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The Metabolism of Ethanol and Its Metabolic Effects*

ROSEMARY D. HAWKINS AND H. KALANT

From the Addiction Research Foundation of Ontario and the Department of Pharmacology, University of Toronto, Toronto, Canada

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I. Introduction

ETHANOL exerts several different types of action in the body. i) It exerts a pharmacological action which gives rise to the physiological and psychological changes characteristic of alcohol intoxication. ii) This state, if sufficiently intense, may secondarily elicit alterations in the activity of certain endocrine glands and of the autonomic nervous system, both of which can induce alterations in metabolism. iii) Ethanol, itself, is fairly rapidly metabolized by enzymatic mechanisms which are common to the metabolic pathways of various foodstuffs and endogenous substrates. The utilization of ethanol therefore modifies the metabolism of these substances. Metabolites produced from ethanol itself may also give rise to metabolic changes in the organism. iv) A growing body of evidence suggests that ethanol *per se* may also produce metabolic alterations by exerting a direct toxic effect on specific target organs other than the nervous system. The nature of this toxicity may vary with the organ involved.

At times it is difficult to decide to which of these categories, or combination

of them, a particular metabolic consequence of the ingestion of ethanol belongs. In addition, the metabolic effects of ethanol vary with the dose given, the composition and amount of the rest of the diet, and the duration of exposure to ethanol. Chronic exposure may lead to adaptive changes, both in the rate and pathways of ethanol metabolism and in the physiological effects of the drug itself. Many of the apparent contradictions and conflicting interpretations found in the literature on this subject are probably the result of failure to consider sufficiently the effects of dose, manner and duration of ethanol administration.

The main purpose of the present review is to examine the significance of these factors in relation to ethanol metabolism and its metabolic consequences *in vivo*. Possible metabolic effects of ethanol on the central nervous system are not covered here. Several comprehensive analyses of the documented biochemical changes occurring in this system have appeared (175, 231, 258, 409, 602, 603, chapters 5–7 of 605) but at this stage of our knowledge it is difficult to distinguish between the changes which are a manifestation of decreased neuronal activity resulting from intoxication and those which may be primary biochemical events leading to the state of intoxication.

The effects of ethanol on zinc and magnesium metabolism and on the blood levels and urinary excretion patterns of the various vitamins have likewise been omitted. Mardones (392) and Wallgren and Barry (605, p. 492) have reviewed much of the earlier work on vitamins and the few papers of major significance which have appeared recently are cited in sections where they seem to be pertinent.

II. The Metabolism of Ethanol and Factors Affecting Its Rate

A. Metabolism of ethanol: suggested pathways

A number of exhaustive reviews on the pathways of ethanol metabolism have appeared in the past 20 years (241, 246, 255, 329, 358–360, 392, 605, 619) and the reader is advised to refer to the more recent of these for the experimental evidence in support of the various reaction mechanisms proposed for the metabolic degradation of this compound. Other recent reviews (259, 604) have examined in detail the factors influencing absorption, distribution and elimination of ethanol, and the effect of these on the apparent rate of metabolism *in vivo*. No attempt will be made here to cover the same ground in detail. Only the main points will be recapitulated, and emphasis will be placed principally upon newer evidence which has helped to clarify the relative importance of different reactions *in vivo*.

Three principal reaction mechanisms, which are capable of oxidizing ethanol to acetaldehyde, have been described. These are the alcohol dehydrogenase (ADH), catalase, and microsomal ethanol oxidizing systems (MEOS). In addition, several other mechanisms, including glucuronide and sulfate conjugation and fatty acid esterification, may make small contributions to the total disposition of ethanol in the body. Each of these reactions will be outlined briefly, and consideration given to the evidence relating to their roles *in vivo*.

1. CATALASE (EC 1-11-1-6)

Keilin and Hartree (277, 278) showed that catalase, in the presence of a H_2O_2-generating system, would catalyze the following reaction:

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

The same type of reaction has been demonstrated with rat liver homogenates containing catalase activity (181, 182, 363). It is not specific for ethanol, since methanol is an equally good substrate (55a, 563) and appears to be oxidized primarily by catalase in the rat (564). Catalase activity in the liver is contained mainly in the peroxisomes, which sediment with the mitochondrial fraction (95); in some other cells, such as erythrocytes, it appears to be present in the soluble cytoplasm.

Because of the large amounts of catalase found in the body, and the presence of various peroxide-generating systems, it seemed reasonable that at least some oxidation of ethanol *in vivo* might occur by this route. However, acute studies involving a single dose of ethanol have lent no support to this view. The rate of metabolism of ethanol in rats was virtually unaffected by treatment with the catalase inhibitor, 3-amino-1,2,4-triazole (293). Even a 90% inhibition of hepatic catalase activity induced by pretreatment with this inhibitor was without effect on the rate of oxidation of ethanol *in vivo* or *in vitro* (542). Lundquist (359) has pointed out that, although a catalase reaction *can* take place under *in vitro* conditions, the possibility of its operation *in vivo* is small, for the rate of formation of hydrogen peroxide would normally be too limited (170a) to permit even a small fraction of ethanol metabolism to take place *via* peroxidation. Under pathological conditions, he suggests, an increase in breakdown products of purine metabolism might give rise (presumably *via* the xanthine oxidase system) to H_2O_2 formation at a rate which might permit the catalase pathway to function.

The possibility cannot be wholly excluded that catalase might participate in ethanol metabolism in subjects chronically exposed to ethanol. Although 3-amino-1,2,4-triazole did not affect ethanol uptake by liver slices of control rats, it did cause a significant inhibition in slices from rats chronically treated with ethanol (592). This result, however, should be viewed with caution. Aminotriazole is apparently not a specific inhibitor of catalase; Kato (274) has reported that it can inhibit a variety of drug-metabolizing enzyme systems as well. Furthermore, Khanna *et al.* (286) found no effect of the drug on the rate of disappearance of a challenge dose of ethanol from the blood of rats chronically fed liquid diets containing ethanol, even though the rate of removal of ethanol was higher in these rats than in controls receiving equicaloric amounts of sucrose.

It would appear that under *in vitro* conditions, some aminotriazole-sensitive reaction can occur, which is not operative in the animal as a whole. One possible explanation is that ethanol uptake by liver slices is usually studied in a system employing 95 to 100% oxygen, rather than air, as the gas phase. Under these conditions, H_2O_2 formation might be enough to sustain some catalatic activity. This might be true especially in rats treated chronically with ethanol, in which

hyperplasia of the hepatic smooth endoplasmic reticulum is associated with increased reduced nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase activity; this is considered further in the next section. Since catalase appears to be present in large excess, increased catalatic activity could be brought about in this way without any increase in catalase itself. Indeed, with one exception (4), most investigators have not found an increase in hepatic catalase levels after chronic administration of ethanol (211, 291, 335, 616). Griffaton and Lowy (181, 182) report an extra oxygen uptake in stoichiometric equivalents when ethanol is metabolized by liver homogenates from animals treated with large doses of ethanol. They interpret this as an indication of a coupled catalase system. Trémolières and Carré (575) and Tephly *et al.* (563) have outlined *in vitro* systems in which blood plasma and erythrocytes, respectively, can cause a peroxidatic oxidation of ethanol; in these cases, alcohol dehydrogenase is absent. However, the quantitative importance of these systems *in vivo* seems very slight, because the necessary peroxide-generating systems have very little activity in plasma or blood, even in chronically alcohol-treated subjects.

In summary, available evidence (605, p. 521) shows only that catalase is capable of carrying out oxidation of ethanol under appropriate conditions, but has not demonstrated the existence of these conditions *in vivo*.

2. MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS)

a. Nature of the MEOS. Orme-Johnson and Ziegler (449) reported that the mixed function oxidase system in mammalian liver microsomes, which plays a major role in hepatic metabolism of many drugs (65), can also catalyze the NADPH- and oxygen-dependent oxidation of methanol and ethanol to their corresponding aldehydes *in vitro*. Lieber and DeCarli (332, 333) have given details of a very similar system, which differed mainly in being sensitive to carbon monoxide and in oxidizing ethanol at a higher rate than methanol. This system, designated MEOS (332, 335), has aroused a great deal of interest because of the important implications of a common pathway for the metabolism of ethanol, barbiturates, meprobamate and other drugs. Much of the interest has centered on the question of whether MEOS activity is indeed the same as the drug-metabolizing system, or represents contamination of the microsomes by another enzyme.

The first possibility is supported by the fact that MEOS, like the drug-metabolizing systems, requires NADPH and oxygen (335), that it is not inhibited by pyrazole at concentrations which inhibit ADH (283, 335), that ethanol can induce changes in the absorption spectra of microsomal hemoproteins which resemble those induced by phenobarbital and other drugs that undergo microsomal metabolism (228, 512), and that MEOS activity is markedly enhanced by chronic pretreatment with phenobarbital (336).

The contrary view is that MEOS activity represents contamination of microsomal preparations with one of the other enzymes known to be capable of oxidizing ethanol, the principal candidate being catalase. The NADPH-dependent

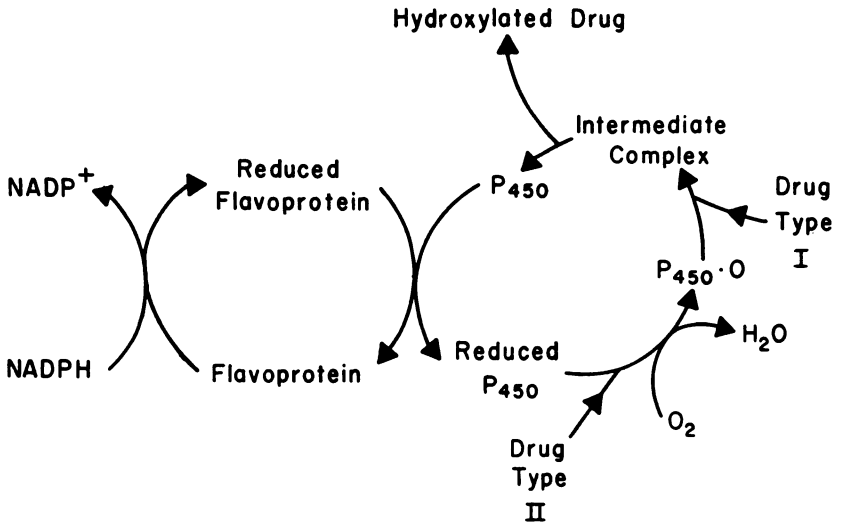

FIG. 1. Pathway of electron flow in the coupled NADPH oxidase-drug metabolizing system in hepatic microsomes. Types I and II refer to drug groups which are thought to bind to the cytochrome P_{450} after and before the formation of the P_{450}·0 complex, respectively.*

drug-metabolizing system in liver microsomes includes a linked sequence of reactions beginning with NADPH oxidase, as shown in the following scheme (adapted from 448, 491a and 526). In the absence of suitable substrates for a hydroxylation reaction, such as metabolizable drugs, the sequence ending with cytochrome P-450 can complete its oxidation-reduction cycle by generating H_2O_2 (166). In the presence of catalase, either as a microsomal constituent or as a contaminant, this system could oxidize ethanol, as already explained in the preceding section. The evidence offered in support of this interpretation of MEOS activity includes the ability of microsomes to oxidize ethanol in the presence of a peroxide-generating system (240, 498) or BaO_2 (283) instead of NADPH and oxygen, the marked reduction in MEOS activity by *in vitro* addition or *in vivo* injection of aminotriazole (240, 283, 498), and the lack of effect of SKF-525A on MEOS activity (283, 335).

It is clear that most of the evidence depends upon the specificity of the various inducers and inhibitors which have been used in these studies. Unfortunately, none is sufficiently specific to permit a definitive answer. Thus, chronic administration of phenobarbital and of ethanol, which both induce increased MEOS activity (330), also induce the formation of many other constituents of the smooth endoplasmic reticulum, including cytochrome P-450 which, as already noted, can act as a peroxide generator. The changes in absorption spectra of hepatic microsomal hemoproteins, induced by alcohols and other substances, are also not sufficiently specific, since cytochrome b_5 and other hemoproteins show closely similar spectral changes in the presence of various substances (447), and only

* This scheme may require revision in the light of the possible intervention of NADH and cytochrome b_5 (see refs. 64b, 165a, 217a).

CO distinguishes cytochrome P-450 from the other hemoproteins, including catalase. Comparison of the data of Imai and Sato (228) with those of Rubin *et al.* (512), with due allowance for differences from one microsomal preparation to another, indicates that the production of comparable degrees of spectral change requires 20 to 30 times as high a molar concentration of ethanol as of aniline. Indeed, Imai and Sato consider the alcohol effect to represent an action on a lipophilic phase of the microsomal membrane adjacent to the hemoprotein, rather than a substrate-like binding to the hemoprotein itself.

On the other hand, aminotriazole is not a specific inhibitor for catalase, nor pyrazole for ADH; both can inhibit a variety of microsomal enzyme activities (274, 344). Conversely, SKF-525A does not affect MEOS activity strongly (283, 335), but it also causes little inhibition of aniline hydroxylase activity, which is known to be an NADPH-dependent system linked to cytochrome P-450 (275). Cholate and azide are also not specific inhibitors of NADPH oxidase and catalase activities, respectively (342).

The differentiation between a separate MEOS and a combination of NADPH-dependent peroxide formation and catalase activity therefore rests entirely on the ability to show differential effects with a single inhibitor (342), and on this the evidence is again conflicting. Cyanide, for example, at a concentration of 10^{-4} M has been reported to inhibit MEOS activity by only 12 to 15% but catalase almost completely (335); however, others have found that 10^{-3} M cyanide does not inhibit the dealkylation of 4-aminoantipyrine by the drug-metabolizing microsomal system (166), and other dealkylating systems (498), but this concentration inhibits MEOS completely (283, 498). Again, 0.1 mM azide added *in vitro*, or pyrazole in a dose of 4.4 mmoles/kg, given shortly before killing the animal, were reported to inhibit drastically the catalase activity of liver microsomes as well as their oxidation of ethanol by H_2O_2 generated *via* the xanthine oxidase reaction, while leaving virtually intact the NADPH-dependent MEOS activity (334, 342). Moreover, microsomal oxidation of ethanol with BaO_2 or an H_2O_2 generating system was abolished by *in vitro* addition of aminotriazole at a concentration which reduced MEOS activity by only 50% (283, 498). This has been interpreted as evidence that the two systems are different (335), but an alternative explanation might be that the active site of the microsomal catalase is more effectively protected by H_2O_2 generated by NADPH oxidase activity within the microsomal particles than by H_2O_2 generated in the soluble phase of the suspending medium. When the aminotriazole was injected *in vivo* 4 hr before removal of the liver, MEOS activity and peroxidatic activity were reduced to the same degree (283).

It has been argued (330) that inhibition of microsomal NADPH oxidase activity and of MEOS activity by cholate (240) does not prove the identity of these systems because cholate is a non-specific disrupter of microsomal structure. It is difficult to see the purpose of this argument, since microsomal NADPH oxidase activity is believed to result from the whole sequence of reactions shown in figure 1 up to the point of cytochrome P-450, which is presumably also an intrinsic part of MEOS activity. The question to be resolved is not whether

NADPH oxidase differs from MEOS, but whether the complex of oxygen and cytochrome P-450 is reduced by transferring the oxygen directly to ethanol (as in the drug-metabolizing activity) or by generating H_2O_2 which then oxidizes ethanol through the intervention of catalase.

This evidence is probably sufficient to indicate that the MEOS cannot yet be definitively identified as either identical with, or distinct from, a combination of microsomal NADPH oxidase plus catalase activities. However, the preponderance of evidence at present available would seem to favor the view that they are identical.

b. Role in vivo of the MEOS. The same factors which create the uncertainty concerning the nature of the *in vitro* MEOS also make it impossible to know with certainty whether or not it plays any role in ethanol metabolism *in vivo*. The only way in which its activity might be recognized *in vivo* would be the observation of the effects of inhibitors and inducers of MEOS and other ethanol-oxidizing enzymes upon ethanol metabolism in the intact liver or the whole organism. Evidence relating to discrepancies between ADH activity and alcohol metabolism will be discussed in section A 4 c. Since the various inhibitors and inducers, as already noted, are not highly specific, the corresponding results *in vivo* are not entirely conclusive.

Pretreatment of rats or mice with SKF-525A, in a dose known to inhibit the hepatic microsomal drug-metabolizing system and to prolong hexobarbital sleeping time, had no effect on the rate of disappearance of ethanol from the blood or the whole body (282), or the rate of conversion of ^{14}C-ethanol to $^{14}CO_2$ *in vivo* (565). Chronic pretreatment with phenobarbital or chlorcyclizine, both of which are highly effective inducers of hepatic microsomal drug metabolism (65) and which have also been shown to increase MEOS activity (285, 417), likewise failed to increase the rate of disappearance of ethanol from the blood (282) or the whole body (285), or the rate of $^{14}CO_2$ production from ^{14}C-ethanol (565). Finally, administration of carbon tetrachloride to rats in a dose which drastically reduced MEOS activity had no effect on ADH activity, on metabolism of ethanol by liver slices prepared from the treated animals, or on disappearance of ethanol from the blood or the whole body (284).

These results appear at first sight to be at variance with those obtained by other investigators (126, 127, 632), but closer scrutiny does not bear this out. Fischer and Oelssner (126, 127) reported faster disappearance of ethanol in barbiturate-pretreated animals, but did not provide evidence of a statistically significant effect. Moreover, hexobarbital appears to have a greater effect than phenobarbital, though the latter is a much better inducer of the microsomal system, and Fischer (125) concluded that ethanol was not being metabolized by microsomal enzymes. Wooles (632) found lower blood ethanol levels in rats pretreated with chlorcyclizine than in controls, during a 4-hr period after alcohol administration. However, even the earliest values were low, so that his results are more consistent with impaired absorption of ethanol than with accelerated metabolism (282).

Although the duration of ethanol-induced sleep was reported to be increased

by SKF-525A in mice (348), others have been unable to confirm this (282). Similarly, chronic pretreatment with chlorcyclizine, which significantly shortened pentobarbital sleeping time, had no significant effect on ethanol sleeping time (282).

The evidence given above suggests that MEOS activity plays little or no role in ethanol metabolism in the normal rat. However, evidence of another type has been offered in support of such a role. Addition of ethanol in concentrations ranging from 3 to 100 mM was found to decrease the aniline hydroxylase, pentobarbital hydroxylase and other drug-metabolizing activities of rat liver microsomes *in vitro* (508). It also decreased the rate of metabolism of meprobamate by liver slices *in vitro*, and a single dose of ethanol *in vivo* lowered the rate of disappearance of pentobarbital from the plasma and from the whole body of rats, and of pentobarbital and meprobamate from the plasma of man (424, 508). These findings are interpreted as evidence that ethanol can be metabolized by the same microsomal enzyme system as these drugs, and thus competes with them for the binding sites. This does not necessarily follow, since ethanol could be an inhibitor, directly or indirectly, without being a competing substrate. An interpretation arising from the suggestion of Imai and Sato (228) would be that nonspecific interaction of ethanol with the hydrophobic milieu of the microsomal hemoprotein, resulting in a change in conformation of the latter, impairs its ability to carry out the drug-hydroxylating reaction. Moreover, meprobamate is a 1,3-propanediol derivative; after hydrolysis of the ester linkages, it would provide an excellent substrate for ADH (28). The hydroxylated metabolites of tolbutamide and of nitrotoluene (165, 404, 494) and possibly of pentobarbital (307), are metabolized further by ADH. Interaction between the metabolism of ethanol and of these drugs *in vivo* has not been proven to occur at the microsomal level rather than at that of ADH. Indeed, pyrazole in a dose of about 1.5 mmoles/kg caused 50% inhibition of ethanol metabolism *in vivo*, and a 65 to 85% reduction in the urinary excretion of metabolites of *p*-nitrotoluene, while the initial microsomal hydroxylation of aminopyrine was not appreciably decreased by pyrazole in concentrations below 10 mM, and the K_i was 25 mM (494). This evidence clearly suggests that metabolism of many drugs might be influenced by ethanol and other substances at stages *after* the initial microsomal hydroxylation reaction. Therefore the above findings, striking as they are, do not prove that MEOS activity occurs *in vivo*.

A greater number of studies have dealt with the *in vivo* effects of chronic administration of ethanol or of known inducers of drug metabolism. In rats fed ethanol-containing diets for 24 days, Lieber and DeCarli (335) found a higher rate of clearance of ethanol from the blood together with higher MEOS activity but unchanged ADH and catalase activities; moreover, the more rapid ethanol clearance *in vivo* was found even when the rats were treated with pyrazole, a potent inhibitor of ADH (167, 326, 566). This finding is consistent with the observation that chronic ethanol treatment causes an increase in the visible amount of smooth endoplasmic reticulum (SER) in the liver (230, 509), and in the activity of certain drug-metabolizing enzymes which are confined to the SER

(509). It has been suggested, therefore, that SER proliferation in response to ethanol ingestion accounts for the metabolic tolerance to ethanol as well as the cross-tolerance to pentobarbital and other drugs which develops in alcoholics (424).

However, other groups have obtained results which suggest a different interpretation. Porta *et al.* (468, 469) also noted increased SER after 2 weeks of ethanol feeding, but not after 4 and 16 weeks. Moreover, a diet in which the ethanol was replaced by an equicaloric amount of fat was also effective in increasing the SER. Khanna *et al.* (285) have corroborated the finding of increased MEOS activity and faster clearance of blood ethanol in rats after chronic feeding of ethanol in a liquid diet similar to that used by Lieber and DeCarli (335). However, when the protein content of the diet was increased to provide 25% rather than 18% of total calories, the rate of metabolism of ethanol remained elevated, but MEOS activity *in vitro* did not (285). Finally, chronic administration of phenobarbital and other inducers of drug metabolism and of MEOS activity was found to have no effect on the disappearance of ethanol from the blood and the whole body (282, 285, 294, 417). Chronic administration of ethanol similarly failed to affect the rate of metabolism of pentobarbital *in vivo* or by liver slices *in vitro* (266).

Estimates of the possible quantitative significance of MEOS in the normal organism, if it is operative *in vivo*, range from less than 10% of the total ethanol metabolism (323, 565) to about 25% (330), depending upon the estimate of completeness of recovery of the microsomal fraction from the liver. In support of the higher figure, it is claimed that pyrazole, a potent inhibitor of ADH, reduces ethanol metabolism in liver slices by only 75% (335). However, others have reported 90 to 100% inhibition *in vivo* (46, 324), so that this is not a conclusive argument.

A further consideration of some importance is that the MEOS, functioning under optimal conditions of NADPH and substrate concentrations *in vitro*, and with acetaldehyde removed by a semicarbazide trap, maintains its maximal rate of activity for only 10 to 20 min (283, 335), after which it rapidly falls to zero. This has not yet been explained, but at least two possibilities must be considered. One is that MEOS is indeed a peroxidative system as discussed earlier. If this is true, the amount of substrate oxidation depends upon the ratio of H_2O_2 to substrate, *e.g.*, ethanol. The mechanism of the catalase reaction involves formation of a complex between the enzyme and one molecule of H_2O_2 followed by further complexing with a hydrogen donor which can be either a second molecule of H_2O_2 (catalatic reaction) or a molecule of substrate (peroxidatic reaction). The affinity of H_2O_2 is much greater than that of ethanol, so that as H_2O_2 generation from NADPH proceeds, one would expect MEOS activity to diminish, as observed. Alternatively, it is conceivable that accumulation of nicotinamide adenine dinucleotide phosphate (NADP), formed from NADPH, somehow inhibits the reaction. In either case, if the system does participate in ethanol metabolism *in vivo*, its rate would be markedly influenced by the rate of H_2O_2 formation or the NADPH/NADP ratio, respectively. Estimates

of the quantitative significance of MEOS which are based on the maximum initial rate *in vitro* under optimal conditions are therefore probably much too high.

The bulk of this evidence, therefore, fails to support the idea of a significant role of MEOS in alcohol metabolism, after either a single dose or chronic administration of ethanol. It has been suggested that MEOS activity represents an artifact of cell disruption (240, 283, 565); those who hold this view also favor the idea that MEOS activity does consist of a combination of H_2O_2-generating system plus catalase. If its rate of activity is ordinarily governed by the rate of H_2O_2 production, then the increase in MEOS activity after chronic treatment with ethanol (285, 333) may simply reflect an increase in rate of H_2O_2 generation under these conditions *in vitro*. This would be consistent with the observation of increased endoplasmic reticulum (230, 509) and increased microsomal NADPH oxidase activity (334) and cytochrome P-450 in the livers of rats treated chronically with ethanol. The explanation of the increased endoplasmic reticulum might then have to be sought in terms of some alteration in lipid metabolism or lipoprotein release (17, 45) or some other microsomal function known to be affected by ethanol. Increased MEOS activity would be seen in these terms as an incidental consequence of increased membrane formation. Some support for this view is provided by evidence that cytochrome P-450 appears to be involved in several reactions related to lipid metabolism (595).

