ETHANOL METABOLISM BY THE LIVER

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1. INTRODUCTION

When ethanol is ingested by mammals it is metabolised through acetaldehyde to acetate. The acetate enters the general metabolic pool through conversion to acetyl CoA. The liver is the tissue primarily responsible for the conversion of ethanol to acetate, but most of the acetate is further metabolised by other tissues. The rate of ethanol removal from the body is essentially linear at ethanol concentrations above 10 mM. (For general reviews of ethanol absorption, distribution and elimination see /1,2,3/.)

With such a simple scenario – a drug metabolised in a linear manner via a 2-step pathway in a single tissue – readers new to the field could be forgiven for assuming that there would be little left to discover about the metabolism of ethanol by the liver. However, the factors which control ethanol metabolism by the liver have been the subject of much debate in the literature in recent years, and the matter is not yet clearly resolved. The main part of this review will be a discussion of the control debate and of factors which influence rates of ethanol metabolism by the liver. The first part will deal briefly with the enzymology of ethanol metabolism, since some background on this is necessary in discussion of control of the pathway. For those interested in reading further there are several recent more detailed reviews on the enzymology of ethanol metabolism /4,5,6,7,8/.

2. ENZYMOLOGY OF ETHANOL METABOLISM

2.1 *Conversion of Ethanol to Acetaldehyde*

2.1.1 *Alcohol Dehydrogenase (ADH)*

The main enzyme responsible for the conversion of ethanol to acetaldehyde is alcohol dehydrogenase (ADH) (EC 1.1.1.1) which catalyses the following reaction:

$$\text{ethanol} + NAD^+ \rightleftharpoons \text{acetaldehyde} + NADH + H^+$$

The enzyme from mammalian liver is a soluble protein (molecular weight 80,000) /4,5/ located in the cytosolic compartment /8,9/. Horse liver ADH was one of the first enzymes to be purified and kinetically characterised and there is now an enormous amount of information available on the enzyme including its sequence, its 3-dimensional structure, its isozyme pattern and the results of detailed mechanistic

studies (for reviews see /6,10/). However, for various reasons, there is less information available about the enzymes from humans or laboratory animals. In the former case, the difficulty in obtaining liver tissue has obviously contributed to a lack of progress, but in addition full characterisation of human liver ADH has been complicated by the number of isozymes present. There are three separate gene loci that code for three structurally distinct polypeptide chains. Genetic polymorphism occurs in at least two of these loci. The enzyme is dimeric, and either homo- or heterodimeric forms may occur. (Reviewed in /4,11,12/.) In addition, two other types of ADH isozymes have been described, π-ADH /4,11,12/ and χ-ADH /11/. Li, Bosron and others are currently engaged in extensive kinetic characterisation of human ADH isozymes /13,14/.

The lack of information about rat ADH is more surprising, considering the wide use of these animals in experimental studies on ethanol metabolism, and the ease with which liver samples can be obtained. The rat enzyme is less stable than the horse enzyme, and has only been purified relatively recently /15,16,17,18,19,20/. Detailed kinetic characterisation has been carried out on purified enzyme /13,17,20/ and on impure enzyme /21/. Differences between some of the kinetic parameters obtained in these studies are still unexplained (see also sections 3.2.3 (b) (i) and 3.6).

The evidence which suggests that ADH is the enzyme primarily responsible for the conversion of ethanol to acetaldehyde has been reviewed previously /3,4,8/. The major supporting facts are: (a) Pyrazole and its derivatives, which are potent inhibitors of liver ADH *in vitro*, inhibit ethanol metabolism *in vivo* and in perfused liver and isolated hepatocytes. (b) Apparent K_M values for ethanol determined *in vivo* or *in situ* are similar to those for ADH *in vitro*. (c) The metabolism of ethanol causes a change in the ratio of free NAD^+/NADH in the cytosol of the liver cell, and (d) The activity of ADH measured *in vitro* is usually sufficient to account for rates of ethanol metabolism *in vivo*. There are claims that non-ADH mediated ethanol metabolism is of significance *in vivo* /22/ and recent studies showing that ethanol is readily metabolised in deermice lacking ADH /23/ have supported this view /22,24/. However, the relevance of this finding to the study of ethanol metabolism in animals with ADH will not be clear until the mechanism of ethanol removal in the deermice lacking ADH has been fully elucidated. (See also section 3.7.1.) It has also been suggested that the pyrazoles may inhibit another ethanol oxidising enzyme system /25,26/ (MEOS, see next section) and that therefore their inhibition of ethanol metabolism

in vivo and in isolated liver preparations may not accurately indicate the contribution of ADH to ethanol metabolism /22/. Counteracting this suggestion is a recent detailed study of the action of a range of pyrazole derivatives on the activity of ADH /27/. The close similarity in action of these pyrazoles *in vitro* and in isolated rat hepatocytes gives strong support for ADH being the major enzyme involved in the oxidation of ethanol.

2.1.2 *Microsomal ethanol oxidising system (MEOS)*

Orme-Johnson and Ziegler /28/ first proposed that microsomal enzymes could oxidise ethanol to acetaldehyde. The proposal has generated a large amount of research, both supporting and contradicting the existence of 'MEOS' as it has become commonly known. Much of the supporting work is by Lieber's group /22,29/ who hold the view that MEOS plays a significant role in ethanol metabolism, particularly after chronic ethanol consumption (see also section 3.7.1).

It is probably generally accepted now that microsomal enzyme systems free of catalase can oxidise ethanol. Exactly how this occurs at the molecular level remains to be established /22,30/. Cytochrome P-450 is involved and NADPH is required as a cofactor /22,30/. It is also possible that hydroxyl radicals could be involved /22,30,31/. However, despite continuing work in the area, there is still no conclusive evidence that the microsomal ethanol oxidising system is of any significance *in vivo* /4,8,32/.

2.1.3 *Catalase*

Catalase can oxidise ethanol to acetaldehyde by the following reaction:

$$CH_3CH_2OH + H_2O_2 \rightarrow CH_3CHO + 2H_2O$$

The activity of this system is probably limited under physiological conditions by the availability of hydrogen peroxide and this factor as well as the results of inhibitor studies suggests that catalase is unlikely to be of much significance in the metabolism of ethanol *in vivo* /4, 8/.

2.2 *Acetaldehyde Removal*

2.2.1 *Aldehyde dehydrogenase (AlDH)*

The primary enzyme responsible for the conversion of acetaldehyde to acetate is aldehyde dehydrogenase (AIDH) (EC1.1.1.3), which catalyses the following reaction:

$$CH_3CHO + NAD^+ + H_2O \longrightarrow CH_3COO^- + NADH + 2H^+$$

The reaction is irreversible under physiological conditions /7/.

Mammalian liver aldehyde dehydrogenases have only been purified and characterised relatively recently /33,34,35,36,37/. The enzyme occurs in cytosolic, mitochondrial and microsomal fractions of liver cells /34,38,39,40,41,42/. Cytosolic and mitochondrial enzymes have been purified from sheep /34,35,43,44/ and horse /45/ liver and two similar isozymes have been purified from human liver /36,46/. All are soluble proteins of molecular weight 200,000-250,000 /5/. Extensive kinetic and mechanistic studies have been carried out on both isoenzymes from horse /4,47/ and sheep /35,43,44,48-61/ liver. There are some published kinetic and mechanistic data for the human isoenzymes /36,62,63,64/ and one human enzyme has recently been sequenced /65/. The sequence of the cytoplasmic horse liver enzyme was published simultaneously /66/.

