Metabolic effects of ethanol

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Ethyl alcohol (in this paper 'ethyl alcohol', 'ethanol' and 'alcohol' are synonymous) is a nutrient, a tranquillizer, a beverage which produces euphoria, but in excess is also a toxic substance responsible for much human degradation and a social plague increasing the death rate among the adult population (Fig. 1). Nevertheless, the consumption of ethanol is widespread. Very curiously, its metabolic effects do not draw the scientific attention they deserve.

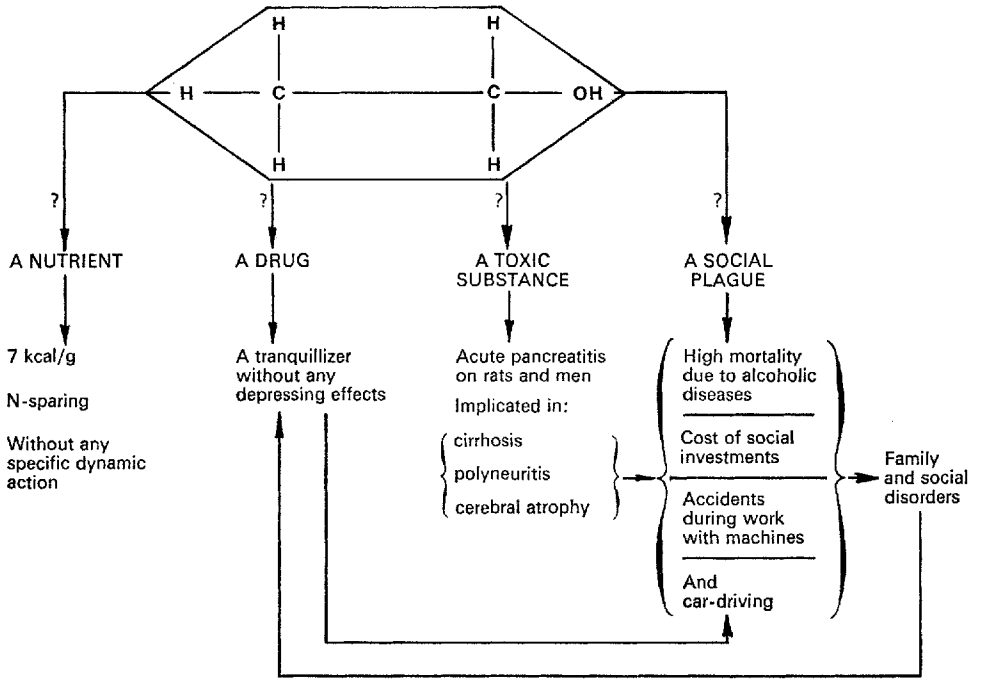

Fig. 1. What is ethanol?

We have already reported (Trémolières & Lowy, 1970) on the physiology of ethanol oxidation in normal doses (less than 2 g/kg body-weight in the non-habituated, well-fed subject) and also in pathological conditions. In this paper, we shall attempt to outline the metabolic effects of ethanol as a background to its

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physiopathological effects. We shall study (1) the enzyme systems which oxidize ethanol; (2) the metabolic effects induced by ethanol oxidation.

Which are the enzyme systems oxidizing ethanol?

It is generally recognized now that three enzyme systems can oxidize ethanol: the best known system, alcohol dehydrogenase (ADH); the most recently discovered, the microsomal ethanol oxidizing system (MEOS) (Orme-Johnson & Ziegler, 1965, in the rat; Lieber & DeCarli, 1968, in man); and the most controversial, catalase, acting as a peroxidase in the presence of an enzymatic generator of hydrogen peroxide.

ADH. This enzyme is Zn^{2+} bound, has NAD^+ as a cofactor, an optimum pH of 10·8, and is found in the cytosol of hepatocytes (Fig. 2). According to some (e.g. Forsander, 1971), ADH can also be found in kidney, lungs, skeletal muscle, brain, retina, adipose tissue and the pancreas. That of the gastro-intestinal tract would come from bacteria and would not exist in germ-free animals.