3. MISCELLANEOUS ROUTES OF ETHANOL METABOLISM

Minor pathways for the metabolism of ethanol have been shown to exist. Urinary excretion of small amounts of ethanol as sulfate and glucuronide conjugates has been established (37, 269) and fatty acid ester formation has been observed *in vitro* (438) and during ethanol metabolism *in vivo* (170). Theoretically, a hydroxylation reaction involving ethylene glycol formation should not be excluded (360) but so far there is no evidence that such a reaction actually occurs.

The quantitative significance of these reactions does not appear to have been studied accurately, but maximum rates observed under favorable conditions *in vitro* (438) suggest that only a minute fraction of ethanol metabolism occurs *via* these reactions. It seems more likely, therefore, that any importance which they may have depends upon the interaction of these pathways or their products with the metabolism of other substances *in vivo*. For example, trichloroethanol produced by enzymatic reduction of chloral hydrate (see section B 2 d) is excreted as a glucuronide in human urine. If ethanol competes for the glucuronide conjugation system, this could conceivably have significance for the pharmacological interaction of the drugs *in vivo*.

4. ALCOHOL DEHYDROGENASE (EC 1-1-1-1)

a. Structure and enzymatic activities. This is one of the most intensively studied enzymes, and a huge literature attests to the research devoted to clarification of its structure and reaction mechanism. The reaction catalyzed by ADH

is represented by the following equation:

$$CH_3 \cdot CH_2OH + NAD^+ \rightleftharpoons NADH + H^+ + CH_3 \cdot CHO$$

This reaction, which is generally considered the rate-limiting step in the total degradation of ethanol (366, 619), has been extensively investigated by Theorell and his associates (566, 569, 570, 572, 601) and others (72, 631, 633). Several recent reviews have dealt with the properties of the enzyme and its reaction mechanism (566–568).

Since the reaction involves the formation of a ternary complex of ethanol, nicotinamide adenine dinucleotide (NAD^+) and ADH, the transfer of hydrogen, and the subsequent dissociation of this complex into enzyme and products, there are a number of possible sequences in which these steps can occur. It is generally agreed that an ordered mechanism following one specific sequence is favored thermodynamically (570, 633), and that other sequences have low probability. For those interested primarily in the metabolism of ethanol *in vivo*, the significance of this ordered reaction sequence lies in its influence upon the possible interactions of the oxidation of ethanol with other metabolic processes which utilize or regenerate the same cofactors, or with substances which affect the binding of substrate or cofactors to the enzyme. Some of these will be considered in the following sections.

The horse liver enzyme contains four atoms of zinc per molecule; removal of two of these by dialysis or by zinc-complexing agents such as *o*-phenanthrolene abolishes enzymatic activity (93, 618). The human liver enzyme appears to contain only the two functional atoms of zinc (28). The active enzyme probably consists of two similar polypeptide chains, each containing a reactive —SH group in a cysteine residue (204). This —SH group, together with zinc and other as yet unidentified amino acid side chains, appears to be involved in binding substrate and coenzyme to each of the active centers within the quaternary structure of the active dimer (567, 569).

Dissociation of the dimer yields two inactive units, designated E and S, which can be recombined in various ways to yield isoenzymes with slightly different properties (253, 254, 461). The existence of a variety of isoenzymes from different plant and animal sources has in fact been reported (67, 103, 180, 426, 520, 541, 586, 605, 615). Apparent electrophoretic inhomogeneity of ADH can be produced by different combinations with NAD^+ and reduced nicotinamide adenine dinucleotide (NADH) (405), and it was suggested that these might be mistaken for isozymes. However, this possible source of confusion can be eliminated by addition of excess NAD^+ to the electrophoretic medium, and after this precaution it is still possible to demonstrate isozymes with distinct electrophoretic and kinetic properties (541). Difference in these properties was in fact the basis for the first recognition of an atypical human liver ADH (613, 615).

It has recently been reported (418) that the kinetic features characteristic of the atypical enzyme can be converted to those of the normal variant by passage through a carboxymethylcellulose column, and that the two variants are immunologically indistinguishable. This does not really call into question the

existence of isozymes *in vivo*, since the typical and atypical enzymes have both been shown to consist of several isozymes (541), some of which are interconvertible by dissociation and recombination (369). The occurrence of typical or atypical kinetic behavior may conceivably depend upon the proportions of the various possible combinations of subunits (541).

Typical ADH purified from human liver exhibits maximal activity *in vitro* at ethanol concentrations of 1.7×10^{-2} M (78 mg/100 ml), with inhibition at higher concentrations (611). Unlike the horse liver enzyme (569, 629), human liver ADH will oxidize methanol, 2-propanol and ethylene glycol (28, 611); methanol is oxidized by human ADH at an even higher rate than is ethanol. This is significant in the treatment of methanol and ethylene glycol poisoning in man, for ethanol will competitively inhibit the oxidation of methanol to formaldehyde and of ethylene glycol to glycolaldehyde by ADH.

ADH is remarkably unspecific: it can catalyze the interconversion of a broad group of primary and secondary alcohols and their respective aldehydes and ketones (566). Although its significance in ethanol metabolism is for the most part accepted, the physiological function of ADH, as Theorell (566) has pointed out, is still unknown. Work on cholanic acid derivatives (601) has demonstrated that crystalline ADH derived from horse liver will catalyze the oxidation-reduction of the primary alcohol groups of sterols with a 3-β-hydroxy or a 3-keto group. Theorell *et al.* (571) have since isolated three ADH fractions, all of which exhibit activity with ethanol, whereas only one manifests steroid-oxidizing ability. This fraction is highly stereo-specific for 3-β-hydroxy steroids, and shows a different K_i for inhibition by pyrazole than the ethanol-oxidizing activity does (495). They postulate that two different binding sites exist in the same molecule of the steroid-oxidizing fraction. Changes in the concentration of steroids in human blood plasma after ethanol ingestion (68) could be either a manifestation of the steroid hydroxylating properties of ADH or of a shift in the keto-hydroxysteroid equilibrium in response to the altered redox potential of the hepatic cell induced by ethanol metabolism.

ADH has also been reported to catalyze numerous other reactions, such as the dismutation of two molecules of formaldehyde or other aldehydes, to yield one molecule of the corresponding alcohol and one of the acid (2, 92, 361). This is now considered to be a coupled reaction involving NAD and acetaldehyde dehydrogenase in the forward direction, and the resulting NADH plus ADH in the reverse direction (73, 362). A different reaction (487) brings about a trans-hydrogenation between an alcohol and an aldehyde if both are substrates for ADH. Through such a system, glyceraldehyde can be reduced to glycerol while ethanol is oxidized to acetaldehyde, the two processes reciprocally facilitating each other. On the basis of identical responses to pH changes and fractionation procedures, it was suggested that ADH and retinene reductase were probably the same enzyme (636). Later work (298, 300) casts doubt upon such a simple interpretation (605) but it is noteworthy that the kinetics of inhibition by pyrazole are closely similar for oxidation of ethanol and retinol (492). Crystalline horse liver ADH also possesses isomerase activity, and will catalyze the NAD-

dependent conversion of glyceraldehyde phosphate to dihydroxyacetone phosphate (587). This latter reaction is much more rapid than the oxidation of ethanol, and is affected differently by various inhibitors. For example, concentrations of certain folic acid analogs which inhibited ethanol oxidation by crystalline horse liver ADH (594) increased the isomerase activity of the same preparation (548). Similarly, *o*-phenanthrolene inhibited the dehydrogenase activity without affecting the isomerase activity, while iodoacetate did the reverse (600). These results suggest that the two activities take place at different locations on the enzyme surface.

In summary, there is no doubt that ADH is capable of converting ethanol to acetaldehyde, but studies *in vitro* suggest that the same enzyme can carry out many other reactions much more efficiently under conditions *in vivo*. It is therefore necessary to examine more closely the evidence relating to the question of whether or not ADH is primarily responsible for ethanol metabolism under all conditions *in vivo*.

b. Evidence for activity in vivo of ADH. Since catalase, MEOS and ADH activities all lead to the formation of the same product, acetaldehyde, the proof of ADH participation in ethanol metabolism *in vivo* is necessarily indirect, as in the case of the other two systems. One type of indirect evidence is the correlation between *in vivo* and *in vitro* effects of known inhibitors and stimulators of ADH. Pyrazole, for example, given to rats in a dose of approximately 4 mmoles/kg shortly before removal of the liver, inhibits ADH activity completely, whereas MEOS activity is virtually unaffected (334); the same dose reduces the rate of ethanol metabolism *in vivo* by 80 to 100% (44, 167, 326, 335). In addition, the ratio between the K_i for ADH *in vitro* and the dose for 50% inhibition of ethanol metabolism *in vivo* was the same for pyrazole and 4-bromopyrazole (493), and proportional degrees of inhibition *in vivo* were produced by doses much too small to affect other ethanol-metabolizing systems (494). Similar agreement between effects *in vivo* and *in vitro* has been observed with various oximes and amides (324). On the other hand, glyceraldehyde, as noted earlier, stimulates the oxidation of ethanol by ADH *in vitro*, probably by reoxidizing NADH to NAD^+ while the coenzyme is still bound to the enzyme (223, 574, 581). Glyceraldehyde and substances from which it is formed metabolically, such as fructose or pyruvate, have been shown also to increase the rate of ethanol metabolism *in vivo* or by liver slices or isolated perfused livers (52, 574, 581, 588); this phenomenon will be considered in more detail in section B 2 a.

As will be discussed in more detail in the next section, the production of NADH during the oxidation of ethanol by ADH can facilitate the reduction of pyruvate to lactate (622). Since the liver cell membrane is permeable to both lactate and pyruvate, the ratio of these substances in the blood of the hepatic vein has been assumed to reflect the redox state of the hepatic cytosol (528). Studies of hepatic vein blood obtained by venous catheterization of human volunteers have shown a significant increase in the lactate:pyruvate and β-hydroxybutyrate:acetoacetate ratios after administration of ethanol (365, 581). Similar findings have been noted with rat liver slices and perfused isolated rat livers (135, 136, 140,

589a). Forsander (137) has converted the experimental values for the ratios obtained in one of these studies (581) into NAD:$NADH_2$ ratios, and has calculated that infusion of ethanol reduces the ratio in the cytosol from a control value of 358:1 to 58:1, and in the mitochondria the ratio was decreased from a control level of 9.7:1 to 3.4:1. This evidence is all consistent with an assumed role for ADH *in vivo*. If catalase or MEOS activities were involved, half as much NADH would be generated per mole of ethanol metabolized, since only the acetaldehyde dehydrogenase reaction would reduce NAD^+. The extent of alteration in the redox state would be limited by the maximum activity of these systems, which has already been discussed in sections II A 1, 2.

In evaluating the effects of ethanol on the redox state of the liver, one should keep in mind the interesting studies of Redetski (488) who found that when human or rabbit erythrocytes are incubated with ethanol (300–900 mg/100 ml), the NAD:$NADH_2$ ratio is reduced, even though these cells lack ADH and no ethanol disappears from the medium. He ascribes the changes in redox level of the medium to the indirect effects of permeability problems created by ethanol. Increased entry of sodium stimulates the cation transport mechanism required for the maintenance of osmotic integrity of the cells; the resultant increase in energy requirement leads to accumulation of reduced cofactors.

Another type of indirect evidence for a major role of ADH in ethanol metabolism is the resemblance between the kinetics of ADH activity *in vitro* and of ethanol disappearance *in vivo*. The great bulk of evidence supports the view that disappearance of ethanol from the blood or from the whole body continues at a constant rate in most species until the concentration in body water has fallen to 2 to 5 mM, depending on the species (259, 385, 605). Calculation of the apparent K_m from the non-linear portions of the blood alcohol curves below these concentrations yields values which agree reasonably closely with those obtained *in vitro* with ADH preparations from the same species (366, 385). The K_m values for other possible oxidative pathways, such as MEOS or catalase, are considerably higher (335).

Related to this is the observation that the concentration of acetaldehyde in the blood and breath during metabolism of ethanol remains essentially constant during the rise and fall of ethanol levels through a wide range of values (161, 260, 383). The acetaldehyde concentration depends upon the balance between its rates of formation and of oxidation or removal. The capacity for acetaldehyde oxidation is quite high, and only when it is impaired by such inhibitors as tetraethylthiuram disulfide (disulfiram, Antabuse®) does substantial elevation of acetaldehyde levels during ethanol metabolism occur (94, 197, 260). Therefore the constancy of acetaldehyde levels during ethanol metabolism is consistent with the constant rate of ethanol disappearance noted above, and with the saturation of ADH at low concentrations of ethanol.

The wide range of substrates which ADH can oxidize or reduce, and the apparent differences in specificity among the isozymes of ADH, suggest that this is not really one enzyme but a collection of enzymes with many features in common. Accordingly, debate continues about the "real" function of ADH *in vivo*

(605). Nevertheless, there is no doubt that one of its functions is the interconversion of alcohols and aldehydes. Small amounts of ethanol can be detected in the blood, tissues and urine of subjects who have not consumed alcohol. Accurate, highly sensitive and specific methods indicate that the concentration of this endogenous ethanol is less than 0.15 mg/100 ml in man and rat (322, 599). According to one study (406), it is not formed by the action of intestinal microorganisms, but appears to be formed in the liver, by decarboxylation of pyruvate to acetaldehyde and subsequent reduction to ethanol by ADH + NADH. However, a recent study (306) appears to contradict this view. Ethanol levels in the portal vein exceeded those in the hepatic venous outflow, and were markedly reduced by oral administration of antibiotics. These findings suggest that hepatic ADH functions as a detoxifying mechanism for the low levels of ethanol which are formed in the intestinal tract.

c. Relation between ADH activity and ethanol metabolism. All of these facts point to a major role of ADH in the metabolism of ethanol *in vivo*. However, Wallgren and Barry (605), in their excellent review of the subject, have gathered a large body of evidence indicating a poor correlation between the measured hepatic ADH activity and the rate of ethanol metabolism in different individuals, species, and stages of growth. There are several factors which may contribute to the discrepancy.

The first is the metabolism of ethanol in tissues other than liver. There can be little doubt that the latter is the main site of ethanol removal. Evidence obtained from eviscerated (367, 423) or hepatectomized (60) animals, and from livers damaged by protein deficiency (224), choline deficiency (420), alcoholic cirrhosis (122, 630), or carbon tetrachloride (310) all supports this conclusion. This view is strengthened by evidence acquired with perfused normal livers from cat (367) and rat (173) and those of rats treated with carbon tetrachloride (111). However, some of the discrepancy between hepatic ADH activity and rate of ethanol metabolism might be explained by the ADH activity in other organs.

ADH has been shown to be present also in kidney (20, 47, 320, 426), gastrointestinal mucosa (426, 550) and lung (426). Raskin and Sokoloff (478), by measuring NAD-dependent transhydrogenation from ethanol to lactaldehyde, have demonstrated a minute amount of an enzyme in the soluble portion of rat brain with kinetic properties similar to, if not identical with, those of liver ADH. No concrete evidence is presently available that this enzyme plays any significant role in the central effects of ethanol, though an adaptive increase in its activity was reported to take place during chronic ethanol ingestion (479). The time course of the appearance and magnitude of this increase was found to be similar to the time course of the acquisition and magnitude of behavioral tolerance to ethanol in rats (316).

Extrahepatic involvement in the metabolism of ethanol was estimated by Larsen (312) to account for approximately 20% of the total ethanol metabolism. But the role of specific organs other than the liver in the total degradation of ethanol still remains relatively unclarified. Studies of $^{14}CO_2$ production from labeled ethanol (20, 612) showed rat kidney slices to be more active than liver

slices. On the other hand, direct measurements of ethanol disappearance from the medium (320) indicated that kidney slices possessed only 5 to 30% of the activity of liver slices. The hind body of the dog, which is 50% muscle, showed the same ability to produce $^{14}CO_2$ from labeled ethanol as does perfused liver (141). Slices of cerebral cortex were reported to degrade ^{14}C-ethanol to $^{14}CO_2$ (561a) even though ADH activity in brain is extremely low (479). Lundquist (359, 360) has pointed out, rightly, that $^{14}CO_2$ production from labeled ethanol depends ultimately upon the rate at which acetaldehyde and acetate are oxidized in the organ examined or in the body as a whole, and therefore is not a reliable measure of the initial step of ethanol oxidation.

Even if extrahepatic metabolism of ethanol does amount to as much as 20% of the total, it would have to vary very widely indeed to account for the erratic relation between liver ADH activity and rate of ethanol metabolism. There is no evident reason why extrahepatic enzymes should vary much more widely than those in the liver, and no evidence has been brought forward to show that they do. Therefore this does not seem to answer the question.

Another possible explanation is that the conditions under which ADH activity is measured *in vitro* bear no relation to the conditions of pH, cofactor availability and substrate concentrations which prevail *in vivo*. This is undoubtedly true. For example, the pH optimum for *in vitro* activity of liver ADH from horse, man, rat and other species is in the vicinity of pH 11 (28, 190, 393, 567), whereas the pH of the cytosol is below 7. However, since the pH-activity curves are similar for all these species, the ratio between maximum activity *in vitro* and rate of ethanol metabolism *in vivo* should be fairly similar for all. Therefore, this does not seem to account for the large variations found.

The existence of atypical variants of ADH with different pH-activity spectra (541, 615) appears to offer one possible explanation. The atypical form has a much lower activity at pH 11 than the usual form does, but a much higher activity at physiological pH. If the enzyme activity is measured under standard conditions, *i.e.*, pH 10 to 11, individuals with the atypical form might be expected to show a much different relation between *in vitro* and *in vivo* rates than the rest of the population. There is little evidence on this point, but one report (101) bears out this assumption in two subjects with the atypical enzyme. The question then arises as to why the individuals with the atypical form do not have abnormally high ethanol metabolism *in vivo*, since the pH optimum of their enzyme is much closer to the normal intracellular pH. The answer must be that the activity of the enzyme itself is not the rate-limiting factor for the whole reaction *in vivo*.

One possible alternative factor is substrate or product inhibition. Though substrate inhibition is observed with purified ADH *in vitro* (569), it does not appear to occur *in vivo*, since the rate of ethanol metabolism is constant over a wide range of ethanol concentrations (360, 605). Product inhibition seems somewhat more plausible, since the affinity of acetaldehyde for ADH is high and the equilibrium constant favors the reduction of acetaldehyde rather than the oxidation of ethanol (569). When ADH activity is measured *in vitro*, an acetaldehyde trap such as semicarbazide is normally employed. However, as noted earlier, accumu-

lation of acetaldehyde during ethanol metabolism is very slight. Perfusion studies with isolated organs and *in vitro* studies with a variety of tissues have shown that acetaldehyde can be oxidized at several sites, with the liver playing the primary role in its degradation. Akabane (5) and Lundquist (359, 360) have reviewed most recently the status of enzymes possibly involved in this process.

In 1949 Racker (473) prepared from ox liver an ADH-free aldehyde dehydrogenase which catalyzed the reaction:

$$NAD^+ + \text{acetaldehyde} \rightleftharpoons NADH + \text{acetate}$$

Büttner (47) and Deitrich *et al.* (82, 83) have examined its kinetic properties and Jacoby (247) has reviewed some of the data available. A wide range of aldehydes, both aromatic and aliphatic, can serve as substrate for this enzyme. In the case of acetaldehyde, affinity of the enzyme for substrate is extremely high (47). An enzyme similar or identical to that of rat liver has been found in human liver (301), rat kidney (47) and bovine, rat and monkey brain (110). Büttner (47) estimates that under optimal conditions of coenzyme and substrate, aldehyde dehydrogenase activity is about 4 to 5 times higher than that of ADH in both liver and kidney. The low Michaelis constant and the high activity relative to that of ADH enable aldehyde dehydrogenase to keep acetaldehyde concentrations low during ethanol metabolism. Liver also contains two molybdenum-dependent flavoproteins, aldehyde oxidase and xanthine oxidase, both of which are capable of degrading acetaldehyde *in vitro* to acetate aerobically. However, they probably take little or no part in the oxidation of acetaldehyde in the concentrations encountered *in vivo*; their Michaelis constants for acetaldehyde are very high (51, 374) and acetaldehyde oxidation is unaltered in molybdenum deficiency (496). Another relatively unspecific aldehyde oxidase, which may actually consist of a group of several similar enzymes, recently reported to be present in the rabbit liver (475–477), was found to contain iron, molybdenum and flavine adenine dinucleotide. Though it is potentially capable of catalyzing the aerobic oxidation of acetaldehyde to acetate *in vitro*, its functional significance has not been established, nor has its reaction mechanism been finally outlined. Its activity is also dependent on high acetaldehyde concentrations, much higher than those reported *in vivo* after ethanol ingestion (161, 383).

Acetaldehyde can undergo a decarboxylation-condensation reaction with pyruvate to yield acetoin (557), and a similar reaction with α-ketoglutarate to yield 5-hydroxy-4-ketohexanoic acid (620). A condensation reaction between glycine and acetaldehyde to form threonine is catalyzed by an enzyme present in mammalian liver but its relevance *in vivo* is doubtful (164, 271); rats fed glycine and ethanol exhibit no decrease in their threonine requirement. In fact, a variety of carboxylases and aldolases can form products other than acetate from acetaldehyde, but, under normal conditions, none of these reactions is considered significant in the removal of acetaldehyde at the levels produced during oxidation of ethanol (161, 260, 325, 362, 383).

The weight of evidence presently available favors Racker's aldehyde dehydrogenase for the paramount role in the removal of acetaldehyde during

ethanol metabolism. Further, its activity appears to be so high that variations in it, with resulting variations in acetaldehyde levels, seem unlikely to explain the discrepancy between ADH activity *in vitro* and ethanol metabolism *in vivo*.

One possibility which cannot be entirely eliminated is the presence *in vivo* of competing substrates for ADH. As noted earlier, this enzyme can act upon a wide variety of alcohols and aldehydes, some with considerably greater affinity for ADH than that of ethanol (567, 605, 611, 613). It is possible that individual and species differences in the equilibrium levels of various endogenous substrates might bring about differing degrees of competitive inhibition of ethanol metabolism, which would not be detected when ADH activity from the subjects is measured *in vitro*. This possibility does not appear to have been explored seriously.

The most important factor governing the rate of ADH activity *in vivo*, however, and the one which most likely accounts for the major discrepancy between *in vivo* and *in vitro* activity, is the rate of reoxidation of NADH. The oxidation of ethanol in the liver results in a decrease in the total NAD/total $NADH_2$ ratio within the hepatocyte (48, 58, 143, 474, 538, 546). This results, primarily, from an increase in the amount of the reduced component, though occasionally the oxidized component may be decreased as well (265). The initial reduction of NAD occurs within the cytoplasmic compartment, where the enzyme ADH is located (445). It is this shift in the redox level of the liver cell which influences many crucial steps in various metabolic pathways; this aspect is considered in later sections of this review.

Forsander (137) has recently provided an exhaustive and excellent review on the influence of ethanol on the redox state of the liver. Krebs (302) has estimated that in the cytoplasm of the liver cell of a fed rat, the ratio of *free* NAD to *free* $NADH_2$ is of the order of 725:1, while that in the mitochondria is around 7.6:1. This ratio, at the moment, must be derived indirectly from an estimation of the concentrations of certain metabolites which form redox pairs, for NAD and NADH exist not only in the free form but also bound to protein in the various cellular compartments. The ratios of the substrates for α-glycerophosphate, lactate and malate dehydrogenase in the cytosol, and for β-hydroxybutyrate and glutamate dehydrogenase in the mitochondria, provide the clues to the redox state at these sites, respectively. Thirty minutes after the infusion of ethanol in a dose of 1.5 g/kg in rats, the hepatic lactate:pyruvate ratio was increased 3-fold, the malate:oxaloacetate ratio 2-fold, and α-glycerophosphate:dihydroxyacetone phosphate, 1.5-fold (480). The intramitochondrial redox state, represented by the ratios, β-hydroxybutyrate:acetoacetate and glutamate:2-oxoglutarate + $[NH_4^+]$, was elevated to twice normal values. Perfusion of isolated rat livers with ethanol also causes a rise in the β-hydroxybutyrate:acetoacetate ratio (140).