The cytosolic and mitochondrial isozymes of sheep and horse liver are distinguished primarily by their differing sensitivity to the inactivator disulfiram, the cytosolic enzyme being extremely sensitive, the mitochondrial much less so /7,67/. The two enzymes also have a difference in charge, and can be separated from one another using ion exchange chromatography /44,45/. Human liver has disulfiram sensitive and insensitive isozymes, but the subcellular localisation of these forms has not been finally confirmed /5,62,68/. The human isozymes are also distinguished on the basis of their K_M value for acetaldehyde, the more disulfiram-sensitive isozyme having a higher K_M (30 μM) than the less sensitive one (3 μM) /5,62,68/. Some have suggested that a low K_M (acetaldehyde), disulfiram sensitive, enzyme located in the cytosol plays an important role in human ethanol metabolism /69,70/. Others have reported that there is no low K_M enzyme in the cytosol /71/. The intracellular localisation of human aldehyde dehydrogenases is an area requiring more study to clarify conflicting reports (see also /72/).

In the rat, mitochondrial and cytosolic aldehyde dehydrogenases cannot be clearly distinguished by their disulfiram sensitivity. There appear to be two or more forms of the enzyme, of differing disulfiram sensitivity and with different K_M's for acetaldehyde in each subcellular compartment /41,73,74/. Although there are some kinetic data available /75,76,77/, the rat enzymes have not been extensively characterised as yet.

The molecular basis of the isozyme patterns of AlDH has not yet

been clarified. On narrow gradient isoelectric focusing there appear to be two or more bands for both major isozymes from sheep and human liver /53,78/ and the banding pattern varies between individual livers. Two bands of a minor liver isozyme have also been reported following isoelectric focusing /79/. The individual bands within each isozyme form are too close to be readily separated preparatively, so that differences between the subforms have yet to be determined. Multiple subforms of human AlDH occurring during ion exchange chromatography have been attributed to oxidation during purification /46/ but this is probably not the explanation in all cases. We have observed multiple banding after isoelectric focusing in fresh autopsy and biopsy samples treated with dithiothreitol (unpublished data). Until the molecular basis of the isozyme pattern of AlDH is elucidated, it is difficult to assess the significance of the differences between rat, sheep, horse and human. Meanwhile caution should be exercised in extending observations on acetaldehyde metabolism in rat liver to the human case.

There is a considerable amount of evidence supporting AlDH as the major enzyme involved in acetaldehyde metabolism. The acetaldehyde concentrations present in the liver during ethanol metabolism are low – in the region of 0-200 μM. In each species studied, there is at least one AlDH with a very low K_M (of the order of 1 μM or less) for acetaldehyde, and many other forms of the enzyme have K_M values for acetaldehyde in the 1-100 μM range. Other enzymes that could possibly be involved in acetaldehyde oxidation (aldehyde oxidase, xanthine oxidase) have high K_Ms for acetaldehyde (of the order of 1-10 mM) /7,80/ and are unlikely to be of significance *in vivo*. Studies using the inactivator disulfiram also support the importance of AlDH in acetaldehyde removal. *In vivo*, disulfiram causes increased acetaldehyde concentrations during ethanol metabolism /81,82,83,84/. A major role for AlDH in acetaldehyde metabolism is also suggested by the finding that oriental subjects apparently lacking one isozyme of liver AlDH show increased acetaldehyde concentrations during ethanol metabolism /85/. A recent report suggests that the isoenzyme is not completely absent in these subjects, but present in a less active form /86/. Further elucidation of this finding may help in establishing the molecular basis of isozyme variation in aldehyde dehydrogenases.

2.2.2 *Other means of acetaldehyde removal*

Acetaldehyde can be oxidised to acetate by aldehyde oxidase and xanthine oxidase /87/. As stated above, K_M values of these enzymes for

acetaldehyde are high /7,80/ and the enzymes are unlikely to be of physiological significance in acetaldehyde metabolism.

Acetaldehyde could also theoretically be reduced by aldehyde reductases /88/. Again, the reaction is unlikely to be of physiological significance because these enzymes have high K_M's for acetaldehyde. Moreover, the reaction would be non-productive in terms of ethanol removal, since the acetaldehyde would be reconverted to ethanol.

It is possible that acetaldehyde may be converted to acetoin by reaction with the hydroxyethyl-thiamine pyrophosphate-pyruvate dehydrogenase complex, although this reaction probably only occurs extrahepatically /89/. Acetoin may be reduced to 2,3-butanediol in the liver /89/, and the occurrence of 2,3-butanediol has been reported in blood from alcoholics /90,91/.

Acetaldehyde is a very reactive compound, and theoretically it could also be removed from tissues by covalent binding – for example to amine groups with formation of Schiff bases. Binding of acetaldehyde to blood proteins has been demonstrated /92,93/, but other than this, there is little evidence in the literature as to whether or not binding reactions play a significant role in acetaldehyde removal.

3. FACTORS AFFECTING RATES OF ETHANOL METABOLISM

3.1 *The Control Debate*

There are two main theories as to what constitutes the major rate determining factor for ethanol metabolism by the liver. The first theory proposes that the rate of ethanol metabolism is limited by the rate at which NADH can be reoxidised /3,94/. This theory arose from the observation that during ethanol metabolism the ratio of free [NAD^+]/ free [NADH] (as indicated by the ratio of [lactate]/[pyruvate]) in the cytosol of liver cells decreases /95/. The decrease in [NAD^+]/[NADH] has been interpreted as showing either that ADH is running out of NAD^+ or being inhibited by excess NADH. The theory depends on the assumption that ADH activity is present 'in excess' and is supported by experiments showing that compounds such as fructose or uncouplers that cause increased reoxidation of NADH also cause increased ethanol oxidation (see section 3.4). Since NADH generated in the ADH reaction cannot be reoxidised in the cytosol, but must be transported into the mitochondria for reoxidation /96/, this theory links regulation of the rate of ethanol metabolism to the rates of operation of the electron transport

chain and of shuttle systems for transfer of reducing equivalents into the mitochondria.

For many years the theory of rate limitation of NADH reoxidation was the only accepted model of control of ethanol metabolism. However, in 1977 it was demonstrated that ADH in rat liver operates at 50-80% of its maximum velocity during ethanol metabolism *in vivo* /97, 98/. Subsequent kinetic analysis demonstrated that rat liver ADH is only inhibited by about 10-15% by physiological concentrations of NADH /21/. These findings led to the proposal of an alternative theory of control, that the rate of ethanol metabolism is primarily regulated by the amount of ADH in the liver /21,98/. This second theory has gained increasing support and acceptance over the last few years /13,99,100, 101,102,103/.

There have been some other recent reviews on the control of ethanol metabolism. Dawson /104/ has given a brief, clear, account of the control debate and Higgins has written a long, detailed review /105/. The control question has also been discussed by Khanna and Israel /94/, Rognstad and Grunnet /8/ and Williamson and Tischler /106/. However, none of these articles includes a comprehensive description of the factors known to influence rates of ethanol metabolism, the first because it was not intended to do so, the others because much of the recent work on factors influencing ADH activity was published after the reviews were written. In the following sections, a wide range of factors known to influence rates of ethanol metabolism *in vitro* and *in vivo* will be discussed, and the experimental evidence will be related to the two theories of control.