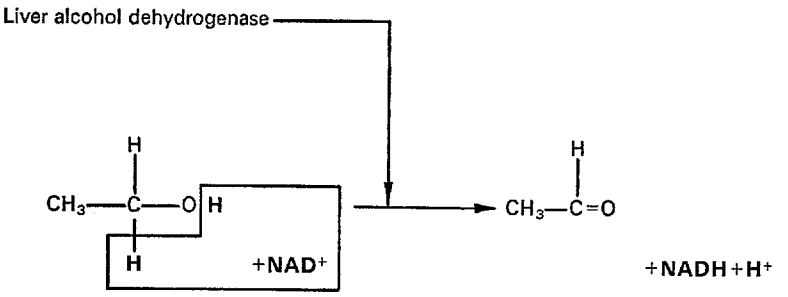

Fig. 2. Oxidation of ethanol: the alcohol dehydrogenase system.

It is known that the thermodynamic equilibrium of the ADH reaction in vitro is in favour of ethanol formation, but oxidation occurs because acetaldehyde and NADH are readily metabolized by other enzymes. The coupled reactions removing these two products are then the limiting factors of ethanol oxidation, at least by this pathway.

MEOS. This system seems to be located exclusively in the liver, and its optimum pH, 6·7–7·4, is more physiological than that of ADH. Its most striking feature is its utilization of NADPH to activate molecular oxygen; neither $NADP^+$ nor NADH can replace NADPH. The systems which can furnish NADPH are glucose-6-phosphate dehydrogenase, soluble isocitrate dehydrogenase, the malic enzyme producing pyruvate from malate, and the glutamate dehydrogenase producing α-ketoglutarate and NH_4^+ (Fig. 3).

Lieber (1971) has calculated from the activity of microsome preparations in vitro that the MEOS would cover more than 20% of the in vivo ethanol oxidation.

The peroxidative pathway of catalase. After Keilin & Hartree (1936), several workers attempted to show that there were experimental or physiopathological

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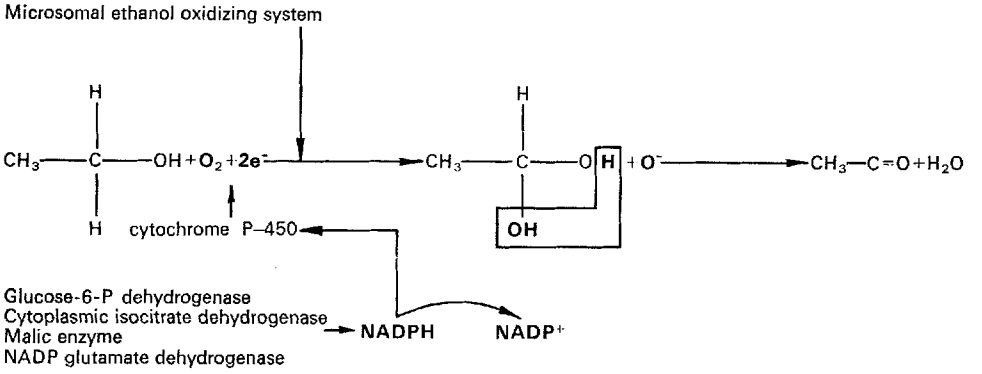

Fig. 3. Oxidation of ethanol: the microsomal ethanol oxidizing system.

conditions in which a peroxidation of ethanol by catalase could be demonstrated (Fig. 4). This opinion was strongly resisted by many people (e.g. Westerfeld & Schulman, 1959).

Trémolières & Carré (1960, 1961) observed that when chronic alcoholics were given either 0·8 g ethanol/kg *per os* or 0·4 g/kg intravenously, there was a 40–50% elevation of the basal energy expenditure; ethanol has a specific dynamic action in these subjects. The oxidation of ethanol was also three to four times more rapid than in normal subjects. Trémolières & Carré (1961) then showed that in the plasma of intoxicated alcoholics there appeared to be an enzyme system able to oxidize ethanol, activated by AMP and D-amino acids, and inhibited by CN^-. These findings were reproduced in the rat (Trémolières & Carré, 1962).