The metabolic consequences of these changes will be considered in section III. Our concern at this point, in relation to the activity of ADH, is with the mechanisms by which NAD is regenerated from NADH, and the rate at which these mechanisms operate. Williamson *et al.* (626) by the use of surface fluorimetry of the liver have shown that the reducing equivalents produced in the cytosol are

rapidly transported into the mitochondria where they are oxidized in the respiratory chain. They observed with perfused rat liver that the addition of ethanol resulted first in a rapid reduction of pyridine nucleotides and an almost equally rapid but relatively smaller reduction of flavoproteins. These results, together with others obtained during brief periods of hepatic anoxia, led to the conclusion that ethanol reduces pyridine nucleotides primarily in the cytosol, whereas the flavin signal was derived solely from mitochondrial oxidation-reduction changes. Furthermore, the fact that reduction of flavoproteins followed that of the pyridine nucleotides with a time lag of less than 1 sec provided evidence in support of a rapid transfer of reducing equivalents across the mitochondrial membrane.

Since this membrane is relatively impermeable to NADH (177, 318), rapid transfer across it might be provided by a shuttle mechanism involving the dihydroxyacetone-phosphate-α-glycerophosphate system, which would deliver hydrogen directly to the mitochondrial flavoprotein. The content of α-glycerophosphate was found to increase in rat liver slices in the presence of ethanol (573) or in the whole liver after ethanol administration *in vivo* (441, 442, 480). However, Hassinen (208) has found very little effect of this redox pair on NADH oxidation by mitochondria of normal rats. A second shuttle mechanism, on the model proposed by Borst (36), exists in the malate-oxaloacetate combination which would deliver the hydrogen to intramitochondrial NAD. Available evidence (42, 221) supports the view that the malate cycle is important in H-transfer. These shuttle systems are indicated in figure 2, but the malate cycle must be modified in the light of later evidence which indicates that it includes a further step, an intramitochondrial transamination between oxaloacetate and glutamate, so that α-ketoglutarate rather than oxaloacetate leaves the mitochondria. The reverse transamination then occurs in the cytoplasm to regenerate oxaloacetate and complete the shuttle (311).

Studies with ^{3}H-labeled ethanol in a reconstituted system containing NAD, ADH, mitochondria and potential shuttle compounds (207, 208) have shown passage of the ^{3}H into the mitochondria as well as its incorporation into malate. This shuttle system seems to be the most important in effecting net regeneration of NAD in the cytosol but it leaves the mitochondrial environment in a highly reduced state, and regeneration of mitochondrial NAD *via* the respiratory chain is dependent on the energy needs of the cell (137). Evidence that this may be the rate-limiting factor in the overall activity of the ADH reaction *in vivo* is provided by the observation that 0.1 mM dinitrophenol increased the rate of ethanol oxidation in normal rat liver slices and *in vivo* by 100 to 160% (235, 591). Forsander (134) has pointed out that the amount of voluntary consumption of ethanol by different individuals and strains of rats correlates well with the degree of hepatic redox change produced by ethanol, rather than with their respective ADH activities *in vitro*. This would appear to support the view that mitochondrial mechanisms for regeneration of cytoplasmic NAD normally govern the rate of ethanol metabolism.

d. Summary and conclusion. On the basis of the preceding discussion, there appears to be substantial evidence in favor of the view that ADH is clearly the

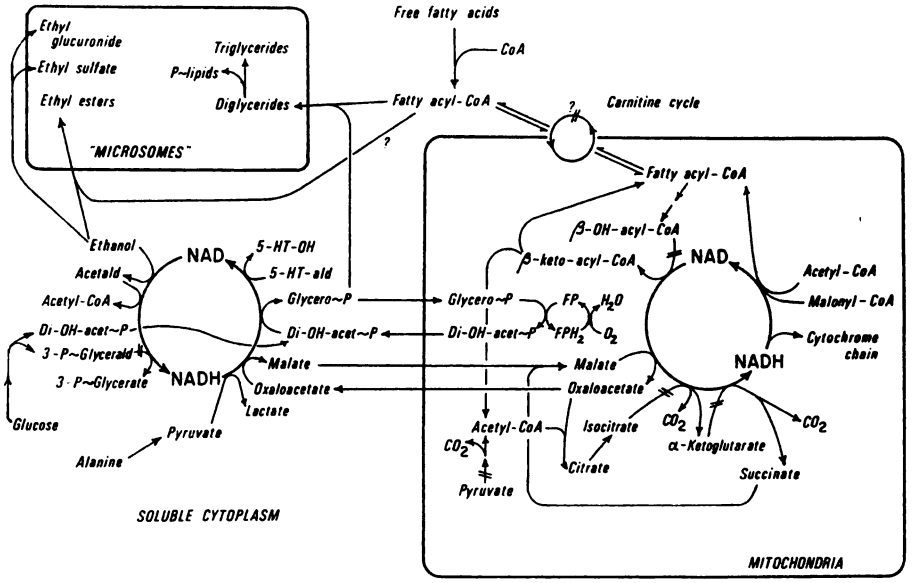

FIG. 2. Scheme of metabolic interrelations of ethanol and acetaldehyde in the liver. ⊣→ denotes pathway impaired during the metabolism of ethanol. (From H. Kalant and J. M. Khanna: Effects of chronic ethanol intake on metabolic pathways. *In* Biochemical and Clinical Aspects of Alcohol Metabolism, edited by V. M. Sardesai, pp. 49–57, Charles C Thomas, Springfield, Ill., 1969. Reprinted with permission of the authors, editor and publisher.)

most important enzyme involved in the metabolism of ethanol *in vivo*. The numerous discrepancies between measured rate of ADH activity *in vitro* and the rate of ethanol metabolism in the same subjects or species are probably attributable to a number of factors. These may include variations in the extent of extrahepatic metabolism of ethanol, and varying degrees of inhibition or stimulation by other substrates, but the most important may well be variation in the efficiency of mitochondrial mechanisms for regeneration of cytoplasmic NAD.

B. Rate of metabolism of ethanol and factors affecting this rate

1. METHODS OF MEASURING RATE OF METABOLISM OF ETHANOL

The rate of removal of ethanol from the body is the sum of its rate of metabolism in the liver and tissues plus the rate of its excretion in breath, sweat and urine. Roughly speaking, excretion accounts for disposal of 2 to 6% of an administered dose, but if blood ethanol has been greatly elevated for a considerable time, the percentage of the total dose which is excreted will increase (see 106, 203, 259, 604 for extensive reviews of this aspect of the subject).

Four main general methods are available for measuring the rate of ethanol metabolism.

i. Measurement of the rate of $^{14}CO_2$ production from an administered dose of

^{14}C-labeled ethanol is a method which, as explained earlier, has inherent defects. Only when a maximum steady state output is maintained for several hours by a large dose of ethanol or by a steady infusion of the drug can the rate of production of labeled CO_2 approximate the rate of ethanol metabolism (see 259).

ii. Total metabolism of ethanol has been studied in small animals by administering a known dose of ethanol and measuring the residual drug in homogenates of whole animals killed at variable times thereafter (282, 284, 396).

iii. With larger animals, a priming dose of the drug is first administered and the concentration in the blood is then maintained by an infusion of the alcohol. The rate of infusion required to keep blood ethanol levels constant over a known period of time will then serve as an approximation of the rate of ethanol metabolism (314).

iv. Calculations of the rate of ethanol metabolism have also been made from data on the rate of ethanol disappearance from the blood. A change in the rate of fall of blood ethanol can reflect a change in the rate of ethanol metabolism (434). However, it can also be caused by other factors which could affect the concentration in the blood, such as alterations in the distribution of body water, distribution of the blood flow among various tissues, and peripheral circulatory flow rates, all of which may be introduced by a variety of physiological and pharmacological means. In fact, a number of reports of conditions or substances ostensibly altering the rate of ethanol metabolism can be explained on the basis of artifacts belonging to this category (259). When the rate of fall of blood ethanol is to be used for calculation of the rate of ethanol metabolism, it is therefore necessary to transform the data by methods outlined by Widmark (624) or by Lundquist and Wolthers (366) (see also 604). Results obtained by this method have been shown to agree well with those obtained by method *ii* (284, 385).

As noted earlier, the fall in blood ethanol levels after completion of absorption is linear until a concentration of 10 to 20 mg/100 ml or thereabouts is reached (see 537, 604); the curve then becomes exponential, presumably because the enzyme system is no longer saturated with substrate (366, 395). However, in the first 30 min after ethanol administration to mice the rate of its metabolism was reported to exceed that found in succeeding intervals of time (396). Some investigators have confirmed this finding (131) but others (431) have not. A suggested explanation is that depletion of available hydrogen acceptors in the hepatic cells during the initial stages of ethanol metabolism is so great that continued action of ADH is limited to a submaximal rate. Conceivably the contradictory results may depend on variation in the initial levels of hydrogen acceptors. Some evidence has been reported (104, 105) that the rate of fall of blood ethanol is greater when initial blood ethanol levels are high; however, as mentioned earlier, the rate of fall of blood ethanol cannot be equated with the rate of ethanol metabolism, since the former is dependent on the distribution volume. Larger doses which can affect the circulatory pattern may well modify the apparent distribution volume. Recent studies provide no corroboration for the theory of an accelerated rate of ethanol oxidation during the early period after its administration (588).

2. FACTORS AFFECTING THE RATE OF METABOLISM OF ETHANOL

Since the initial oxidation reaction catalyzed by ADH appears to be the rate-limiting step in the overall metabolism of ethanol, any condition (prolonged malnutrition, cirrhosis) which decreases the amount of this enzyme in liver would be expected to decrease the rate of ethanol metabolism. Conversely, an adaptive increase in the amount of the enzyme could accelerate the process. If the overall rate of the ADH reaction *in vivo* is governed by the rate of reoxidation of NADH after its dissociation from the enzyme, it seems reasonable to assume that anything which accelerates this regeneration or which by-passes the dissociation of enzyme and cofactor will also accelerate the rate of ethanol metabolism. Alterations in the blood supply to the liver cells can induce artifactual changes and these must be considered in evaluating experimental results.

Caution must be applied in extrapolating results obtained from studies of the rate of ethanol metabolism in artificial systems to rates presumed to occur *in vivo*. Build-up of reaction products, substrate concentrations used, availability of cofactor and the means of regenerating it and sequestration of possible components *in vivo* by the barrier membranes of subcellular elements may not be duplicated *in vitro*. Moreover, if ethanol metabolism in liver slices is examined by following the rate of its disappearance or the rate of acetaldehyde production, one is presumably following the rate of the ADH reaction or of the regeneration of NAD, which may resemble the rates pertaining *in vivo*. In contrast, if $^{14}CO_2$ production from labeled ethanol is followed, the situation does *not* mimic that prevailing *in vivo* because the liver is normally only fractionally responsible for the oxidation of the 2-carbon residues derived from ethanol (630). Conversely, the rate-limiting step in slices and in perfused organs is not necessarily identical to that operating *in vivo*; Gordon's studies (173) as well as those of Videla and Israel (591) and of Van Harken and Mannering (588), for example, have demonstrated that the degree of oxygenation of the environment may alter the overall picture.

All of these considerations must be borne in mind when one examines the effects of the following factors on the rate of ethanol metabolism.

a. By-pass of dissociation of ADH-NADH complex. Theoretically, any NADH-requiring system which could oxidize the ADH-NADH complex directly would tend to enhance the rate of the ADH reaction, since the dissociation of this complex appears to be the rate-determining step in the action of the isolated enzyme (570). Acceleration of ethanol metabolism by fructose (25, 26, 52, 61, 213, 295, 357, 366, 453, 463, 464, 558, 574, 581) might be explained on this basis. It has been suggested (223) that the glyceraldehyde formed from fructose could serve as a substrate for ADH-NADH, reoxidizing the NADH directly so that the ADH-NAD complex would be immediately available to oxidize more ethanol. Fructose and glyceraldehyde have both been found to increase the rate of ethanol metabolism in liver slices (574). Normally, glyceraldehyde would be oxidized to glycerate, rather than reduced to glycerol by ADH, but the oxidative pathway is blocked when ethanol and fructose are being metabolized (581).

It is thought that the acetaldehyde formed from ethanol will competitively appropriate the enzyme, aldehyde dehydrogenase, which would be necessary for glycerate formation from glyceraldehyde. In support of this explanation, glycerol production was greatly enhanced when liver slices were incubated with fructose and ethanol (482, 574). Studies with the perfused rat liver (173, 588) have shown that the rate of removal of ethanol from the perfusate at first approximated that observed *in vivo*, but later declined with time. This was at first ascribed to the decreasing concentration of ethanol (173). However, addition of pyruvate or fructose to the perfusion fluid prevented or delayed this decline (588). As Van Harken and Mannering point out (588), the NADH-reoxidizing mechanisms are not being maintained at a steady state in the perfused organ as they are in the intact animal, where no such decline in rate of ethanol metabolism is observed.

b. NAD regeneration. As mentioned earlier, recent work has focussed attention on the availability of NAD as the major factor in regulating the rate of metabolism of ethanol in structurally intact hepatic tissue (410). Studies of the metabolism of ethanol in rat liver slices suggest that the rate of mitochondrial oxidation of NADH is the primary limiting step (591). The acceleration of ethanol metabolism by uncoupling agents, which increase mitochondrial ability to oxidize NADH, suggests that neither the ADH-ethanol reaction nor the efficiency of the mitochondrial "shuttle system" of redox pairs plays the rate-determining role under normal conditions.

c. Chronic ethanol intake. i. Effects on rate of ethanol metabolism. The effect of chronic ingestion of ethanol on its rate of metabolism has received a good deal of attention, since the finding of an enhanced removal after such treatment might help to explain the development of tolerance to the drug. Von Wartburg and Papenberg (614), in evaluating the biochemical and enzymatic changes induced by chronic ethanol intake, concluded that there is very little evidence in animals for a significant increase in the capacity to oxidize ethanol after chronic intake. In their opinion, the maximum rate found in normal non-fasting subjects at high blood ethanol levels is sufficient to account for removal of the excessive quantities consumed by some alcoholics. On the other hand, Pringsheim (471), as early as 1908, reported an increased ability of rats to metabolize ethanol after a period of daily ingestion and concluded that this metabolic adaptation contributed to the development of tolerance. Similar results were reported in man (201, 229). In male rats which had received ethanol daily for 6 to 12 weeks, the rate of disappearance of a test dose of ethanol from the blood increased by over 50% (211). A similar increase was observed in the uptake of ethanol by liver slices from rats kept on a prolonged intake of ethanol (591).

On the other hand, a number of investigators have reported that the rate of ethanol metabolism by rats *in vivo* was unchanged by chronic intake (20, 178, 291, 616). In three studies (20, 178, 616) the elimination of labeled CO_2 after administration of ^{14}C-labeled ethanol was measured before and after a period of chronic ethanol intake, though the extent and duration of exposure to ethanol were different in the three cases. Comparable studies involving administration

of ^{14}C-labeled ethanol to man, including abstinent alcoholics and non-alcoholics, before and after chronic exposure to alcohol (408, 412, 414, 415), indicate that after 14 days of heavy intake there was a 4-fold increase in the amount of $^{14}CO_2$ expired during the 20-min period after oral administration of the test dose. This increase in rate of ethanol metabolism occurred both in the alcoholics and the non-alcoholics, though the latter had a lower rate of metabolism of labeled ethanol in the pre-drinking period than their alcoholic counterparts. To what extent variations in absorption rates may have contributed to these findings is unknown. Recent studies by Kater *et al.* (272, 273) showed a more rapid removal of ethanol from the blood of recently drinking alcoholics as compared with non-alcoholics.

ii. Effects on ethanol-metabolizing enzyme systems. The situation regarding possible changes in ADH activity is even more obscure. After daily intake of small amounts of ethanol, ADH activity was said to be increased not only in the liver but also in the cerebral cortex (1, 479). A gradual increase in both ADH and acetaldehyde dehydrogenase was observed in the livers and sera of male rats drinking 20% ethanol for up to a maximum of 26 weeks (70); however, Redmond and Cohen (489) found no increase in aldehyde dehydrogenase after a 4-day course of ethanol injections, but did find it after similar treatment with phenobarbital. In a strain of mice with high preference for ethanol, an increase in liver ADH occurred upon forced ingestion of alcohol (402) and a decrease occurred during subsequent abstinence; the evidence did not permit any conclusion as to whether the elevation in enzyme activity represents *de novo* synthesis or release from inhibition. In male mice receiving 15% ethanol as their only fluid, liver ADH was increased significantly after 3 and 5 months of continued ingestion but not after 12 months (421, 422). In a similar experiment with male rats given 10% ethanol to drink, an elevation in liver ADH occurred which could be suppressed by chronic treatment with puromycin, an agent known to interfere with protein synthesis (413). The livers of male mice receiving 10% ethanol as their sole drinking fluid for 90 to 104 days showed a significant elevation of liver ADH activity, but the activities of hexokinase and xanthine oxidase were either unchanged or decreased (529). Only those enzymes concerned in ethanol metabolism were increased by prolonged ethanol intake.

In contrast to the above findings, Figueroa and Klotz (123) reported no change in liver ADH activity in female rats injected intraperitoneally with moderate doses of ethanol for 12 to 16 weeks, but their animals showed no weight gain and were in poor condition; this is not uncommon when ethanol is given repeatedly by the intraperitoneal route. Other investigators (4, 178, 616) also failed to note any significant change in liver ADH levels after prolonged oral intake. Videla and Israel (591) observed no change in liver ADH activity of rats chronically treated with ethanol, yet an enhanced rate of metabolism of ethanol was displayed by liver slices obtained from these animals. Greatly reduced levels of liver ADH have been reported in biopsy specimens from patients with alcoholic cirrhosis (122, 531), and a 33% decrease was found in specimens from alcoholics with normal liver histology.

Two processes may be involved in the effect of prolonged ethanol intake on

levels of liver ADH: an adaptive process involving a specific induction of ADH, and a non-specific decrease of enzyme level as a manifestation of depressed protein synthesis resulting from liver impairment. Differences in the relative susceptibility of species and sexes with respect to these two factors have failed to explain some of the conflicting results which have appeared, but variations in dose of ethanol, route of administration, duration of exposure and composition of the diet make it difficult to resolve the controversy.

The activity *in vitro* of MEOS (332, 333, 449) has also been reported (339, 509, 510) to be increased after chronic ethanol intake in rats and man. Though this increase has been confirmed in other studies (284, 335), the significance of MEOS in the degradation of ethanol *in vivo* has been challenged by several groups, as mentioned in section II A 2 b. However, the possibility remains that chronic ethanol intake may lead to changes in other pathways which normally are not implicated in removal of the drug but which, under the influence of continued exposure, may assume such a role (330). The concentration of the coenzyme NADP has been found (626) to fall significantly in the perfused rat liver when ethanol was added, so that the ratio NADPH:NADP was increased. The NADPH:NADP ratio in the liver was reported to increase gradually during a period of chronic ethanol treatment (263, 265); this might represent a stimulation of NADH-NADP transhydrogenation reactions. Moreover, NADPH oxidase is reported to exhibit enhanced activity (334) after chronic ethanol intake. Both the coenzyme and the oxidase have been implicated in MEOS activity, yet their role *in vivo* may be in some reaction independent of this, possibly in relation to lipid metabolism. Changes in hepatic levels of the adenine nucleotides have been examined, but aside from opposing sets of results on the effect of ethanol on levels of adenosine 5′-diphosphate (ADP) and adenosine 5′-triphosphate (ATP) under acute conditions (153, 626), few data are available on possible changes during chronic exposure. In any event, it would be changes in their intramitochondrial ratio, rather than in total tissue levels, which would be significant.

iii. Effects on regeneration of NAD. Chronic intake of ethanol for 2 weeks has been reported to be accompanied by the same change in the ratio of total NAD to total NADH as occurs acutely (263, 265). After 6 weeks the sum of total NAD + total NADH in the liver was increased, but the ratio was still depressed. Sixteen hours after ethanol withdrawal, the sum of total NAD + total NADH was still elevated, but the ratio had returned to normal. Moreover, the change in nucleotide ratio at 1 and 4 hr after a test dose of ethanol given at that time was similar to the change seen in control animals. On this basis, chronic ethanol ingestion does not appear to produce adaptive changes resulting in more rapid reoxidation of hepatic NADH. Similar findings have been noted by others (see 263). One must keep in mind, however, that these studies relate only to changes in total NAD and NADH, *i.e.*, the sum of both bound and free forms of each in *all* cellular compartments.

To date, no one has examined a possible change in the efficiency of any of the mechanisms for H-transfer across the mitochondrial membrane or a shift in

their relative significance after chronic exposure to ethanol. The well documented changes in mitochondrial permeability and fragility encountered after chronic ethanol (154, 155, 158) and the significant decrease in mitochondrial phospholipids (157) might be indicative of structural changes which would allow an increase in the rate of movement of the "shuttle" compounds.

Of more importance, perhaps, is the observation (591) that dinitrophenol no longer increased the rate of ethanol metabolism by liver slices from animals treated chronically with ethanol. This implies either a loss of respiratory control in the mitochondria, or an adaptive increase in the activity of the electron transport chain, so that some other portion of the whole sequence, such as the ADH reaction or the "shuttle system," had become rate-limiting. There are many unanswered questions in relation to mitochondrial function in subjects given ethanol chronically (263), and it is not yet clear which changes are adaptive and which represent degeneration. The subject deserves much further investigation.

d. Miscellaneous agents. Many reports have appeared on the efficacy of various treatments for enhancing the rate of disappearance of ethanol *in vivo* or *in vitro*. These can be grouped roughly under two headings: i) Physical and physiological factors, and ii) Pharmacological agents.

i. Physical and physiological factors. Much of the early work on these has been reviewed by Newman (434), by Harger and Hulpieu (203) and by Mardones (392).

Exercise: Recent work (19, 454) has corroborated most of the earlier findings that physical exercise, unless complicated by hyperventilation, produces no significant alterations in the rate of ethanol metabolism. Krebs *et al.* (304) found a depressed rate of fall of blood ethanol after severe exercise, but admit that splanchnic circulation and absorption may be altered by the severity of the experimental conditions involved, so that prolonged absorption would obscure the rate of disappearance from the blood.

Hypothermia and shock: Hypothermia had no effect on the rate of fall of blood ethanol level in dogs (373), though readjustments of the circulatory system in these animals may have tended to mask or compensate for any changes in the rate of the drug's elimination. In rats, hypothermia was found to retard ethanol clearance (96). Traumatic shock induced by hind limb fracture was reported (371) to depress the rate of ethanol metabolism; it was suggested that this might be due to depression in hepatic circulation which accompanies shock (556).

Exposure to high environmental temperature: This has been reported to accelerate the removal of alcohol from the blood in man (18) but the role of hyperventilation and sweating in these experiments makes it difficult to assess the significance of the results.

Prolonged exposure of rats to cold: This has been reported to increase the rate of $^{14}CO_2$ production from labeled ethanol beyond that observed in control animals maintained at room temperature (462). However, this might reflect a change in the metabolism of acetyl-coenzyme A (acetyl-CoA) rather than of ethanol. Alterations in the relative blood supply to the liver could be involved here also.

High altitude: After a standard dose of ethanol, a lower peak in the blood alcohol curve was found in sheep raised at high altitudes than in others of the same fold raised at sea level (640). But again, alterations in blood volume in response to higher altitudes, with correspondingly greater dilution of the administered ethanol, may be responsible for the observed results (183).

Elevated oxygen tension: Exposure to elevated oxygen pressure produced no alteration in the rate of ethanol removal in rats (400) or cats (314). Mattie (400) reported similar findings *in vitro*. However, other *in vitro* studies with rat liver slices (591) or perfused rat livers (173) showed a higher rate of ethanol removal when higher O_2 tension is used. If regeneration of NAD is the rate-limiting step in ethanol removal under these conditions, it may be, as Videla and Israel (591) propose, that either the transfer of reducing equivalents from NADH to flavoproteins with subsequent oxidation by O_2 is operative at high O_2 tension, or the peroxisomal lactate oxidase electron shuttle of de Duve and Baudhuin (95) could be involved within the cytosol. Either would explain the observed failure of dinitrophenol to augment the rate of ethanol oxidation at high O_2 tension (591).

Fasting and undernutrition: The rate of clearance of ethanol from the blood of man and the rate of uptake by rat liver slices *in vitro* have been shown repeatedly to be decreased by fasting (212, 434, 451, 546, 593), or by feeding a protein-free diet (280). However, this has not been confirmed in mice (396) nor in dogs (292). The reasons for these discrepancies are unknown.