3.2 *Substrates and Products of the ADH Reaction*

3.2.1 *Ethanol*

The K_M values for ethanol of most liver ADH's are in the region of 0.5-2.0 mM /12,17,21,107/. This means that at concentrations of ethanol above 10 mM the rate of oxidation of ethanol by these ADH's should be essentially constant. At ethanol concentrations below 10 mM the rate of ethanol oxidation will depend on the ethanol concentration. It has been shown that rates of ethanol metabolism *in vivo* follow this pattern /3,107,108/ which in itself is support for the activity of ADH being rate-determining /107/. However, a number of other factors must be considered. Firstly, ADH's are inhibited by high ethanol concentrations /17,109/. For the rat enzyme this substrate inhibition becomes

significant at ethanol concentrations above 15 mM /13,17/. This suggests that if ADH activity is rate-determining, rates of ethanol metabolism *in vivo* should be decreased at very high ethanol concentrations. Such a decrease has been observed /99/, and the magnitude of the decrease was found to agree reasonably well with that predicted from the kinetic properties of rat liver ADH /13,17/. In contrast with these findings, other studies in rats /110,111/, humans /110,112/ and baboons /113,114/ have shown increased rates of ethanol clearance in the presence of high ethanol concentrations, and a number of different explanations have been proposed. The microsomal ethanol oxidising system (section 2.1.2) has a high K_M for ethanol, and this system may cause increased clearance rates with high ethanol concentrations /22, 110,113,114/. Alternatively, an ADH-mediated mechanism called the swift increase in alcohol metabolism (SIAM; see section 3.8) has been implicated /111,115/. In human subjects the occurrence of an ADH isozyme with a high K_M for ethanol could provide an explanation for high clearance rates observed with high ethanol concentrations /12/.

At this stage, more studies are needed to define the effects of high ethanol concentrations on rates of ethanol metabolism, both in rats and in humans. It could be pointed out, however, that the study showing decreased rates in rats at high ethanol concentrations /99/ was very detailed. Each animal was used as its own control, and blood was sampled directly from the post-hepatic vena cava rather than the tail. The latter point is critical, as circulation to the tail is affected by high ethanol concentrations /116/. Calculation of total body water for each animal was carried out to check that the slopes used to determine ethanol clearance rates were valid. To date, studies showing increased clearance rates at high ethanol concentrations have not been as detailed.

3.2.2 *Acetaldehyde*

The ADH reaction is readily reversible, and ADH is subject to product inhibition by acetaldehyde. The magnitude of the inhibitory effect of acetaldehyde in rats has been illustrated by calculation using kinetic parameters for rat liver ADH /21,100/ and in experiments using rat liver homogenates /117,118/. It has also been demonstrated that acetaldehyde can accumulate to potentially inhibitory levels in rats metabolising ethanol *in vivo* /99/, or in perfused rat livers or isolated rat hepatocytes /119,120/. Thus in any study of factors regulating rates of ethanol metabolism in rats, the importance of measurement of acetaldehyde concentrations should be considered. The same probably

applies for other experimental animals, where acetaldehyde concentrations during ethanol metabolism have not yet been well-defined. A recent study in mice has shown a high correlation between the K_M of one AlDH isozome for acetaldehyde and ethanol elimination rates /121/.

In humans, the current estimates of acetaldehyde concentrations occurring during ethanol metabolism are very low. In normal subjects, blood levels are close to zero /122/ and liver concentrations are probably no higher than 10 μM. Unless human liver ADH's prove to be much more sensitive than rat ADH's to inhibition by acetaldehyde, which appears to be unlikely on the basis of current data /14/, the normal concentrations of this metabolite would not have much effect on rates of ethanol clearance. In alcoholics /122,123,124,125/, some oriental subjects /122,126/, or people taking disulfiram or calcium carbimide /83,122,127/ acetaldehyde concentrations may be increased because of reduced AlDH activity and could begin to play a role in reducing ethanol clearance rates. A preliminary report of studies in Japanese subjects suggests that elevated acetaldehyde may be influencing ethanol elimination rates in subjects deficient in one AlDH isozyme /128/.

The occurrence of increased acetaldehyde concentrations in subjects lacking one AlDH isozyme /122,126/ or being administered AlDH inhibitors /83,122,127/ shows that acetaldehyde concentrations in humans can be influenced by changes in the activity of AlDH. Studies in rats indicate that acetaldehyde concentrations are regulated by the balance between the activities of ADH and AlDH /119,129/. Thus any factors which alter AlDH activity may play a role in regulation of ethanol clearance rates. Factors affecting the activity of AlDH's have been reviewed recently /81,130/ and will be referred to again in section 3.3.2.

3.2.3 *NAD^+ and NADH*

The observation of a change in ratio of [NAD^+]/[NADH] in the liver during ethanol metabolism is not of itself evidence supporting a rate-determining role for NADH reoxidation, although it is often assumed to be so /94/. Some incorrect assumptions concerning the significance of the change in [NAD^+]/[NADH] ratio have been discussed previously /131/ but the question will be readdressed here as it is of vital importance to understanding the control of ethanol metabolism.

In order to correctly assess the significance of the change in [NAD^+] [NADH] ratio, two factors must be considered: (a) The actual changes in concentrations of NAD^+ and NADH and (b) the effects of these changes on ADH. All of the following discussion is based on information

available for rat liver. It can be assumed that in many respects a similar situation will apply in human liver, but confirmation of this assumption will be difficult due to the problems in estimating cytosolic [NAD^+]/[NADH] redox state and free NAD^+ and NADH concentrations in human liver, and due to the complexity of human liver ADH isozyme patterns.

(a) Concentrations of NAD^+ and NADH

Since liver ADH is a cytosolic enzyme, its activity will be affected by changes in the free cytosolic concentrations of NAD^+ and NADH. Measurements of total tissue contents of NAD^+ and NADH are not a guide to the free cytosolic concentrations of these metabolites. The mitochondrial membrane is impermeable to NAD^+ and NADH, and the levels of these nucleotides will be different in each subcellular compartment. Also, a proportion of NAD^+ and NADH is bound within the cell, so that free concentrations are less than the total concentrations.

Bucher *et al* /132/ have estimated the free cytosolic NAD^+ concentration in rat liver as about 0.5 mM. The ratio of free cytosolic [NAD^+] to free cytosolic [NADH] in rat liver, determined from [lactate]/[pyruvate] ratio, is normally about 1000 /133,134/. If the free NAD^+ concentration is 0.5 mM, then the free [NADH] must be about 0.5 μM. When ethanol is being metabolised, the cytosolic [NAD^+]/[NADH] drops to about 200-500 /133,135/. The best way to explain this drop is by an increase in free [NADH] to 1.0-5.0 μM. To explain the change in ratio by a decrease in [NAD^+] would require a large alteration in the total free [NAD^+] + [NADH] and it is difficult to envisage a mechanism for this. Because the ratio of free [NAD^+]/[NADH] is so large, increasing free [NADH] to 1-5 μM would not significantly decrease the free [NAD^+].

Thus we have an estimate of the actual changes in free cytosolic [NAD^+] and [NADH] occurring during ethanol metabolism. The NADH concentration increases from 0.5 to 1-5 μM, and the NAD^+ concentration is essentially unchanged (e.g. decreases from 0.5 to 0.498 mM). This illustrates that assumptions that the cell runs out of NAD^+ during ethanol metabolism are probably incorrect. The effects of the increase in [NADH] depend on the kinetic parameters of ADH, as discussed in the next section.

