ALCOHOL METABOLISM

For the most part, alcohol in the context of this lecture means ethanol. Ethanol arises as the natural product of the fermentation of carbohydrates. Most societies seem to concoct alcoholic beverages of some form or another, and except for ritual occasions seem to consume them with moderation and good sense. Only when distillation, affluence and advertising come together does excessive alcoholic intake seem to become a problem.

Even, if by natural inclination or because of some religious conviction, one avoids the ingestion of alcohol by mouth, the fact that the gastrointestinal tract contains microorganisms means that there is a constant potential for the production of ethanol following the ingestion of a high carbohydrate containing meal. The amount of ethanol produced is usually very small, but it does vary depending on the amount and type of carbohydrate consumed, and on the nature of the bacteria, yeasts and other fungi which are found on the gut.

Since material absorbed from the gut passes into the portal vein and goes to the liver, the metabolism of ethanol occurs largely in the liver. Most of the alcohol (greater than 80% ) is metabolised by the pathway I'm going to present, and less than 20% by a microsomal detoxification pathway that I won't be discussing.

The first enzyme in the pathway, which really only has 3 enzymes in it, is alcohol dehydrogenase, and this enzyme has a very high activity in liver compared to other tissues in the body.
Thus, alcohol absorbed by the gut passes into the portal vein and onto the liver. The first enzyme in the pathway, the alcohol dehydrogenase, converts the ethanol to acetaldehyde in a reversible reaction which involves the conversion of NAD to NADH.

The alcohol dehydrogenase is
- cytoplasmic
- relatively non-specific, will react with other alcohols including methanol and glycerol
- human liver enzyme has a $K_m$ of about 1.2mM
- the equilibrium is in the direction of ethanol formation rather than ethanol oxidation

The $K_m$ of 1.2mM is quite low when one considers that the present legal driving limit is 80 mg of ethanol per 100ml of blood, or about 18mM, so at the legal driving limit the enzyme would be expected to be pretty well saturated. However, at concentrations above about 10mM the enzyme is subject to inhibition by its own substrate.

The next step is the oxidation of the acetaldehyde to acetic acid, again using NAD
This reaction is irreversible, and the enzyme, aldehyde dehydrogenase, is again in highest activity in the liver, but also again to some extent in most other tissues. This enzyme is cytosolic in man, but is mitochondrial in rats.

Although theoretically the activity of this enzyme is high enough to cope with the rate at which alcohol is oxidised by alcohol dehydrogenase, there is a limit to the rate at which NADH can be converted back to NAD in order to allow the reaction to continue. (This is dependent on the various shuttle systems, particularly the malate-aspartate shuttle, which effectively transfer cytosolic NADH into the mitochondrion where it can interact with the electron transport chain. This is shown below. **Figure 4-malate-aspartate shuttle.**

This series of reactions allows NADH to be converted into NAD on the cytosolic side of the mitochondrial membrane by linking this to the conversion of cytosolic oxaloacetate to cytosolic malate. Malate is then transferred across the inner mitochondrial membrane by a
specific transporter and the reaction reversed in the inner mitochondrial space generating NADH from NAD. The NADH then has access to the electron transport chain. The rest of the scheme involves regenerating the cytosolic oxaloacetate from the mitochondrial pool of oxaloacetate.)

As a result of the limitations in regenerating NAD, there is always a build up of acetaldehyde which passes out from the liver into the blood, and this acetaldehyde is responsible for some of the unpleasant symptoms of alcohol excess, ie. headache, nausea and vomiting. A genetic deficiency of this aldehyde dehydrogenase occurs in 45% of the Chinese and Japanese population, which makes them particularly prone to the unpleasant effects of ethanol intake.

