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An n.m.r. study of the tricarboxylic acid cycle

Jans *et al.* (1989) report a study by n.m.r. of the metabolism of [3-¹³C]citrate by rabbit renal-proximal tubules incubated in a Ringer bicarbonate medium. They claim to calculate from the enrichment of carbons in glucose and amino acids the relative flux rates of glutamate dehydrogenase, glutamine synthase and pyruvate kinase (relative to gluconeogenesis). Obviously, the synthesis of glutamate and glutamine from oxoglutarate requires ammonia. None was added, and there was virtually no endogenous ammonia in the cells. What the authors observed was labelling of cell glutamate by an exchange reaction catalysed by transaminase between the large glutamate pool and oxoglutarate. Glutamine labelling also represents an exchange reaction. In tubules, as in most cells, the concentration of glutamate and glutamine exceeds greatly that of the tricarboxylic acid cycle intermediates, and the extent of labelling reflects largely the size of the intracellular amino acid pool and is not a measure of flux.

In Table 1 the authors present a number of equations to calculate flux rates from the labelling patterns in glucose and amino acids. They report that the ratio of labelling in C-5+C-2/C-1+C-6 in glucose is 2.3 and equals the labelling ratio of C-2/C-3 in oxaloacetate. As is well known, the incorporation of C-3 of citrate into malate and oxaloacetate proceeds via fumarate, which causes complete equilibration of C-2 and C-3 of these acids; they are equally labelled and the ratio is 1. How the authors obtained their value of 2.3 is obscure.

The authors claim to measure the rate of pyruvate kinase from the labelling ratio of C-2/C-3 in alanine. The rate of pyruvate cycling (not 'pyruvate kinase flux') may be measured from such a ratio with [2-¹⁴C]- or [2-¹³C]pyruvate as substrate, but never from [3-¹³C]citrate. As mentioned above, the flux of glutamate dehydrogenase and glutamine synthase is virtually zero, rather than 1.2 times the rate of glucose synthesis, as claimed by the authors.

The labelling patterns in the tricarboxylic acid cycle intermediates, glucose and glutamate were worked out with ¹⁴C substrates in the 1950s (Strisower *et al.* 1952; Katz & Chaikoff, 1955). Recent, more complete quantitative analyses of labelling patterns in tricarboxylic acid

Strisower, E. H., Kohler, G. D. & Chaikoff, I. L. (1952) *J. Biol. Chem.* **198**, 115–123

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The fate of [3-¹³C]citrate in renal cells monitored by ¹³C n.m.r.

Usually, ¹³C-n.m.r. studies on metabolism and metabolic fluxes in cells and perfused organs are carried out by using ¹³C-labelled substrates like alanine, lactate, glucose, or pyruvate [1–4]. In a recent ¹³C-n.m.r. study, we showed the application of the new substrate [3-¹³C]citrate for metabolic studies of rabbit renal proximal convoluted tubular cells [5]. This study showed a labelling pattern of the metabolic intermediates which is rather different from the labelling pattern observed in experiments with [3-¹³C]pyruvate [6]. This discrepancy led to some comments by Katz [7].

Initially we have to discuss what type of information can be obtained from the ¹³C-n.m.r. spectrum as represented in Fig. 1. First, the relative enrichments of glutamate and glutamine reflect the labelling pattern of intermediate 2-oxoglutarate, which is formed from the substrate [3-¹³C]citrate. Thus, the ¹³C-labelled glutamate pool reflects the efflux of ¹³C label from the tricarboxylic acid cycle into the glutamate pool. In this sense, 'efflux' is related to the fraction of 2-oxoglutarate which is transformed into glutamate and glutamine, where we cannot determine the exact pathways involved in this process. In addition, it should be noted that the natural abundance contribution of the glutamate pool in control spectra is too low to be detected. Therefore, we exclude a simple isotopic exchange as being responsible for this labelling.

Second, the ratio of 2.8 for the C-3 and C-2 of the glutamate pool is the consequence of formation of C-3-labelled glutamate and glutamine from [3-¹³C]citrate, redistribution via the tricarboxylic acid cycle, and a subsequent equalization of label distribution at the stage of succinate, resulting in an equal labelling of C-2 and C-3 of the glutamate pool after each turn of the cycle.

Third, rather remarkable is the unequal C-2,5/C-6,1 labelling of glucose in the ratio of 2.3, which equals the labelling of C-2/C-3 in oxaloacetate. This is in contrast to experiments with [3-¹³C]pyruvate, where a ratio of 1 can be expected.

The higher pool of intermediate [2-¹³C]oxaloacetate can only be explained in terms of a reversible oxaloacetate + acetyl-CoA → citrate reaction under our experimental conditions. This conclusion is confirmed by the observation that the ratio Ala C-2/Ala C-3 is determined as 2.23, indicating a higher pool of [2-¹³C]oxaloacetate. This is not so strange in view of the use

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Jans, A. W. H., Winkel, C., Buitenthuis, L. & Lugtenberg, J.
(1989) *Biochem. J.* **257**, 425–429

Katz, J. (1985) *Am. J. Physiol* **248**, R391–R399

Katz, J. & Chaikoff, I. L. (1955) *Biochim. Biophys. Acta* **18**,
87–101

Malloy, C. R., Sherry, A. D. & Jeffrey, F. M. H. (1988) *J. Biol. Chem.* **263**, 6964–6971