# Alcoholism, Alcohol, and Drugs

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Alcoholism is a major public health problem in the United States, as well as in most of the western world. Precise figures are impossible to obtain in a problem of this type, but it appears reasonable to assume that in the United States there are at least 6 to 8 million alcoholics, or more than 5 percent of the population at risk. In terms of addiction, therefore, ethyl alcohol (*1*) is of far greater social and medical importance than all other drugs combined. The federal government, recognizing the importance of this problem, has created the National Center for Prevention and Control of Alcoholism, first as a division, and now as an institute within the National Institute of Mental Health. In light of the current interest in drug addiction generally, and the particular emphasis being accorded the problem of alcoholism, it seems appropriate to inquire into the status of alcohol as a drug and its relationship with other drugs.

Owing to the widespread and increasing use of pharmaceutical agents of all kinds, the interaction of drugs is increasingly being recognized as a hazard (*2*). The high incidence of alcoholism and the fact that many alcoholics are not recognized as such, magnify the importance of this problem. More than half of the fatal automobile accidents in the United States directly involve intoxicated drivers (*3*), many of whom may suffer from a synergism between alcohol and other commonly ingested drugs such as barbiturates and tranquilizers (*4*). An interaction between alcohol and other drugs may also contribute to accidental or suicidal deaths in individuals who have consumed barbiturates while they were inebriated. Indeed, more alcoholics die from drug intoxication than from acute alcohol intoxication (*5*). Both acute alcohol intoxication and alcoholism may affect the dosage requirements in many clinical situations that require the use of drugs, for example, anesthetics, oral hypoglycemic agents in the treatment of diabetes, anticonvulsants in the therapy of epilepsy, and anticoagulants after myocardial infarction (*4*).

The well-known clinical interactions of ethanol and drugs appear paradoxical. Inebriated individuals, whether they are alcoholics or not, display a striking sensitivity to a wide variety of drugs, including sedatives and tranquilizers (*4*). On the other hand, alcoholics, when sober, are unusually tolerant of many drugs, particularly sedatives such as barbiturates (*6*). These effects of acute and chronic ethanol intoxication have been attributed, respectively, to additive and to adaptive phenomena in the central nervous system (*4*). Although ethanol is utilized as a source of calories, the results of ethanol ingestion are strikingly similar to those of acute and chronic administration of drugs. For example, acute administration of phenobarbital to an individual who has been given dicoumarol prolongs the effectiveness of the dicoumarol (*7*). Conversely, chronic administration of phenobarbital decreases the response to other drugs (*7*). These effects of drug administration may be attributed, in the first case, to competition for oxidation by hepatic microsomes when two drugs are given simultaneously; and, in the second case, to induction of drug-detoxifying enzymes in the hepatic microsomes.

We therefore embarked on a series of experiments to determine whether, in addition to effects on the central nervous system, the influence of acute and chronic ethanol ingestion on the response to drugs might be explained by an interaction of ethanol and hepatic microsomes. In other words, we posed the question of whether ethanol could be considered to be a drug in the same way that phenobarbital is.

Drugs that are metabolized by aerobic, nicotinamide adenine dinucleotide phosphate (NADPH)–dependent enzyme systems in hepatic microsomes exhibit the following characteristics (*7*).

1) Chronic administration of a drug leads to (i) hypertrophy of hepatic smooth endoplasmic reticulum (SER); (ii) acceleration of its own metabolism; (iii) induction of a variety of other microsomal drug-metabolizing enzymes; and (iv) increase in cytochrome P450, the hemoprotein that is the terminal acceptor in the electron transport chain leading to drug oxidation.

2) Acute administration of a drug, or addition of a second drug in an in vitro system, inhibits other microsomal drug-metabolizing systems.

3) These drugs bind to hemoprotein in the hepatic microsomes, which results in a characteristic change in spectra (*8*).

The relation of ethanol and drug metabolism cannot be adequately studied clinically in alcoholics, since in these individuals nutritional status, previous ingestion of drugs, amount or duration of ethanol consumption, and intercurrent disease cannot be accurately evaluated. To test whether the effects of ethanol on the liver are comparable to those of a drug such as phenobarbital, we examined both the action of ethanol in vitro and the response of rats and human volunteers to controlled ethanol administration.