(b) The effects of NAD^+ and NADH concentrations on ADH

(i) NAD^+

Although the free NAD^+ concentration probably does not change much during ethanol metabolism, the concentration of NAD^+ may be less than saturating for ADH. Three published studies giving complete kinetic parameters for rat liver ADH /17,20,21/ give different estimates of the K_M (K_a) for NAD^+. The first estimate of 0.15 mM /21/ would allow ADH to operate at about 0.8 V_{max} at physiological NAD^+ concentrations (0.5 mM, see above). This has been suggested as the main reason why rates of ethanol metabolism *in vivo* are less than the V_{max} of ADH /98/. The second major kinetic study reported a K_M (K_a) of rat liver ADH for NAD^+ of 0.033 mM /17/. With this K_M, ADH would be over 90% saturated at physiological NAD^+ concentrations and other factors (substrate inhibition by ethanol, inhibition by NADH and acetaldehyde) are postulated to account for the fact that ADH does not operate at V_{max} *in vivo* /13/. The third complete kinetic study gave a K_M for NAD^+ of 0.046 mM /20/. In other partial kinetic characterisations, differing K_M values for NAD^+ have also been reported. In one study a value of 0.176 mM was given /18/ and in another study four ADH isozymes were purified and showed K_M values for NAD^+ ranging from 0.044 to 0.115 mM /19/. The reason for the differences in reported K_M values for NAD^+ is unknown (but see section 3.6). There are also differences in other kinetic parameters reported in these studies.

An attempt has been made to influence rates of ethanol oxidation *in vivo* by increasing NAD^+ concentrations through feeding nicotinamide /136/. The total liver NAD^+ concentration was increased, but the rate of ethanol clearance in the animals did not change. This experiment does not prove that the concentration of NAD^+ is not a rate determining factor, however, as it is not known whether the free cytosolic NAD^+ concentration was affected by nicotinamide feeding. Final assessment of the significance of NAD^+ concentration in regulating rates of ethanol clearance will require further experimental studies.

(ii) NADH

The K_i of rat liver ADH for NADH is in the region of 1-2 μM /17, 21/ and concentrations of NADH occurring during ethanol metabolism *in vivo* will inhibit the enzyme by about 10-20% /13,17,21/. Therefore, even if all the increase in NADH concentration which normally occurs during ethanol metabolism was prevented, the rate of ethanol oxidation

would only be increased by 10-20%. In other words, by increasing the rate of NADH reoxidation it should only be possible to increase the rate of ethanol metabolism by a maximum of 20%.

Nevertheless, it is often claimed that NADH reoxidation is the major rate determining factor for ethanol metabolism, and increases in rates of ethanol clearance of up to 3 fold have been reported in the presence of substances giving increased NADH reoxidation (e.g. fructose, pyruvate). As discussed later (section 3.4) most of these findings can be explained in terms of the experimental conditions used. It has yet to be conclusively demonstrated *in vivo* that rates of ethanol metabolism can be increased by more than 20% by increasing rates of NADH reoxidation. It is of course possible that the kinetic parameters determined for rat liver ADH *in vitro* do not reflect those *in vivo* /105/. However, there are at present no experimental data which directly support such a possibility.

3.3 *Compounds Inhibiting Ethanol Metabolism*

3.3.1 *Inhibitors of alcohol dehydrogenase*

The most widely-used inhibitors of ethanol metabolism are the pyrazoles /137/. These compounds are potent and specific inhibitors of liver alcohol dehydrogenases /6,137/ (with the exception of π-ADH and χ-ADH from human liver /11/). The mechanism of inhibition of ADH by pyrazole has been established and its competitive nature with respect to ethanol means that the degree of inhibition is dependent on ethanol concentration /6,8/. Experiments in rats *in vivo* /138,139,140/ and in perfused liver /141/ or isolated rat hepatocytes /97,142/ have shown that 80-100% of ethanol metabolism may be inhibited by pyrazole or 4-methylpyrazole, which are the most commonly used inhibitors in the group. Some studies have been carried out in human subjects /143,144/ but the toxicity of the inhibitors means that the maximum effect on ethanol clearance rates cannot be determined /94/.

A study using a wide range of pyrazole derivatives has established a structure-activity relationship for the action of these inhibitors *in vitro* and in isolated hepatocytes /27/. It appears that the inhibitors react similarly with ADH in each case, which supports the usefulness of these compounds in physiological experiments. The fact that inhibition of ADH by the pyrazoles is competitive with respect to ethanol causes problems in interpretation of some experiments, for example those at high ethanol concentration /8/ or those where apparent K_M values for

ethanol are determined *in vivo* /145/, but there are few non-toxic alternatives. Some studies on 2 groups of uncompetitive (with respect to ethanol) inhibitors, sulfoxides and amides, have suggested that these compounds should be useful in inhibiting ethanol metabolism via the ADH pathway /146,147,148/ but they have not as yet been widely tested. The experiments so far carried out on rats *in vivo* support the conclusion that ADH activity is a major rate-determining factor in ethanol metabolism in rats /147/. Inhibitors of ADH have been used in attempts to quantitatively assess the contribution of ADH to rates of ethanol metabolism /8/, but this approach has not yet been extended as far as possible in the analysis of control of ethanol metabolism (see section 4).

3.3.2 *Inhibitors of aldehyde dehydrogenase*

Since acetaldehyde acts as an inhibitor of the ADH reaction, inhibitors of AlDH which cause increased acetaldehyde concentrations may decrease rates of ethanol metabolism. Two aldehyde dehydrogenase inhibitors, disulfiram and cyanamide, are widely used in the treatment of alcoholics /149,150/.

The mode of action of disulfiram has been extensively studied. It is a potent inhibitor of some isozymes of AlDH *in vitro* /5,7,62,149/ and the mechanism of its interaction with the enzymes from sheep /44,67,151,152/ and human /62/ liver has been investigated in detail. Disulfiram has often been used in experimental studies in animals where it consistently gives increased acetaldehyde concentrations during ethanol metabolism /7,81,82/. Inactivation of liver AlDH following disulfiram administration to live rats has been observed /153/.

The mode of action of cyanamide is not known. Administration of the compound to rats or humans gives increased acetaldehyde concentrations during ethanol metabolism /82,127,154,155/ and inactivation of AlDH following cyanamide treatment has been demonstrated in rats /82/. However, cyanamide is not a strong inhibitor of AlDH *in vitro* /156/. The compound must be converted to an active metabolite *in vivo*, but this metabolite has not yet been identified /156,157/. A recent brief report suggests that catalase is involved in this conversion /158/.

A number of other compounds (e.g. pargyline, coprine) have been shown to inhibit aldehyde dehydrogenase following administration *in vivo* but not *in vitro* /7,62/. Other compounds have been shown to act as inhibitors of AlDH *in vitro* but have not been used *in vivo* /62/. It is

interesting to note that some thiol reagents that have been studied give activation of AlDH under some conditions /159,160,161/. Steroid hormones and related compounds may also influence the activity of AlDH /162/ but the physiological significance of these findings is uncertain.

3.3.3 *Inhibitors of NADH reoxidation*

Compounds which inhibit the reoxidation of cytosolic NADH, either at the level of shuttle systems or the mitochondrial electron transport chain, will inhibit ethanol metabolism /106/. Such inhibition does not, however, indicate that these systems are rate limiting under normal, uninhibited, conditions /8/, although it does show that they are essential steps in the pathway of ethanol metabolism.