Foodstuffs and metabolites: The well documented effect of fructose in enhancing the rate of ethanol metabolism (25, 26, 61, 213, 295, 357, 366, 453, 463, 464, 558, 591), and the probable role of a coupled ADH reaction between ethanol and glyceraldehyde in this effect, have been dealt with earlier (see section II B 2 a). The action of pyruvate (320, 546, 621, 623) and of alanine (320, 546) may be partially explainable in the same terms. However, any NADH-linked system might contribute to an increased rate of ethanol metabolism by regenerating NAD more rapidly. In the cytoplasmic compartment, for example, pyruvate may be converted to lactate by the action of lactic dehydrogenase and NADH. Coupling of this reaction with the ADH reaction would increase the rate of the latter. This has been demonstrated in mammalian liver (361, 622). However, others believe that the presence or absence of an augmenting effect of pyruvate or alanine *in vivo* may depend on the initial rate of metabolism of ethanol when treatment is administered, a suboptimal rate being enhanced by increased supply of carbohydrate metabolites, while an already maximum rate would be unaffected (219, 620a).

Though succinate was reported to enhance ethanol disappearance *in vitro* (320), single, repeated or continuous intravenous injection of succinate failed to change the rate of disappearance of ethanol *in vivo* (227).

Attempts have been made to enhance the rate of ethanol metabolism by other methods of increasing the supply of NAD available. Pretreatment of mice with nicotinamide to elevate NAD in the livers was found (545) to be without effect on the rate of fall of blood ethanol. In contrast, studies *in vitro* with slices

and homogenates from treated and untreated animals showed that nicotinamide would enhance ethanol removal when NAD was presumably the limiting factor (363).

Insulin: No agreement concerning the effect of insulin on blood alcohol levels has been reached. Insulin alone or in combination with glucose was found to increase the rate of ethanol disappearance *in vivo* in some studies (290, 437), but inconsistently in others (435). Mardones (392) and Harger and Forney (202) provide additional references on the early work on this subject. More recently, Rawat (481) reported that the rate of ethanol metabolism *in vitro* by liver slices taken from rats with hyper- and hypoinsulinism, was augmented and decreased respectively. Variations in levels of free fatty acids (FFA), secondary to changes in insulin levels, have been implicated in this action; ethanol oxidation by the perfused rat liver was inhibited during fatty acid oxidation (626).

Glucagon: In dogs, the intravenous injection of glucagon, 2 hr before administration of ethanol, caused over 60% increase in the rate of disappearance of ethanol from peripheral blood (430). It was suggested that this effect was due to increased presentation of ethanol to the liver for oxidation, since glucagon has been reported to increase hepatic blood flow by 43 to 204% in dogs. This is difficult to reconcile with the known zero order kinetics of ethanol oxidation *in vivo*, unless the relevant effect of increased hepatic blood flow is an increase in the supply of oxygen available for NADH reoxidation. This seems improbable, however, because increased rates have been reported only with markedly elevated oxygen *tension* rather than supply, as mentioned earlier. One alternative explanation of the effect of glucagon may lie in its ability to enhance gluconeogenesis with resulting increase in the hepatic levels of pyruvate and of phosphoglyceraldehyde. A different suggestion is that low doses of glucagon lead to reduced mobilization of FFA, possibly indirectly by stimulating insulin secretion (483); this would diminish the inhibitory influence of fatty acids in the liver, as suggested above. This is consistent with the observation (483) that similar increases in ethanol utilization by liver slices were produced by *in vivo* administration of insulin and of glucagon.

Adrenaline: Widmark (625) reported that adrenaline, though it can alter ethanol distribution temporarily because of its circulatory effects, especially in muscle, induces no significant change in the rate of ethanol disposal.

Adrenocorticoids: The slope of the descending portion of the blood ethanol disappearance curve (see 624) was reportedly increased after injection of either methylprednisolone or dexamethasone (62). It was suggested that the effect may be due to an increased conversion of $NADH_2$ to NAD, as a result of enhanced gluconeogenesis.

Thyroid hormones: Although tri-iodothyronine was reported to enhance the rate of disappearance of blood ethanol in alcoholics and the rate of their return to sobriety (168), other investigators failed to note any such effect of the hormone in dogs (436, 540) or in man (267). Liver slices obtained from rats pretreated with thyroxine or with methylthiouracil metabolized ethanol at a rate comparable to that seen with slices from control animals (485). Yet treatment

with thyroid hormone diminishes, and thyroidectomy increases, the effect of ethanol on the redox state of the cytoplasm (209, 484, 634). These findings appear to detract from the importance of NAD regeneration as a rate-limiting factor *in vivo*. However, it must be remembered that they were obtained with liver slices or perfused livers gassed with 95% oxygen, *i.e.*, under conditions in which dinitrophenol had no effect on ethanol metabolism (591).

Vitamins: Berg, Stotz and Westerfeld (24) found no significant effect of thiamine deficiency on the rate of ethanol elimination. Subcutaneous doses of thiamine as high as 10 mg/kg have been reported to cause no change in the rate of disappearance of ethanol from the blood (see 203). As mentioned earlier, the administration of nicotinamide to mice on an adequate diet had no effect on the rate of disposal of ethanol (22).

ii. Pharmacological agents. Forney and Hughes (130) and Wallgren and Barry (605, p. 621) have reviewed the subject of the interaction of alcohol and psychopharmacological drugs, and Polacsek (466) has prepared a comprehensive annotated bibliography. The present review will not attempt to deal with this subject exhaustively.

Barbiturates and sedatives: Fischer and Oelssner (127) found that pretreatment of mice for 5 days with hexobarbital or phenobarbital resulted in a 25% increase in the speed of disappearance of ethanol. These authors interpreted their findings in terms of changes in the NAD/$NADH_2$ ratio in the liver which, according to Fischer (125), underwent an elevation of approximately 30% after hexobarbital treatment. However, their results are not presented in sufficient detail to permit an evaluation of the statistical significance of the reported differences. Others (282) have found no effect of chronic pretreatment with phenobarbital or chlorcyclizine on the rate of ethanol metabolism. Ethanol given together with chloral hydrate disappeared from the blood of man at the same rate as ethanol alone (270). Chloral hydrate is reduced to trichloroethanol by ADH together with NADH, so that a mutual facilitation of metabolism of ethanol and chloral hydrate might be anticipated, as in the case of ethanol and glyceraldehyde. Yet, at low concentrations of ethanol, chloral hydrate reduced the rate of ethanol metabolism in the mouse (162) even though ethanol increased that of chloral hydrate (49). The answer appears to lie in the relative affinities of the two substrates and their respective products for the enzyme-cofactor complex. This should lend itself readily to kinetic analysis by computer. No influence of either glutethimide or secobarbital on the rate of disposal of ethanol has been observed (407).

Chlorpromazine: A number of papers have appeared on the effect of this compound on the rate of alcohol metabolism. Chlorpromazine was reported to inhibit liver ADH *in vitro* (287). However, it was found to have no effect on *beta*, the slope of the blood alcohol disappearance curve in man (54) or rats (544) *in vivo*, or on uptake by rat liver slices *in vitro* (544). Like adrenaline, chlorpromazine can bring about significant changes in peripheral blood flow and distribution, as well as on body temperature, which must be borne in mind when its effects on ethanol disappearance are studied (259).

III. Effect of Ethanol on Metabolic Processes

A. Introduction

Derangements in a variety of metabolic processes can occur when ethanol undergoes oxidative degradation. Kalant and Khanna (263) have emphasized the necessity of distinguishing metabolic effects secondary to the physiological actions of ethanol from those concerned with the metabolism of ethanol itself. Unfortunately, the literature on the subject reveals many instances where such a distinction has not been made.

There are at least two main causes of the metabolic derangements induced by alcohol. One arises from the fact that ethanol itself can give rise to a large supply of 2-carbon residues which enter common metabolic pools that are also supplied by other pathways. The latter are therefore "hydraulically" influenced by the input from ethanol. The second is that ethanol, in the course of its oxidative breakdown, preempts the supply of cofactors which are normally shared, in a series of delicately counterbalanced reactions, by the oxidative processes involved in the degradation of a host of endogenous substances. The hepatocyte, as a consequence, undergoes a change from an oxidative to a reductive internal environment. This change occurs in the cytoplasmic compartment where the oxidation of ethanol takes place (445), in the microsomal fraction where the magnitude of certain specific reductive processes will be enhanced, and in the mitochondrial compartment where disposal of the extra reducing equivalents must be primarily effected. The changes in the redox state of the various cellular compartments and the mechanisms available for NAD regeneration have already been discussed (see section II A 4 c).

B. Metabolism of 2-carbon residues derived from ethanol

Regardless of whether ADH, catalase or MEOS activity is involved, the first step of ethanol metabolism leads to the production of acetaldehyde. This in turn is metabolized by an NAD-dependent aldehyde dehydrogenase which has already been described. This enzyme is present in the liver cytosol but some activity has been reported in washed mitochondria from rat tissues (209, 598) and from bovine, rat and monkey brain (110). The mitochondrial enzyme resembles that in the cytosol with respect to K_m for acetaldehyde, broad substrate specificity, pH optimum and sensitivity to sulfhydryl reagents and appears to be distinct from the NADP-linked enzyme described by Brady *et al.* (39). It has been calculated that 60% of the total acetaldehyde dehydrogenase activity in brain resides in the mitochondrial fraction (110). Freezing and thawing of the brain causes all the enzyme to appear in the 30,000 $\times$ *g* supernatant fraction, presumbably because of leakage from the mitochondria. The question therefore remains open, whether separate enzymes are normally present in the mitochondria and the cytosol. It might prove interesting to re-examine the distribution within the liver cell under conditions in which mitochondrial integrity is maintained as carefully as possible.

Ingestion of ethanol by subjects pretreated with tetraethylthiuram disulfide

(disulfiram, Antabuse®) has been shown to result in the accumulation of acetaldehyde in the blood and alveolar air (160, 197, 245, 576). This is associated with inhibition of aldehyde dehydrogenase activity (82, 176), the inhibition being competitive with respect to NAD and non-competitive with respect to substrate. The pharmacological actions of disulfiram and other inhibitors of acetaldehyde metabolism will not be considered here, because it is expected that they will form the subject of another review shortly.

It is still unclear whether free acetate is a direct product of acetaldehyde metabolism or is formed indirectly, the acetaldehyde being converted to acetyl-CoA which can then be hydrolyzed to yield acetate. Lundquist *et al.* (363) found with rat liver suspensions that the amount of acetate produced corresponded to the decrease in ethanol in the medium. Moreover, in experiments in man infused with ethanol (365), differences in levels found in blood from hepatic vein and femoral artery indicated that the major part of ethanol is oxidized to free acetate, with the latter accounting for more than 50% of the ethanol metabolized. Perfusion of labeled ethanol through the isolated rat liver has been shown to yield large amounts of labeled acetate (141) and acetate was the main metabolite accumulating when ethanol was metabolized by the perfused rat liver (142). However, these findings do not really settle the matter. It is well known that oxidation of ethanol in the liver almost completely suppresses the activity of the mitochondrial tricarboxylic acid cycle (see 137, as well as section III D). Under these conditions, acetyl-CoA formed from acetaldehyde might have no other route to follow except hydrolysis to free acetate.

While ethanol and acetaldehyde are metabolized primarily in the liver, acetate, whether free or in the form of acetyl-CoA, is mainly disposed of in the extrahepatic tissues (365, 581, 630). The human myocardium will increase its uptake (and, presumably, utilization) of acetate 5-fold during ethanol infusion (351), with a concomitant decrease in fatty acid utilization. Skeletal muscle also utilizes a large part of the acetate released from the liver (359).

The evidence available from experiments in which ^{14}C-labeled ethanol has been employed suggests that the acetyl component of acetyl-CoA passes through the tricarboxylic acid cycle (71, 527) with the final conversion of ethanol to carbon dioxide and water through the mediation of the mitochondrial flavoprotein-cytochrome system. The appearance of traces of the label in glycerol and glycogen (527), cholesterol (398, 514), lipids and fatty acids (53, 141, 514, 543, 547) fits in with the concept that degradation of ethanol yields acetyl-CoA and the latter's pathway of metabolism would permit such labeling to appear at the sites mentioned. The fate of ethanol is thus reflected in the fate of the 2-carbon residues derived from it.

C. Effects of ethanol on gluconeogenesis, blood glucose and hepatic glycogen levels

Within the past 10 years, numerous studies on the effect of ethanol on various aspects of carbohydrate metabolism have helped to clarify some of the confusion which had existed in this field. For a résumé of these studies and the hypotheses arising from them, the reader is referred to a number of reviews (10a, 11, 137, 138, 148, 241, 303, 329, 377).

Human subjects in the postabsorptive state have been shown to exhibit an increase in blood sugar levels 30 to 60 min after drinking 150 ml of whisky (144); later, however, blood sugar fell to hypoglycemic levels more rapidly than in control tests on the same subjects without whisky. In the morning after intoxication, hypoglycemia was encountered; the more the intake of ethanol, the lower the blood sugar level (589). It was concluded that an initial rise in blood sugar after ethanol probably reflects glycogenolysis, while later on, when glycogen stores are depleted, the ingestion of ethanol leads to hypoglycemia. Dogs given single doses (3 g/kg) of ethanol by stomach tube developed hyperglycemia 1 to 2 hr later; on chronic ethanol intake, no hypoglycemia was seen when food was available, but when fasted, all dogs showed hypoglycemia by the 5th to 11th day, even though fasted controls maintained normal blood glucose levels (63). In rabbits, infusions of low doses of ethanol induced hyperglycemia, but the elevation could be prevented by ganglionic blockade, suggesting that the hyperglycemia was probably of adrenomedullary origin (459, 460). All these investigations pointed to the fact that when hepatic depots of carbohydrate were adequate, ethanol would initially cause a hyperglycemia, if the dose of the drug were sufficiently high, presumably as a result of adrenaline response to the stress of intoxication.

Glucose output by surviving liver slices of rats subjected to prolonged ethanol intake is depressed (64, 422) and, since hepatic glycogen stores were significantly lower than those of the controls, the reduction in glucose output was ascribed to the reduced level of hepatic glycogen resulting from ethanol pretreatment (64). Decreased hepatic glycogen levels after ethanol infusion, despite normal blood glucose, have been reported by others (627). But the fact that addition of ethanol to the medium caused glucose output from liver slices to decrease, irrespective of whether these slices were derived from alcohol-treated or control rats, suggested that some other factor aside from depressed glycogen levels was also involved.

Since 1941, numerous clinical reports of the occurrence of postalcoholic hypoglycemia have appeared (41, 69a, 146, 186, 250, 394, 429, 562a). The subjects generally presented a history of poor dietary intake, and hypoglycemia symptoms usually appeared 6 to 36 hr after cessation of ethanol intake, often when ethanol was no longer detectable in the blood. Liver biopsies revealed that glycogen content was low or missing. The condition was not confined to the chronic alcoholic; young children appeared particularly susceptible to such an attack. The hypoglycemia was attributed to a hepatotoxic action of ethanol in livers already damaged by a deficient diet, with the result that glycogen reserves were exhausted and gluconeogenesis inadequate (429). However, normal adults also can exhibit the symptoms of alcoholic hypoglycemia when they ingest beverage alcohol or pure ethanol after fasting for 36 to 72 hr (151).

The underlying pathogenesis and clinical features of alcoholic hypoglycemia have been outlined in an excellent series of papers by Freinkel and others (12, 29, 121, 149–152). When hypoglycemia was produced by ethanol in fasted normal subjects, immuno-assay studies revealed no rise in plasma insulin levels. Studies in chronic alcoholics who exhibited the condition showed that though their

potential for mobilization of pancreatic insulin and their adipose tissue responses were preserved, they generally exhibited a decrease in the counter-regulatory response to fasting, and in their autonomic reaction to hypoglycemia induced by exogenous insulin (152); furthermore, intravenous glucagon failed to interrupt the hypoglycemia, suggesting that glycogen stores were depleted.

It has been claimed (169) that the hypoglycemia observed in dogs infused with ethanol after a 5-day fast could be prevented by the daily administration of a small amount of crystalline zinc insulin during the fasting period. This effect of non-hypoglycemic amounts of insulin has been attributed to its ability to maintain a high carbohydrate turnover, thereby preventing a shift to the use of fat as the primary fuel. An alternative explanation might be that the cessation of administration of exogenous insulin could lead to a short period of temporary insulin insufficiency, and the resulting tendency to hyperglycemia could offset the hypoglycemic action which ethanol could have under the existing conditions. In any event, insulin does not appear to be centrally involved in the phenomenon of alcoholic hypoglycemia.

Evidence of a direct inhibitory action of ethanol on hepatic gluconeogenesis has been found in studies of the metabolism of surviving liver slices of fed and fasted rats, incubated with uniformly labeled alanine in substrate (10 mM) or tracer amounts (0.01 mM), under conditions of high or low pyruvate turnover (29, 152). Labeled alanine provided a slow generation of labeled pyruvate and thus simulated the delivery of 3-carbon gluconeogenic precursors *in vivo*. Where pyruvate turnover and availability were limited and gluconeogenesis would be marginal, ethanol inhibited the formation of glucose-^{14}C and decreased the total glucose recovered in the medium at the end of incubation. With adequate pyruvate turnover, glucose-^{14}C formation was increased by ethanol when slices had an adequate supply of endogenous substrate (fed rats) and was either increased, unaffected or decreased in slices from fasted animals. Accumulation of labeled aspartic acid always occurred in the presence of ethanol. It was postulated that the variable effects upon glucose formation might be the result of an alteration in the ratio of NAD^+ to NADH in the liver which would occur as the result of ethanol degradation; gluconeogenesis would increase when the reoxidation of NADH occurred primarily in the cytoplasm as a result of adequate pyruvate supply. Conversely, when the supply of pyruvate at that site is limited and mitochondrial oxidative mechanisms play the major role, gluconeogenesis would be impaired (358a). The divergent effect of ethanol on gluconeogenesis thus would represent the extremes of a continuum, the overall direction of which would depend on the mechanisms available for the disposal of extra electrons. The biochemical lesion induced by ethanol was localized to the renewal of glucose 6-phosphate from small carbon fragments, with the intrahepatic availability and turnover of pyruvate playing the pivotal role. The pre-emption of an already marginal supply of pyruvate by reduction to lactate could compromise the renewal of phosphoenolpyruvate and arrest gluconeogenesis.

In experiments with perfused rat liver, addition of ethanol to the perfusate caused a decrease in glucose production, with a 50% reduction in urea output,

though production of amino acids was relatively unchanged (121). This would indicate that, while protein degradation was not altered by ethanol, deamination of amino acids was affected and the decreased urea formation would lead, in turn, to decreased gluconeogenesis and thereafter to decreased release of glucose from liver. Krebs *et al.* (305) have also found that ethanol impairs gluconeogenesis from alanine, serine and proline. However, Freinkel *et al.* (152) reported no difference in results with liver slices when labeled pyruvate was substituted for labeled alanine, suggesting that deamination of alanine is not impaired by ethanol. The same may not obtain for other deaminating mechanisms. Certainly this apparent discrepancy requires resolution.

The findings outlined above have been substantiated and complemented by *in vivo* studies. With fasted dogs with chronic portocaval shunt to permit measurement of hepatic glucose balance, Lochner *et al.* (352, 353) found that an infusion of ethanol decreased hepatic glucose output. Amounts producing blood ethanol levels of 10 to 20 mg/100 ml caused a 58% to 68% decrease in mean hepatic glucose output (378). A concomitant infusion of fructose, which can give rise to glucose without participation of NAD-dependent mechanisms, raised this output to or above control levels, whereas the infusion of either α-ketoglutarate or glutamate failed to alter the ethanol-induced suppression. Administration of methylene blue, a redox dye capable of oxidizing $NADH_2$ to NAD, prevented the decline in hepatic glucose output which is otherwise produced by ethanol in fasted dogs. On the basis of these results, it was concluded that the pathway from fructose 1,6-diphosphate to glucose is not blocked by ethanol, but the conversion of glutamate and α-ketoglutarate to glucose is diminished and conversion of citric acid cycle intermediates to oxaloacetate is depressed during ethanol metabolism (378).

Krebs *et al.* (305) have confirmed and extended this work by studies on the perfused rat liver and incubated kidney cortex slices. Ethanol sharply inhibited gluconeogenesis in the liver only, and from lactate but not from pyruvate. The organ specificity of the ethanol effect, its relation to ethanol concentration, and the effects of pyrazole and of acetaldehyde upon it, all suggest that the inhibition results from impaired conversion of lactate to pyruvate as a result of NADH accumulation during the oxidation of ethanol by liver ADH. Ethanol also inhibited to some extent the formation of glucose from glycerol, dihydroxyacetone and fructose; this effect is probably also attributable to increased $NADH/NAD^+$ ratio, as explained later (sections III E 5 and III G 5 d).

An interesting observation regarding factors influencing gluconeogenesis is that an increase in the $NADH/NAD^+$ ratio will inhibit the conversion of glutamate to α-ketoglutarate by glutamic dehydrogenase (159). The increase in NADH resulting from the metabolism of ethanol would thus tend to depress gluconeogenesis from those amino acids which can enter the citric acid cycle. Measurements of levels of gluconeogenetic intermediates in livers of fasted rats 4 hr after the ingestion of ethanol revealed significantly increased levels of malate and glutamate and a decrease in aspartate. Although oxaloacetate levels were too low to allow accurate measurement, the findings with aspartate suggest that oxaloacetate was shunted to malate (638). In Zakim's opinion (638) the primary action

of ethanol is to inhibit *entry* of the gluconeogenetic substrates into the gluconeogenetic pathways. At the same time, the increase in glutamate level would assist the function of the malate "shuttle" mechanism for mitochondrial reoxidation of cytoplasmic NADH (section II A 4 c).

In the presence of ethanol, the fall in blood lactate levels after a period of severe exercise is significantly delayed (304). The time course of this fall is consistent with the hypothesis that the drug impairs the metabolic disposal of lactate. According to Krebs (303), one must therefore consider the elevated blood lactate levels seen at low levels of blood ethanol (<10 mM or 46 mg/100 ml) as attributable *not* to an excessive production of lactate by the liver, but rather to a defective resynthesis of glucose from lactate. Since only net changes in blood lactate levels were being measured, it seems more appropriate to conclude that both processes may contribute to the elevation.

D. Effect of ethanol on the citric acid cycle

In addition to its effects on gluconeogenesis, ethanol has been found consistently to reduce the formation of CO_2 from a variety of isotopically labeled and unlabeled substrates by isolated liver slices (29, 132, 133, 152, 384), perfused livers (142, 589a, 626) and intact animals (534a). With ^{14}C-labeled glucose or fructose, the inhibitory effect of ethanol was greater when the isotope was in the C-6 rather than the C-1 position, suggesting that metabolism *via* glycolysis and the citric acid cycle was inhibited, rather than *via* the hexose phosphate shunt. From these and subsequent studies it was concluded that the function of the tricarboxylic acid cycle was inhibited by ethanol, and it has been calculated that this inhibition amounts to about 75% (626). Studies of the metabolism of various alcohols by rat liver slices (136), and of the effects of dinitrophenol on the metabolism of ethanol by the perfused rat liver (589a), indicate an excellent correlation between the effects of ethanol on the redox state and on the production of CO_2. It is specifically the change in intramitochondrial $NAD^+/NADH$ ratio which leads to this inhibition of tricarboxylic acid cycle activity (589a).

Two mechanisms must be considered in relation to this effect. As noted earlier, NADH accumulation during ethanol metabolism tends to lower the steady-state concentration of pyruvate in the cytosol; this, in turn, lowers the rate of the pyruvate carboxylase reaction which produces oxaloacetate. The latter can either condense with acetyl-CoA to enter the citric acid cycle, or follow a gluconeogenetic pathway *via* phosphoenolpyruvate. Ethanol metabolism, by lowering the pyruvate level, can thus impair both gluconeogenesis and mitochondrial production of CO_2 (137, 150, 303). A reduction in intramitochondrial pyruvate level after ethanol administration has not been definitely demonstrated (137). The second mechanism, relating to the shift in redox state within the mitochondria, involves the increase in malate:oxaloacetate ratio which has been mentioned earlier. This would also contribute to the decrease in activity of the tricarboxylic acid cycle (136, 138). Most of the malate and oxaloacetate in the liver cell is located in the cytoplasm, rather than the mitochondria (see 222). This probably explains the fact that oxaloacetate levels in rat liver *in vivo* were not significantly changed after ethanol infusion (480).