It is remarkable that ethanol in vivo induces a ribonucleic and nucleotide catabolism in some tissues, especially pancreas (Decloître & Lowy, 1963; Trémolières, Carré, Scheggia, Delcroix & Potet, 1963; Trémolières, Carré, Scheggia, Potet &

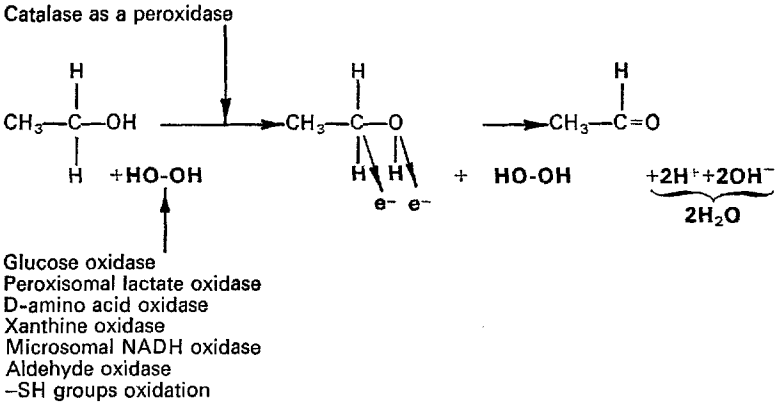

Fig. 4. Oxidation of ethanol: the peroxidative pathway of catalase.

Martin, 1963; Lowy & Griffaton, 1965). However, the mechanisms which trigger this catabolism are unknown. The evidence for the peroxidative pathway of ethanol oxidation is summarized in Fig. 5.

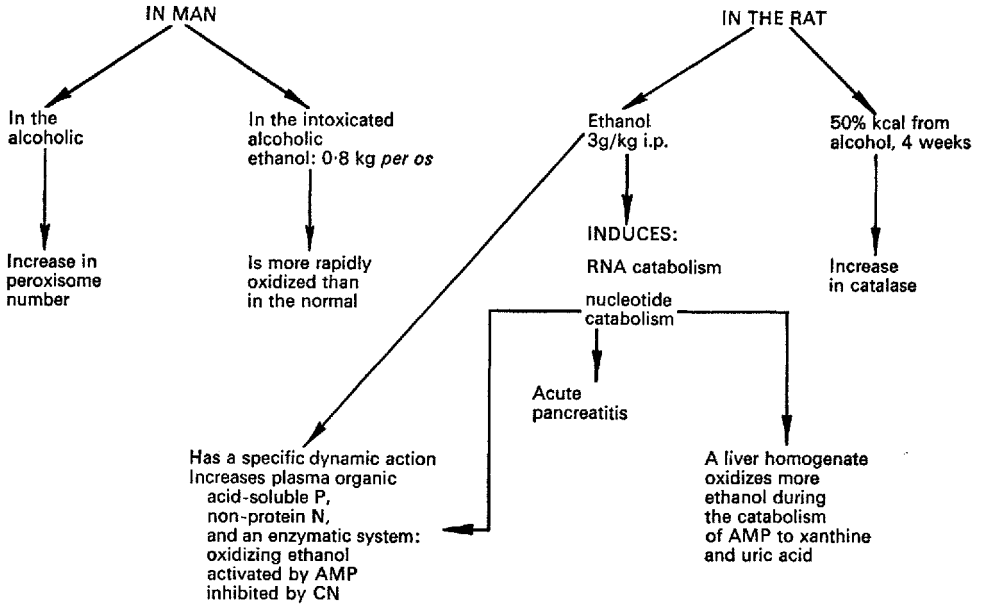

Fig. 5. Evidence for a peroxidative pathway of ethanol oxidation.

Metabolic effects of ethanol

The introduction of ethanol into an organism or into the perfusion medium of an isolated liver increases the redox potential of tissues and results in an increase of reduced compounds in the liver, blood or the perfusion liquid.

According to classical authors (e.g. Forsander & Räihä, 1960; Forsander, Mäenpää & Salaspuro, 1965), ethanol administration produces the following effects: an increase in pyruvate and lactate, and an increase in the lactate:pyruvate ratio; an elevation of β-hydroxybutyrate, acetoacetate and the ratio β-hydroxybutyrate: acetoacetate; an increase in succinate, fumarate and acetate; and in experiments with 1–^{14}C-labelled ethanol, acetate becomes highly labelled, whereas pyruvate and β-hydroxybutyrate poorly incorporate the label.