3.4 *Compounds Accelerating Ethanol Metabolism*

The possibility of finding a drug which could increase rates of ethanol metabolism has been the driving force behind a considerable amount of research (for reviews of this topic see /107,163/). However, to date there is no compound which has been consistently shown to give a significant increase in the rate of ethanol metabolism *in vivo*, although a number have been claimed to do so, as discussed in the following sections.

3.4.1 *Fructose*

It is often stated without qualification that fructose increases rates of ethanol metabolism. This is certainly true in isolated hepatocytes /164,165,166/ and in perfused liver /167,168/, where increases of 2-3 fold have been observed. However, in these preparations, rates of ethanol clearance in the absence of fructose are less than those observed *in vivo*, and fructose simply restores the rates to a physiological level /120, 169/. In these experiments the mode of action of fructose is similar to that of other substrates (e.g. lactate, pyruvate) which increase rates of ethanol metabolism by increasing NADH reoxidation through replenishment of intracellular metabolites lost during preparative procedures /21,103/.

In vivo, many studies have shown that fructose gives no acceleration of ethanol metabolism. In other studies, claims of increased rates in the presence of fructose are not borne out by the experimental evidence (for a full discussion see /170/). A recent, carefully executed study showed that rates of ethanol clearance in humans were increased by

14-17% in the presence of fructose /123/. Another recent study shows about 60% increase in overall rates of ethanol clearance following fructose infusion /171/. Thus the status of fructose as an accelerator of ethanol metabolism *in vivo* is still unclear, although it is obvious that the effects are much less than the up to 3 fold acceleration that may be observed *in vitro*.

Much of the impetus in studying fructose as a possible accelerator of ethanol metabolism has stemmed from the belief that the reoxidation of NADH is the major rate-determining step for the pathway, as there are a number of means by which fructose could possibly increase the rate of NADH reoxidation /172/. If NADH reoxidation is not a major rate-determining step for ethanol metabolism, there is less reason to assume that fructose should be effective *in vivo*. However, even if the major rate-determining factor for ethanol metabolism is the amount of ADH, there are other mechanisms by which fructose could theoretically increase rates of ethanol metabolism. Fructose is converted to glyceraldehyde, which could then bind to the ADH-NADH complex and reoxidise this to ADH-NAD^+, thus circumventing the rate limiting step of the enzyme reaction (NADH dissociation) and effectively increasing the maximum velocity /172/. This mechanism has not been demonstrated to be of significance *in vivo*, and probably requires higher concentrations of glyceraldehyde than those occurring during fructose metabolism /173/. Covalent binding of fructose to horse liver ADH, with subsequent activation of the enzyme, has been reported /174/. The authors indicate that this effect is unlikely to be a viable explanation for fructose effects *in vivo*, but the possibility could be further investigated.

3.4.2 *Pyruvate*

Pyruvate gives marked acceleration of ethanol metabolism in perfused livers and in isolated hepatocytes /120,166,173/ (see also previous section) and it has been claimed that the compound is effective *in vivo* /111/. However, re-examination of this finding led to the conclusion that pyruvate does not accelerate ethanol metabolism in rats *in vivo* /99/.

3.4.3 *Uncouplers*

Compounds which uncouple oxidative phosphorylation (2,4-dinitrophenol, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP)) have been shown to give increased rates of ethanol metabolism in liver

slices /175/, perfused liver /176,177/, isolated hepatocytes /178/ and in rats *in vivo* /179/. These findings have been explained in terms of uncoupling leading to increased NADH reoxidation /175,178/ and are frequently quoted as evidence supporting a rate-limiting role for NADH reoxidation /3,94,179/. The experiments in liver slices and isolated hepatocytes are of dubious significance, because the initial rates of ethanol metabolism are low. Experiments using dinitrophenol *in vivo* show increases in ethanol removal of about 15-20% after correction for loss in expired air /179/. This is consistent with the figure of 10-20% calculated for the degree of inhibition of ADH by concentrations of NADH occurring during ethanol metabolism *in vivo* /17/. These results suggest that the administration of dinitrophenol to rats *in vivo* may have abolished the accumulation of NADH which normally occurs during ethanol metabolism, and thereby increased ethanol clearance slightly. It should be noted that although the effects of dinitrophenol *in vivo* suggest that the rate of NADH reoxidation may contribute to determination of the rate of ethanol metabolism, this does not preclude the amount of ADH from being a rate determining factor.

3.4.4 *Other compounds giving acceleration*

Clofibrate has been reported to increase the rate of ethanol clearance in rats *in vivo* /180/ and this effect has been explained by the increase in liver size caused by this compound /181/. However, the latter study also showed an increased activity of liver ADH in clofibrate-treated rats, and increased activity of AlDH has been reported /182/. Thus there are three factors which may contribute to increased ethanol metabolism in the presence of clofibrate. A further study has indicated that increased NADH reoxidation may be of significance in the early stages of clofibrate treatment /183/ but since neither ADH nor AlDH activity was monitored the possible contribution of these enzymes at early stages is unknown.

Propionaldehyde has been shown to increase rates of ethanol metabolism by 3-fold in perfused rat liver in the presence of pyruvate /173/. This has been ascribed to circumvention of the rate limiting step of ADH (i.e. NADH dissociation) by the reaction of propionaldehyde with the ADH-NADH complex /173/. Compounds giving increased AlDH activity could act as accelerators of ethanol metabolism by giving decreased acetaldehyde concentrations. The enzyme may be induced by phenobarbital and tetrachlorodibenzo-p-dioxin /130,184/ and is activated by some steroids and related compounds /162/.

3.5 *Diet*

Starvation has been shown to decrease rates of ethanol clearance in rats, and this has been accounted for by decreased activity of liver ADH /99,101,102/. A conclusion that rates of ethanol metabolism are limited by different factors in fed and starved rats /178/ is not valid as it was based on experiments carried out using isolated liver cells depleted of substrates and metabolising ethanol at low rates. Another study has shown that the main difference between hepatocytes isolated from fed and starved rats is that cells from fed animals have higher acetaldehyde concentrations during ethanol metabolism /120/. Higher acetaldehyde concentrations have also been observed in fed rats *in vivo* /99,185,186/. In some cases this was caused by the presence of AlDH inhibitors in the diet /185/. Thus while starvation decreases ADH activity, the effects of this on ethanol clearance rate may be partially offset by a decrease in acetaldehyde concentration. A reason for higher acetaldehyde concentrations occurring in fed rats, other than the possible presence of unrecognised dietary AlDH inhibitors, is not clear. It may simply be due to the higher activity of ADH in fed animals.

3.6 *Hormonal Influences*

In a number of recent studies the rate of metabolism of ethanol has been shown to be subject to hormonal influences. Castration increases both rates of ethanol metabolism and liver ADH activity in male rats /187,188,189,190/. This was first observed in spontaneously hypertensive rats /187/ but has subsequently been shown to occur in Sprague-Dawley rats as well /188,189,190/. Administration of testosterone reverses the increases in ethanol metabolism and ADH activity caused by castration /187,188,189,190/. We have attempted to reproduce these results, but in our Sprague-Dawley animals castration produced only a slight increase in ADH activity /191/. However, ADH activity in the control animals in our experiments (5.0 μmol/min/g wet weight liver) was much higher than the control activities in other experiments (1-3 μmol/min/g wet weight) /188,189,190,192/, and as high as or higher than the 'induced' activity following castration in the other studies. These findings show that factors other than testosterone status play a role in determining ADH activity in Sprague-Dawley rats, (as also concluded by Cicero *et al.* /190/) and suggest that ADH induction following castration is more likely to occur if initial ADH activities are low.