With the perfused liver, no accumulation of citrate was found in the absence of exogenous fatty acids when ethanol was added, yet a fall in citrate levels was noted in the presence of oleate (626). These findings have been interpreted as evidence of a coordinated inhibition by ethanol of both citrate synthetase and isocitrate dehydrogenase. When oleate was added, total activity of the tricarboxylic acid cycle was increased, and only the effect of ethanol at the citrate synthetase stage was seen; therefore this was considered the primary site of the inhibitory action of ethanol. At the present time, no information is available about the relative distribution of the metabolites in the cytoplasmic and mitochondrial compartments. The NAD-linked isocitrate dehydrogenase is strongly inhibited by NADH; therefore the increase in intramitochondrial NADH:NAD^+ ratio induced by ethanol could exert a direct effect on its activity. In contrast, this shift in redox state would alter citrate synthetase activity only *via* its effect on the availability of oxaloacetate.

A diminution in the oxidation of ^{14}C-labeled acetate and palmitate to $^{14}CO_2$, encountered in the presence of ethanol (486), also provides evidence of a depression in citric acid cycle activity. In fact the accumulation of acetate itself when ethanol is ingested can be considered a reflection of depressed activity of the cycle (135).

In summary, most investigators feel that the main effect of ethanol on carbohydrate metabolism resides in its inhibition of gluconeogenesis, with a possible resultant deficit in hepatic glucose output. The inhibition of gluconeogenesis is in some way related to the rise in NADH/NAD^+ ratio. The decrease in carbon dioxide production by the liver, with little or no change in its oxygen uptake, indicates an interference with the functioning of the tricarboxylic acid cycle in the mitochondria. Though numerous hypotheses have been advanced to account for the depressed activity within this cycle, the precise reaction most vulnerable to the alteration in the mitochondrial environment induced by ethanol in its metabolism has not yet been proven.

E. Ethanol and carbohydrate utilization

1. GLUCOSE UTILIZATION

Studies in dogs with portocaval shunt showed that ethanol infusion, after a 2- to 3-day fast, led to a decrease in peripheral glucose utilization (352, 353). The decrease was not due to decreased arterial glucose concentration, since the greatest change in peripheral utilization occurred in dogs with little or no fall in arterial glucose levels. In most studies, the decrease in hepatic glucose output exceeded the decreased glucose utilization, and arterial glucose concentrations fell. It has been suggested that acetaldehyde or acetate, rather than ethanol itself, may be the factor responsible for the decreased utilization.

2. PYRUVATE AND LACTATE METABOLISM

As noted earlier, reduction of pyruvate to lactate in the cytoplasm of the hepatocyte provides a means of reoxidizing some of the NADH resulting from the metabolism of ethanol. Disposal of the additional reducing equivalents is then

accomplished by transport of the lactate in the blood to the peripheral tissues, primarily muscle, where it is oxidized. Thus, the load placed by ethanol on the oxidative capacity of the mitochondrial system of the hepatocytes is partially alleviated, but the excess lactate will affect the redox state of the peripheral tissues (58).

The possibility that competition between alcohol dehydrogenase and pyruvate dehydrogenase for available NAD could impair pyruvate decarboxylation has been raised. The increased production of CO_2 by rat liver slices on the addition of pyruvate to the medium has been found to be competitively inhibited by ethanol (132). In dogs pretreated with oxythiamine the infusion of ethanol led to marked hypoglycemia, though neither drug by itself affected blood glucose levels (627). This effect of ethanol could be prevented by administration of thiamine, a fact which again points to the central role of pyruvate in the metabolic effects of ethanol.

3. COENZYME A AND ACETYL-COENZYME A LEVELS

Reduction of CoA levels in liver and brain has been reported as an aftermath of acute or chronic ethanol intake (7–9). Between the 1st and 2nd hr after ethanol infusion, CoA levels in liver fell more than 90%, but gradually rose thereafter, reaching control levels after 20 hr. This may not be a direct effect of ethanol but of acetaldehyde formed from it (9). Addition of acetaldehyde to homogenates of liver and brain lowered the levels in both. Acetaldehyde is not formed to any extent in brain, but it can be carried there *in vivo* by the blood after its formation in the liver. Pretreatment with disulfiram, before administration of ethanol *in vivo*, caused a more pronounced depletion of hepatic CoA than in animals given ethanol alone; this again suggests that acetaldehyde, rather than ethanol, is the agent responsible for the changes observed (9). The possible formation of a semimercaptide or mercaptide bond between acetaldehyde and the SH-group of CoA is believed to result in a blockade of the active site of the coenzyme and interference with its transacetylating property. The isolation and identification of the mercaptide would provide more concrete evidence for this interesting hypothesis.

In contrast, others have reported an increase in hepatic levels of CoA and its fatty acid esters, 2 hr after acute administration of a high dose of ethanol, as well as in rats on chronic ethanol treatment (32). The reason for these opposing results is not yet known, but various possibilities must be considered. Ammon *et al.* (9) found the reduction in hepatic CoA level to vary with the dose of ethanol. Since both doses which they used were sufficient to maintain linear fall of blood ethanol level during the 1st hr, the production of acetaldehyde by ADH should have been the same. Therefore the dose-dependence of the change in CoA levels may reflect either a secondary disturbance related to the degree of intoxication, or the production of additional acetaldehyde by another route of alcohol metabolism active at high blood levels in the mouse and not in other species (131, 396). The second suggestion seems a little more probable, because Bode *et al.* (32) also gave a large dose of ethanol, but used rats rather than mice.

An increase in the acetyl-CoA content of liver has also been reported after ethanol ingestion (32, 139). The accumulation could result from activation of acetate, derived from ethanol, from impairment of end-oxidation of fatty acids or from impaired inflow into the citric acid cycle. Further data obtained from studies employing radioactive labeled substrates are needed to pinpoint the cause of the accumulation. Yet, Rawat (480) observed a highly significant *fall* in hepatic acetyl CoA levels 30 min after ethanol infusion. Here again the discrepancy needs to be resolved. It may be that variations in sampling times are responsible for the contrasting results.

4. GALACTOSE METABOLISM

Rapid removal of galactose from the circulation has long served as a measure of the functional efficiency of the liver. Normal subjects, upon ingesting ethanol, display reduced rate of removal of galactose from the circulation (555, 580). In contrast, ethanol caused no further impairment in cirrhotic subjects beyond that already seen (179). Ethanol is also reported to cause *less* inhibition of galactose oxidation in patients with a fatty liver than in normal subjects (515).

Experimental studies of the $^{14}CO_2$ content of the expired air after infusion of ^{14}C-galactose in human volunteers have shown that the intake of as little as 10 to 20 ml of ethanol led to a clear-cut reduction in the rate of carbon dioxide formation, a drop in the rate of disappearance of galactose from the blood, and an increased excretion of this sugar in the urine (534). These effects were not offset by pyruvate or fructose. The postulated explanation was that NADH formation in the course of the oxidation of ethanol and acetaldehyde blocks the activity of the enzyme uridine diphosphate-galactose epimerase. This has been confirmed by studies of the effect of ethanol on galactose metabolism *in vitro* (242, 243). The inhibition was increased by factors which increase the rate of ethanol metabolism (added NAD) and decreased by those which decrease the concentration of NADH (lactic dehydrogenase and pyruvate). Addition of sorbitol (which, like ethanol, leads to augmented levels of hepatic NADH) also produced a significant inhibition of galactose oxidation by a soluble rat liver system. In hemolysates, which lack alcohol dehydrogenase, ethanol did not inhibit galactose utilization, but inhibition did occur upon addition of ADH. No direct inhibition of any of the enzymes involved in galactose metabolism was noted when ethanol alone was added, but addition of ethanol together with yeast ADH caused an 80 to 90 % depression of the activity of the key enzyme in galactose metabolism, uridine diphosphate-galactose 4-epimerase. This enzyme requires catalytic amounts of NAD for its activity but is extremely sensitive to inhibition by NADH (401). Thus, the cellular concentration of NADH, as well as of NAD, is an important factor in the regulation of galactose metabolism.

The elimination of intravenous galactose in man has been studied by calculating the galactose elimination capacity [(amt. injected − amt. excreted)/$t_{c\to 0}$, where $t_{c\to 0}$ is the intercept on the time axis of the extrapolation of the linear part of the declining blood galactose curve] (580). The ingestion of 20 g of ethanol caused this function to decrease from the mean of 510 mg/min found in control

runs to 200 to 250/min after ethanol. In cirrhotic patients, the value fell only from a control value of 200 mg/min to 160 mg/min after ethanol. With continuous slow intravenous infusion of galactose, it was found that after ethanol (10–20 g orally) the steepness of rise in the blood galactose level was independent of the blood ethanol level as long as the latter was above 10 mg/100 ml. These data are in agreement with the hypothesis that ethanol blocks galactose conversion due to its effect on the NAD/NADH ratio in the liver; but this only becomes important when blood ethanol exceeds 10 mg/100 ml, the level at which hepatic utilization of ethanol is maximal. The failure of ethanol to exert any great effect on galactose utilization in cirrhotics may be due either to a deficiency in NADH-oxidizing enzymes in their livers, with the result that even the control rate of galactose utilization is low, or the cirrhotic liver may not be able to metabolize ethanol as rapidly as the normal liver, with the result that the level of NADH is not altered to the same extent by the presence of ethanol. Probably the latter explanation is best.

5. FRUCTOSE AND SORBITOL METABOLISM

The effect of fructose on the rate of ethanol metabolism has been discussed earlier (see section II B 2 a) but ethanol, in turn, will alter the metabolism of fructose, for the shift in the redox potential of the liver cytosol, induced by the metabolism of ethanol, will favor the reduction of a greater than normal amount of fructose to sorbitol. This may be the reason for the reduction in gluconeogenesis from fructose in the perfused liver on the addition of ethanol (305).

Sorbitol metabolism is also impaired by ethanol. This has been observed in studies of the rate of sorbitol disappearance from the blood in man (590), as well as of sorbitol oxidation by liver slices and in intact animals (218). Sorbitol is normally oxidized to fructose in the liver, by the NAD^+-dependent sorbitol dehydrogenase. Presumably the increase in NADH:NAD^+ ratio, which favors the reduction of fructose, also impairs the oxidation of sorbitol.

F. Protein and amino acid metabolism

Data on the effect of acute and chronic intake of ethanol on protein and amino acid metabolism are relatively sparse. Isselbacher and Greenberger (241) and later Forsander (138) have commented on the relatively small number of studies in this field. The effect of ethanol on protein synthesis has been studied mainly in the liver and pancreas, and in both cases enzyme levels have been used extensively as indices of synthetic activity.

1. PROTEIN SYNTHESIS

The rate of incorporation of ^{14}C-leucine into pancreatic proteins *in vivo* has been measured in rats after varying periods of feeding with ethanol (517–519). As the time of exposure to ethanol increased, the specific activity of the pancreatic proteins decreased, indicating a progressive reduction in the rate of protein synthesis which was independent of age. Pancreas slices from the chronic ethanol-fed rats were also incubated with an amino acid mixture containing labeled leu-

cine and an ATP-generating system; the longer the preceding duration of exposure to ethanol, the greater was the decrease in rate of tissue respiration, in specific activity of the pancreatic protein, and in trypsin and ribonuclease activity. It is possible that the decrease in protein synthesis seen in these experiments is a reflection of a general debility, due to nutritional imbalance resulting from prolonged ethanol consumption, rather than a specific inhibition of protein synthesis by ethanol *per se*. Repetition of the experiments with ethanol incorporated into a liquid diet (339), and the use of paired feeding to prevent group differences in body weight, might help to dissociate these two factors.

In contrast to these results, others have reported that gavage with fairly large doses of ethanol for 2 to 7 days, combined with either a complete or a protein-poor diet, was without effect on the uptake of ^{14}C-labeled leucine into various subcellular fractions of the pancreas (577). Still more confusing is the report (64a) that, 12 hr after the intraperitoneal injection of ethanol in a dose of 4 g/kg, the Na^+-dependent uptake of lysine, proline and methionine by slices of the pancreas *in vitro* was impaired. As pointed out elsewhere (257), ethanol must be present for inhibition of active transport of Na^+ and K^+ and amino acids to occur, and in these experiments ethanol was not added *in vitro* nor would it still be present *in vivo* under the conditions of testing. The effect might be the result of unspecific local damage within the peritoneal cavity after injection of ethanol in the high concentration used.

In another study of incorporation of labeled leucine into rat liver proteins *in vivo* it was found that a moderate dose of ethanol (0.45 g/kg) increased the specific activity and total recovery of radioactivity in total liver protein. The elevation appeared at the 30-min point after leucine administration and persisted for at least 2.5 hr (13). No significant differences were noted in the specific activity of the hepatic ribonucleic acid (RNA)-protein fractions or in the total recovery of radioactivity in the amino acid fraction, but the specific activity and total radioactivity of total serum proteins, serum albumin and *beta*-globulins were significantly increased at 1.5 and 2.5 hr in the ethanol-fed rats. Thus, a small dose of ethanol evidently caused no impairment in leucine uptake or incorporation, or protein secretion. The increased incorporation of radioactivity into *beta*-globulins after ethanol may even reflect an increased secretion of this moiety as *beta*-lipoprotein by the liver (147). The results agree with those from studies of hepatic lipoprotein production after the administration of ^{14}C-lysine (16, 17) to rats treated *chronically* with ethanol. Although all serum lipoprotein fractions increased, the main increase occurred in the fraction with a density of less than 1.019. The ^{14}C-labeling of this fraction increased by 270%, while that of liver and of residual serum proteins was unaffected, as compared to levels found in controls fed a carbohydrate replacement in equicaloric amounts. Blockade of lipoprotein utilization with Triton WR1339 indicated that increased lipoprotein production, rather than impaired peripheral utilization, was responsible for the findings. Moreover, the disappearance of doubly labeled chylomicrons from the circulation was similar in animals from both groups, but in the ethanol-fed group it was followed by a more prominent increase in the radioactivity of the d <

1.019 lipoprotein fraction. Thus, chronic ethanol intake appears to cause an increased production and secretion of the lipoprotein moiety and no deficit in its protein content.

In contrast, studies with much higher ethanol concentrations in the isolated perfused rat liver yielded results opposite to those discussed above (536). Although experimental details are meager, it was concluded that ethanol interferes with the incorporation of labeled leucine into perfusate lipoproteins and other plasma proteins, causing a significant preferential reduction of the amino acid incorporation into low-density lipoproteins. No reduction in incorporation occurred in the protein of the liver itself, despite high concentrations of ethanol, suggesting that the impaired labeling of the lipoproteins was not due primarily to a decrease in protein synthesis but was secondary to an impaired release of the lipoprotein from the liver.

A recent report (506) indicates that in the isolated perfused liver, albumin synthesis is depressed by 65 to 75% in the presence of 50 mM ethanol. Partial reversal of this depression could be achieved by 10 mM tryptophan. On the other hand, livers from animals receiving chronic ethanol treatment synthesized albumin at the same rate as those from sucrose-fed controls, when the perfusion fluid did not contain added ethanol. The seeming disparity between the acute and chronic effects of ethanol must still be explained, but the most likely cause is the difference in ethanol level. At the moment, more emphasis on the *in vivo* results appears warranted, especially in view of the findings (171, 173, 626) that the experimental conditions involving aeration and availability of metabolites can profoundly affect the metabolism of ethanol itself by the isolated organ.

Recent findings indicate that ethanol *in vitro* reduces the rate of incorporation of amino acids into the protein of isolated normal hepatic mitochondria (507). No such depression was seen in mitochondria from rats which had received a single dose of ethanol *in vivo*, when incubated in the absence of ethanol. In contrast, chronic exposure to the drug led both to morphological alterations in hepatic mitochondria and to depressed incorporation of labeled leucine into mitochondrial protein during incubation in the absence of ethanol. This finding of decreased mitochondrial protein synthesis has been interpreted as a direct effect of ethanol on mitochondrial membrane formation. Concomitant alterations in mitochondrial respiration, succinic dehydrogenase activity, content of cytochromes and sensitivity to swelling at high K^+ concentrations suggest that chronic exposure to ethanol leads to mitochondrial injury rather than to adaptation (467). The site of injury appears localized to the inner membrane, where intramitochondrial protein synthesis is believed to occur (22, 23).

2. AMINO ACID OXIDATION, TRANSPORT AND DEAMINATION

In the isolated perfused rat liver a 60 to 70% inhibition in the oxidation of an amino acid such as leucine was noted (536) in the presence of ethanol. It was suggested that the effect may be partially related to the caloric action of ethanol, since glucose will also inhibit the oxidation of amino acids by the liver to the same extent. No further elaboration of the mechanism involved was presented.

The acute effects of ethanol on the transport of a variety of ^{14}C-labeled amino acids by everted sacs of rat small intestine have been examined by several groups of investigators. In one study (56), a concentration of 1% (217 mM) ethanol had no effect on this process, whereas 3% ethanol decreased it in all cases. Another group (550) reported that the active transport of glycine and L-proline was inhibited by ethanol, but the concentration required to bring this about was very high (4.6%); no significant decrease in the transport was produced by 0.1 M ethanol (460 mg/100 ml). In contrast, Israel *et al.* (236) reported 60% inhibition of active transport of ^{3}H-L-phenylalanine in everted jejunal sacs exposed to 0.5% ethanol (0.11 M); 2% ethanol caused 84% inhibition of phenylalanine transport and 100% with L-methionine. Studies *in vivo* by the same group showed 50% inhibition of absorption of L-phenylalanine when ethanol was administered *per os* in a dose of 2.5 g:kg body weight, though absorption of D-phenylalanine, which is not actively transported, was unaffected. An inhibition of the net uptake of ^{14}C-labeled α-aminoisobutyric acid by the isolated perfused rat liver has also been observed when ethanol was present in the perfusion fluid in concentrations as low as 50 mg/100 ml (55); only inward transport of the amino acid was found to be altered, as would be expected if active transport is involved.

The reason for these marked differences in effective inhibitor concentrations of ethanol is not apparent. However, all agree that some inhibition occurs. This action of ethanol is consistent with the fact that active transport of amino acids is in some way dependent on the existence of a sodium gradient between the cell and the extracellular medium. Inhibitors of the active transport of Na^+ that abolish this gradient are known to decrease the active transport of amino acids (552). Ethanol has been shown to inhibit both ion transport and ATPase in a variety of tissues (232–234, 238, 262). The important question is whether such inhibition occurs to a significant degree at ethanol concentrations compatible with life. The study *in vivo* by Israel *et al.* (236), together with the demonstration that appropriate concentrations of ethanol are actually found in the duodenum of human subjects for up to an hour after oral ingestion of 20% ethanol (237), indicate that it does. Temporary inhibition of amino acid uptake might explain the observation of impaired hepatic protein synthesis at relatively high ethanol concentrations (506, 507, 536), while at lower concentrations the stimulation of hepatic release of lipoproteins predominates.

The effects of a 2-week intake of ethanol on the activity of four enzymes involved in methionine metabolism have also been studied in rat liver (124). Methionine-activating enzyme and cystathionase activity were increased within 5 days of onset of treatment. Methionine and choline administration were ineffective in preventing the increase in activity of either enzyme, though puromycin and actinomycin D did so. The authors suggest that the induced increase in methionine-activating enzyme may lead to increased utilization of methionine for synthesis of S-adenosylmethionine; this would facilitate transmethylation reactions, thereby diverting the amino acid from participation in protein synthesis. This possibility will be discussed later in relation to lipid metabolism.

The inhibitory influence of ethanol on deamination of amino acids, and on

gluconeogenesis from the deaminated residues, has already been described in section III C. Though the mechanism of this effect of ethanol on amino acid metabolism is not yet fully clarified, Forsander (138) has suggested that the change in redox level of the liver may favor the formation of amino acids from keto acids by shifting the equilibrium of the NAD-dependent oxidative deamination reactions.

Studies on the effects of ethanol on the amino acid metabolism of rat brain have shown that the effect of ethanol *in vivo* could be reproduced by addition *in vitro* to stimulated slices. With fractions of brain homogenates, the ethanol effects could be seen only after ethanol had been given to the living animal (192, 195). These effects included an increase in γ-aminobutyrate (GABA), glutamic acid and aspartic acid, and a decrease in glutamine content; alanine, glycine, serine and taurine levels were unchanged, whereas keto acids were decreased. The significance of these changes in the central response to ethanol has not been elucidated. However, the fact that they are seen only in functionally intact tissue raises the possibility that they are indirect consequences of the pharmacological actions of ethanol on neuronal activity.

Various investigators have examined the effect of ethanol on the brain content of GABA and their results in turn have been varied. Ferrari and Arnold (119) found changes ranging from an 18% decrease to an 8% increase in the total brain content of GABA 1 hr after ethanol, and concluded that the drug had no significant effect. Most other investigators have also found no significant change in GABA content of whole brain after acute or chronic administration of ethanol (128, 188, 217) but one group (191–194, 196) has consistently reported an elevation in rat brain after an intoxicating dose of ethanol, and a greater release of GABA into the medium by electrically stimulated slices of rat brain cortex slices when ethanol was added. These results were attributed to an inhibition of GABA breakdown, but the effect was not considered to be a direct one (196). Gordon (172) measured GABA levels in the cerebral hemispheres and the cerebellum of rats at various times after the ingestion of ethanol. Whereas the content of the cerebral hemispheres was not significantly decreased, cerebellar levels showed a marked drop 3 hr postethanol and still had not returned to control values at 5 hr. In this experiment immediate fixation in liquid nitrogen was used to prevent rapid postmortem change in amino acid levels. GABA levels are reported to rise rapidly if the brain is not immediately frozen with liquid air; an increase of the order of 50% within a space of 2 min has been observed (356), and it appeared that this might account for some of the variation in reported results. However, Flock *et al.* (128) used the same technique and found no effect of ethanol on GABA levels. Their suggestion that the difference in results may depend on specificity of the method of measuring GABA (enzymatic *vs.* chromatographic) seems quite reasonable.

Amino acid levels in the blood have been examined in patients in various stages of acute alcoholic hepatitis and in patients with other forms of liver disease (444). Patients without liver disease showed minor or no change in blood amino acid patterns during ethanol infusion, but in those with acute alcoholic hepatitis the

ratio of non-essential to essential amino acids was increased. Low values of the branched-chain amino acids, leucine, isoleucine and valine, together with high values for glutamate were encountered. In patients with inactive cirrhosis, a normal blood amino acid pattern was found. These changes presumably reflect an alteration by ethanol of the membrane permeability of cells already affected by an inflammatory process. Therefore, they cannot be considered a primary metabolic effect of ethanol.

3. HEPATIC ENZYME LEVELS

Investigations into the effect of ethanol on a variety of enzymes in the liver have yielded a wide spectrum of results. Mention has already been made of the results of investigations of hepatic alcohol dehydrogenase during continued exposure to ethanol (see section II B 2 c) and of the complications in interpretation of the data which can arise from impairment of hepatic function. A similar situation exists when other hepatic enzymes are examined after prolonged exposure to ethanol. Hepatic levels of isocitric acid dehydrogenase are reported to be somewhat reduced in liver biopsy specimens from patients with alcoholic cirrhosis, with a concomitant elevation in its activity in the serum; yet glutamic-oxaloacetic and glutamic-pyruvic transaminase levels in plasma were increased considerably, though hepatic levels of these enzymes were within normal range (122).

A second problem of interpretation arises in connection with the finding of a significant increase in hepatic succinic dehydrogenase activity after chronic ethanol intake (158, 591). It has been suggested that this increased activity is an artifact, arising from enhanced accessibility of the substrate to the enzyme *in vitro* as a result of altered mitochondrial permeability (158). Particularly confusing are the observations (432, 433, 488) that within 10 to 60 min of the per oral or intravenous administration of ethanol, hepatic levels of glucose 6-phosphatase in normal fed rats increased significantly, whereas the activities of fructose 1,6-diphosphatase, β-glucuronidase and gulonolactone oxidase were either unchanged or decreased. Experiments *in vitro* with liver slices exposed to high concentrations of ethanol suggested that a direct chemical effect of ethanol might be implicated (432). In subsequent experiments, homogenates of livers from control and ethanol-treated animals were incubated with or without deoxycholate (DOC). DOC enhanced enzymatic activity in both preparations (433). The apparent K_m of glucose 6-phosphatase was decreased by both DOC and pretreatment with ethanol *in vivo*, and their effects appear additive up to the same maximal value. The effect of ethanol thus seems similar to that produced by surface active agents. In contrast, Isselbacher (see reference 241) reported no significant effect of acute ethanol on the activity of several hepatic microsomal enzymes, including glucose 6-phosphatase, over a 16-hr period.

These miscellaneous and apparently contradictory findings are included here only to illustrate the difficulties which exist at present in attempting to interpret the functional significance of changes in hepatic enzyme activities after administration of ethanol. Such changes may reflect direct action of ethanol on enzyme synthesis, indirect effects resulting from alterations in substrate or product pools,

or remote effects of alteration in diet or of hepatic pathology. Very little work has been done to date, to sort out these various types of change.