These facts would be associated with an inhibition of the tricarboxylic acid cycle in the liver, where ethanol is oxidized to acetate. Acetate itself would then not be oxidized in the liver but in other tissues.

It is thought that mitochondrial inhibition results from the NADH transfer from cytoplasm to mitochondria. Various shuttles have been proposed: the dihydroxyacetone–α-glycerophosphate pair (Thieden & Lundquist, 1967); the malate–oxaloacetate system (Borst, 1961; Hassinen, 1967); and at the present time, the shuttle of Whereat, Orishimo, Nelson & Phillips (1969) by which fatty acids

pass from the cytoplasm into the mitochondria and out again, a cycle which is accompanied by a transfer of NADH and acetyl-CoA from the cytoplasm to the mitochondria.

Another important property of ethanol metabolism is the inhibition of the Embden–Meyerhof pathway of glycolysis. The mechanism would be either the increase in the NADH:NAD^+ ratio, inhibiting the enzyme, glyceraldehyde-3-phosphate dehydrogenase, or the competitive inhibition of the same enzyme with ADH for their common factor, NAD^+.

Metabolic effects of ethanol in the liver

The following facts have been observed (Baron, Griffaton & Lowy, 1968, 1969; Lowy, Baron & Griffaton, 1970): (1) an increase in α-glycerophosphate and citrate (more than 200%), in glutamate (50%) and especially in malate (almost 700%), and in NADH and NADPH (in the total tissue); (2) a decrease in aspartate, acetoacetate, NAD^+ and $NADP^+$, and in the concentration of NH_4^+ (as estimated according to Williamson, Lund & Krebs, 1967); (3) a lowering of the ATP level by 39% without any elevation of ADP, AMP or inorganic phosphate; (4) no change in the concentrations of pyruvate, dihydroxyacetone phosphate, oxaloacetate, α-ketoglutarate, alanine and urea; (5) an occasional insignificant increase in lactate and β-hydroxybutyrate, although the β-hydroxybutyrate: acetoacetate ratio is always raised because of the decrease in acetoacetate.

Inhibition of the tricarboxylic acid cycle and the need for oxaloacetate. Among the possible redox pairs of the liver cell, it is the oxaloacetate system which reacts the more strongly to the presence or to the metabolism of ethanol or both. This results in an increased need for oxaloacetate. Further evidence of this increased need is the decrease in aspartate without any symptom of urea cycle activation. But oxaloacetate does not seem to be used only to reoxidize NADH to NAD^+: there is an accumulation of citrate which cannot be entirely explained by the tricarboxylic acid cycle inhibition. An increased citrate synthesis could be accounted for by the high affinity of acetaldehyde for coenzyme A, that is the formation of acetyl-CoA during ethanol catabolism. The cycle might then be blocked at the α-ketoglutarate oxidation step, which is inhibited by NADH (Greville, 1965).

The requirement of oxaloacetate for malate and citrate synthesis prevents the formation of phosphoenolpyruvate (PEP) from oxaloacetate. The PEP concentration has been shown to be lowered during alcohol intoxication (Nordmann & Nordmann, 1969).

NADPH production. During ethanol metabolism, glutamate accumulates without change of α-ketoglutarate concentration. Glutamate may be formed from citrate via the soluble $NADP^+$-isocitrate dehydrogenase when a rise of NADPH concentration is actually observed. This is very interesting, since NADPH is the obligatory co-factor of the MEOS.

NADPH can also arise from other sources, for example from the malic enzyme pathway. The hexosemonophosphate shunt may also produce NADPH, for citrate

is an allosteric inhibitor of phosphofructokinase. However, it must be kept in mind that the ATP concentration is lowered, and the regulation of phosphofructokinase by ATP, ADP, AMP, citrate, fructose-6-phosphate and fructosediphosphate is very complex.

