondria. Although the pH differences (7.2 versus 7.0) and incubation conditions were different from those used in Smith et al. (7 versus 6.6), this contrary finding must surely put in doubt the major conclusions of the latter work, or at least the work should maybe be repeated using the more direct assay system and perhaps also more suitable incubation conditions (see below).

McCormack (1985) also obtained similar results using the ¹⁴CO₂ technique, but with [1-¹⁴C]glutamate, except that the observed *K_m* values were around 5 times those obtained with 2-oxoglutarate. This would be consistent with the expected physiological equilibrium status of these two substrates through glutamate dehydrogenase. However, this finding is again at variance with the suggestion by Smith et al. (1992), based on their data and methodology with exogenous glutamate, where a preference for this as a substrate was shown, that 2-oxoglutarate generated by transamination has preferential access to 2-OGDH.

Smith et al. (1992) also reported modest activatory effects of Ca²⁺ on 2-OGDH in intact mitochondria from both tissues under some incubation conditions. Again, it is surprising that earlier reports were not quoted, where very marked demonstrations of the Ca²⁺-sensitivity of this enzyme, and indeed the other two matrix Ca²⁺-sensitive dehydrogenases (NAD⁺-isocitrate dehydrogenase and pyruvate dehydrogenase) were fully described within intact liver (McCormack, 1985; Johnston and Brand, 1987) and kidney (Tullson and Goldstein, 1982; McCormack et al., 1988) mitochondria under many different conditions. Another unquoted work of relevance was the observations by Johnston and Brand (1989) that Ca²⁺ also stimulated citrulline synthesis by intact liver mitochondria.

Consultation of these early works and adoption of some of the approaches used could clearly have helped the work of Smith et al. (1992) and may have led to different conclusions. For instance, the latter workers could observe no effects of Ca²⁺ in the presence of ADP or using the oxygen electrode, and therefore concluded that at high ADP/ATP ratios neither Ca²⁺ nor H⁺ could regulate the enzyme. In contrast, McCormack et al. (1988) clearly demonstrated effects of Ca²⁺ using the oxygen electrode and other techniques with kidney mitochondria incubated with ADP, although McCormack (1985) did report difficulty in demonstrating such effects with liver mitochondria under these conditions; nevertheless, effects were clearly evident under other conditions using several other techniques, including a 'State 3.5' respiration condition.

However, Johnston and Brand (1987) were able to show clear effects of Ca²⁺ on liver mitochondrial ADP-stimulated respiration, by simply using an oxygen electrode, and with flux through all three of the matrix Ca²⁺-sensitive dehydrogenases. The critical difference here was that the temperature of incubation was the physiological 37 °C, and this clearly influences what step becomes rate-limiting for the overall process. The experiments of Smith et al. (1992) were carried out at 28 °C, and it is important to ask what would have been found, and what would be the resultant hypothesis, if they had been carried out at 37 °C. It may also be useful to make further use of the observations of Johnston and Brand (1987) on oxygen uptake with isocitrate as substrate, as NAD⁺-isocitrate dehydrogenase is also regulated by pH in a similar manner to 2-OGDH (McCormack and Denton, 1981),

but would not be influenced by GOT or the glutamate–aspartate carrier, and thus could be used to assess the specificity of the purported effects of these enzymes in masking the inherent pH-sensitivity of 2-OGDH.

There also appear to be some discrepancies within the data of Smith et al. (1992), which could influence the conclusions drawn. As noted, the effects of increasing $[Ca^{2+}]$ or $[H^+]$ on 2-OGDH are brought about by their causing decreases in its K_m for 2-oxoglutarate. On p. 821 of Smith et al. (1992), it is argued that the V_{max} , obtained from O_2 -uptake experiments for liver mitochondria (17.9 nmol/min per mg of protein) is a valid estimate of maximal flux through 2-OGDH in intact mitochondria. Even if the maximal flux that could be achieved were nearer that found with extracted mitochondria (about double this value), it is still difficult to reconcile this with the data in Figure 2 of Smith et al. (1992), where in State 3 conditions rates of around 25–35 nmol/min per mg of protein are shown, but where it is claimed that the concentrations of 2-oxoglutarate (0.5 mM, with 1 mM glutamate) chosen were sub-saturating (and no effects of Ca^{2+} or H^+ were seen). It would obviously be of interest to see if effects would be observed at lower 2-oxoglutarate concentrations which were more clearly within the K_m range.

Overall, therefore, although the proposition of Smith et al. (1992) is attractive and apparently backed up by their data, there are earlier data which contradict many aspects of the work, and which also provide alternative means and conditions for measuring the desired parameters, which may yield different results and hence conclusions. Until some of the points raised are answered, there thus remains some doubt over the conclusions obtained by Smith et al. (1992).