4. ETHANOL AND GROWTH

For a review of the experimental data available on the effect of prolonged exposure to ethanol on growth, the reader is referred to the excellent review of Wallgren and Barry (605, p. 482).

G. Ethanol and fat metabolism

1. INTRODUCTION

A large proportion of the papers on metabolic actions of ethanol which have appeared within the past few years have dealt with its effect on fat metabolism. Many investigations have been concerned with the elevation of hepatic triglyceride (TG) produced in two different ways: i) by a single large intoxicating dose of ethanol; ii) by continued ingestion of ethanol as part of the diet. Whether the fatty liver in these two conditions is produced by the same underlying mechanism or by two separate processes is still only incompletely resolved. The controversy regarding the relation between fatty liver and the development of hepatic cirrhosis is beyond the scope of this review. No definitive work has yet appeared on the relationship between steatosis, necrosis and cirrhosis. The reader is referred to the review by Scheig (523) for a discussion of the possible relationship between the metabolic alterations induced by ethanol and ethanol-related liver disease.

A variety of agents including ethanol can induce an abnormal accumulation of fat, primarily TG, in the parenchymal cells of the liver. Hepatic accumulation of TG, as many authors have pointed out (112, 239, 328, 329, 355), represents the end product of several concurrent processes, each one of which involves several variables and possible interactions. Reduced to its most simple terms, it can be considered the result of an imbalance between the rate of synthesis and the rate of utilization of hepatic TG. Some of the factors involved can themselves induce TG accumulation, while others may operate secondarily to influence the extent of accumulation, only when the primary factors are operating.

Progress in unraveling the underlying pathogenesis of hepatic lipid accumulation, and of the effect of ethanol on it, has been dependent on progress in elucidation of the basic mechanisms involved in lipid synthesis, utilization and transport. The problem is a complex one because of the multicompartmental organization of the cell, and the functional localization of different metabolic processes in different compartments. Moreover, the metabolism of ethanol itself adds a further complication, since it yields metabolites (*e.g.*, acetate) which can themselves be involved in fatty acid synthesis by liver. In any study of the incorporation of labeled precursors into hepatic TG, this may introduce a factor of isotopic dilution which must be taken into account in the experimental design.

Some recent studies have taken cognizance of the multicompartmental nature of the hepatic TG pool and have attempted to devise methods of analyzing its total turnover. Multicompartmental models have been set up (15, 43, 440, 530)

to permit computation of rates of individual steps in TG metabolism on the basis of data obtained from samples of blood and liver removed at timed intervals after administration of one or two labeled precursors. From such studies, it seems clear that hepatic TG is contained in at least two separate pools, and that these pools mix poorly, with only one of them secreting TG into the plasma (15, 114, 210, 440, 554, 628). Bustos (43) has pointed out that theoretically a fatty liver may develop if TG are preferentially directed towards the slow turnover pool. The bulk of the increase in hepatic TG after chronic ethanol intake is accounted for by the increase in the floating fat fraction of liver homogenates, rather than in the endoplasmic reticulum which gives rise to the microsomal fraction (45). Morphologically the floating fat fraction has its counterpart in free cytoplasmic lipid droplets (251) and it may represent a slow turnover compartment or, according to Havel *et al.* (210), a storage reservoir for hepatic TG.

Baker (14) has evaluated critically the methods and conclusions of the main studies on lipid metabolism which have employed a computer technique. These interesting and provocative studies underline the fact that fatty acid incorporation into hepatic and plasma TG is a complex process, with time curves containing several different rate constants.

The effects of ethanol on fat metabolism can be examined under three main headings:

a—its effect on hepatic fatty acid synthesis, a process which occurs in the cytoplasm of the parenchymal cells (597) and involves, among a variety of processes, the carboxylation of acetyl-CoA; both NADPH and NADH can act as hydrogen donors in the essentially reductive process (503, 597).

b—its effect on fatty acid esterification, a process occurring in the endoplasmic reticulum which depends on the availability of the substrates, long chain acyl-CoA derivatives and α-glycerophosphate. Both are believed to play a role in the physiological regulation of triglyceride synthesis (226, 279, 372, 582). α-Glycerophosphate can be formed from glycerol through the action of a glycerokinase or can be derived from glycolysis (553). In the reductive environment induced by ethanol metabolism it can arise from dihydroxyacetone phosphate (442, 637).

c—its effect on the intrahepatic utilization of triglyceride, which in the main takes two forms: i) incorporation into very low density lipoproteins (199, 214, 251, 380, 551) and subsequent secretion of these into the circulation (see 147 and 355), and ii) lipolysis, with subsequent oxidation of the fatty acid components in the mitochondria of the hepatocyte.

Great difficulty is encountered when one attempts to sift out, from the mass of available data, the relative significance of the effects which ethanol may have on one or more of these processes in terms of the overall picture. Slices, homogenates, organelles, perfused livers, intact animals, normal human subjects and currently abstinent alcoholics have all been subjected to experimental studies, and the conditions *in vivo* and *in vitro* are frequently not comparable. Moreover, varying sampling times have been employed after administration of the drug and these do not always reflect a similar situation with respect to the kinetic process

involved. To cite an example: after a moderate dose of ethanol, the NAD:NADH ratio in the liver may be altered for 3 to 4 hr and any metabolic derangement resulting therefrom will be in evidence during that time. In contrast, at 12 to 16 hr after the ethanol is ingested, the nucleotide ratio will have returned to normal, and adaptive changes of a secondary nature which may be found may not represent the significant metabolic alteration caused by ethanol. This situation is illustrated by the finding that the rate of fatty acid esterification was normal or increased in rats on chronic ethanol treatment, but only while ethanol was being *actively* metabolized (43, 45). When the blood ethanol level fell to zero, the rate of triglyceride synthesis was less than in control animals.

Another source of confusion is the nutritional state of the subjects. A diet high in fat tends to suppress fatty acid synthesis in the liver and a low-fat diet stimulates it (397). Under different dietary conditions, therefore, the effect of ethanol may be more or less evident. Finally, some effects on lipid metabolism may be apparent only at high doses of ethanol, sufficient to call into play a number of neural and endocrine responses which are not really specific to ethanol. All of these factors must be kept in mind during the evaluation of the experimental results.

2. EFFECT OF ETHANOL ON HEPATIC FATTY ACID SYNTHESIS

Rat liver slices *in vitro* were able to incorporate tracer quantities of ^{14}C-ethanol or ^{14}C-acetate into fatty acids with equal ease. Yet when substrate quantities were added, fatty acid synthesis from ethanol was much greater than from acetate or glucose (337, 345, 346). This suggested that the metabolism of ethanol facilitated the incorporation of 2-carbon fragments into fatty acids, a hypothesis which was confirmed by the finding that unlabeled ethanol (10 mM) stimulated the incorporation of trace amounts of labeled acetate into fatty acids in liver slices but not in adipose tissue, which contains no ADH. The stimulatory effect of ethanol on hepatic fatty acid synthesis was ascribed to the excess $NADH_2$ generated by the oxidation of ethanol. Earlier studies (470) had in fact shown that $NADH_2$ stimulates fatty acid synthesis in cell-free liver extracts. This explanation was confirmed by the finding that a second $NADH_2$-generating process, the oxidation of sorbitol to fructose, also enhanced the incorporation of labeled acetate into fatty acids, whereas addition of the H acceptor, methylene blue, decreased the stimulatory effect of ethanol. The excess $NADH_2$ formed by ethanol was assumed to alter the relative disposition of acetyl-CoA in such a way that more acetate was incorporated into fatty acids and less was oxidized *via* the tricarboxylic acid cycle. In the presence of unlabeled ethanol, liver slices produced less $^{14}CO_2$ from labeled acetate, as compared with the output in the presence of unlabeled acetate. Majchrowicz (382) repeated the experiments but concluded that when appropriate controls for isotopic dilution were added, the results indicated that ethanol suppressed rather than stimulated hepatic fatty acid synthesis. This interpretation is difficult to accept; $^{14}CO_2$ and ^{14}C-fatty acids are derived from the same precursor pool. Therefore, if the results are expressed as a ratio of ^{14}C incorporation into the two products, they are unaffected by isotope dilution.

Hepatic synthesis of fatty acid *in vivo* from acetate-1-^{14}C was found to be en-

hanced in fasted rats given a single dose of ethanol and more radioactivity was incorporated into hepatic lipids than in livers of controls given isocaloric glucose or saline (486). Pretreatment for 3 days with nicotinamide (500 mg/kg) prior to ethanol administration prevented the decrease in NAD/$NADH_2$ ratio and reduced the incorporation of labeled acetate into lipids, but the extent of hepatic lipid deposition was unaffected. These results would make it appear that enhanced fatty acid synthesis does not play a primary role in hepatic lipid accumulation.

From the results of experiments on intact rats given an acute dose of ethanol or an isocaloric amount of glucose, and on slices, homogenates and microsomal fractions obtained from the livers of such rats and incubated in a glucose medium with or without added ethanol, Scheig and Isselbacher (524, 525) concluded that *in vitro* ethanol enhances fatty acid synthesis. However, no increase in fatty acid synthesis was detected *in vivo under the conditions employed*, and ethanol appeared to act primarily on the microsomes, promoting the preferential esterification of fatty acids to TG. Little or no work seems to have been done on the effect of chronic ethanol administration on hepatic fatty acid synthesis.

3. EFFECT OF ETHANOL ON HEPATIC TRIGLYCERIDE SYNTHESIS

The *in vivo* incorporation of fatty acids and glycerol into hepatic lipids shows an increase after acute intake of ethanol, with the TG fraction accounting for most of this increment (441–443). The enhanced esterification is associated with an elevation in hepatic level of α-glycerophosphate (442, 637), one of the substrates for TG synthesis, and parallels a shift in the hepatic ratio of NAD: $NADH_2$. Studies *in vitro* with liver homogenates from rats which had ingested an intoxicating dose of ethanol showed almost twice as much incorporation of fatty acid into TG and only half as much into phospholipids as in homogenates from saline-treated controls (388, 596). Enhanced esterification *in vitro* has also been found with microsomal preparations from fasted rats given a single large dose of ethanol (525), yet addition of ethanol to the medium failed to alter the incoporation of palmitate into TG by liver homogenates or microsomes from either ethanol-fed rats or glucose-fed controls. Since substrate concentrations and cofactor ratios *in vivo* at the time of sacrifice would have little significance in reconstituted systems to which an excess of both has been added, these findings suggest an induced change in enzyme activities which was probably brought about indirectly *in vivo*, through changes in substrate levels, pituitary-adrenal activation, or other as yet unidentified means.

An increased incorporation of glycerol not only into hepatic TG but also into hepatic phospholipid, primarily phosphatidylcholine, has been reported in rats receiving an ethanol-containing liquid diet for 4 to 6 weeks (416). The turnover rate of TG was increased in the ethanol-treated animals, so that the elevation in TG content was attributed to a relatively greater increase in the rate of synthesis than in the rate of removal. Essentially the same conclusion has been reached on the basis of a similar study in which ^{14}C-palmitate was used as the tracer (43, 45). An apparent difference in results was that incorporation of labeled fatty acid into hepatic phospholipids was similar in the ethanol group and the sucrose controls;

this is not a major point, however, because Mendenhall *et al.* (416) found only a small effect of ethanol on phospholipids compared to that produced on TG. In the study by Bustos *et al.* (45), palmitate incorporation into hepatic and microsomal TG was below that of the controls when ethanol was withheld for 7 to 8 hr, but when ethanol was being actively metabolized, TG synthesis in the ethanol-rats was normal or increased. Since the turnover rate in serum FFA was decreased (see section III G 5 b), it would appear that ethanol tends to direct a larger proportion of FFA into TG formation. On this basis it was suggested that when ethanol was withheld from the chronically treated animals, the excess of TG already present in the liver might inhibit synthesis *de novo* of TG, whereas during the active metabolism of ethanol the fall in cofactor ratio would favor TG synthesis by increasing α-glycerophosphate production. During chronic ethanol intake the two effects are balanced; but upon withdrawal of ethanol the nucleotide ratio reverts rapidly to normal (265) and only the inhibitory effect would be evident.

The accumulation of reducing equivalents during ethanol metabolism tends to favor reduction of dihydroxyacetone phosphate to α-glycerophosphate (see section II A 4 c). Several workers have reported such an accumulation (442, 443, 574) in the presence of ethanol given in a single dose. Ethanol also appears to favor the reduction of triosephosphates to potential lipid precursors, rather than their condensation for the biosynthesis of hexoses (516), and this preferential reduction is also believed to reflect the more reduced state of the hepatic cytosol attendant upon oxidation of ethanol (364, 442, 516). The report that glycerol 3-phosphate of itself will stimulate synthesis *de novo* of long chain fatty acids by rat liver microsomes (226) would add further support to the idea that increased TG formation results from this change in intermediary metabolism.

We are aware of only one study published to date (44), concerning the effects of chronic administration of ethanol on α-glycerophosphate levels. These were not elevated in the livers of rats receiving an ethanol diet for 2 or 3 weeks, and long-chain acyl-CoA levels were elevated only after 3 weeks, though TG levels were definitely increased at both times. This is not inconsistent with an increased rate of production and turnover of α-glycerophosphate, but it implies that the primary factor responsible for stimulation of esterification was something other than raised levels of TG precursors.

4. EFFECT OF ETHANOL ON HEPATIC TRIGLYCERIDE UTILIZATION

Turnover of hepatic TG is dependent on a) its formation into very low density lipoproteins and release of these into the circulation, and b) intrahepatic lipolysis with subsequent oxidation, or re-esterification.

a. Formation and release of lipoproteins. Blockade of the ecretory process appears to be the mechanism underlying the steatogenic action of a variety of hepatotoxic agents: with carbon tetrachloride, ethionine, puromycin and white phosphorus, the primary defect appears to be the suppression of synthesis of the protein moiety of the very low density ($d < 1.019$) lipoproteins (VLDL), with a resultant decrease in lipid transport to plasma (74, 113, 354, 499, 500,

530, 532, 533, 583). The fatty liver resulting from ingestion of a choline-deficient diet seems to be due primarily to a deficiency in the synthesis of the phospholipid component of the lipoprotein complex (355).

The majority of available reports concerning ethanol suggest that it differs from both carbon tetrachloride and choline deficiency in its effects on TG secretion from the liver. Although ethanol does not inhibit the synthesis of the protein moiety of lipoproteins in the liver, as noted in section III F 1, a few reports have indicated a depression of lipoprotein secretion. The VLDL content of rat serum, as well as the levels of its protein and lipid components, was depressed at 4 hr after a large dose (6 g/kg) of ethanol (379). A high concentration (350 mg/100 ml) of ethanol added to the fluid perfusing the isolated rat liver was also reported to decrease the rate and extent of incorporation of ^{14}C-palmitate into the TG of the perfusate, while increasing the incorporation into the hepatic TG (521). One study with isolated liver slices from ethanol-fed rats found no evidence of impaired incorporation *in vitro* of ^{14}C-leucine into hepatic lipoproteins, but a disturbed release of high-density lipoproteins (299); the authors suggested that these may play a role in the rat analogous to that of VLDL in human lipid metabolism, but this has not yet been corroborated.

Most other studies, in contrast to these, have indicated either no impairment of lipoprotein release by ethanol, or even an increase. Seakins and Robinson (533) found no interference by ethanol with the incorporation of ^{14}C-leucine or ^{32}P-orthophosphate into the protein and phospholipid components of plasma lipoproteins at various times up to 16 hr after administration. Instead, they found a significant *increase* in the incorporation of ^{32}P into the liver and plasma phospholipids in the ethanol-treated group 16 hr after the ethanol was administered. They interpreted this as part of the recovery phase concerned with increased removal of excess TG from the liver. Others have reported the finding of increased incorporation of ^{14}C-palmitate into plasma TG in rats fed an ethanol-containing diet for 3 weeks (43, 45). Low concentrations of ethanol (60–200 mg/100 ml) in the perfusion fluid also increased the rate of release of newly-formed TG by the isolated perfused rat liver (174). The increase in plasma TG by ethanol in all these studies cannot be attributed to impaired peripheral utilization, because it occurred in isolated liver preparations, and also in intact animals in which peripheral utilization was blocked by the use of Triton WR-1339, an inhibitor of lipoprotein lipase (17).

The apparent discrepancy between the results of all these studies and of those mentioned earlier can probably be explained by two points of technique. Inhibition of lipoprotein release was seen only with large doses or high concentrations of ethanol, which may have produced a non-specific depression of many membrane functions, including secretory processes. Many membrane effects of ethanol show a biphasic relation to concentration, with stimulation at low concentrations and inhibition at high (259, 604). The second point is that results obtained with the perfused isolated liver may sometimes give a distorted picture of the metabolic changes occurring *in vivo*. As Forney and Harger (129) have pointed out, ketone levels in the intact animal would be much lower because of their oxidation

in muscle tissue, and acetate formed from ethanol in liver would also normally be oxidized in the periphery. The lack of this compensating factor might well result in a greater disturbance within the isolated liver than would occur at the same ethanol level *in vivo*.

It is worth noting, however, that the increased rate of release of lipoproteins from the liver under the effect of ethanol does not appear to keep pace with the increased synthesis (45, 416, 533), so that hepatic TG accumulation occurs. One possible explanation is that the secretory mechanism is working at maximum capacity (504). An alternative explanation arises from the concept, based on computer models for kinetic analysis of lipid metabolism (15, 43, 440, 530), that total hepatic TG is distributed among at least two pools with very different turnover rates. This is borne out by kinetic studies of the effects of ethanol on lipid turnover in the isolated perfused liver (174) and in the intact animal (45). Newly-formed TG appears rapidly in the perfusate or plasma, while previously formed TG is secreted relatively slowly. The slow turnover fraction, which accounts for most of the TG in the alcoholic fatty liver (45), is the fraction which floats to the top of a liver homogenate and is the fraction which is probably derived from free lipid droplets in the cell cytoplasm (199). Bustos (43) has proposed that ethanol stimulates esterification in the endoplasmic reticulum, as well as the migration of membrane-enclosed lipoprotein droplets from this to the cell surface where the contents are released by exocytosis. During the migration, he suggests, a certain proportion of the droplets pass into the cytoplasm to form the slow turnover pool. In the alcohol-free situation, this pool is kept to a small size by intrahepatic utilization, but in the presence of ethanol the lipid accumulates because utilization is impaired, as discussed in the next section. If this hypothesis is correct, the increase in endoplasmic reticulum in subjects given ethanol chronically might represent a response to the stimulation of TG release by ethanol. The increase in cytochrome P-450, MEOS activity, and drug metabolism *in vitro* noted in section II A 2 b might then be seen as incidental consequences of the effect on lipid metabolism.

b. Intrahepatic oxidation of fatty acids. It has been shown repeatedly, both *in vivo* and *in vitro*, that ethanol decreases the rate of fatty acid oxidation by liver, and of utilization of chylomicron fatty acids (86, 281, 345, 347, 416, 465, 486). Studies of acute ethanol intoxication in rats indicated that fatty acid uptake by the liver proceeded at a normal rate, but little of that which had been taken up was oxidized (120). In the perfused liver, addition of ethanol was followed by a block in CO_2 production from both long chain (C_{16}) and medium chain (C_8) fatty acids (639); but whereas the C_{16}-fatty acids caused TG accumulation, the C_8-fatty acids, which are poorly esterified under normal circumstances, not only did not induce TG accumulation, but did not accumulate themselves. This finding suggested that entry of the C_8-fatty acids into the *beta*-oxidation scheme was not blocked, even though production of CO_2 is inhibited at a later stage of the pathway (see section III D). It was therefore postulated (639) that depressed oxidation was the result rather than the cause of a shunt to esterification. However, as noted earlier, in the same type of preparation ethanol suppressed *beta*-oxidation

of both endogenous and exogenous long chain fatty acids, by competition at the level of the flavin enzymes in the respiratory chain (626). It seems likely, therefore, that ethanol depresses fatty acid oxidation and increases esterification by independent effects at two different sites of action within the cell.

The suppression of *beta*-oxidation of fatty acids has generally been explained in terms of the increased supply of reducing equivalents originating in the cytoplasm when ethanol is oxidized, and translocated into the intramitochondrial environment for disposal by the electron transport chain (see section II A 4 c). This large influx of H-equivalents appears to supplant the citric acid cycle as the source of H-equivalents for the flavoprotein-cytochrome electron transport chain, and, in so doing, suppresses fatty acid oxidation. While this may be true during the time ethanol is being actively metabolized, it does not explain results obtained after ethanol is withdrawn and cofactor ratios have returned to normal (265). Alterations in mitochondrial morphology and permeability (see section III G 5 e) offer a more likely basis for the depressed utilization observed under these conditions.

5. MISCELLANEOUS EFFECTS OF ETHANOL ON FAT METABOLISM

a. Mobilization of depot fat. An additional complicating effect of ethanol on fat metabolism may be introduced when the dose administered is sufficiently large to induce intoxication. The fatty liver observed under these conditions (84, 390) occurs only in the presence of adrenocortical and pituitary hormones. This suggests that the TG accumulation may be a non-specific result of a stress reaction to intoxication, rather than a specific action of the drug itself. The concomitant rise in plasma FFA (389) is also consistent with enhanced mobilization from the depots. This view is further strengthened by the fact that under these conditions the hepatic TG contain a proportionately larger amount of linoleic acid, a fatty acid not synthesized in the body (225, 347, 387). One series of studies on fatty acid mobilization from fat pads after a large dose of ethanol (107, 108, 465) showed that although depot fat was indeed the source of the fatty acid components in hepatic TG, mobilization from these depots was not enhanced by ethanol. However, another study with similar techniques (281) failed to corroborate this finding.

Activation of adipose tissue lipase in the epididymal fat pads, leading to increased fatty acid output from these depots, occurred after acute administration of ethanol to rats with an intact sympathetic nervous system (40). Pretreatment with sympathetic blocking agents or "chemical sympathectomy" (adrenodemedullation plus treatment with reserpine) prevented the acute ethanol-induced hepatic steatosis. Hence, it would seem that activation of the pituitary-adrenal axis triggers fatty acid mobilization and initiates a chain of events leading to a fatty liver. Single large doses of ethanol have been shown to cause release of catecholamines and corticosteroids (109, 261, 456). Indirect evidence of several types is also consistent with the view that the acute model of alcohol-induced fatty liver results in part from increased mobilization as distinct from impaired intrahepatic utilization. The first is that small doses of ethanol do not appear to cause

TG accumulation even though they are still large enough to disturb the hepatic cofactor ratio (265). The second is that after large doses of ethanol the degree of accumulation is related to the dose administered (386), even though the ethanol-oxidizing system is saturated (section II A 4). Finally, administration of pyrazole in a dose large enough to suppress ethanol oxidation did not prevent TG accumulation (46). A similar result was obtained when ethanol metabolism was markedly reduced by a different method, the feeding of a protein-deficient diet (31a). Despite a clear decrease in other metabolic consequences of ethanol metabolism, the accumulation of hepatic TG after a single large dose of ethanol was unchanged. Unfortunately the interpretation of this finding is complicated by the possible effect of dietary protein deficiency on the formation or secretion of lipoproteins by the liver.

Two other studies have yielded results which conflict with these: pyrazole (425) and 4-methylpyrazole (30) were both reported to block completely the accumulation of hepatic TG after a single large dose of ethanol. However, one of these studies (30) had no controls without ethanol, so that the effect of 4-methylpyrazole alone on hepatic TG could not be judged; this is important because other pyrazole derivatives are evidently able to inhibit mobilization of fatty acids from adipose tissue (27a, 50a).

In the other study (425) all the hepatic TG levels were unusually low and the blood ethanol levels were much lower than would be predicted from the dose administered, perhaps because the use of 40% ethanol solution often leads to delay in gastric emptying and slow absorption (261). This would also alter the balance between the adrenergic response to intoxication and the possible antimobilization effect of pyrazole.

A recent report by Johnson *et al.* (250a) provides support for the hypothesis that two different actions may be involved in the production of the acute fatty liver. Pretreatment with pyrazole did prevent the elevation of hepatic TG observed 6 hr after the administration of a large dose of ethanol but did not prevent the increase seen at 16 hr.