In addition, it is this second enzyme, the aldehyde dehydrogenase, that is inhibited by the drug disulpharim or 'Antabuse'. This is a drug which is given to alcoholics in what amounts to a chemical aversion therapy. Following the administration of 'Antabuse', and hence the inhibition of the aldehyde dehydrogenase, the drinking of alcohol results in the accumulation of acetaldehyde in the body resulting in severe nausea, vomiting and hypotension. It is a rather dangerous drug, and death has been recorded after drinking only 30ml or so of rum in an individual who was given the standard dose of 'Antabuse' for several days. The effects of 'Antabuse' are not entirely due to the acetaldehyde alone. If acetaldehyde is administered exogenously, rather than generating it endogenously with alcohol plus 'Antabuse' then instead of hypotension a state of hypertension develops. The 'Antabuse' must therefore do something in addition to raising the acetaldehyde concentration of the blood. The 'Antabuse' is metabolised by the liver by a mechanism that utilises reduced glutathione and in which the 'Antabuse' is cleaved into 2 molecules of diethyl dithiocarbamate. The diethyl dithiocarbamate is a potent chelator of metal ions, particularly copper, and will effectively complex the copper ions associated with the enzyme dopamine _-hydroxylase, which is a key enzyme in the synthesis of nor-adrenalin. This is outlined below.
If one gives acetaldehyde alone this clearly doesn't happen. With acetaldehyde alone there is a release of nor-adrenalin in response to the acetaldehyde and this causes vasoconstriction of the arterioles and an increase in blood pressure (ie hypertension). In the case of 'Antabuse' plus alcohol generating acetaldehyde endogenously, the nor-adrenaline release does not occur because of the inhibitory effect of diethyl dithiocarbamate on the activity of dopamine _-hydroxylase preventing nor-adrenalin synthesis. The result here is that the blood vessels dilate and there is a consequent fall in blood pressure (ie hypotension). Thus, it is usual to give noradrenalin injections to anyone suffering excessive reaction from taking alcohol whilst on 'Antabuse' therapy.
The last step in the pathway is the conversion of acetic acid to acetyl CoA by one of the thiokinase enzymes.

**Figure 6- acetate thiokinase reaction.**

![Acetate Thiokinase Reaction](image)

The pyrophosphate is rapidly converted to inorganic phosphate by pyrophosphatase.

This pathway of ethanol metabolism is not by itself particularly striking, but it is the interactions of this pathway with other pathways which can produce unfortunate results when there is an overload of ethanol.

**Ethanol Overload.**

A major contribution to the adverse effects of ethanol, as far as interaction with other pathways is concerned, arises from the change in redox state, due to the increase in NADH and decrease in NAD. This altered redox state occurs in the cytosol, where NADH can be produced in excess of the capacity of the aspartate-malate shuttle system to transfer reducing equivalents across the mitochondrial membrane, and within the mitochondrion itself. The effects are reportedly more difficult to induce in rats than in humans, because in the rat the acetaldehyde dehydrogenase is a mitochondrial enzyme (rather than cytosolic as in man) and hence the NADH generated from this reaction has a more ready access to the electron transport chain of the mitochondrion than the NADH produced cytosolically in man, where transport into the mitochondrion may be rate limiting.

**1). Effects on Gluconeogenesis.**

Overconsumption of ethanol can lead to hypoglycaemia, ie a reduction in the concentration of glucose in the blood, because the metabolism of ethanol can interfere with gluconeogenesis in the liver. Krebs showed many years ago that in the isolated perfused rat liver, the rate of gluconeogenesis from lactate fell as the ethanol content of the perfusate was
increased. This is shown on the next slide. (Although the effect is shown for gluconeogenesis from lactate, similar inhibition would be expected for other gluconeogenic substrates such as alanine and other amino acids, and glycerol.)

**Figure 7- effect of EtOH on perfused liver gluconeogenesis.**

![Figure 7](image)

The inhibitory effect of ethanol increases up to about 10mM and then falls off. The decrease in inhibitory effect is due to the inhibitory effect of ethanol on the alcohol dehydrogenase at concentrations above about 10mM. Maximum inhibition therefore occurs at concentrations which in physiological terms are not very high. For example, 3 glasses of sherry, (about 200ml,) will produce a blood alcohol level in excess of 7mM, and for the next 2 hours it will remain between 5 to 10mM.

