The Intermediary Metabolism of Alcohol

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THE probable metabolic pathway for alcohol (Fig. 1) involves an initial oxidation to acetaldehyde, a conversion of the latter to acetyl co-enzyme A (CoA) either directly or via acetic acid, and the complete combustion of acetyl CoA in the citric acid cycle. The evidence for this pathway is reasonably substantial, and no data have been obtained so far which cast any serious doubt upon it.

ALCOHOL TO ACETALDEHYDE

The enzyme which carries out the oxidation of alcohol to acetaldehyde, alcohol dehydrogenase, has been studied extensively *in vitro*, and the appearance of acetaldehyde in body fluids during alchohol metabolism demonstrates that this reaction takes place *in vivo*. This enzyme is localized within the liver and kidney, and most of the oxidation of alcohol *in vivo* takes place in the liver. Catalase plus hydrogen peroxide are capable of oxidizing alcohol to acetaldehyde *in vitro*, but this has no metabolic significance.[1, 2] The metabolic removal of acetaldehyde is faster than its formation from alcohol, so this initial oxidation of alcohol is the rate-limiting reaction in its over-all removal. Since this reaction is not influenced by the over-all metabolic needs of the organism, the rate of alcohol metabolism is not affected by hyperthyroidism, exposure to cold or muscular exercise.

Alcohol dehydrogenase is a zinc-containing enzyme[3, 4] which utilizes DPN as the hydrogen acceptor, and is dependent upon free SH groups in the protein for activity.[5, 6] The zinc is one of the binding sites of DPN with the enzyme.[7, 8] The liver enzyme has a molecular weight of 84,000, contains 2 atoms of zinc and 2 molecules of bound DPN[9]; the yeast alcohol dehydrogenase is approximately twice as large and contains 4 or 5 atoms of zinc plus an equal number of DPN units. The equilibrium for the oxidation of alcohol to acetaldehyde *in vitro* is markedly in favor of the alcohol, and the reaction stops when relatively little acetaldehyde has been formed unless the latter is removed from the sphere of action. The DPNH formed in this reaction must also be removed in order to allow the reaction to proceed.

In an isolated system the oxidation-reduction (OR) potential for the DPN:DPNH system is in the neighborhood of −0.3 volts. However, when this system combines with the alcohol dehydrogenase at a pH of 7 to 7.4, the OR potential increases to −0.20 to −0.22 volts.[10] The potential usually given for the ethanol:acetaldehyde system is approximately −0.16 volts. Hence the alcohol is being oxidized by a system of lower potential. This is not unique for alcohol. Most substrate systems which are oxidized by DPN or TPN enzymes appear to have higher potentials than the oxidant system. This undoubtedly provides a good mechanism for controlling the rate of oxidation of the substrate and preventing its explosive combustion. However, such conditions are unfavorable for the oxidation of alcohol in an isolated system, and can be utilized biologically only because the products of the reaction (acetaldehyde and DPNH) are removed continuously.

The oxidation of alcohol by the alcohol dehydrogenase-DPN system theoretically and actually occurs at a low ratio of acetaldehyde:-alcohol. Liver normally contains a DPN:-DPNH ratio of 1.7 to 1.8:1.[11, 12] During the

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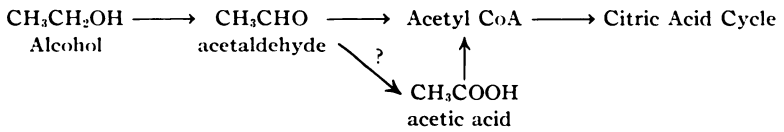

FIG. 1. The probable pathway for the metabolism of alcohol.

metabolism of alcohol the DPN:DPNH ratio falls to 1 to 1.2:1,[11,12] and the corresponding acetaldehyde:alcohol ratio is about 1:40. This ratio is considerably higher than the 1:500 ratio found by Lundquist et al.[13] for liver homogenates, and suggests that the OR potentials of the two systems are further apart than indicated. Irrespective of the exact values, it is evident that the oxidation of alcohol stops when relatively little acetaldehyde has been formed. Alcohol could be a relatively effective substrate for providing a more reduced atmosphere in the animal body since it requires no pretreatment to be a hydrogen donor, and no controlling mechanism could be interposed to prevent its acting as a hydrogen donor.