The weight of available evidence indicates that the acute alcoholic fatty liver is at least partly an indirect metabolic byproduct of intoxication, and that it is not identical in mechanism with the TG accumulation produced by chronic ingestion of ethanol. The latter does not appear to be accompanied by increased mobilization of fatty acids from depot fat (43, 45, 347).

b. Ethanol and plasma free fatty acids. Plasma FFA levels can be altered in two different directions by ethanol, depending on the dose. When a highly intoxicating dose is given to fasted rats, plasma FFA increases (389); as noted above, depot fat mobilization occurs and activation of the pituitary-adrenal axis is involved (see section III G 5 a for further discussion). In chronic alcoholics who ingested ethanol in increasing amounts, no increase in plasma FFA occurred while plasma ethanol levels were moderate (200 mg/100 ml), but when levels of 400 mg/100 ml were achieved, plasma fatty acid levels were elevated and gas chromatographic examination indicated a composition consistent with origin in adipose tissue (522).

In contrast, human subjects infused with a low dose of ethanol exhibited an initial *fall* in plasma fatty acids, followed by a slow return to the original baseline (340). These results suggest either a decreased release of fatty acids from adipose tissue or an increased uptake by muscle or other tissues. Later experiments demonstrated that acetate derived from ethanol was directly responsible for the reduction in fatty acid level (69). The reduction in plasma levels of FFA was accompanied by a decreased turnover rate (252), which is more consistent with decreased mobilization than with increased peripheral utilization. In another study carried out in man, but with a longer sampling time after ethanol administration, the initial fall in FFA was followed by a subsequent overshooting of the baseline. The former was intensified and the latter blocked by nethalide, a β-adrenergic blocking agent (38).

These studies indicate that the net effect of ethanol on plasma FFA depends upon a balance of two indirect effects: reduction of mobilization by the acetate formed as a result of ethanol metabolism, and increased mobilization as a result of an adrenergic response to intoxication. The intensity of these two effects, and their order of appearance, will obviously depend upon the dose and the route of ethanol administration. In the last experiment mentioned (38), peripheral mobilization of fatty acids was introduced later and tended to mask the initial lowering effect on plasma FFA levels. With chronic administration of ethanol to rats by means of a liquid diet, the second phase does not appear to occur. Under these conditions, any change in FFA turnover was in the direction of reduced flux (43, 45).

c. Ketogenesis and ethanol. Both in rats and in abstinent alcoholics, chronic ethanol intake leads to an increase in blood ketone levels in both the fed and fasting state (317). The fat content of the diet influences markedly the alcohol effect. In human subjects receiving 46% of their calories from ethanol, definite ketosis was seen when dietary fat provided 36% of calories but not when fat contributed only 5% (317). Liver slices from ethanol-fed rats produced significantly more acetoacetate than did slices from carbohydrate-fed controls, and the conversion of palmitate to acetoacetate was greater in the ethanol-fed animals in both the fed and fasting state. The elevation in blood ketone levels by ethanol did not involve any change in acetoacetate utilization, though production of $^{14}CO_2$ from labeled β-hydroxybutyrate by the isolated rat diaphragm was somewhat reduced (317). Neither lack of carbohydrate nor elevated acetate levels seemed to be implicated in the ethanol effect. These results were essentially in agreement with those obtained by surface fluorimetry studies in the perfused rat liver (626), which showed that ethanol stimulated ketogenesis despite a 37% inhibition of *beta*-oxidation of oleate. The data support the concept that ketogenesis increased as a function of acetyl-CoA concentration (which is elevated by reduced entry of acetyl-CoA into the tricarboxylic acid cycle) and decreased as a function of free CoA level. Ketogenesis during ethanol oxidation was enhanced only when the ratio of acetyl-CoA:CoA increased, *i.e.*, in the presence of exogenous fatty acids.

In contrast to these results, studies of hepatic arteriovenous differences in

ketone body concentrations in man revealed no effect of ethanol in 9 out of 10 subjects (365). In juvenile diabetics deprived of insulin and showing incipient ketoacidosis, ethanol slowed or stopped the rise in ketonemia (12), though it increased the ratio of β-hydroxybutyrate to acetoacetate. The changes were not parallel to changes in plasma FFA levels.

These discrepancies, together with the observed lag time between the administration of ethanol and the increase in ketone body production (317), indicate that the effect of ethanol on ketogenesis is indirect. It probably reflects a balance between the actions of ethanol on acetate formation, mitochondrial oxidation, and gluconeogenesis, and is therefore undoubtedly affected by the previous nutritional state of the subject.

d. Glycerol metabolism. In 1946, Lundsgaard (368) reported that after the ingestion of glycerol by human subjects the rate of its disappearance from the blood was markedly decreased by administration of ethanol. This observation might be explained either by decreased removal by liver and possibly other organs, or by an increased release of glycerol into the blood stream. As a result of adrenomedullary stimulation which would lead to activation of adipose tissue lipase (381), glycerol and FFA would be released into the circulation from the fatty depots.

The first explanation is supported by the observation of a consistent reduction in the splanchnic consumption of glycerol when ethanol is infused into man (364). This decrease in glycerol uptake by liver has also been noted *in vitro* when ethanol is added (484, 573). It has been suggested that the reduced utilization is due to an inhibition of glycerokinase induced by the elevation in glycerophosphate levels during ethanol metabolism (see section II A 4 c). In addition, the concentration of adenosine monophosphate (AMP), a known inhibitor of the glycerokinase reaction (184, 501), is increased in the tissue. Rawat (482) has examined glycerol utilization by liver slices *in vitro* under different hormonal conditions in which AMP and α-glycerophosphate levels are known to be altered, and has confirmed that an increase in either of them results in an inhibition of glycerol utilization.

The second explanation does not appear to be valid, at least *in vivo*. Plasma levels of FFA and glycerol fall simultaneously in man during the first 0.5 hr after oral ethanol (116). In view of the decreased hepatic clearance of glycerol during ethanol infusion (364), these changes could only result from a decreased release of glycerol into the blood, presumably as a result of a depression of lipolysis in adipose tissue either by a metabolite from ethanol (perhaps acetate) or a metabolic alteration resulting from oxidation of the drug locally, which does not seem to occur.

e. Action on membranes. Aside from its action on the specific processes listed above, there is evidence that ethanol may exert direct or "toxic" effects on subcellular functions which may lead to the production of hepatic steatosis. This may take the form of a physicochemical action on membranes of the cell surface, mitochondria or the endoplasmic reticulum. Morphological alterations and altered fragility in mitochondria after chronic ethanol intake have been well

documented (155, 158, 230, 468, 511), but there seems to be no *in vitro* evidence available that concentrations of ethanol which would result from amounts voluntarily ingested can cause such effects. Altered oxidative capacity (59, 289, 608) of isolated mitochondria has been reported to occur in the presence of ethanol, but in some of these studies the concentrations used were very high. The significance of these biochemical and morphological changes in the production of hepatic steatosis has yet to be determined. It is of interest that the mitochondrial structural changes appear later than the steatosis in rats (230). This suggests that the changes are not related to the onset of TG accumulation; however, despite normal morphology, permeability of the mitochondria might already be altered in the early stages.

f. Peroxidative effects. In the case of hepatic steatosis resulting from acute administration of ethanol, DiLuzio and his colleagues (85–91, 268) have amassed considerable evidence of mitochondrial peroxidation which they ascribe to free-radical attacks on the lipoprotein membrane. The ability of a variety of antioxidants (coenzyme Q, α-tocopherol, N,N′-diphenyl-*p*-phenylenediamine) to ameliorate or prevent the steatosis is attributed to their ability to prevent lipoperoxide formation. The toxic effect of acute ethanol is envisaged as a diminution in the antioxidant levels of the liver cell. However, this hypothesis has not received universal support (206). Lipoperoxidation could equally well cause inhibition of enzymes concerned with TG hydrolysis (584). Antioxidants have proved only partially effective or of little value in preventing or decreasing the lipid accumulation from chronic ethanol intake (89, 91, 331), though problems of solubility and absorption could be involved.

g. Hepatotoxicity versus nutritional imbalance. A controversy has raged for years regarding the role of nutritional imbalance in the hepatic steatosis resulting from chronic intake of ethanol. One view is that hepatic steatosis is primarily the result of disturbances in intermediary metabolism resulting from the oxidation of ethanol; another is that it is produced by nutritional imbalance brought about by inadequacy of the dietary protein or lipotropic factors relative to the caloric contribution of the ethanol. It is beyond the scope of this review to evaluate critically the voluminous literature dealing with this subject. The reader is referred to reviews by Lieber (329), Rubin and Lieber (511), Hartroft and Porta (205) and Scheig (523) for the evidence favoring one side or another in this argument.

In the light of recent findings these may not be the only two alternatives. A third possibility which must be considered is a direct hepatotoxic effect of ethanol *per se*. From experiments with rats receiving chronic ethanol treatment plus pyrazole, Lelbach (319) concluded that blockade of the indirect metabolic effects of ethanol was unable to prevent the hepatic changes seen with chronic ethanol alone. These experiments do not, however, exclude the possibility that chronic pyrazole itself may exert a hepatotoxic action; some studies have indicated such an effect (31, 344). As noted earlier, pyrazole has been reported to have no effect on the development of the acute fatty liver 16 hr after a single dose of ethanol (46, 250a), which appears to result primarily from increased mobilization of

FFA. Recent studies have shown that in rats on a chronic ethanol diet the addition of pyrazole in a dose which reduced ethanol oxidation by about 40% caused a large increase in hepatic TG accumulation, whereas pyrazole itself caused no change or even a decrease (264). These are only initial exploratory studies; much more work is required before the hypothesis of ethanol hepatotoxicity can be evaluated.

6. SUMMARY

i. Acute ethanol intoxication results in an enhanced mobilization of adipose tissue lipids and consequent elevation in plasma FFA levels. Preferential esterification of FFA into hepatic TG ensues. Whether this is the result of depressed intrahepatic utilization of fatty acids or its cause is still undecided.

ii. With chronic ethanol intake, the observed alterations in fat metabolism will depend on whether or not the sampling time(s) involve the period when ethanol is being actively metabolized.

During this period, a larger than normal proportion of fatty acid is directed towards TG formation, primarily in the floating fat fraction; this distribution suggests that much of the newly formed TG is deposited in the cytoplasm as lipid droplets.

During intervals when ethanol is absent, intrahepatic TG utilization is decreased, and this decrease must be explained in terms of transesterification, hydrolysis or oxidation of TG fatty acids. A depression in fatty acid oxidation is well documented, but an action on the other two processes has not yet been excluded. There seems to be no concrete evidence that the formation or secretion of hepatic TG into the circulation as lipoprotein is reduced *in vivo*; there is even some evidence that a larger than normal proportion of microsomal TG, its precursor, is disposed of by this route.

H. Ethanol and intermediary metabolism in kidney and heart

Although the initial stages of ethanol metabolism take place mainly in the liver, the final steps in its degradation occur primarily in the periphery. From an examination of ethanol metabolism in the human subject with catheterized hepatic vein, it was calculated that approximately 65% of the ethanol removed by the liver appears as acetate in the venous outflow (365); in cirrhotic patients with portocaval anastomoses, the proportion rose to 85 to 90% (630). It would, therefore, be expected that oxidation of this acetate in the periphery would cause alterations in the metabolic patterns of extrahepatic tissues. Moreover, the rise in blood lactate which may accompany ethanol oxidation also places a demand on the periphery, if homeostasis is to be maintained. Very little research has been devoted to this aspect of the metabolic effects of either acute or chronic administration of ethanol, yet it is one which merits investigation. Kidney and heart have received some attention, but even here information is meager.

1. KIDNEY

In 1938, results of two studies (320, 370) showed that ethanol could be oxidized by the kidney. Later it was calculated that approximately 9% of the over-

all elimination of ethanol occurred in this organ (313). The ADH and acetaldehyde dehydrogenase activities of rat liver and kidney homogenates have been compared and it has been calculated that 3 to 4 % of the total oxidation of alcohol and acetaldehyde could be brought about by the kidney (47).

A decrease in the NAD^+:NADH levels of rat kidney 4 hr after the oral ingestion of varying doses of ethanol has been reported (58). At first sight, this might seem to be a natural consequence of renal ADH activity. However, ingestion of sodium lactate likewise depressed the cofactor ratio, and an increase in blood lactate was observed after a dose of ethanol as low as 2 g/kg. The change in renal nucleotide ratio was related to the dose of ethanol over a wide range; therefore it seems unlikely to be the result of activity of renal alcohol and acetaldehyde dehydrogenases. It is much more likely to have been caused by a combination of hypoxia due to respiratory depression, and oxidation of lactate brought to the kidney. Chronic ethanol intake, at a rate which did not produce deep intoxication, caused no significant difference in the renal levels of the reduced or oxidized forms of NAD and NADP, or in their sum or ratio (265).

Renal TG levels were found to be increased after a single large dose of ethanol (386), and histological examination indicated fine droplets of lipid at the base of the epithelium of the convoluted tubules. The renal changes are comparable in severity to those seen in the liver and are similarly blocked b autonomic blocking agents, suggesting that the TG are derived from fatty acids mobilized from fat depots under the influence of adrenergic stimulation. No studies appear to have been done on renal TG levels after acute administration of ethanol in doses which do not trigger a sympathoadrenal response. After chronic ethanol intake, renal TG levels are greatly enhanced (347a); dietary provision of TG containing fatty acids of medium chain length, rather than the usual long chain fatty acids, reduced the extent of TG accumulation in the kidney just as it did in liver. Unlike the liver, however, the kidney of the adult rat does not show enhancement of TG accumulation by deficiency of dietary protein or lipotropic factors.

Thus, two mechanisms could account for the changes in intrarenal TG metabolism induced by ethanol. One involves the possible changes brought about by the intrarenal metabolism of acetate or of ethanol itself; the other, the elevated levels of lipoproteins released from the liver as a result of intake of ethanol, and their subsequent uptake by the kidney from the circulation. Both possibilities warrant investigation.

Few studies have been carried out on the effect of acute or chronic ethanol administration on renal tubular transport mechanisms. As noted earlier, there is ample evidence for an effect of the drug on active transport of amino acids in the small intestine (236, 237) and on electrolyte transport in the central nervous system (231, 258, 605). Studies *in vitro* with renal cortex slices have indicated that the inhibitory effect of ethanol on electrolyte transport is not confined to the central nervous system (238). There is a need for additional studies on the effect of ethanol on the uptake not only of amino acids but of a variety of other metabolites which are reabsorbed by active transport mechanisms within the renal tubules.

2. HEART

Any participation of cardiac muscle in the initial stages of ethanol metabolism appears to be minimal and no significant activity of ADH has been detected in this organ (58). It would therefore appear that any alteration in the metabolic pattern of cardiac muscle results from either a direct effect of ethanol on myocardial membrane permeability or work load, or an indirect effect *via* metabolites carried in the circulation from the sites of ethanol metabolism. The NAD^+ level in the heart was generally decreased, that of NADH always increased, and the NAD^+:NADH ratio decreased, in rats receiving varying doses of ethanol (58, 115). The change in cofactor ratio was attributed to increased utilization of metabolites with reducing potential (58). Though lactate is one such compound, no effect of oral lactate on the cofactor ratio in the heart was noted at the time investigated. Possible alterations in myocardial metabolism induced by the slow infusion of ethanol have been studied in intact dogs (490, 491). At plasma ethanol levels of 150 to 215 mg/100 ml, a progressive reduction in the uptake of FFA was noted, while TG extraction, minimal in the control period, increased progressively and appeared to contribute to the TG accumulation in the myocardium. Lactate extraction was not significantly affected. Others have also noted a significant increase in the glyceride content of ventricular muscle of dogs on a chronic intake of ethanol, but no significant changes in the enzyme profile of the myocardium were observed (185).

The effect of ethanol on the myocardial utilization of acetate, lactate and FFA in man has been examined in the postabsorptive state (351). From calculations of the oxygen extraction ratio for each substrate metabolized, it was found that ethanol infusion raised the blood levels and cardiac uptakes of lactate and acetate 2- to 5-fold while the oxygen extraction ratio for FFA decreased by almost half. Essentially similar findings with respect to lactate uptake and fatty acid utilization have been obtained in dogs with controlled respiration which were infused with a moderate dose of ethanol (1.5 g/kg) (585). However, in this study oxygen utilization was less than would be required for the total aerobic metabolism of all substrates examined and the authors suggest than ethanol may interfere with aerobic metabolism. Thus, as blood lactate and acetate concentrations increase the myocardium appears to use proportionately more of them and less of FFA. This is reminiscent of the reduction by ethanol of the utilization of FFA in the liver, despite the fact that no appreciable oxidation of ethanol occurs in the heart. It is possible, therefore, that ethanol may exert a direct pharmacological effect on mitochondrial oxidation of fatty acids. In this connection, it is noteworthy that carnitine was reported to produce less stimulation of palmitate oxidation *in vitro* with liver mitochondria from ethanol-fed rats than from controls (276).

Uptake of labeled palmitate by the isolated perfused rat heart was unchanged in the presence of 100 to 200 mM ethanol, but with 200 mM the percent incorporated into cardiac lipids was increased, and lactate production and $^{14}CO_2$ formation were depressed, though pyruvate oxidation was not affected. Hearts from rats maintained on a chronic intake of ethanol showed an uptake of labeled

palmitate similar to that of control animals but, again, recovery of label in tissue lipids was significantly higher (351a). Since no indirect metabolic effects of ethanol can occur in the isolated organ, it would appear that the changes seen in the presence of ethanol must be due to a direct drug action. However, the concentrations of ethanol required to exert the above effects are much higher than those encountered *in vivo*, and their significance is difficult to evaluate.

Participation of the sympathoadrenal system in the changes in peripheral metabolism induced by acute ethanol intoxication is suggested by the report that cardiac lipoprotein lipase levels are elevated 3 to 4 hr after an intoxicating dose of ethanol, or after catecholamine injections (391). This action of ethanol is considered to be indirect; intoxication probably promotes release of endogenous catecholamines, and the latter in turn enhance the lipoprotein lipase activity in the heart.

Chronic ethanol ingestion may alter the susceptibility of the myocardium to damage by various drugs (98, 99). Cobalt has been considered to have a low myocardial toxicity, but in combination with inadequate food intake and large amounts of ethanol, it may cause serious myocardial derangements. High levels of cobalt have been demonstrated in hearts obtained at autopsy from patients dying of "beer-drinker's heart" (559).

To summarize: Ethanol causes a shift in the pattern of cardiac metabolism and a significant elevation in cardiac TG levels. Since intracardiac oxidation of ethanol is insignificant, these changes must be ascribed to one of two mechanisms: a *direct* effect of ethanol on cell activity or membrane function, leading to an alteration in cardiac intermediary metabolism in response to an increased work load, or an *indirect* metabolic action resulting from alterations in the level of hormones or circulating metabolic substrates presented to the heart. The latter could arise as a consequence of metabolic modifications originating in the liver or as a result of endocrine release. At this point it is impossible to decide which mechanism applies in the case of each of the metabolic changes noted.

I. Ethanol and metabolism of biogenic amines

Numerous publications within the last decade have indicated a correlation between changes in the levels or metabolism of biogenic amines (noradrenaline, dopamine, 5-hydroxytryptamine) in the central nervous system and changes in mood or behavior elicited by various psychotropic drugs. Ethanol, as a well known mood modifier, has also received its share of attention. The current views on the effect of ethanol on the metabolism of biogenic amines can be found in several excellent reviews (79, 137, 231).

1. ETHANOL AND SEROTONIN (5-HT) METABOLISM

Alcoholics have been reported to display not only subnormal basal excretion of 5-hydroxyindoleacetic acid (5-HIAA) but also a deficient response to a test load of tryptophane (446).The question naturally arose as to whether these changes represented a metabolic anomaly preceding the alcoholism, a metabolic effect of ethanol or a consequence of nutritional disturbance associated with alcoholism. Later, chronic alcoholics who were abstinent at least 10 days were

found to show no difference in the 24-hr urine output of 5-HIAA, as compared with normal subjects (428). Subsequent investigations have attempted to resolve this conflict by studying the acute effects of ethanol in healthy subjects.

In one study in healthy young men, no alteration was found in the urinary excretion of 5-HIAA over a 3-hr period after the ingestion of moderate doses of ethanol (457). However, most investigators have found definite alterations, both *in vivo* and *in vitro*. In mice pretreated with a single injection of ethanol, the rate of metabolism of exogenous 5-hydroxytryptophane, as indicated by the formation of 5-HIAA, was appreciably depressed, possibly by some competitive mechanism operating at the aldehyde stage of the oxidative metabolism of the amine (505). In normal men, when ^{14}C-5-HT was administered 1, 2 or 4 hr after a dose of ethanol the total recovery of ^{14}C in the urine was not altered, but only 30 to 34% of the injected dose was recovered as ^{14}C-5-HIAA compared to 76% in the absence of ethanol. When the ^{14}C-5-HT was given 24 hr after the ethanol, the urinary excretion of ^{14}C-5-HIAA was again normal (117). It was suggested that the decreased 5-HIAA excretion could occur either through competition between acetaldehyde and 5-HT aldehyde (resulting from the action of monoamine oxidase on 5-HT) for the common enzyme, aldehyde dehydrogenase, or through depletion of NAD^+ stores, as a result of the oxidation of ethanol.

Aldehyde dehydrogenase is present in liver, brain and other tissues. It is noteworthy that monoamine oxidase (MAO) is also present in all areas of the brain and is also located in the mitochondria (33). Aldehydes produced by oxidation of biologically active amines, including 5-hydroxyindole-3-acetaldehyde, can serve as substrates for acetaldehyde dehydrogenase (83, 110). Acetaldehyde has been found in brain tissue after ethanol administration (288), and an accumulation of it might successfully compete with the amine derivatives for the active site of the enzyme, thereby blocking their metabolism locally (110, 607). Some support for this idea is provided by the finding that addition of acetaldehyde to liver slices *in vitro* depressed 5-HIAA formation and resulted in an accumulation of 5-hydroxyindole-acetaldehyde (309). Increased availability of NAD^+ failed to overcome this inhibition. Thus it appears that the effect of ethanol on 5-HT metabolism is mediated primarily *via* acetaldehyde, with competition for acetaldehyde dehydrogenase being the primary mechanism involved.

Metabolism of ^{14}C-labeled 5-HT by rat liver homogenates has been studied and the metabolites identified chromatographically (118). In the presence of added NAD^+, 80% of the 5-HT appeared as 5-HIAA, and less than 10% as 5-hydroxytryptophol (5-HTOH). When NADH was added instead of NAD^+, the formation of 5-HIAA was reduced and the conversion of 5-hydroxy-indole-acetaldehyde to 5-HTOH by ADH was enhanced, accounting for 51% of the amine metabolized (118). Results fully compatible with this action of ethanol have also been demonstrated in man (76, 78). Thus, under normal conditions where NAD^+ availability is high, formation of 5-HTOH may not be important, but when a reduction in NAD^+/NADH ratio occurs, this pathway of 5-HT metabolism may be significant.

Later studies with ^{14}C-5-HT in man showed a very similar effect of ethanol on the distribution of metabolites in the urine. The total excretion of 5-HIAA

plus 5-HTOH was unchanged, but the proportion of ^{14}C excreted as 5-HTOH increased from 2% in controls to 42% after ethanol, while the proportion as 5-HIAA fell from 82% in the control series to 42% in the presence of ethanol (76). Thus, under the influence of ethanol, 5-HT metabolism was not inhibited but was diverted to a reductive pathway. Very similar results have been reported with liver slices incubated with 5-HT and increasing concentrations of ethanol in the range of 10^{-5} to 10^{-1} M (97). With increasing concentrations of ethanol there would be increasing shift in the NAD^+:NADH ratio until the maximum rate of ADH activity was reached.

The evidence concerning brain, however, has been much more ambiguous. A single dose (2 g/kg) of ethanol was reported to deplete the 5-HT content of the rabbit brainstem by 50% (187), but several other reports indicated either no change (94, 102, 189, 472, 579) or even an elevation (35) of 5-HT level after the same or larger doses of ethanol. Since the level at any given time reflects the balance between synthesis and release, the resolution of this discrepancy required a separate examination of the effects of ethanol on these processes. When ^{14}C-5-HT was injected into the lateral ventricle of the rat brain (79), ethanol administration was followed by a small but significant increase in the percentage of ^{14}C appearing in 5-HTOH and 5-hydroxyacetaldehyde. This could reflect the influence of acetaldehyde, either produced in the brain by the action of the small amount of ADH which it contains (478) or carried to the brain from the liver. Unfortunately the procedure used does not exclude the possibility that some of the labeled 5-HT was leaving the brain and being metabolized in the liver. When ethanol was added to rat brain slices *in vitro*, changes in 5-HTOH and 5-hydroxyacetaldehyde formation were of doubtful significance and the formation of 5-HIAA was unaltered, but the percentage of total 5-HT metabolized decreased with increasing concentrations of ethanol (97). A similar lack of effect of ethanol was noted *in vivo* when ^{14}C-5-HT was injected directly into the caudate nucleus by micropipette, in both hepatectomized and intact animals (578). The changes in excretion patterns of 5-HT metabolites caused by ethanol (76) therefore appeared to be due primarily to metabolic effects in the liver.