Stress due to repeated immobilisation has been shown to increase ADH activity and rates of ethanol metabolism in rats /193/. It has been suggested that this effect may be due to increased levels of growth hormone, since increased ADH activity was found following injections of growth hormone and stress caused elevated plasma levels of this hormone in rats /194/. A similar mechanism has been proposed to explain the elevation in ADH activity after experimental uremia /194,195/.

Rates of ethanol metabolism and liver ADH activity are higher in female rats than in male rats /196/. Administration of oestradiol to female rats caused an increase in total liver ADH activity, apparently without change in the rate of ethanol elimination /196/. Although the authors did not regard it as significant, data in the paper show an increase in the K_M of ADH for NAD^+ from 0.045 to 0.16 mM with oestradiol treatment. Such a change is interesting in view of differences in published values of K_M of rat liver ADH for NAD^+ (see section 3.2.1) and could explain why an increase in total ADH activity with oestradiol treatment did not increase ethanol clearance rates at physiological NAD^+ concentrations.

It has been suggested that the rate of ethanol metabolism is increased in hyperthyroid animals /197/ (see also section 3.7.2). However, other studies have shown no increase in rates of ethanol metabolism following triiodothyronine treatment /198/. Decreased rat liver ADH activity has been observed following triiodothyronine administration /198,199, 200/. Thyroxine and triiodothyronine are inhibitors of horse liver ADH /201,202/ and rat liver ADH /200/ *in vitro*. In this context, it is interesting to note that hyperthyroid rats show an increased sensitivity to ethanol poisoning /116/. Although the results suggest that increased rates of ethanol absorption and equilibration contribute to this effect, it is apparent that other factors may also be involved. Thyroidectomy has been shown to increase the activity of liver ADH /200/, without an accompanying increase in the rate of ethanol metabolism. The reason for the lack of correlation between ADH activity and ethanol clearance rate in this instance is not clear. It could be argued that thyroidectomy decreases oxygen consumption, and hence decreases the rate of NADH reoxidation, so that ADH is inhibited. However, the calculation of similar cytosolic $[NAD^+]/[NADH]$ ratios for control and thyroidectomised animals /200/ does not support this possibility.

It appears that further studies are necessary to clarify the interaction between thyroid status and rates of ethanol metabolism. A comprehensive study would need to take into account the effect of thyroid status

on: (1) the activity of ADH; (2) the rate of NADH reoxidation, as monitored by [lactate]/[pyruvate] ratio (since this is an index of free cytosolic NADH, which liver ADH responds to rather than to rates of oxygen consumption) and (3) absorption and distribution of ethanol.

3.7 *Chronic Ethanol Treatment*

It has frequently been observed that chronic ethanol ingestion increases rates of ethanol metabolism /3,94,203/. There are three main explanations of this 'metabolic tolerance' to ethanol, as follows:

3.7.1 *Microsomal ethanol oxidising system (MEOS)*

The observation that microsomal membranes proliferate during chronic ethanol treatment led to the suggestion that the microsomal ethanol oxidising system could be responsible for development of metabolic tolerance to ethanol /204,205/. However, inhibitor studies have shown that a major part of the effect is ADH-mediated /138,206/. It has also been shown that rates of ethanol metabolism return to control levels more rapidly than the activity of MEOS following ethanol withdrawal /207/. On the basis of these and other findings the role of MEOS in development of metabolic tolerance has been questioned /94/.

This is still an area of active research (for example see /22,30/). The present case supporting the participation of MEOS in the development of metabolic tolerance rests on observations of changes in cytochrome P-450 following chronic ethanol treatment and on the observation of increased acceleration of ethanol elimination at high ethanol concentrations in ethanol-fed animals /22/. Support has also been claimed to come from experiments using deermice lacking ADH /24,32/. These animals show increased rates of ethanol metabolism and increased MEOS activity following chronic ethanol treatment. Assessment of the data on a quantitative basis, however, suggests that MEOS activity has little to do with the development of metabolic tolerance. Chronic ethanol treatment increased MEOS activity in ADH^- deermice by 4.67 fold, when expressed in μmoles per kg body wt per minute /24,32/. At ethanol concentrations of 5-10 mM, where an enzyme system such as MEOS with a K_M for ethanol of 13.2 mM would operate at 20-40% of its maximum velocity, ADH^- deermice showed no increase in ethanol clearance rates with chronic ethanol treatment /24,32/. At ethanol concentrations of 40-70 mM, where MEOS should be 70-80% saturated, chronic ethanol treatment increased ethanol clearance rates by only

30%. Until the nature of the 'non-ADH, non-MEOS' pathway /32/ which accounts for ethanol metabolism at low concentrations in ADH^- deermice is defined, the significance of experiments on chronic ethanol treatment of these animals must remain uncertain. An earlier study using ADH-negative deermice has also shown that caution must be used in equating increases in MEOS activity *in vitro* with increased ethanol elimination rates *in vivo* in these animals /23/.

At the present stage, there is no direct, conclusive evidence to show that MEOS plays a significant role in the development of metabolic tolerance to ethanol. On the other hand, there is no doubt that chronic ethanol treatment often produces significant changes in microsomal proteins and in the microsomal metabolism of other drugs /22,94/.

3.7.2 *Hypermetabolic state*

It has been suggested that increased rates of ethanol metabolism following chronic ethanol administration are caused by the development of a hypermetabolic state /94,203/. It is presumed that the hypermetabolic state leads to increased reoxidation of NADH by the electron transport chain, which then leads to increased ethanol oxidation by ADH. The theory was initially proposed following the observation of increased rates of oxygen consumption in liver slices from ethanol-treated rats /175,208/. These findings have been reproduced in one subsequent study using perfused rat liver /206/ but not in others using isolated rat hepatocytes /209,210/ or live rats as well as perfused liver /211/. The initial studies using liver slices and perfused liver /175,206, 208/ are of dubious significance as all the rates of oxygen consumption and ethanol oxidation were very low. Oxygen consumption has been shown to increase in perfused livers from male spontaneously hypertensive rats following chronic ethanol treatment /212/. Rates of ethanol metabolism were also increased, but it has subsequently been shown (by the same group /187/) that this was due to an increase in ADH activity (see next section also).

It has been suggested that the basic mechanism underlying the increase in oxygen consumption in livers from rats treated chronically with ethanol is an increase in the activity of sodium-potassium-activated ATPase /206/. This mechanism depends on the supply of ADP, as influenced by the ATPase, being the rate-limiting factor for operation of the electron transport chain. However, the factors which regulate the rate of operation of the electron transport chain under physiological conditions have yet to be fully defined /213,214/.

The possibility of increased NADH reoxidation occurring after chronic ethanol consumption is supported by reports of attenuation of the change in liver cytosolic [NAD^+]/[NADH] ratio under these conditions /215,216/. This attenuation could, however, also be related to decreased ADH activity /216/. It is unfortunate that rates of ethanol clearance were not measured at the same time as cytosolic [NAD^+]/[NADH] ratios were assessed in these studies. If the assumption that ethanol clearance rates were increased, as found in other experiments, is correct the data provide support for the operation of a non-ADH pathway.