A consideration of OR potentials as they apply to an equilibrium reaction *in vitro* cannot provide any information about reaction rates *in vivo*. Alcohol seems to be metabolized as rapidly in the presence of the high levels of acetaldehyde caused by Antabuse® as in the presence of the relatively low levels of acetaldehyde found in the absence of Antabuse. A more important factor in determining the rate of alcohol metabolism is the rate at which DPNH is reoxidized to DPN.[12]

There are potentially two methods by which DPNH can be reoxidized to DPN. One is by the classic electron transport chain: the flavoprotein-cytochrome system. This chain is localized in the mitochondria and seems to be responsible for the bulk of the oxygen utilization in the body irrespective of the substrates being oxidized. Alcohol dehydrogenase is present in the cytoplasm (soluble supernatant fraction) of the cell, and the DPNH which is formed during alcohol metabolism must therefore be transported to the mitochondria before being oxidized. This would seem to represent another biological means of slowing down and controlling a metabolic oxidation. Alcohol cannot be oxidized any faster than the DPNH is regenerated, and the DPN carrier must shuttle back and forth between the electron supply and the electron furnace. Again, this situation is not unique for alcohol since other substrates are similarly oxidized in the cytoplasm by DPN or TPN enzymes. While the total amount of DPN:DPNH could theoretically be limiting for this reaction, the rate of alcohol metabolism is not decreased by a niacin deficiency[14] or increased by the higher levels of DPN resulting from the administration of nicotinamide.[15]

DPNH can also be oxidized by using some electron acceptor other than oxygen, i.e., some other substrate. The addition of pyruvate to an anaerobic liver homogenate reoxidizes the DPNH.[13] In this type of reaction the hydrogen is removed from the alcohol and passed by way of DPN to another substrate, such as pyruvate. Such a coupled oxidation-reduction reaction can take place because the DPN carrier is required for both the alcohol-acetaldehyde and the pyruvate-lactate interconversions; the DPN can therefore act as a shuttle between the two substrate systems. This coupled oxidation-reduction reaction would cause the accumulation of another substrate (such as lactate), and would therefore tend to be self-limiting. However, it provides a possible means whereby the oxidizing capacity of other tissues could be borrowed by the liver. Any lactate formed by this type of reaction which escaped from the liver to the muscles would be reoxidized to pyruvate at that site. In effect the liver would be oxidizing alcohol by utilizing lactate as a carrier of hydrogen to the cytochrome chain in the muscles. A similar use of the dihydroxyacetone phosphate-α-glycerophosphate system could transfer hydrogens from the cytoplasmic DPNH to the mitochondrial chain within the liver cell itself.

There is another type of coupled oxidation-reduction which does not have the same self-limitation as the utilization of the lactate-

pyruvate system. This is the synthesis of fat. The formation of a fatty acid from acetyl CoA is a reductive process, and the hydrogens used in this process must come from the dehydrogenation of other substrates. In this coupled reaction the reduction product (newly synthesized fatty acid) can accumulate without limit because it is deposited as neutral fat in an open-ended system.

It is not known how extensively fat synthesis utilizes the hydrogens derived from the oxidation of alcohol. The hydrogens from both DPNH and TPNH are utilized at different points in the synthesis of fatty acids. However, the amount of TPNH available seems to be the limiting factor in this process. Excessive amounts of DPNH could not automatically lead to increased fat synthesis unless (1) an adequate supply of TPNH were available simultaneously from the oxidation of some suitable TPNH-requiring substrate, or (2) the liver transhydrogenase shifts some of the hydrogens from the DPN to the TPN system.

A number of substances have been reported to speed the rate of alcohol metabolism. These substances include insulin plus glucose,[16] fructose,[17] pyruvate, and alanine.[12] This area remains controversial[18,19] for reasons which are not entirely clear. Positive effects have been obtained most consistently when the initial rate of alcohol removal was slow, but small increases have also been observed when the initial rate was relatively high. Since the oxidation of alcohol to acetaldehyde is the rate-limiting reaction in alcohol metabolism, the effect of these substances must be on this reaction. The large daily variation in the rate of alcohol metabolism in the same animal[20] strongly suggests that some factor other than the concentration of alcohol dehydrogenase is involved in determining the rate of this reaction. The only mechanism which is apparent at present is through some influence on the reoxidation of DPNH.[12] This could involve a coupled oxidation-reduction reaction between alcohol and some substrate such as pyruvate or the coupled oxidation-reduction reactions involved in fat synthesis. Moreover, insulin and fructose could stimulate the pentose pathway of carbohydrate metabolism and thereby provide additional TPNH for increased fat synthesis. This might also explain why alcohol metabolism causes a simultaneous disappearance of carbohydrate.

ACETALDEHYDE METABOLISM

The metabolic fate of acetaldehyde has not been clearly established. Like alcohol, it is metabolized primarily in the liver. There are at least two molybdenum-containing (Mo) enzymes (xanthine oxidase and aldehyde oxidase) and one DPN-enzyme present in liver which can oxidize acetaldehyde to acetic acid *in vitro*, but the Mo-enzymes are relatively unimportant in this process.[21] Liver slices, homogenates and rat liver mitochondria all oxidize acetaldehyde to acetic acid by means of the nonspecific DPN-requiring aldehyde dehydrogenase.[13,22] This enzyme is inhibited by Antabuse, and might be expected to carry out the oxidation of acetaldehyde to acetic acid *in vivo*. However 90 per cent of this aldehyde dehydrogenase activity can be removed from rat liver by feeding the animals low protein diet; this depletion has relatively little effect on the metabolism of acetaldehyde *in vivo*. In the dog the feeding of a low protein diet has no effect on the metabolism of acetaldehyde. Hence there is some doubt about the role of this enzyme in acetaldehyde metabolism in the intact animal. There is actually no evidence that free acetic acid is formed during the course of alcohol or acetaldehyde metabolism *in vivo*, and it is possible that acetaldehyde is converted to acetyl CoA by a more direct process which bypasses acetic acid.