In another study, the turnover of 5-HT and its metabolites in the rat brain was studied by measuring the rates of accumulation of 5-HT and of disappearance of 5-HIAA after administration of pargyline, a MAO inhibitor (579). Ethanol was found to cause a small but significant decrease in both of these rates, compatible with the reduction in 5-HT metabolism which it produced in isolated brain slices (97). This might be a non-specific effect, due to depression of all neuronal activity, or a more specific effect related to central metabolic actions of ethanol or acetaldehyde. Further experiments will be required to clarify the matter.

2. ETHANOL AND CATECHOLAMINE METABOLISM

Reports of changes in the pattern of adrenaline* and noradrenaline† excretion after the ingestion of ethanol at various dose levels have appeared frequently.

* NE = epinephrine, adrenaline.
† NE = norepinephrine, noradrenaline.

Such changes are difficult to interpret. An increased output could represent stimulation of adrenal medullary secretion or release from binding sites, but it could also involve decreased uptake of the amine by the adrenal medullary granules, alteration in the metabolism of the amines once released from storage granules, or differences in renal clearance. These possibilities require exploration before conclusions can be drawn regarding the effect of ethanol on catecholamines in the periphery. The array of conflicting reports on the effect of ethanol on endogenous catecholamine excretion (3, 10, 163, 296, 399, 455–458, 609, 610) is impossible to evaluate critically. The variety of species used, of doses of ethanol given, of distribution of dose over time, as well as the inclusion or absence of controls for the diuretic effects of the drug and the questionable adequacy of some of the methods used for the separation and identification of the amines and their metabolites, all add to the confusion. Certainly, dose is of vital importance here, since activation of the adrenomedullary system as a side-effect of intoxication would only complicate the situation further. Moreover, the significance of the findings, once these conflicts are resolved, is unknown. The excretion probably reflects changes in the amines located in the periphery and would therefore bear little relationship to amine release or catabolism occurring centrally.

Alterations in NE metabolism after ingestion of ethanol, similar to those observed in 5-HT metabolism, have been reported (77, 79). This is not unexpected, because similar alternate routes for metabolism of the aldehyde derivatives are available. When ^{14}C-labeled NE was infused in two subjects, and 8-hr urine samples were collected, the ingestion of ethanol caused a decreased excretion of ^{14}C-3-methoxy-4-hydroxymandelic acid but an increased output of ^{14}C-3-methoxy-4-hydroxyphenylglycol. No alteration was noted in the excretion of ^{14}C-labeled NE, normetanephrine, 3,4-dihydroxymandelic acid or 3,4-dihydroxyphenylglycol. No parallel changes were observed in the pattern of excretion of the deaminated catechols, which represent only about 10% of the NE metabolites. The principal effect of ethanol appeared to be a marked diversion of the intermediate glycolaldehyde from an oxidative to a reductive pathway (see fig. 3). In chronic alcoholics infused with tritium-labeled NE, ethanol caused a similar decrease in 3-methoxy-4-hydroxymandelic acid and a transient reversal of the ratio of this compound to 3-methoxy-4-hydroxyphenylglycol (539). Moreover, when a large amount of ethanol was ingested, the excretion rate of unchanged NE during the 1st-hr collection appeared to be nearly twice as great as the control; this reflected either higher plasma levels of the amine or diuresis induced by the drug.

Just as in the case of 5-HT, these changes in catecholamine metabolism may have at least two possible explanations, *i.e.*, a diversion from an oxidative to a reductive pathway by the increased NADH:NAD^+ ratio resulting from ethanol metabolism, or competition between acetaldehyde and the glycolaldehydes for the aldehyde dehydrogenase. Recent evidence favors the latter explanation (607).

Again, as in the case of 5-HT, ethanol infusion was reported to cause a reduction in NE level in the brainstem of the rabbit (187). Other investigators have

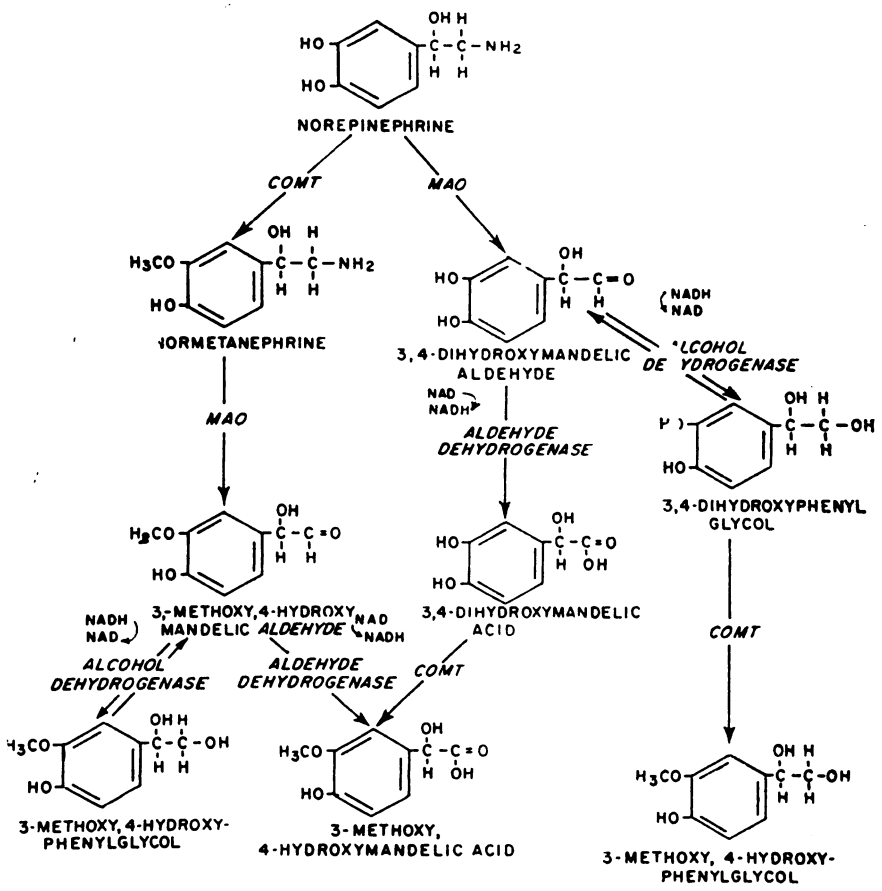

FIG. 3. Alternate pathways for the metabolism of norepinephrine.* COMT, catechol-O-methyl transferase; MAO, monoamine oxidase; NAD, nicotinamide adenine dinucleotide; and NADH, reduced nicotinamide adenine dinucleotide. (From V. E. Davis, J. A. Huff and H. Brown: Alcohol and biogenic amines. *In* Biochemical and Clinical Aspects of Alcohol Metabolism, edited by V. M. Sardesai, pp. 95–104, Charles C Thomas, Springfield, Ill., 1969. Reprinted with permission of the authors, editor and publisher.)

consistently been unable to repeat these results after either acute or chronic treatment with ethanol in mice, rats or rabbits (102, 189, 472, 513). But in rats pretreated with L-methyltyrosine-methylester, an inhibitor of NE and dopamine synthesis, ethanol (2 g/kg) did significantly lower the brain NE level. Two such combined treatments led to a decrease of fluorescence in the specific NE systems of the brainstem, but not in that of the dopamine-containing neurons (66). Thus,

* Recent evidence (see reference 562) indicates that the NADPH-linked aldehyde reductase may also be involved in the reduction of the aldehyde derivatives to the corresponding glycols.

the acute effects of ethanol appear to lead to specific activation of central NE-containing neurons, whether by direct or indirect means.

Israel (231) has pointed out that these findings could also be interpreted in terms of inhibition of amine reuptake, one of the main mechanisms for the physiological removal of noradrenaline. Reuptake, he notes, is depressed by ouabain, a known inhibitor of the active transport of Na^+ and K^+, and ethanol itself has also been shown to function as an inhibitor of ion transport in brain tissue (234).

Within the past 2 years, Davis and her colleagues (81, 606) have pursued and expanded their observation (80) that, in brainstem and liver homogenates, a condensation reaction may take place between a biogenic amine and the aldehyde formed as an intermediary in its metabolism. In the case of dopamine, the alkaloid tetrahydropapaveroline (THP) is formed, and the amount produced is dependent on the supply of NAD^+ which is normally used in the alternative pathway for the degradation of the aldehyde to its corresponding acid. Studies *in vitro* on dopamine metabolism showed that an augmented production of THP occurred when ethanol or acetaldehyde was present, as long as a supply of NAD^+ was available; in its absence, however, acetaldehyde condensed with dopamine to form salsolinol, and THP generation was depressed as a result of substrate removal. The data suggest that *in vitro*, at least, an ethanol-induced, acetaldehyde-mediated modification of dopamine metabolism can occur. The question has naturally been raised whether this type of modification in amine metabolism could be involved in the pharmacological effects of ethanol. As yet, no evidence has been presented that amines other than dopamine can be diverted in their metabolism along condensation pathways, or that such reactions occur *in vivo* under the conditions produced by the use of non-lethal doses of ethanol. At this point of knowledge, the idea that such condensation products may be related to a common mechanism underlying the ability of ethanol and morphine to give rise to dependence (80) can be regarded only as interesting speculation.

J. Effect of ethanol on iron and porphyrin metabolism and hematopoiesis

1. IRON UTILIZATION

Disorders of iron metabolism are frequently associated with over-indulgence in ethanol (215). Several possible mechanisms exist whereby ethanol might lead to an excess of iron in the tissues (see 315), but no experimental assessment of their relative significance has been carried out.

In normal subjects alcohol enhances the absorption of ferric iron from the gastrointestinal tract (57), probably by stimulating HCl secretion (244). Two pathological sequelae of excessive ethanol intake, chronic pancreatitis and chronic liver disease, may also contribute to iron overload (50, 75). In folate-deficient patients the hematopoietic response to pteroylglutamic acid supplements is suppressed by ethanol, and the serum iron level is elevated (560); these findings suggest that ethanol can impair iron utilization. A gradual rise in serum iron levels has also been demonstrated during experimentally induced chronic

intoxication in man (350), though the magnitude of the alterations did not correlate with the level of alcohol intake (617).

It is difficult to decide whether the changes in serum iron levels reflect primarily enhanced absorption of iron, impaired utilization, or altered distribution and storage, but a recent study may help to elucidate a possible site of interference by ethanol with iron utilization (220). Two of three alcoholics, during a prolonged period of ethanol intake, showed a 90% increase in the saturation of serum iron-binding capacity, and a 3-fold increase in plasma iron turnover. In all three there was an increase in storage iron, and serum pyridoxal phosphate (PLP) levels decreased progressively. The response of serum PLP to pyridoxine loading was greater after ethanol withdrawal, but did not approximate that seen in normal subjects until 9 weeks after alcohol removal. Administration of PLP itself caused reversion of the bone marrow to normal and serum iron levels decreased markedly, but pyridoxine plus folic acid had no such effect. It was suggested that ethanol may produce the changes in iron metabolism secondary to its effect on B_6 metabolism, possibly by interfering with the conversion of pyridoxine to pyridoxal phosphate.

This latter suggestion conflicts with an earlier report (321) that abnormally high urinary excretion of xanthurenic acid, after administration of a load of tryptophane to alcoholics suffering withdrawal convulsions, was quickly corrected by an intramuscular dose of pyridoxine hydrochloride. In rats on chronic ethanol intake, the activity of dihydroxyphenylalanine (DOPA) decarboxylase in the liver was depressed because of a reduction in the level of the active coenzyme, PLP (156), but this information does not enable one to decide whether the defect was in the supply of pyridoxine or in its conversion to PLP. In either case, the reaction involving the formation of δ-aminolevulinic acid is one of the first steps in heme synthesis and PLP plays an essential role as coenzyme in the reaction. A decrease in its availability could therefore be reflected in a decreased utilization of iron, and ensuing proerythroblastic abnormalities. This is an interesting hypothesis but a great many auxiliary experiments are needed to place it on a firm foundation.

The activity of hepatic δ-aminolevulinate synthetase, which is believed to be rate-limiting for heme synthesis, is reported to be increased 30 to 50% in rats within 3 hr after the administration of a single dose of ethanol intraperitoneally, and to return gradually to normal levels by 5 to 6 hr (535). This elevation in enzyme activity was ascribed to enzyme induction as postulated by Labbe (308), but more experiments are needed before such a conclusion can be considered valid. Conceivably, an alteration in mitochondrial permeability induced by ethanol might facilitate access of components in the reaction *in vitro* to the mitochondrial site, in much the same way as French observed in his studies with succinic dehydrogenase (158).

2. PORPHYRIN METABOLISM

The observation that the administration of ethanol increases urinary excretion of coproporphyrins (145) has been confirmed and extended, both in healthy sub-

jects and in chronic alcoholics (34, 450, 561, 641). The increased excretion persists beyond the cessation of alcohol ingestion, and generally parallels the degree of alcoholism. It was considered to be due to a block in conversion of coproporphyrinogen to protoporphyrin, rather than to an overproduction of precursors, and could be explained by a temporary deficiency of NAD^+ and a consequent accumulation of coproporphyrinogen. This was then slowly oxidized and subsequently excreted in the urine. Hence, competition by ethanol and coproporphyrinogen for the available NAD^+ was put forward as a tentative hypothesis to explain the findings (450). Time relationships in the sequence of events might prove an obstacle to the acceptance of this theory as it now stands. Competition for NAD^+ is confined to a relatively short period of time required for the metabolism of ingested ethanol (265), yet the elevated urinary output of coproporphyrin by alcoholics continues during many months of abstinence (450). An extremely slow release from tissues might be postulated to satisfy the observed results, though the lag in the elevation of urinary coproporphyrin output could be explained on the basis of a slow oxidative process. It may also be that the prolonged elevation of coproporphyrin excretion reflects the course of secondary liver disease, rather than a continuing direct effect of ethanol. Additional experiments are needed to delineate more clearly the significance of these time factors in relation to the observed findings.

It has been pointed out that many drugs which induce changes in porphyrin metabolism are inhibitors of NADH-oxidase (308) and alterations in porphogenesis have been tentativly linked to decreased oxidation of mitochondrial NADH or even to a less specific impairment in mitochondrial terminal electron transport. The influence of ethanol on porphyrin metabolism has been ascribed to a reported decrease in hepatic ATP level (452). However, there is no unanimity of opinion on the effect of acute ethanol intake on hepatic ATP (8, 153). The variation in drug dose, route of administration and time elapsed after administration may be critical factors in determining the direction of measured change in ATP levels.

3. ETHANOL AND HEMATOPOIESIS

The results of several studies suggest that ethanol acts as a suppressant of hematopoiesis, though the site of its action is not clear (21, 248, 249, 349, 403, 617). The reader is referred to a recent review (100) for a summary of the clinical and experimental evidence on the subject. The changes have been ascribed to a direct toxic effect of ethanol on the bone marrow (617) but the role played by associated nutritional complications in alcoholics cannot be excluded. Experimental studies, in which nutritional factors have been controlled, have yielded variable results; some support the concept that ethanol suppresses hematopoiesis (220, 350), while others do not (411).

The possibility that a deficiency of folic acid may be involved in the abnormalities of bone marrow function observed in alcoholics has also been studied (216, 297). As mentioned earlier, ethanol impaired the reticulocyte response to pteroylglutamic acid supplements in anemic folate-deficient patients (560). The

reticulopenia lasted as long as ethanol was being ingested, but was reversed by alcohol withdrawal. The possibility of malabsorption of folate was excluded; yet elevation of the supplement, although it increased serum folate levels, did not completely overcome the suppressant effect of ethanol. This study suggests an impairment of folate utilization by ethanol, but the experimental design does not permit an examination of the site of action of ethanol nor of the mechanism whereby impairment is effected.

After an oral dose of tritiated crystalline folic acid (PGA), peak levels of PGA in the plasma of alcoholics were significantly lower than those of the non-alcoholic controls, though both had received a tissue-saturating dose of unlabeled PGA earlier (198). Urinary excretion of the label was similarly depressed. Results after one dose of ethanol were not consistent, but a 10-day intake caused a flattening of the ^{3}H-PGA absorption curve and a depressed excretion. Three days after ethanol withdrawal, folate absorption had returned to normal. Since crystalline PGA was used, it would appear that either its conjugation or metabolic conversion must be the factor altered. This study does not allow an assessment of whether ethanol might also affect critical steps in the digestion and absorption of the naturally-occurring polyglutamic forms of the vitamin.

Studies *in vitro* of the folate-dependent transfer of 1-carbon units in rat bone marrow and liver appear to indicate that the ^{14}C-formate incorporation into nucleic acids of bone marrow cells was significantly inhibited by ethanol, whereas ^{3}H-thymidine incorporation was unaffected (27). Formyltetrahydrofolate synthetase purified from rat liver was inhibited by a concentration of ethanol comparable to that used in the formate incorporation studies. The ethanol appeared to compete with formate for the active site of the enzyme. The concentrations of ethanol required for a significant effect were higher than those likely to be encountered *in vivo*, but it was suggested that suppression of hemopoiesis by ethanol might be mediated at least in part through a reversible inhibition of the activity of this enzyme. Confirmation of this hypothesis would require the results of additional studies, with lower concentrations of ethanol.

K. Ethanol and uric acid metabolism

A high ethanol intake has long been considered a precipitating or predisposing factor in attacks of gout, but experimental evidence of a relationship between ethanol ingestion and uric acid metabolism has only recently accumulated. Lieber has reviewed some of the evidence in this field (327, 329).

Serum uric acid concentrations in a group of alcoholic patients were significantly higher when they were intoxicated than during a subsequent period of sobriety (338). Blood lactate levels were also elevated during the stage of intoxication, returning to normal when sobriety was restored. An infusion of ethanol caused an increase in serum uric acid levels concomitant with the rise in serum ethanol, and the rise continued beyond the period of drug administration. In subjects showing this increased serum urate level after ingesting ethanol, the urinary uric acid output decreased to 20 to 50% of control values, but returned to normal after cessation of ethanol intake. The deficit in urate output during the

period of ethanol intake corresponded to the rise in the serum urate levels. Prolonged intake of large amounts of beverage alcohol by a group of alcoholics on an adequate diet also caused a marked decrease in urinary uric acid output leading to the development of a secondary hyperuricemia (339, 341).

Qualitatively, at least, it has been demonstrated that a decreased urinary urate excretion accompanies an increase in blood lactate, whether the latter is achieved by administration of racemic lactate (338, 419, 635) or results from metabolic causes such as exercise (439), toxemia of pregnancy (200) or glycogen storage disease (6). Experimental evidence is consistent with the idea that the decrease in uric acid excretion induced by ethanol is also secondary to elevation of blood lactate levels (338). However, the underlying mechanism by which hyperlactacidemia affects urate excretion is unknown.

Mudge (427) has raised the question of a possible alteration in renal metabolism by ethanol, leading to changes in the endogenous level of lactate within the kidney itself. ADH is known to be present in the kidney and it is conceivable that alcohol might induce local production of lactate in this organ. This is a theoretical possibility which merits experimental scrutiny. An effect of ethanol in the glomerular filtrate on urate transport across the tubular epithelium is also conceivable.

It is clear that many questions still require examination before a final explanation of this phenomenon can be given. It has been suggested (375, 502) that, in the fasting state, the elevation of serum urate by ethanol is the combined consequence of the rise in both blood lactate and β-hydroxybutyrate, and this possibility invites further exploration. It has also been demonstrated that ethanol and fasting may exert additive or mutually potentiating effects on uric acid metabolism and on the rapid changes in serum urate levels frequently associated with gouty attacks (376). Thus, evidence presently available appears to indicate that elevated lactate levels can play an important role in the changes in uric acid metabolism induced by ethanol, but other factors may be involved as well.

IV. General Summary

Despite a large amount of research on the metabolism of ethanol in the past decade, the picture has not changed radically with respect to the routes of this metabolism and the factors which control it. Catalase can certainly oxidize ethanol *in vitro*, and potential H_2O_2-generating systems exist within the cell, but no clear evidence has so far been presented to support the idea that ethanol is metabolized by a catalase system *in vivo*.

Similarly, hepatic microsomal enzymes can oxidize ethanol *in vitro* by a reaction involving NADPH and oxygen (MEOS), but a substantial body of evidence suggests that this reaction plays no significant role *in vivo*, after either single or repeated administration of ethanol. Increase in MEOS activity after chronic ethanol intake is a reflection of increased smooth endoplasmic reticulum in the liver cell, but this may be related to the effect of ethanol on lipid metabolism rather than to the metabolism of ethanol itself.

Miscellaneous pathways involving esterification or condensation of ethanol

with glucuronate, fatty acids or other substances, play a quantitatively trivial role in ethanol metabolism. The significance of these reactions lies in the interaction between ethanol and other substances which may compete for the same metabolic pathways.

Alcohol dehydrogenase (ADH) seems unquestionably the most important enzyme in the oxidation of ethanol. It probably detoxifies the small amounts of ethanol produced in the gastrointestinal tract, yet its kinetic properties suggest that its major role *in vivo* involves some other substrate than ethanol. Indeed, ADH appears to be a group of enzymes, each of which may have a different primary role *in vivo*. The discrepancy between ADH activity measured *in vitro* and the rate of ethanol metabolism *in vivo* probably depends upon a variety of factors including the presence of competing substrates, differences in pH, extrahepatic metabolism of ethanol, changes in hepatic and other regional blood flows, and differences in rate of mitochondrial reoxidation of NADH. The last may be particularly important in relation to adaptive increases in ethanol metabolism after chronic ethanol ingestion. At the same time liver damage or malnutrition, which frequently accompanies ethanol intake, tends to reduce the ADH activity so that the final effect on ethanol metabolism reflects a balance of opposing tendencies.

The metabolic effects of ethanol are of at least three different types: those resulting from alterations in metabolite pools and cofactors produced by the metabolism of ethanol itself, those resulting from neuroendocrine disturbances secondary to the state of intoxication, and those produced directly by the pharmacological action of ethanol on specific cells and processes. In almost every major area of metabolism these various types of effect contribute in different degrees. Many of the apparent disagreements concerning the metabolic effects of ethanol arise from differences in experimental conditions, which cause the relative contributions of these factors to vary.

Effects of the first type, resulting from ethanol metabolism, have been studied most intensively. The fundamental effect is the increase in NADH:NAD^+ ratio within the cytoplasm and mitochondria of the liver cell. This in turn affects the availability of pyruvate and oxaloacetate, thus bringing about a wide-ranging series of disturbances in mitochondrial oxidation of fatty acids and other substrates, gluconeogenesis and carbohydrate utilization. In addition, the change in nucleotide ratio directly affects many other NAD^+-dependent reactions involved in metabolism of amino acids, biogenic amines, glycerol, carbohydrates, porphyrins and compounds of other classes. Finally, the large amounts of acetate, lactate and lipids, and the smaller amounts of acetaldehyde, which leave the liver during ethanol oxidation produce indirect effects on the metabolism of other tissues.

Effects of the second type, related to the degree of intoxication, have been much less thoroughly studied. Sympathetic and adrenomedullary responses are involved in hepatic glycogenolysis produced by large doses of ethanol, and probably in the mobilization of fatty acids from peripheral adipose tissue which appears to play an important role in hepatic steatosis after a single large dose of ethanol. Hypoxia and disturbances in blood flow through various organs may

account for much of the variability in metabolism of ethanol itself, as well as in its effects on metabolism of other substances.

Effects of the third type, resulting from a direct pharmacological action of ethanol, have been the least well explored of all, even though they may prove to be of considerable significance. Very incomplete evidence suggests that ethanol may reduce active transport of amino acids in the liver, gastrointestinal tract and elsewhere. It may also have a direct effect on processes involved in synthesis and exocytosis of lipoproteins in the liver, on renal tubular transport mechanisms, and on permeability of mitochondrial and cell membranes. All of these, if verified, would have important implications for metabolism.

Because of the peculiar kinetics of ethanol oxidation *in vivo*, effects of the first type predominate at low concentrations of ethanol in body fluids, while those of the second and third types become progressively more important at higher concentrations. This variation, together with the complicating factors of nutritional imbalance and hepatic pathology which may occur during chronic ethanol ingestion, must be taken into account in explaining or predicting the metabolic consequences of ethanol under any given set of conditions.

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