Considering the known kinetic parameters of rat liver ADH, it is difficult to see how increased NADH reoxidation caused by a hypermetabolic state could account for increases in ethanol clearance rate of more than 20% in rats. The increase in ethanol metabolic rate after chronic ethanol treatment observed in a recent study by Britton and Israel was 16-17% /217/. This increase was reversed by treatment with the antithyroid drug propylthiouracil, which supports the concept of a thyroid-mediated hypermetabolic state as an explanation of metabolic tolerance in this instance. It is disappointing that liver cytosolic [NAD^+]/[NADH] ratios were not determined in this study. For humans there are not enough data available to assess the probable effects of increased NADH reoxidation on ethanol clearance rates.

In summary, liver oxygen consumption may be increased by chronic ethanol treatment (see also /218/), but further work is needed to confirm whether there is any link between this and the development of metabolic tolerance to ethanol.

3.7.3 *Increased ADH activity*

Some early, carefully controlled, studies carried out by Khanna and others /219,220/ showed that increased rates of ethanol metabolism following chronic ethanol treatment were closely correlated with increased activity of ADH, and this suggested that increased ADH activity explained metabolic tolerance to ethanol. A later, much less extensive study by the same group discounted these findings, and it was assumed that the amount of enzyme was of little significance as it was not rate-limiting /138/. Findings that the increase in ethanol metabolism was pyrazole-sensitive and therefore ADH mediated were explained on the basis of increased NADH reoxidation leading to the increased ADH activity /94/. However, in some very recent studies, again by the same group, it was shown that in spontaneously hypertensive rats increased rates of ethanol clearance after chronic ethanol consumption were

closely paralleled by increases in ADH activity /187/. The induction of ADH appeared to be related to a decrease in testosterone levels caused by chronic ethanol intake. In other words, ethanol is effectively acting as a chemical castrating agent (see section 3.6). The Toronto group did not find the same effect in Wistar rats, and predicted that it would be unique to the spontaneously hypertensive animals which had very low ADH activity /192/. However, similar findings have subsequently been reported by other workers using Sprague-Dawley rats /190/. In our own studies, we have been unable to find induction of ADH in Sprague-Dawley rats following chronic ethanol feeding, but our control animals had higher ADH activity than those in studies showing induction, and ADH was not induced by castration /191/ (see section 2.6). It seems probable that in our studies some other factor, possibly a hormone change due to environmental stress, was inducing ADH prior to the experimental period. The activity of ADH was not reduced by testosterone administration in our experiments. That testosterone status is not the only effector of ADH activity during chronic ethanol treatment is also shown by the results of Cicero *et al.* /190/ who demonstrated that the increase in ADH activity following ethanol consumption was not completely reversed by the administration of testosterone.

Khanna *et al.*/221/ have reported that the extent of metabolic tolerance in some rat strains is dependent on initial rates of ethanol metabolism. Rats with low initial rates show a greater degree of metabolic tolerance than rats with high initial rates. This suggests, as do our results, that induction of ADH following chronic ethanol treatment is more likely to occur in animals with low initial ADH activity. The effect of chronic ethanol consumption on ADH activity in rats and mice has been measured in many studies over the last 20 years. In addition to those already cited, other results have shown increases /222-229/, no change /175,204,205, 230-235/ or decreases /230,235,236/ in enzyme activity with chronic ethanol treatment. In view of the complexity of hormonal controls on the enzyme, which is just now becoming apparent, and the finding that factors such as environmental stress may influence enzyme activity, such variation in results is not surprising. The ADH activities obtained in most of these studies cannot be directly compared since different extraction and assay procedures were used. Because of the difficulty in obtaining consistent results, it is obvious that any study on the possibility of a connection between chronic ethanol intake, rates of ethanol metabolism and ADH activity must be carefully controlled. It should be noted that chronic ethanol intake has

been reported to increase acetaldehyde concentrations /237,238,239/. This would tend to decrease rates of ethanol oxidation by ADH, which would complicate the analysis of relationships between ADH activity and rates of ethanol metabolism.

In summary, there is a considerable amount of evidence which shows that metabolic tolerance to ethanol can, in some animals, be accounted for by increased ADH activity. The basic mechanism of the effect has not been fully defined, although it appears to be related to the decrease in testosterone level caused by ethanol.

3.8 *Swift Increase in Alcohol Metabolism (SIAM)*

It has been reported that a large, acute dose of ethanol (5.0-6.5 g/kg) causes an increase in the rate of metabolism of ethanol in rats /115/ and mice /240/ and in ADH^+ deermice /241/. The phenomenon has been named Swift Increase in Alcohol Metabolism (SIAM). Studies using perfused liver have indicated that the increase in the rate of ethanol metabolism is accompanied by increased oxygen uptake by the liver /242,243/. It has also been shown that both the increased rates of ethanol oxidation and of oxygen consumption can be abolished by the antithyroid drug propylthiouracil /244/. In some studies, it has been claimed that there is a genetic difference in the tendency to show a SIAM effect, and 'SIAM' or 'non-SIAM' strains of rats have been selected /115,245/. In other studies, unselected rats have apparently all demonstrated the SIAM effect /242/. Response to a high ethanol dose has been shown to vary in different strains of mice /240,246/. To date, a majority of the work concerning SIAM has been carried out by the group of Thurman and others. An unsuccessful attempt to reproduce SIAM in rats *in vivo* /99/ has been criticised by Thurman /240/ on the basis that the question of genetic susceptibility was not considered and that experimental conditions could have been inappropriate. We have used isolated hepatocytes in a further reassessment of the SIAM effect. In cells isolated from fed rats, oxygen consumption was not affected by acute ethanol administration (5.0 g/kg, 2.5h prior to isolation of cells) /247/. In cells from starved animals, rates of oxygen consumption were increased by 10-20% /247/. Rates of gluconeogenesis from lactate and pyruvate were also increased, but ethanol oxidation rates were unchanged /247/. Thus although our data do not support the occurrence of a swift increase in alcohol metabolism, they do support the suggestion that acute ethanol treatment can increase oxygen consump-

tion. The occurrence of the effect in cells from starved animals but not from fed does not support the theory that such increased oxygen consumption is caused by inhibition of glycolysis /242/.

It has been suggested that the increased rates of oxygen consumption and ethanol clearance occurring with acute ethanol treatment are caused by the same mechanism as that giving similar increases following chronic ethanol treatment /248/. In view of the variation in findings related to oxygen consumption following chronic ethanol treatment /175,206,208,209,210,211/ the validity of this suggestion is difficult to assess. The effects of acute and chronic ethanol treatment on oxygen uptake in fed and starved rats require further study. One thing is clear, however – increased ethanol oxidation need not be an automatic consequence of increased oxygen consumption.

3.9 *ATP demand*

It is often stated that the rate of NADH reoxidation limits rates of ethanol metabolism, and that both are regulated by the rate of operation of the electron transport chain, which is in turn regulated by the demand for ATP synthesis /172,206,249/. Such reasoning lies behind basic mechanisms proposed to account for SIAM /242/, metabolic tolerance to ethanol produced by a hypermetabolic state /249/ and the fructose effect /172/. However, evidence linking rates of ethanol metabolism to ATP demand under physiological conditions is hard to find. The experiments of Scholz /172/ on the fructose effect in perfused liver are difficult to interpret in relation to the situation *in vivo* as the rate of ethanol metabolism in the absence of fructose was very low. In another study in hepatocytes metabolising ethanol at rates similar to those *in vivo* the addition of fructose increased rates of glucose synthesis and decreased ATP concentrations, but did not accelerate ethanol oxidation /120/. It has been claimed that inhibition of gluconeogenesis by quinolinate decreases rates of ethanol oxidation in isolated rat hepatocytes /178/. However, this finding has been contradicted by the results of another study, where rates of gluconeogenesis were inhibited by quinolinate or tryptophan without affecting ethanol oxidation /250/. The reason for disagreement between the two studies is not known. In the latter study /250/ it was also shown that maximal acceleration of ethanol oxidation by lactate in isolated hepatocytes occurred at lactate concentrations of 0.5-2.0 mM, where rates of gluconeogenesis were very low. With higher lactate concentrations (2-10 mM) rates of gluconeo-

genesis increased in a linear fashion, whereas rates of ethanol oxidation were almost unchanged. Thus there is in fact evidence suggesting that rates of ethanol metabolism are not regulated by ATP demand under physiological conditions.