Acetyl CoA is considered to be an intermediate in alcohol metabolism for two reasons: (1) there is no other known pathway for the metabolism of this type of 2-carbon unit, and (2) isotopic alcohol is found in the acetyl group of a metabolically acetylated amine. The latter point would be conclusive evidence for conversion to acetyl CoA if it were also certain that acetyl CoA is the only acetylating agent in the body; it is the only one known at the present time.

Animal tissues contain other enzymes which are capable of utilizing acetaldehyde as a substrate and forming products other than acetate or the acetyl CoA unit. These include (1) different aldolases which can combine acetaldehyde with dihydroxyacetone phosphate or glyceraldehyde phosphate to form pentose derivatives'[23] (2) carboxylase which can combine acetaldehyde with a decarboxylated pyruvate to form acetoin, and (3) an aldolase which condenses acetaldehyde with glycine to form threonine.[24] To date there is no evidence to suggest that any of these potential pathways is utilized significantly in the metabolism of acetaldehyde; there is good evidence that the threonine[24] and acetoin pathways are not used *in vivo*, and none of these condensation reactions take place in an anaerobic liver homogenate metabolizing acetaldehyde.[13]

Acetaldehyde is not a major metabolite in the breakdown of the usual foodstuffs. Small amounts of acetaldehyde and alcohol apparently arise normally from pyruvate[25] and some acetaldehyde might be formed from the β-alanine produced as an intermediate in pyrimidine degradation.[26] But from a quantitative standpoint, alcohol and acetaldehyde are relatively unique to alcohol metabolism. Antabuse produces a relatively specific block in acetaldehyde metabolism and exhibits an effect only during alcohol metabolism. Both the alcohol and the acetaldehyde are metabolized in the presence of Antabuse, but the process takes place at higher concentrations of blood acetaldehyde than would otherwise obtain.[27]

ACETYL CoA METABOLISM

There are quantitatively several major and several minor pathways for the disposal or utilization of acetyl CoA. The major oxidative pathway is the citric acid cycle. It is by means of this cycle that the alcohol is completely burned to CO_2 and H_2O. It is also by means of this cycle that traces of the alcohol carbon atoms are believed to be incorporated into proteins and carbohydrates.

Acetyl CoA is the starting point for the synthesis of fatty acids and cholesterol. Quantitatively the former is without limit, while the total amount of cholesterol synthesized per day is circumscribed. Alcohol, acetaldehyde and acetic acid are all good precursors of fatty acids and cholesterol, presumably because they are metabolized via acetyl CoA. Alcohol and acetaldehyde appear to be better precursors than acetic acid, but the reason for this is not yet known and might be artifactual due to experimental technics.[28,29]

In diabetes, the acetyl CoA units are formed from fatty acid degradation in the liver faster than they can be metabolized, and they accumulate as ketone bodies. Acetoacetate can also be formed from the acetyl CoA units derived from alcohol, and more ketones are produced from alcohol by a glycogen-poor (fasted) than by a glycogen-rich liver.[11] There is also less accumulation of liver lipid following the administration of alcohol to fasted rate than to well fed rats.[30] Ketone bodies do not accumulate in the normal animal metabolizing alcohol, and no extra ketones would be expected in the diabetic animal if the alcohol simply replaced fatty acids as the source of the acetyl CoA units.

The acetoacetate formed by the liver can normally be metabolized by muscle and other tissues at a rate which prevents its accumulation. The conversion of alcohol to acetoacetate provides a mechanism whereby alcohol can contribute energy to those tissues (e.g., muscle) which are incapable of utilizing alcohol itself. Some such relationship would seem to be essential in those situations in which alcohol contributes the bulk of the daily calories.

STUDIES IN INTACT ANIMALS

While most metabolic reactions are studied and developed in *in vitro* system, such studies merely demonstrate that a given reaction is possible. They must be supplemented with studies in the intact animal in order to prove that a given reaction or a postulated sequence of reactions actually does take place.

The relatively few such studies available in the alcohol field at present are consistent with the postulated pathway. They show

essentially that (1) alcohol and acetate metabolism are parallel, i.e., the 2-carbon unit from alcohol is handled by the body in essentially the same way as the 2-carbon unit from acetate;[31] and (2) the distribution of the alcohol carbons found in glycerol and glycogen is consistent with the passage of acetyl CoA through the citric acid cycle.[32]

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