An over-production of NADPH can favour fatty acid synthesis. Ethanol metabolism creates other conditions that promote lipogenesis, besides the decrease in ATP concentration: citrate (Goodwin, 1968) and α-glycerophosphate are both allosteric activators of acetyl-CoA carboxylase, the enzyme which controls the first step of fatty acid synthesis. Moreover, α-glycerophosphate esterifies the fatty acids formed, which are inhibitors by mass action and by allosteric regulation. On the other hand, ethanol prevents fatty acid activation and oxidation. Even if the synthesis of fat is depressed because of the low ATP concentration, an ethanol-intoxicated liver will poorly oxidize the endogenous fat and the fatty acids mobilized from the peripheral depots.

Origin of the α-glycerophosphate accumulation. Ethanol or acetaldehyde or both stimulate the adrenal production of epinephrine. This can lead to a peripheral lipolysis and, in the liver, to a phosphorolysis of glycogen. Glycolysis is inhibited, as we have said above, and the redox state of the cell causes the phosphorolysed glycogen to be entirely changed into α-glycerophosphate. It seems that the synthesis of this compound quantitatively corresponds to the incorporation of inorganic phosphate from the broken-down ATP.

Modifications of the metabolic effects of ethanol

The effects of administering simultaneously with ethanol doses of other metabolites have been studied by Baron, Griffaton & Lowy (1970, 1971) and Griffaton, Lowy and Baron (in preparation). Briefly, fructose and pyruvate protect the tricarboxylic acid cycle against ethanol inhibition and, with α-ketoglutarate, provide compounds that are reduced in the presence of NADH, so contributing to its reoxidation into NAD^+. Fructose and pyruvate are precursors of oxaloacetate and prevent aspartate from being lowered after ethanol administration. The case of α-ketoglutarate is more complicated: it would compete with acetaldehyde for CoA, and this would result in a decrease of the oxaloacetate requirement. Pyruvate stops the phosphorolysis of glycogen: the tricarboxylic acid cycle, being activated, takes up again the inorganic phosphate liberated by the breakdown of ATP. It is interesting that fructose and pyruvate enhance the NADPH production, so that they might be able to trigger the MEOS.

But it would be an illusion to believe that these compounds are only beneficial. Fructose (and perhaps also pyruvate) provokes a nucleotide catabolism that adds up with the one from ethanol and leads to hyperuricaemia in man. Fructose also considerably increases the risk of lipogenesis, since the syntheses of citrate, α-glycerophosphate and NADPH are very important, and there is no longer a lack of ATP as there is when ethanol is administered alone. Fig. 6 summarizes the effects of fructose.

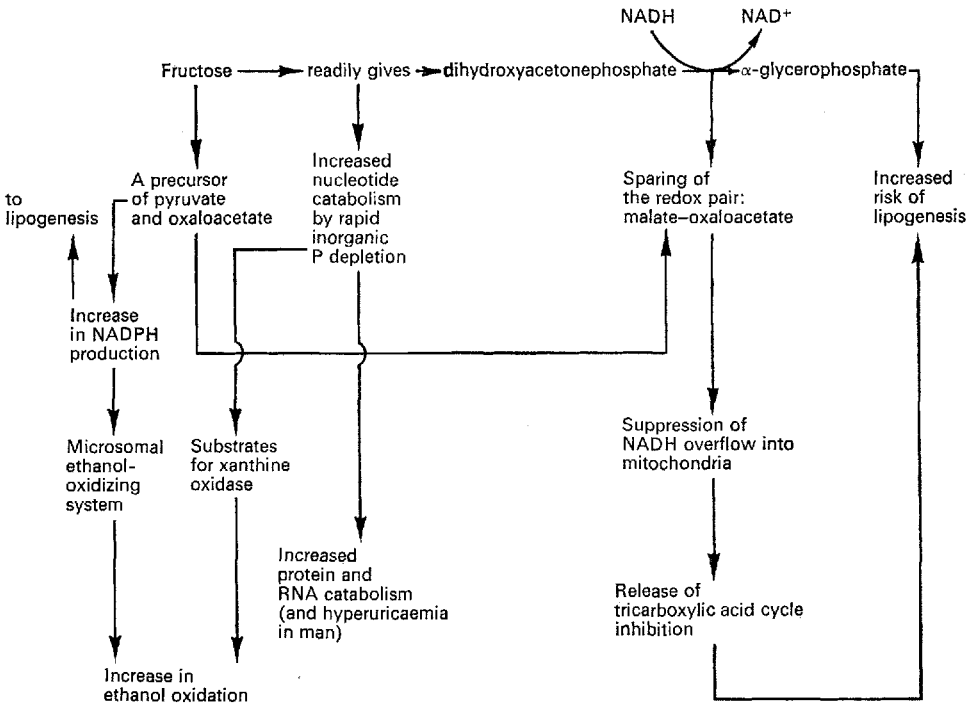

Fig. 6. Fructose as a modifier of ethanol effects.