The inhibitory effect of gluconeogenesis occurs because the rapid oxidation of ethanol to acetaldehyde converts NAD to NADH, and this altered NAD/NADH ratio affects a number of NAD-linked enzyme reactions. Since there is less NAD available the capacity of lactate dehydrogenase to convert lactate to pyruvate is reduced.

\[ \text{Lactate} + \text{NAD} \rightarrow \text{Pyruvate} + \text{NADH} \]

Since this step is the first step in committing lactate to glucose formation then gluconeogenesis is inhibited.

The altered cytosolic redox state would also be expected to act against gluconeogenesis at 2 other points involving NAD linked enzymes.
Firstly at the level of glycerol phosphate dehydrogenase, which is involved in feeding glycerol into the gluconeogenic pathway, and would be expected to act specifically against the conversion of glycerol to glucose, and secondly at the level of cytosolic malate dehydrogenase. This enzyme is involved in conversion of cytosolic malate to oxaloacetate, which is then converted by PEPCK to PEP as part of the reversal of pyruvate to PEP. The inhibitory effect of the altered redox state at this step has a general inhibitory effect on gluconeogenesis from all substrates which involve converting pyruvate to PEP, which means all substrates other than glycerol.

Consequences of Ethanol Overload

Gluconeogenesis

Lactate Dehydrogenase

\[
\text{Lactate} + \text{NAD} \rightleftharpoons \text{Pyruvate} + \text{NADH}
\]

Glycerol to Dihydroxyacetone phosphate

\[
\text{Glycerol} \rightarrow \text{Glycerol 1-phosphate} \rightarrow \text{Dihydroxyacetone phosphate}
\]

\[
\begin{align*}
\text{ATP} & \quad \text{ADP} \\
\text{NAD} & \quad \text{NADH}
\end{align*}
\]

Cytosolic Malate Dehydrogenase

\[
\text{Malate} + \text{NAD} \rightleftharpoons \text{Oxaloacetate} + \text{NADH}
\]

Theoretically at least the altered mitochondrial redox state may also contribute to a decrease in Krebs cycle activity in the liver since the isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehydrogenase are all opposed by the increase in NADH. However, the extent to which TCA cycle activity is reduced depends on the rate at which the NADH derived from the cytosolic compartment can transfer electrons to the electron transport chain.

2). Development of a fatty liver.
A longer term, more chronic, consequence of sustained ethanol overload is the development of a fatty liver. If more than 30 to 40% of the daily calorie intake is in the form of ethanol then a fatty liver is likely to result. From a biochemical standpoint, the development of a fatty liver requires the provision of both glycerol 1-phosphate, (as backbone for the triglyceride), and long chain fatty acids, and then some mechanism by which the synthesised triglyceride is contained within the liver rather than exported.

The pathway for triglyceride formation is summarised below.

(i) **Provision of glycerol 1-phosphate**.

As already outlined, the change in cytoplasmic redox state, the increase in NADH, will affect any NAD-linked enzyme. The enzyme which forms glycerol 1-phosphate from dihydroxyacetone phosphate, ie glycerol phosphate dehydrogenase, is one such enzyme. In normal liver perfused with lactate the concentration of glycerol 1-phosphate is about 0.24mM, but if one includes ethanol in the perfusate medium at a concentration of 10mM (about the level expected after 3 glasses of sherry) the level of glycerol 1-phosphate will increase to about 1.6mM within about 45 minutes. This is because the increase in cytosolic
NADH pushes the glycerol 1-phosphate dehydrogenase reaction in the direction of glycerol 1-phosphate formation and this increase in glycerol 1-phosphate favours triglyceride synthesis.

