*Leading article***Measuring the damage—ethanol and the liver**

Acute or chronic administration of large amounts of ethanol causes morphological damage not only to the liver but also to many other organs, together with various metabolic changes, such as accumulation of triglycerides in the liver.¹⁻³ It is not known if ethanol is the primary toxin, or only indirectly toxic through the damaging effects of its metabolites, nor whether alcoholics are genetically more susceptible to tissue damage by ethanol than others who drink seemingly with impunity.¹⁻⁶ These two problems are addressed in this issue of *Gut* by Matthewson *et al.*,⁷ who combine measurement of hepatic enzyme activities *in vitro* with the blood concentrations of acetaldehyde after an ethanol load.

After rapid absorption from the stomach and small intestine ethanol not bound to plasma proteins is distributed in body water and efficiently removed from blood almost solely by the liver. More than 60% of ethanol is rapidly oxidised in hepatocytes⁸ to acetate and thence to carbon dioxide and water: the accompanying obligatory reduction of NAD to NADH alters the hepatic intracellular redox state, which can adversely affect many other metabolic reactions in the liver.¹⁻³ These biochemical changes may contribute to the hepatotoxicity of ethanol.

Acetaldehyde, the first metabolite of alcohol is, however, itself toxic and may augment the widespread tissue damage that follows ethanol ingestion.⁹ Probably 80% of ethanol entering the liver is oxidised in the cytosol and microsomes of the hepatocyte to acetaldehyde,¹⁻³ and the resulting NADH shuttles into the mitochondria. Acetaldehyde is then oxidised to acetate. After administration of ethanol acetaldehyde can be detected in blood, so presumably its rate of transfer to the mitochondria and/or the speed of its oxidation are rate-limiting. Blood concentrations of acetaldehyde after administration of ethanol are higher in alcoholics than in normal subjects,¹⁰ perhaps because of chronic ethanolic liver damage, although it has also been suggested that this difference is caused by a genetic inability of alcoholics to metabolise, and hence detoxify, large amounts of acetaldehyde.¹¹ There are differences in the rate of ethanol metabolism and susceptibility to liver damage between and within species and so, even if such a genetic hypothesis is at first unattractive, it is possible that the alcoholic is inherently more prone to tissue damage. But how can this be shown so that at least doctors' sympathy for their patients is increased?

The most direct method is to measure the *in vitro* activity of the various ethanol metabolising enzymes in liver biopsy specimens from alcoholics while they are drinking and later when abstinent, and to compare the results with those from patients with comparable non-alcoholic liver disease. This is not only ethically and technically difficult to do on 10-20 mg

of tissue, but also the results of much work are difficult to interpret. Firstly, which enzyme activity should be measured? There are several enzymes and isoenzymes that oxidise ethanol and acetaldehyde, and their affinities for their substrates (K_m values) differ, as therefore do their abilities to function at low ethanol concentrations.^{1,2} These enzymes are also present in different subcellular fractions and their pH optima are dissimilar. Thus ethanol in the liver is oxidised by at least three enzyme systems;^{1,3} chiefly (80%) by the zinc metalloenzyme alcohol dehydrogenase in the cytosol, but also partly by a microsomal ethanol oxidising system, which can be induced like other enzymes in this fraction by drugs and by ethanol itself, and finally to a small extent by the haem containing enzyme catalase. At normal intakes of ethanol alcohol dehydrogenase is the most active and quantitatively the most important enzyme, but the ethanol microsomal oxidising system may be more important in the alcoholic without severe liver damage at high ethanol concentrations,¹ while its induction may explain the increased clearance of ethanol from the blood and oxidation in actively drinking alcoholics.¹²⁻¹⁴ Indeed, there is some evidence that hepatic alcohol dehydrogenase activity is decreased in alcoholics even without liver damage.¹⁵

The acetaldehyde formed from ethanol is then oxidised to acetate by several isoenzymes of acetaldehyde dehydrogenase present in mitochondria and cytosol, and the acetate is distributed to the tissues. It is not decided whether the mitochondrial fraction is quantitatively the more important.^{16,17} The cytosolic fraction, which was measured by Matthewson *et al.*,⁷ may be more prone to damage by liver disease.¹⁸⁻²⁰

Recent measurements of acetaldehyde dehydrogenase activity in human needle liver biopsies from patients with alcoholic and non-alcoholic liver disease have been contradictory,^{11,18,19} even when done by the same investigators, and so the important question whether alcoholics are prone to liver damage through some inherited metabolic weakness is not yet answered. Matthewson *et al.*⁷ join the fray by measuring the activities of alcohol dehydrogenase and the cytosolic fraction of acetaldehyde dehydrogenase. These authors show that these enzyme activities were grossly diminished in patients with liver disease due to alcohol and in patients with non-alcoholic active chronic hepatitis, or in those recovering from paracetamol overdose. They therefore conclude that liver damage *per se* causes this effect, rather than a specific genetic or acquired defect in alcoholics. In addition they found a negative correlation between the activity of acetaldehyde dehydrogenase and mean blood acetaldehyde concentration after oral alcohol suggesting that this enzyme was rate limiting for acetaldehyde metabolism. Similar results have been obtained in the baboon.²¹ Is the controversy therefore settled? Unfortunately not.