Summary

The effects of ethanol in the hepatic metabolism of the young fasted rat are based upon two essential points: (1) production of NADH by either ADH or aldehyde dehydrogenase or both, arising in the cytosol but readily transferred into the mitochondria; (2) formation of acetyl-CoA resulting from the high affinity of acetaldehyde for CoA. The consequences are: an inhibition of glycolysis, of the tricarboxylic acid cycle and of gluconeogenesis, and a production of NADPH, stimulating the further oxidation of ethanol by the MEOS and the syntheses of fatty acids and cholesterol. The situation is summarized in Fig. 7.

The effects of giving the animals other metabolites at the same time vary according to the experimental conditions and the nutritional status.

A better knowledge of the metabolic effects of ethanol would give us a sounder basis for determining the level and the rhythm of ingestion at which ethanol is deleterious and the nutritional association with which it is most harmful. We know now the coupled reactions which control the speed of ethanol oxidation through the ADH system. We do not know the mechanism of nucleotide catabolism responsible for acute pancreatitis and the conditions triggering the two dangerous peroxidative and microsomal oxidative pathways.

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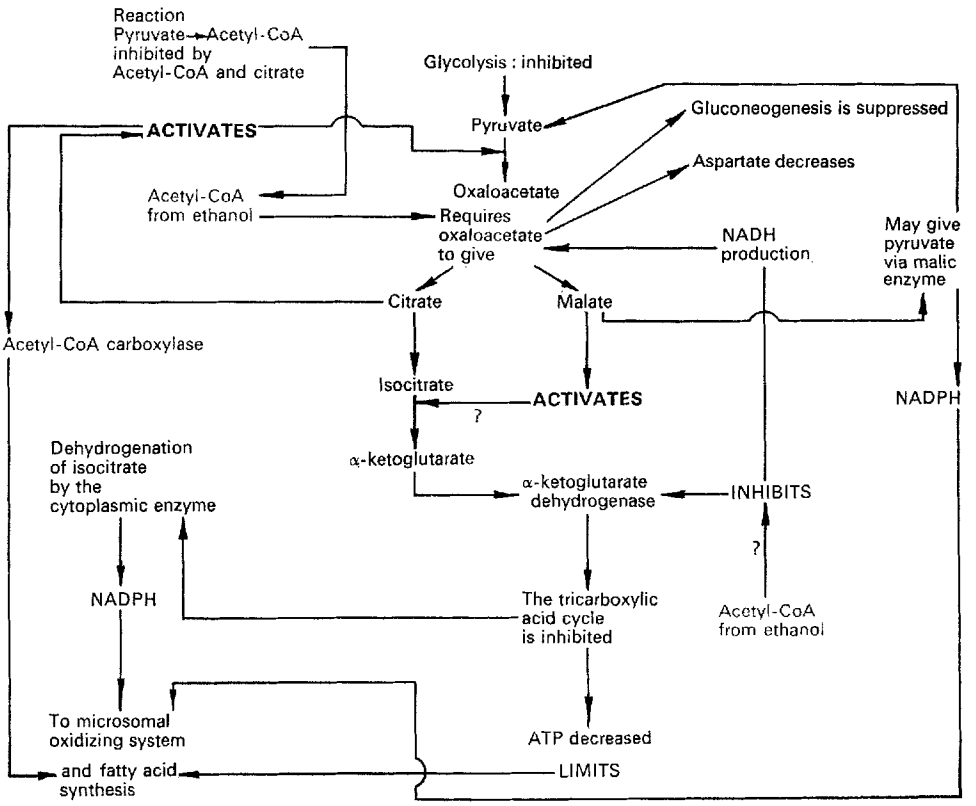

Fig. 7. Ethanol, the tricarboxylic acid cycle and gluconeogenesis.

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