(ii) **Provision of fatty acids.**
Although it is well established that excessive ethanol consumption leads to fatty livers the source of the fatty acids was uncertain for some time. There was some debate as to whether the fatty acids were formed from the acetyl CoA produced as a result of the ethanol metabolism, or whether they were derived from adipose tissue. Direct production of fatty acids from ethanol seemed unlikely since the activity of the thiokinase enzyme is insufficient to account for the rate at which triglyceride can be formed in the liver. The fatty acids are now thought to be derived from the blood stream. Ethanol does not markedly increase the level of fatty acids in the blood (although one might anticipate that the hypoglycaemic effect of ethanol might result in a lowered circulating insulin concentration and consequent hydrolysis of triglyceride and release of fatty acids ), but ethanol does increase blood flow through the liver. A dose of ethanol of 0.7g of ethanol/kg body weight (equivalent to about 3 to 4 pints of beer ) increases blood flow in the liver by about 50%. This is associated with an increase in the number of sinusoids perfused and an increase in the absorptive surface and uptake of fatty acids from the blood.

(iii) **Stimulation of phosphatidic acid phosphatase.**
In addition to providing more glycerol 1-phosphate, and increasing the supply of fatty acids, ethanol also increases the activity of a key enzyme in the formation of triglycerides. The activity of the phosphatidic acid phosphatase is increased more than 4 fold when large doses of ethanol are taken. This enzyme commits the phosphatic acid to the formation of diglyceride and then triglyceride, rather than the formation of other phospholipids, which is the alternative route for phosphatidic acid metabolism.

Thus, ethanol increases the formation of glycerol 1-phosphate, increases the provision of fatty acids by the blood supply, and increases the activity of one of the enzymes of triglyceride synthesis, the overall effect being to promote the synthesis of triglyceride and generate a fatty liver. It is not clear from the literature why the triglyceride formed is not exported from the liver as VLDL, since this would be the normal consequence of hepatic
triglyceride formation, but clearly this does not happen with chronic alcohol consumption. One of the problems in understanding the effects of chronic high ethanol intakes is that such a diet is often associated with overall poor nutrition, and this poor nutrition can give rise to a number of other complications which are not directly attributable to the ethanol.

3). Interference with the visual process
A further consequence of ethanol overload is an effect on Vitamin A metabolism. As mentioned earlier, the alcohol dehydrogenase is relatively non-specific and will act on a number of alcohols converting them to the corresponding aldehydes. Apart from liver, most tissues usually see very little ethanol. However, in the retina of the eye, the alcohol dehydrogenase is an important component in the visual process where it converts retinol (or Vitamin A) into retinal (the aldehyde form), a reaction which is particularly important in night vision. (The same reaction is also important in the process of sperm formation in the testis.)

Consequences of Ethanol Overload

If the system is flooded with ethanol this is preferentially oxidised and prevents the formation of retinal from retinol, and in so doing interferes with both the visual process and sperm formation. This relates to the fact that chronic alcoholics suffer from night blindness and are usually sterile. In experiments with homogenates of the testis it has been demonstrated that the conversion of retinol to retinal is inhibited at an ethanol concentration about 1/100th that of the legal driving limit.

Methanol Consumption.
The enzymes which convert ethanol to acetaldehyde to acetic acid will also act on methanol to generate firstly formaldehyde and then formic acid.

ie Ethanol -------> Acetaldehyde -------> Acetic acid
and

Methanol------> Formaldehyde -------> Formic acid

The formaldehyde and formic acid are much more reactive chemically than acetaldehyde and acetic acid (formaldehyde is used to preserve tissues, and formic acid is used as a poison by some stinging insects, you wouldn't put it on chips instead of vinegar --acetic acid !!). Subjects who, by accident or design, consume methanol put themselves at severe risk of tissue damage and in extreme cases death. If formaldehyde and formic acid are generated in any concentration within tissues they cause severe damage, a sort of in situ fixation, an irreversible denaturation which leads to severe liver damage and blindness.

The treatment for anyone who is known to have consumed methanol is to flood them out with ethanol. This is an attempt to prevent damaging concentrations of formaldehyde and formic acid being formed. The enzymes involved preferentially react with ethanol and acetaldehyde and so the provision of ethanol diverts enzyme activity away from the metabolism of the methanol, stops formaldehyde and formic acid generation and allows the body to lose the methanol through the kidneys into the urine, or because methanol is very volatile it can be lost via the lungs.