Comparing enzyme activities *in vitro* with *in vivo* blood clearances is not easy. Enzyme proteins are expected to perform in incubation tubes as they did in intact cells. Detergents are added to maximise their performance, and the concentration of their substrates and products may differ greatly from those *in vivo* within domains of the cell. Moreover, enzyme activities are usually expressed as specific activities with reference to protein concentration in the tissue, but seldom is the protein content of the whole liver determined in order to estimate total organ activity, which is one of the factors that controls blood levels of ethanol and acetaldehyde. This

problem is particularly relevant in liver disease, when a sample of 1×10^{-5} of the liver is unlikely to be representative of the remaining diseased tissue. Furthermore, the protein concentration of oedematous, fatty, inflamed or fibrotic liver will differ from the normal and hence distort the results. Perhaps erythrocyte acetaldehyde dehydrogenase activity will better reflect the activity in the liver.²²

In theory mathematical analysis of blood concentration *versus* time curves of ethanol will indicate the rate of metabolism of ethanol in the liver. Oral clearance, which is derived from the area under the peripheral blood curve after an oral dose, is approximately equivalent to the intrinsic hepatic clearance, which is the maximal clearance possible when blood flow is not limiting. Intrinsic hepatic clearance, however, cannot be estimated in this way when there is systemic shunting of portal blood, which severely impairs the efficiency of hepatic clearance and particularly the clearance of an oral dose, as shunting then prevents the liver cells and their enzymes from achieving their maximum rate of removal. Thus impaired clearance will not accurately reflect enzyme activities.

The peak blood concentration of a drug is also critically dependent on the rate of intestinal absorption, which in turn depends on the rate of delivery to the intestinal mucosa and hence on gastric emptying. Ideally gastric emptying should be measured simultaneously in oral kinetic studies – for example, by adding another marker such as 3-0-methyl glucose that is absorbed, but not metabolised. Absorption of ethanol has indeed been shown to be highly variable.²³ A further problem is that blood concentrations of alcohol and its metabolite depend on their volumes of distribution, which are likely to change in liver disease.

Intravenous administration of alcohol is therefore probably preferable. Even better if the drug is given by constant infusion to steady state⁸ so that blood (systemic) clearance can be calculated more accurately without being influenced by altered volumes of distribution, although if high concentrations of ethanol are used they are painful,¹⁴ and may stimulate hepatic alcohol metabolism¹³ and alter liver blood flow.¹

An alternative approach to assessing hepatic metabolism is to measure the excretion of ¹⁴C-labelled carbon dioxide derived from acetate in breath after administering a tracer dose of ¹⁴C-ethanol.^{14 24} The rate of excretion in breath should be proportional to the hepatic metabolic rate, as has been shown for the similar aminopyrine breath test. Ethanol metabolism increases in alcoholics, but eventually falls below normal if there is severe liver disease.^{25 26}

The blood level of acetaldehyde is more difficult to measure and to interpret, because it depends on the rate of efflux of the compound from liver cells, and its rates of re-uptake from the blood back into liver cells and into other tissue. Efflux and uptake can be calculated from model dependent analysis of blood curves after administering the compound intravenously (as has been shown for bilirubin, bile acids, and some drugs), but may be more difficult for a compound such as acetaldehyde that enters the blood primarily by efflux from liver cells. Moreover, some individuals possess isoenzymes of alcohol dehydrogenase that metabolise alcohol faster and cause unusually high blood acetaldehyde concentrations and flushing.²⁷

A further problem in this field is the difficulty of obtaining comparable

disease groups of sufficient numbers of patients and of assessing and matching their relative degrees of liver dysfunction. Most liver blood tests do not test liver function and the results in patients with histologically diagnosed cirrhosis differ markedly. Thus it is well known that in primary biliary cirrhosis, in which cholestasis predominates, liver cell function is preserved better than in alcoholic cirrhosis. Unfortunately it is not easy to find enough patients matched for type and degree of liver disease in one centre.

In addition, malnutrition is common in alcoholism¹⁷ and affects the activity of alcohol dehydrogenase,¹ while it is never possible to assess reliably present alcohol intake in outpatients. It is thus difficult to assume that alcoholics are abstaining without admitting them to hospital.

The concentrations of ethanol and acetaldehyde²⁸ in blood and even the rate of gastric emptying differ between the sexes, and alcoholic liver disease predominates in men. This sex imbalance decreases the comparability of groups being studied, as in the paper by Matthewson *et al.*⁷ The rate of ethanol blood clearance rapidly falls during the first days of alcohol withdrawal,¹³ and the activity of the microsomal ethanol oxidising system perhaps more slowly.¹² Ideally therefore, studies should be done either in alcoholics who are still drinking and therefore on the day of admission to hospital, or after several weeks' abstinence.

Analyses of blood curves or of breath excretion are therefore more likely than *in vitro* studies to reflect accurately whole body metabolism, which is ultimately what is important. Enzyme activities, however, do allow us to study qualitative effects, although the results cannot easily be quantitatively extrapolated to the whole organ, let alone the whole animal. This might be achieved in man by calculating whole organ enzyme activities by measuring liver mass using ultrasonography.

At present the evidence favours those who believe that alcohol causes liver damage in many of those who drink heavily, frequently, and for a long time, and that this occurs regardless of their genetic inheritance.^{7 11} The evidence, however, may yet turn around and then make us more understanding of the alcoholic's plight.

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