



United States Patent [19]

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- [54] **¹³C ISOTOPOMER ANALYSES IN INTACT TISSUE USING (¹³C) HOMONUCLEAR DECOUPLING**
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Related U.S. Application Data

- [63] Continuation of Ser. No. 220,102, Mar. 30, 1994, abandoned, which is a continuation-in-part of Ser. No. 555,270, Jul. 18, 1990, Pat. No. 5,413,917.
- [51] **Int. Cl.⁶** **A61K 51/04; C12Q 1/00; C12Q 1/02; C12Q 1/04**
- [52] **U.S. Cl.** **424/9.3; 435/35; 435/4; 435/14; 435/29; 435/30; 436/59; 436/57; 436/63; 436/173; 424/9.35; 424/1.81; 128/653.4; 128/654**
- [58] **Field of Search** **435/4, 35, 14, 435/30, 29; 436/173, 57, 56, 63; 424/9, 9.35, 1.11, 9.0, 1.17, 1.81, 9.3, 1.21; 324/300, 307, 311, 316; 128/653.4, 654**

References Cited

PUBLICATIONS

Avison et al., "Detection of Human Muscle Glycogen by Natural Abundance ¹³C NMR," *Proc. Natl. Acad. Sci. USA*, 85:1634-1636, Mar. 1988.

Behar et al., "Detection of Metabolites in Rabbit Brain by ¹³C NMR Spectroscopy following Administration of [1-¹³C] Glucose," *Magnetic Resonance in Medicine*, 3:911-920, 1986.

Bottomley et al., "Proton-Decoupled, Overhauser-Enhanced, Spatially Localized Carbon-13 Spectroscopy in Humans," *Magnetic Resonance in Medicine*, 12:348-363, 1989.

Brown et al., "Delineation of Myocardial Oxygen Utilization With Carbon-11-Labeled Acetate," *Circulation*, 76(3):687-696, 1987.

Chance et al., "Mathematical Analysis of Isotope Labeling in the Citric Acid Cycle with Applications to ¹³C NMR Studies in Perfused Rat Hearts," *The J. of Biolog. Chem.*, 258(2):13785-13794, 1983.

Chatzidakis and Otto, "Labeled Oxidation Produces from [1-¹⁴C], [U-¹⁴C] and [16-¹⁴C] Palmitate in Hepatocytes and Mitochondria," *Lipids*, 22(9):620-627, (1987).

Cohen, "Simultaneous ¹³C and ³¹P NMR Studies of Perfused Rat Liver," *The J. of Biolog. Chem.*, 258(23):14292-14308, 1983.

Heerschap et al., "Broadband Proton Decoupled Natural Abundance ¹³C NMR Spectroscopy of Humans at 1.5 T", (1989).

Jue et al., "Natural Abundance ¹³C NMR Spectrum of Glycogen in Humans," *Magnetic Resonance in Medicine*, 5:377-379, 1987.

Keifo and Peuhkurinen, "Regulation of the Tricarboxylic Acid Cycle Pool Size in Heart Muscle," *J. Mol. Cell. Cardiol.*, 16:487-495, 1984.

Kornberg, "Anaplerotic Sequences and their Role in Metabolism," *Essays in Biochem.*, 2:1-31, 1966.

Le Cocq and Lallemand, "Precise Carbon-13 N.M.R. Multiplicity Determination," *J.C.S. Chem. Comm.*, 150-152, 1981.

Lerch et al., "Localization of Viable, Ischemic Myocardium by Positron-emission Tomography with ¹¹C-Palmitate," *Circulation*, 64(4):689-699, 1981.

Liedtke et al., "Changes in Substrate Metabolism and Effects of Excess Fatty Acids in Reperfused Myocardium," *Circulation Research*, 62(3):535-542, Mar. 1988.

London, "¹³C Labeling in Metabolic Regulation Studies," *Prog. in NMR Spectroscopy*, 20:337-383, 1988.

Magnusson et al., "Increased Rate of Gluconeogenesis in Type II Diabetes Mellitus; A ¹³C Nuclear Magnetic Resonance Study," *J. Clin. Invest.*, 90:1323-1327, Oct. 1992.

Malloy, "Analysis of Substrate Utilization by ¹³C NMR Spectroscopy," *Structural and Organizational Aspects of Metabolic Regulation*, 363-374, 1990.

Malloy et al., "Contribution of Exogenous Substrates to Acetyl Coenzyme A: Measurements by ¹³C NMR Under Non-Steady-State Conditions," *Biochemistry*, 29(29):6756-6761, (1990).

Malloy et al., "Evaluation of Carbon Flux and Substrate Selection Through Alternative Pathways Involving the Citric Acid Cycle of the Heart by ¹³C NMR Spectroscopy," *J. of Biolog. Chem.*, 263(15):6964-6971, 1988.

Mickle et al., "Exogenous Substrate Preference of the Post-Ischaemic Myocardium," *Cardiovascular Research*, 20:256-263, 1986.

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[57] ABSTRACT

Entry of ¹³C-enriched acetyl-CoA into the citric acid cycle results in scrambling of ¹³C into the various carbon positions of all intermediate pools. The eventual result is that the ¹³C resonances of all detectable intermediates or molecules exchanging with those intermediates appear as multiplets due to nearest neighbor spin-spin couplings. Isotopomer analysis of the glutamate ¹³C multiplets provides a history of ¹³C flow through the cycle pools. Relative substrate utilization and relative anaplerotic flux can be quantitated. A major limitation of the method for in vivo applications is spectral resolution of multi-line resonances required for a complete isotopomer analysis. It is now shown that (¹³C)homonuclear decoupling of the glutamate C3 resonance collapses nine-line C4 and C2 resonances into three line multiplets. These three-line ¹³C multiplets are well resolved in isolated, perfused rat hearts and present steady-state equations which allow an isotopomer analysis from data obtained in intact tissue. This advancement shows for the first time that ¹³C isotopomer methods may be extended to complex metabolic conditions for resolution of carbon-carbon coupling, and particularly to in vivo measurements.