

ULTRASONIC PROPAGATION PROPERTIES (at 100 MHz) IN LIVER OF RAT EXPOSED TO ETHANOL OR CARBON TETRACHLORIDE

P. Tiernan^a, K. McCauley^b, T. Hebner^a, J. Erdman, Jr.^{b,c}, W. D. O'Brien, Jr.^{a,b}

The Bioacoustics Research Laboratory^a, the Division of Nutritional Sciences^b and the Department of Food Sciences^c, University of Illinois, Urbana IL 61801

ABSTRACT

The effects of the ultrasonic propagation properties of livers of rats exposed for five weeks to ethanol or carbon tetrachloride were examined. The lipid concentration nearly doubled for both treatments while protein content remained unchanged. The attenuation coefficient and speed in 51 samples were measured at 100 MHz with the scanning laser acoustic microscope. In the ethanol groups an increase of the attenuation coefficient (76%) and a decrease of speed (1.3%) compared to the pair-fed controls were observed. In the CCl₄ study there was a 46% increase of the attenuation coefficient and a 0.7% decrease of speed. Results were significant to 5% by the student's t-test.

INTRODUCTION

The purpose of this study was to evaluate quantitatively the effect of ultrasonic propagation properties (speed and attenuation coefficient at 100 MHz) on tissue properties (lipid and protein concentrations). In the case of alcohol, a previous study [2] with adult male Sprague Dawley rats showed that the attenuation coefficient increased and the speed decreased as the liver lipid concentration increased. In the case of carbon tetrachloride, there is no known comparable study.

METHODS

Fifty-two male weanling Sprague Dawley rats initially weighing 29 to 44 gms were randomly placed on one of the four experimental diets. Group A was fed an experimental diet with ethanol added; Group B was fed a control diet

without ethanol. Groups C and D were fed an identical experimental diet, but Group C was treated with CCl₄ and Group D without. After five weeks the rats were sacrificed with ether. Livers were immediately removed, cleaned, wrapped in aluminum foil, coded, frozen, and placed in a freezer at -70°C. Representative liver sections were taken for histological analysis prior to freezing.

The coded samples were removed from the freezer, prepared, and measured on the 100 MHz scanning laser acoustic microscope (Sono microscope 100®, Sonoscan Inc., Bensenville, IL). Attenuation coefficient and speed for each sample were measured using existing ultrasound methods previously published [2,5,6,7]. Attenuation coefficient measurement techniques used the insertion loss technique [2,6]. Speed measurements used the scanning laser acoustic microscope's interferometric mode [2,5]. For each of four thicknesses, 35 speed values were determined across the section, for which a mean value was computed. The reported speed for each specimen was determined from the mean of each section's mean speed.

Total liver lipid was determined gravimetrically by the method of Folch [9]. Total protein was determined by the Biuret method [10].

Grouped means for treatment and control were compared using the one way analysis of variance t-test procedure. Comparisons were considered significant at the $p < 0.05$ level.

RESULTS AND DISCUSSION

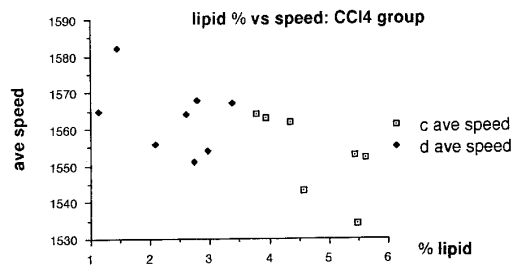
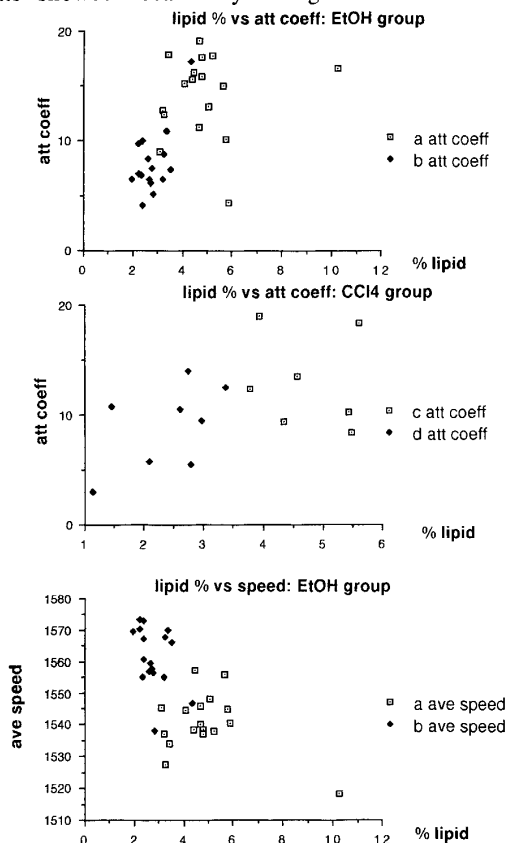
Compared to their respective controls, the percentage liver lipids were increased 75% and 83% for the ethanol treatment and the carbon tetrachloride treatment, respectively. There was no statistically significant difference in the

percentage liver protein between either the ethanol or the CCl₄ groups and controls.

