USE OF $^{13}$C NMR FOR INVESTIGATION OF ETHANOL METABOLISM IN PERFUSED LIVER

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ABSTRACT

Time courses of $^{13}$C labels from alanine or lactate and ethanol in perfused mouse livers have been followed by NMR. The enrichment at specific carbons of glucose, glutamate, glutamine, aspartate, acetate, acetoacetate, $\beta$-hydroxybutyrate and lactate has been measured. The specific labeling of glutamate in the presence of labeled alanine and labeled or unlabeled ethanol shows that under these conditions alanine enters the tricarboxylic cycle almost exclusively through pyruvate carboxylation, whereas ethanol is the exclusive source of acetyl-CoA. By comparing the randomization of $^{13}$C between C3 and C2 of glutamate it is possible to estimate the mitochondrial fumarase activity; the C6-to-C5 ratios in glucose give the additional scrambling by cytosolic fumarase exchange. These two ratios provide the basis for a continuous study of the activity of the malate-aspartate cycle during the metabolism of ethanol.

Recently we have shown how $^{13}$C NMR experiments can be used to determine the intermediates and end products of gluconeogenesis from labeled glycerol in suspensions of isolated rat liver cells (Cohen et al., 1979a). In a single spectrum it was possible to measure the
flux through the major pathway, e.g. from [2-^{13}C] glycerol into C2 and C5 of glucose, and simultaneously to estimate the pentose cycle activity. The present paper is a report of $^{13}$C NMR studies of gluconeogenesis from [3-^{13}C] alanine and from [2-^{13}C] lactate in perfused mouse liver. The competition between ethanol and alanine into the tricarboxylic acid cycle is investigated and a preliminary account of the isotopic approach for studying the importance of the malate-aspartate cycle mechanism during the metabolism of ethanol is given. A more detailed report of the studies involving [3-^{13}C] alanine appears elsewhere (Cohen et al., 1979b).

Male Swiss-Webster mice (25-35g), fasted 24 h, were used. The recirculating perfusion fluid was Krebs bicarbonate buffer containing 3% dialyzed bovine serum albumin and equilibrated with 95% O$_2$/5% CO$_2$. The perfused liver was positioned in a 15 mm diameter NMR tube; $^{13}$C NMR spectra were measured at 35 ± 1°C on a Bruker WH-360 spectrometer at 90.5 MHz.

**Competition of Alanine and Ethanol into the Tricarboxylic Acid Cycle**

Figure 1 shows the $^{13}$C NMR spectra of two different perfused livers recorded during the same period, 90-120 min, after the addition of [3-^{13}C] alanine and either unlabeled or [2-^{13}C] ethanol. In both spectra the glucose end products have similar label distributions. The relative $^{13}$C enrichment of individual glucose peaks can be determined by comparison with the same peaks in non-enriched glucose, which is equally labeled at all carbons by the 1.1% natural abundance $^{13}$C. These intensities relative to the most intense $\alpha$C6 + $\beta$C6 peak are given in Table 1. Qualitatively the results show that there is appreciable $^{13}$C labeling at C1, C2, C5 and C6 while there is less $^{13}$C at C3 and C4. The notable difference is that in Figure 1b there are additional intense $^{13}$C NMR peaks from glutamate C4, glutamine C4 and acetate C2, as well as a strong peak from unused ethanol. Whereas acetate is a direct product of ethanol oxidation, the increased intensities of glutamate C4 and glutamine C4 give information about the relative fluxes into the tricarboxylic acid cycle. To a first approximation, the spectra in Figure 1 show that the C4 labels of glutamate and, hence, glutamine come almost exclusively from ethanol, whereas the C2 and C3 labels of glutamate and glutamine come from [3-^{13}C] alanine.

Figure 2 is a simplified gluconeogenic pathway from alanine showing the label distribution expected in glucose and glutamate from several extreme pathways. After transamination of alanine to pyruvate, pyruvate carboxylase moves the original [3-^{13}C] alanine label to the methylene carbon of oxaloacetate. If Pathway I is followed, then in the absence of any fumarase scrambling only C1 and C6 of glucose would be labeled as shown in Figure 2. With the addition of full fumarase exchange, the label is randomized within each triose unit and in glucose is found with equal probability at C1, C2, C5, and C6.