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- Johnston, J. D. and Brand, M. D. (1987) *Biochem. J.* **245**, 217–222
 Johnston, J. D. and Brand, M. D. (1989) *Biochem. J.* **257**, 285–288
 McCormack, J. G. (1985) *Biochem. J.* **231**, 581–595
 McCormack, J. G. and Denton, R. M. (1979) *Biochem. J.* **180**, 533–544
 McCormack, J. G. and Denton, R. M. (1981) *Biochem. J.* **196**, 619–624
 McCormack, J. G., Bromidge, E. S. and Dawes, N. J. (1988) *Biochim. Biophys. Acta* **934**, 282–292
 Smith, B. C., Clotfelter, L. A., Cheung, J. Y. and LaNoue, K. F. (1992) *Biochem. J.* **284**, 819–826
 Tullson, P. and Goldstein, L. (1982) *FEBS Lett.* **150**, 197–200

Received 26 March 1993

Differences in 2-oxoglutarate dehydrogenase regulation in liver and kidney: a reply

McCormack (1993) raises several concerns regarding our recently published work (Smith et al., 1992), on the basis that his earlier data contradict certain aspects of our work. He suggests that we should repeat our studies using the alternative conditions of the earlier studies in order to substantiate our conclusions.

We do not believe that the earlier studies contradict our recent work. The fact that alternative conditions were used in those studies does explain some differences in results, while in fact leaving our conclusions intact. Fortunately, in some respects, the earlier data tend to confirm our conclusions.

Our protocols have the advantage that they provide more detailed information than those of previous studies. We directly compared liver and kidney mitochondria under identical conditions where both were exposed simultaneously to low, non-saturating, concentrations of 2-oxo[U- ^{14}C]glutarate (2-OG) and [3H]glutamate.

We measured 2-OG flux by two methods which were in good agreement with each other. One analysed changes in 2-OG and aspartate to obtain the sum disappearance of 2-OG, and the other measured the sum of the ^{14}C and 3H products. The $^{14}C/^3H$ ratio of the products indicated whether the dehydrogenase was using 3H -labelled 2-OG generated inside the mitochondria or ^{14}C -labelled 2-OG transported across the membrane on the 2-OG transporter. Aspartate production provided a measure of the rate of [3H]2-OG appearance in the matrix space. Exchange of [^{14}C]2-OG for [3H]2-OG in the media gave information about minimal rates of transport.

First, McCormack (1993) criticizes us because we did not quote previous data (McCormack, 1985; Johnston and Brand, 1987) on the kinetic parameters of the liver and kidney 2-OG dehydrogenase (2-OGDH), but instead measured the effect of Ca^{2+} and H^+ on the K_m of both enzymes in permeabilized mitochondria ourselves. It appeared reasonable to us to evaluate the activities of these two enzymes, under identical conditions in the same laboratory, since the main theme of the paper was a direct comparison. In fact, it is necessary, since neither McCormack (1985) nor Johnston and Brand (1987) reported the effect of pH on the K_m of the liver enzyme. McCormack (1985) did report the effect of pH on flux at a single 2-OG concentration. At saturating concentrations of Ca^{2+} and 0.1 mM 2-OG, he found that a shift from pH 7.0 to 7.2 caused a 42% increase in flux through the enzyme. The focus of his paper was on the control by Ca^{2+} , not by pH. There is no information at all about the effect of pH on kinetic parameters in Johnston and Brand (1987). Data concerning the influence of pH on the kidney enzyme are more complete (McCormack et al., 1988).

Table 3 of McCormack (1985) has the only data in the entire paper which relate to the effect of pH on 2-OG oxidation in intact mitochondria. A 50% decrease in K_m of external 2-OG is observed when pH changes from 7.3 to 7.1 independent of Ca^{2+} . The change in K_m with pH is small compared with the Ca^{2+} -induced change, and even small when compared with that produced by replacing 0.5 mM malate with 0.5 mM malonate in the medium. We do not refute these observations, since the conclusion drawn from our paper (Smith et al., 1992) was that 2-OG generated inside the mitochondria by transamination is the preferred substrate of the dehydrogenase, and the preference for transaminase-generated 2-OG gives the flux through 2-OGDH the same pH-sensitivity as the transaminase (but only when the transaminase is active).

In a recent preliminary report (Smith and LaNoue, 1992), we show that adding an inhibitor of transamination (amino-oxy-acetic acid) to the incubation media reverses the pH-sensitivity of the enzyme flux in the liver but not in the kidney, just as our hypotheses would predict. Thus, the results of McCormack (1985), studying $^{14}CO_2$ produced during incubation with [^{14}C]2-OG, are in good agreement with our hypotheses.

Although McCormack (1993) states that he found similar results with [^{14}C]glutamate, we could find no data in his earlier paper (McCormack, 1985) on the effect of pH on oxidation of glutamate. The data reported on glutamate are in the text and in Table 4. The text states: "The stimulation by Ca^{2+} of the rate of $^{14}CO_2$ production from [^{14}C]glutamate could also be observed by this technique, although the K_m values for glutamate were approximately five times those observed for 2-oxoglutarate