

# Mitochondrial enzyme activities in liver biopsies from patients with alcoholic liver disease

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**SUMMARY** The hypothesis that mitochondrial damage is a significant factor in the pathogenesis of alcoholic liver disease (ALD) was investigated by enzymic analysis of mitochondrial fractions isolated from needle biopsy specimens from control patients, patients with fatty liver due to chronic alcoholism, and from patients with other forms of liver disease. Enzymes associated with the inner and outer mitochondrial membranes showed normal levels in ALD. Enzymes associated with the mitochondrial matrix, glutamate dehydrogenase, malate dehydrogenase and aspartate aminotransferase showed significantly raised levels in ALD, but the levels in patients with non-alcoholic liver disease were normal. In addition, analysis of the mitochondria by sucrose density gradient centrifugation revealed no differences between control tissue and liver from patients with alcoholic liver disease. These results do not indicate that there is significant mitochondrial damage in ALD. The raised mitochondrial matrix enzymes may represent an adaptive response to the ethanol load.

Alcoholic liver disease (ALD) is a major clinical problem of increasing importance, and alcoholic cirrhosis is now the third main cause of death between the ages of 25 and 65 years in the USA (Lieber, 1975). However, it is not known how ethanol damages the liver, or why there are marked variations in the extent of hepatic damage between individuals consuming amounts of ethanol.

It has been claimed that mitochondrial damage is an early and characteristic feature of ALD and that it is important in the pathogenesis of hepatic damage (Rubin *et al.*, 1970). Electron microscopic studies have shown swollen mitochondria with disorientated cristae (Svoboda and Manning, 1964), and experimental animal studies feeding large quantities of ethanol to rats causes a small but significant decrease in the functional capabilities of hepatic mitochondria (Rubin *et al.*, 1970). Increased serum levels of hepatic enzymes, particularly the mitochondrial enzyme glutamate dehydrogenase, are frequently found in alcoholics, even when steatosis is the only histological abnormality (Kontinen *et al.*, 1970), and this has strengthened the suggestion of mitochondrial injury.

Although there have been several animal studies, little is known of the mitochondrial enzyme changes in patients with ALD. In order to assess hepatic mitochondrial damage more directly in chronic

alcoholics the levels of marker enzymes for mitochondrial inner and outer membranes, and matrix were assayed in liver biopsies from patients with ALD, and compared with controls and with patients with other forms of liver disease.

## Methods

### PATIENTS

The thirteen controls were patients undergoing diagnostic liver biopsy, but who had normal serum liver function tests and whose liver biopsies were subsequently shown to be histologically normal. Fourteen chronic alcoholics, who had raised serum aspartate aminotransferase levels (range 44-135 IU/l), and whose liver biopsies showed only steatosis, were studied within 36 hours of stopping drinking alcohol. Those with cirrhosis or alcoholic hepatitis were excluded from this study. Six patients with other forms of liver disease were studied later to see whether the changes found were confined to ALD. This group consisted of two patients with chronic active hepatitis; one with chronic persistent hepatitis; two with cholelithiasis; and one with granulomatous hepatitis. All had raised serum aspartate aminotransferase levels (range 63-1500), and none had recently taken alcohol.

The liver biopsies were obtained percutaneously with a Menghini needle. The tissue was immediately washed in ice-cold 0.15 M NaCl. A portion was separated for histology, and the remainder homo-

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genised in 0.25 M sucrose containing 20 mM ethanol and 1 mM Na<sub>2</sub> EDTA, pH 7.2 (Leighton *et al.*, 1968). Nuclear, mitochondrial, and post-mitochondrial fractions were prepared by differential centrifugation as described by Peters *et al.* (1975). Analytical subcellular fractionation was performed as described by Seymour *et al.* (1974).