Both the ultrasonic speed and ultrasonic attenuation coefficient were significantly different between the ethanol groups using the t-test. Compared with the control, the speed was decreased by 1.3% and the attenuation coefficient was increased by 76%.

In the CCl₄ group, a statistically significant increase of the attenuation coefficient (46.4%) and a statistically significant decrease of the speed (0.71%) compared to the pair-fed controls were observed. One CCl₄ treated rat liver was removed from the statistical analysis because its lipid content was less than the mean of the control group.

Although quantitative collagen analyses were not performed, histological examination of the livers revealed the CCl₄ group to have both fatty change and periportal fibrosis with formation of regenerating nodules suggestive of early cirrhosis[1]. Light microscopic examination of liver sections from ethanol-fed rats showed focal fatty change without fibrosis.



- 1 K McCauley, M Prabhudesai, JW Erdman Jr, "Effects of EtOH and CCl₄ upon Vitamin A status of rats," *Alcoholism: Clin and Expt Res* (sub).
- 2 KMU Tervola, MA Grummer, JW Erdman Jr, WD O'Brien Jr, "Ultrasonic attenuation and velocity properties in rat liver as a function of fat concentration: A study at 100 MHz using a scanning laser acoustic microscope (SLAM)," *J Acoust Soc Am* **77**, 307-313, 1985.
- 3 MA Grummer, JW Erdman Jr, "Effect of Chronic EtOH consumption and moderate or high fat diet upon tissue distribution of vitamin A in rats fed either vitamin A or β carotene," *J Nutr Res*, **113**, 350-64, 1983.
- 4 MA Grummer and JW Erdman, Jr, "Effect of chronic EtOH consumption and moderate or high fat diet upon tissue distribution of vitamin A in rats fed either vitamin A or β -carotene," *Nutr Res*, **6**, 61-73, 1986.
- 5 PM Embree, SG Foster, G Bright, WD O'Brien, "Ultrasonic velocity spatial distribution analysis of biological materials with the SLAM," *Acoustical Imaging*, MKaveh et al, (eds), vol 13, pp 203-16, Plenum Press 1983.
- 6 KMU Tervola, SG Foster, WD O'Brien, "Ultrasonic attenuation measurement using 100 MHz SLAM," *IEEE Transactions on Sonics Ultrasonics*, **su-32**, 259-65, 1985.
- 7 WD O'Brien Jr, JW Erdman Jr, TB Hebner, "Ultrasonic propagation properties (at 100 MHz) in excessively fatty rat liver," *J Acoust Soc Amer*, **83**, 1159-66, 1988.
- 8 DL Steiger, WD O'Brien Jr, JE Olerud, MA Rieder Henderson and GR Oland, "Measurement uncertainty assessment of the SLAM and application to canine skin and wound," *IEEE Trans Ultrasonics, Ferroelectrics and Frequency Control*, in press.
- 9 J Folch, M Lees, G Sloane-Stanley, "A simple method for the isolation and purification of total lipids from animal tissues," *J Biol Chem*, **226**, 497-509, 1957.
- 10 JM Clark, RL Switzer, *Exp. Biochemistry*, 2nd ed p 12 WH Freeman Co, SF CA, 1964.
- 11 Statistical Analysis System Inst Inc SAS Users' Guide: Statistics, Version 5 ed, pp 656-709, SAS Inst, Inc, Cary NC 1985.
- 12 KJ Parker and TA Tuthill, "CCl₄ induced changes in ultrasonic properties of liver," *IEEE Trans Biomed Eng*, **BME-33**, 453-60, 1986.
- 13 National Research Council, *Nutrient Requirements of Laboratory Animals*, 3rd rev ed, National Academy of Sciences, Wash, DC 1978.

Author - Pete Tiernan, MD, Department of Surgery, University Hosp, HSC Floor 19, SUNY-Stony Brook, Stony Brook NY 11794-8191, Ph (516) 444-2492