The fact that ouabain (an inhibitor of sodium plus potassium-activated ATPase) inhibits ethanol metabolism has also been accepted as evidence that ATP demand is an important rate-controllng factor, particularly following chronic ethanol treatment /206/. Other evidence suggests, however, that the inhibition of ethanol metabolism by ouabain is caused by non-specific inhibition of the respiratory chain /251/. In light of this evidence, studies using ouabain cannot provide direct support for ATP demand being a rate-determining factor. The role of ATP demand in regulation of ethanol metabolism needs to be reinvestigated, taking into account current work on the regulation of the respiratory chain /213,214/.

4. THE PRESENT STATUS OF THE CONTROL DEBATE

The evidence discussed in the preceding sections, particularly those concerning hormone effects, chronic ethanol treatment, and diet, makes it clear that the activity of ADH is an important factor in determining rates of ethanol metabolism. Kinetic studies have also shown that the NADH concentrations occurring during ethanol metabolism should be inhibitory to ADH, to such an extent that increased NADH reoxidation should cause about a 20% increase in the rate of ethanol oxidation. In addition, acetaldehyde may accumulate, at least in rat liver, to concentrations sufficient to inhibit ADH, and so the activity of aldehyde dehydrogenase may play a part in limiting rates of ethanol metabolism. Therefore it is likely that there is no single rate determining step for the pathway of ethanol metabolism in the liver.

If it is accepted that the activity of ADH can regulate the rate of ethanol metabolism, a problem remains in explaining the increase in free cytosolic NADH concentration which occurs in the liver during ethanol metabolism. In an alternative model, where the rate of NADH reoxidation was the only rate determining step, the increase in NADH concentration could be explained by an absolute limitation on the rate of NADH reoxidation. NADH would accumulate until the rate of ethanol oxidation by ADH was reduced to the same as the rate of NADH reoxidation. In such a model, an increase in the activity of ADH would lead to increased NADH concentrations but not to an increase in the

rate of ethanol metabolism. However, increases in ADH activity often do lead to increased rates of ethanol metabolism, as well as to increased NADH concentrations.

It has now been shown that the increase in free cytosolic NADH concentration during ethanol metabolism can instead be explained by the kinetic properties of cytosolic malate dehydrogenase /100, 131/, which is the first enzyme involved in the malate-aspartate shuttle mediated reoxidation of cytosolic NADH. This enzyme is not saturated with NADH at physiological concentrations, and responds to increased NADH concentrations by increasing the rate of NADH reoxidation. Therefore the extent of NAD^+/NADH redox state change in the cytosol of the liver cell during ethanol metabolism is influenced by malate dehydrogenase, and responds to rather than regulates the rate of ethanol metabolism. The significance of malate dehydrogenase in relation to metabolic regulation has probably been underestimated, since it is often regarded as a 'near equilibrium enzyme' and traditionally such enzymes are not 'control enzymes'. The relevance of the near equilibrium concept as applied to cytosolic malate dehydrogenase is placed in doubt by the results of Cronholm /252/. Although the activity of malate dehydrogenase does not have much influence on flux through the pathway of ethanol metabolism, it is clear that it can regulate the concentration of NADH /100/ and would therefore influence rates of reactions which are very responsive to this parameter.

At present, no quantitative assessment is available of the relative contribution of each potential control step to the regulation of ethanol metabolism. Attempts have been made to 'quantitate' pathways of ethanol metabolism using inhibitors or isotopic labelling. Such studies have generally been directed towards calculating the contribution of non-ADH pathways to overall rates of ethanol metabolism. For example, two isotopic labelling studies /253,254/ have shown that non-ADH pathways account for only 11% or less of total ethanol metabolism, while the results of another study /255/ contradict these, indicating that 20-30% of ethanol metabolism is non-ADH mediated. However, Rognstad and Grunnet /8/ (page 73) have used inhibition of ethanol metabolism in isolated hepatocytes, by varying concentrations of pyrazole, in an attempt to assess the role of ADH in regulation of ethanol metabolism. Their approach is as described by Rognstad /265/ for using a wide range of inhibitor concentrations to identify rate-limiting steps in metabolic pathways. Rognstad suggests that a linear plot of 1/v vs [I] indicates that the inhibited enzyme is rate determining, and that a

curved plot with a 'null' region at low inhibitor concentrations indicates a non-rate determining enzyme. Rognstad and Grunnet /8/ obtained a curved 1/v vs [I] plot, and concluded that ADH is present in excess, at least 35% above the capacity of the system, and that until the enzyme is inhibited by this amount no effects on the overall system will be seen. Although this 35% excess correlates well with predictions based on kinetic parameters for the rat enzyme /13,98,103/ this is probably fortuitous. The experiments were carried out in hepatocytes incubated in the presence of ethanol alone. Cornell /103/ has shown that supplementation of hepatocytes with lactate, pyruvate and lysine gives a linear plot when the reciprocal of the rate of ethanol metabolism is plotted against pentylpyrazole concentration. This suggests, using Rognstad's approach, that ADH is rate-determining in supplemented cells, although it is not in unsupplemented cells. The data of Cornell also show that even in supplemented cells ADH must be operating at only 63-78% of its maximum velocity. Therefore, a linear plot of 1/v vs [I] does not necessarily mean that ADH is working at its maximum velocity, and that the assumption that if ADH works at 35% less than V_{max} it must be inhibited by 35% before affecting the overall rate of ethanol metabolism is incorrect. In view of this the inhibitor experiments of Rognstad and Grunnet /8/ do not provide a quantitative determination of the role of ADH in regulation of ethanol metabolism.

As pointed out by Kacser in a recent article /257/, theoretical frameworks do exist /258,259,260,261/ within which quantitative assessment of the relative significance of control steps can be made if appropriate experiments are carried out. These theoretical models can readily account for the occurrence of multiple rate-determining steps in a pathway. Kacser's approach to analysis of metabolic control relies on the alteration of the activity of one enzyme in a pathway by small amounts and measurement of the effect of the alteration on flux through the pathway. One of the main limitations of the approach is the availability of suitable means of specifically altering the activity of a single enzyme. In this regard, the work already carried out by Cornell *et al.* /27/ and by Plapp *et al.* /147/ should be particularly helpful in determining suitable inhibitors for ADH. It is to be hoped that in the near future, assessment of factors controlling rates of ethanol metabolism will be carried out on the basis suggested by Kacser /257/ and a clear solution to the control debate will be provided. Such experiments would be difficult to perform in humans, but extension of studies to animals other than the rat would help to confirm whether the control of the pathway is similar

in all mammals. A recent study of ethanol metabolism in guinea pigs /262/ suggests that these animals could be particularly useful since in several respects they show closer metabolic similarity to humans than rats do.

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