The following mitochondrial enzymes, with locations shown between parentheses, were assayed by micromodifications of standard techniques: monoamine oxidase (Wurtman and Axelrod, 1963) (outer membrane); succinic dehydrogenase (Prospero, 1974) and cytochrome c oxidase (Cooperstein and Lazarow, 1951) (inner membrane); glutamate dehydrogenase (Ellis and Goldberg, 1972) (matrix); and malate dehydrogenase (Lowry *et al.*, 1957) and aspartate aminotransferase (Karmen, 1955) located both in the mitochondrial matrix and cytosol. Protein was assayed by the Lowry method (Lowry *et al.*, 1951). Enzyme specific activities in the different groups were compared by the Wilcoxon rank sum test.

The studies reported in this paper have been approved by the Local Ethical Committee.

## Results

Table 1 shows the enzyme levels expressed as mUnits/mg mitochondrial fraction protein in the different patient groups. There is no significant difference between controls and alcoholics in the specific activities of monoamine oxidase, succinic dehydrogenase, and cytochrome c oxidase. However, the levels of glutamate and malate dehydrogenase and aspartate aminotransferase are raised in the alcoholic group, whereas the levels in patients with other forms of liver disease do not differ from controls.

Table 1 *Enzyme specific activities and protein content in mitochondrial fractions of liver biopsies*

Enzyme	Controls	Alcoholics	Other liver disease
Monoamine oxidase	53.3 ± 4.6 (11)	62.7 ± 7.6 (9) P > 0.1	—
Succinic dehydrogenase	42.1 ± 4.4 (6)	40.3 ± 3.5 (6) P > 0.1	—
Cytochrome oxidase	580 ± 109 (7)	552 ± 92 (6) P > 0.1	—
Glutamate dehydrogenase	103 ± 12 (11)	214 ± 15 (12) P < 0.01	90 ± 3 (6) P > 0.1
Malate dehydrogenase	2350 ± 370 (8)	3910 ± 390 (10) P < 0.05	2210 ± 290 (5) P > 0.1
Aspartate aminotransferase	800 ± 60 (9)	170 ± 120 (8) P < 0.02	870 ± 120 (5) P > 0.1
% Total protein in mitochondrial fraction	29.4 ± 0.8 (13)	31.6 ± 1.3 (14) P > 0.1	25.2 ± 2.1 (6) P > 0.1

Activities ± SEM expressed as mUnits/mg protein. Cytochrome c oxidase is expressed as mUnits/mg protein as defined by Cooperstein and Lazarow (1951). Numbers studied shown between parentheses. P values refer to significance of differences between normal subjects and patient groups.

There is no significant difference in the protein content of the mitochondrial fraction in the three patient groups. Table 2 shows the specific activities of malate dehydrogenase and aspartate aminotransferase in the post-mitochondrial supernatant indicating that the alteration in the levels of these enzymes is confined to the mitochondrial fraction.

The Figure shows the density distributions for three mitochondrial marker enzymes after sucrose gradient centrifugation. There is no significant difference in the distribution of these enzymes or of protein between the two groups.

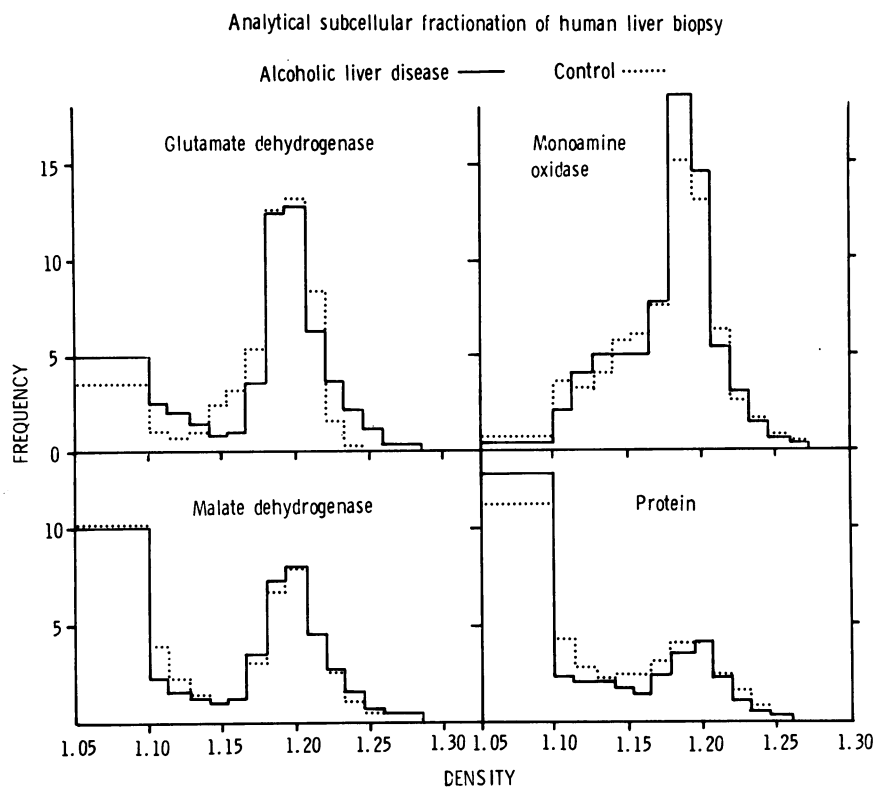
Table 2 *Enzyme specific activities in the post-mitochondrial supernatant of liver biopsies*

Enzyme	Controls	Alcoholics
Malate dehydrogenase	1460 ± 120 (8)	1790 ± 130 (10) P > 0.1
Aspartate aminotransferase	350 ± 40 (8)	405 ± 68 (10) P > 0.1

Activities ± SEM expressed as m Units/mg protein. Numbers studied shown between parentheses.

## Discussion

These studies do not support the hypothesis that mitochondrial damage is important in the pathogenesis of alcoholic liver disease. Damage to mitochondria is usually associated with a decreased activity of its marker enzymes or in an alteration in its centrifugation properties due to permeability changes of its inner membrane (Collot *et al.*, 1975). Rubín and colleagues (1970) reported a decrease of 17% in liver mitochondrial succinate dehydrogenase in rats fed a 36% ethanol diet for 24 days. However, our alcoholic patients not only had normal levels of some mitochondrial enzymes including



**Figure** Isopycnic centrifugation of 8000 g-min supernatant from liver biopsy homogenate. Graphs show frequency-density histograms for mitochondrial marker enzymes and for protein. Frequency is defined as fraction of total recovered activity present in the subcellular fraction divided by the density span covered. The activity over the density span 1.05 to 1.10 represents soluble enzyme activity remaining in the sample layer. The control data is shown by the interrupted line and is the mean of five to eight experiments. The data from alcoholic liver disease is shown by the continuous line and is the mean of four to six experiments. Recovered enzyme activity is 83-104%.

succinic dehydrogenase but also had increased levels of mitochondrial matrix enzymes. Patients with other forms of liver disease had values within the normal range indicating that the increases are specific to alcoholic liver disease. It is possible that the raised serum aspartate aminotransferase and glutamate dehydrogenase frequently found in alcoholics are not simply a result of enzyme leakage from damaged hepatocytes, but may be at least partly due to the increased liver mitochondrial enzyme activity.

The reason for this increase in mitochondrial matrix enzyme activities is not entirely clear. Ethanol metabolism is associated with the production of strikingly raised levels of reduced pyridine nucleotides in the cytoplasm (Forsander, 1970). Reoxidation of the nucleotides involves the transport of reducing equivalents to the mitochondria mainly

by the malate-aspartate shuttle (Ylikahri *et al.*, 1971; Digerness and Reddy, 1976; Tischler *et al.*, 1976). Malate dehydrogenase and aspartate aminotransferase are involved with this shuttle and the increased enzyme levels may represent a further example of hepatic enzyme induction by ethanol. Glutamate dehydrogenase is known to be induced by dietary and hormonal alterations in the experimental animal (Swick *et al.*, 1968) and the increased mitochondrial activity may result from the chronic ethanol load.

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