## Effects on Hepatic SER of Chronic Administration of Ethanol

Iseri *et al.* (*9*) showed in rats that chronic administration of ethanol, as an isocaloric replacement for carbohydrate (36 percent of total calories), leads to an increase in hepatic SER. This finding was subsequently confirmed morphologically (Fig. 1) and chemically by the demonstration of increased protein in the hepatic microsome fraction, the chemical counterpart of the SER (*10, 11*). It seemed likely that chronic administration of ethanol also leads to hypertrophy of SER in man, since examination of liver biopsy specimens from patients with alcoholic liver disease revealed an increase in SER (*12*).

As mentioned previously, factors other than ethanol consumption play a role in alcoholics. We therefore administered ethanol, as an isocaloric replacement for carbohydrate (42 to 46 percent of total calories), to alcoholics who had abstained from alcohol for 2 to 4 months in the hospital and to healthy, normal (not alcoholic) volunteers. These volunteers consumed a variety of nutritionally adequate diets, including 16 to

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25 percent of total calories as protein, 5 to 36 percent of total calories as fat, choline chloride (10 grams daily), and vitamins, minerals, and folic acid supplementation (*11, 13*). Comparing liver biopsy specimens obtained before and after ethanol feeding, we found that, in all instances, administration of ethanol produced hypertrophy of SER in 9 to 16 days.

### Chronic Consumption of Ethanol and Hepatic Drug-Metabolizing Enzymes

The induction of microsomal drug-metabolizing enzymes is generally characterized by its lack of specificity. Thus, phenobarbital not only induces its own metabolism, but also increases the activities of systems that metabolize unrelated compounds such as aniline hydroxylase, aminopyrene demethylase, nitroreductase, and many more (*7*). In order to determine whether ethanol is similar in this respect to phenobarbital, we studied the effect of its chronic administration on a number of drug-metabolizing systems. These included the hydroxylation of aniline (an aromatic amine), pentobarbital (a sedative), and 3,4-benzopyrene (a polycyclic hydrocarbon carcinogen). The rate of nitroreduction of *p*-nitrobenzoic acid, an anaerobic system, was also measured. When rats were fed ethanol with the adequate diet, aniline hydroxylase activity was increased about sevenfold (*10*) (Fig. 2). A diet deficient in protein and choline accentuated this change. Ethanol significantly increased the activities of pentobarbital hydroxylase, benzopyrene hydroxylase, and nitroreductase, although not to the extent of aniline hydroxylase (*10*) (Fig. 2). Cytochrome P450 was doubled by chronic administration of ethanol (*10, 14*). These data were subsequently confirmed by others (*15*).

Since the induction of drug-metabolizing enzymes is, to some extent, dependent on species and age, we investigated this phenomenon in human volunteers. Three young, healthy, normal volunteers, two men and one woman, were fed ethanol for 12 days. Aspiration liver biopsies were performed before and after administration of ethanol. In these volunteers, ethanol consumption produced an increase of two to three times the initial activity of hepatic pentobarbital hydroxylase (*16*) (Fig. 2). To our knowledge, this was the first direct demonstration in man of the induction of an increase in hepatic drug-metabolizing enzyme activity.

Another effect of drug administration is the induction of hepatic δ-aminolevulinic acid synthetase (ALAS), the rate-limiting enzyme in biosynthesis of porphyrins. In this respect, it is interesting to note that this induction may be an adaptive response of the liver, which serves to increase the production of the porphyrin-containing protein cytochrome P450. However, this concept is controversial (*17*). Acute administration of ethanol to rats increased hepatic ALAS activity more than threefold within 3 hours (*11*) in one study, and 45 percent in another (*18*). This may explain why ethanol consumption increases urinary coproporphyrin excretion (*19*) and precipitates porphyric crises in individuals with congenital hepatic porphyria (*20*). Rats that had consumed ethanol for 24 days displayed increased ALAS activity when they were killed within a few hours after termination of ethanol ingestion. However, when animals were killed 18 hours after termination of ethanol consumption, no difference in ALAS activity was found between chronically treated rats and pair-fed controls.

These findings may be explained by the very rapid turnover of ALAS (with a half-life, $t_{1/2}$, equal to 70 minutes) (*21*) and the rapid oxidation of the inducer, ethanol. In contrast, drugs such as phenobarbital continue to induce ALAS for several days, probably because the drug persists in the circulation. Prior treatment of rats with puromycin or actinomycin prevented induction of ALAS activity, an indication that ethanol stimulates synthesis of new enzyme protein. Hepatic ALAS can also be induced in man by acute administration of ethanol (*22*).

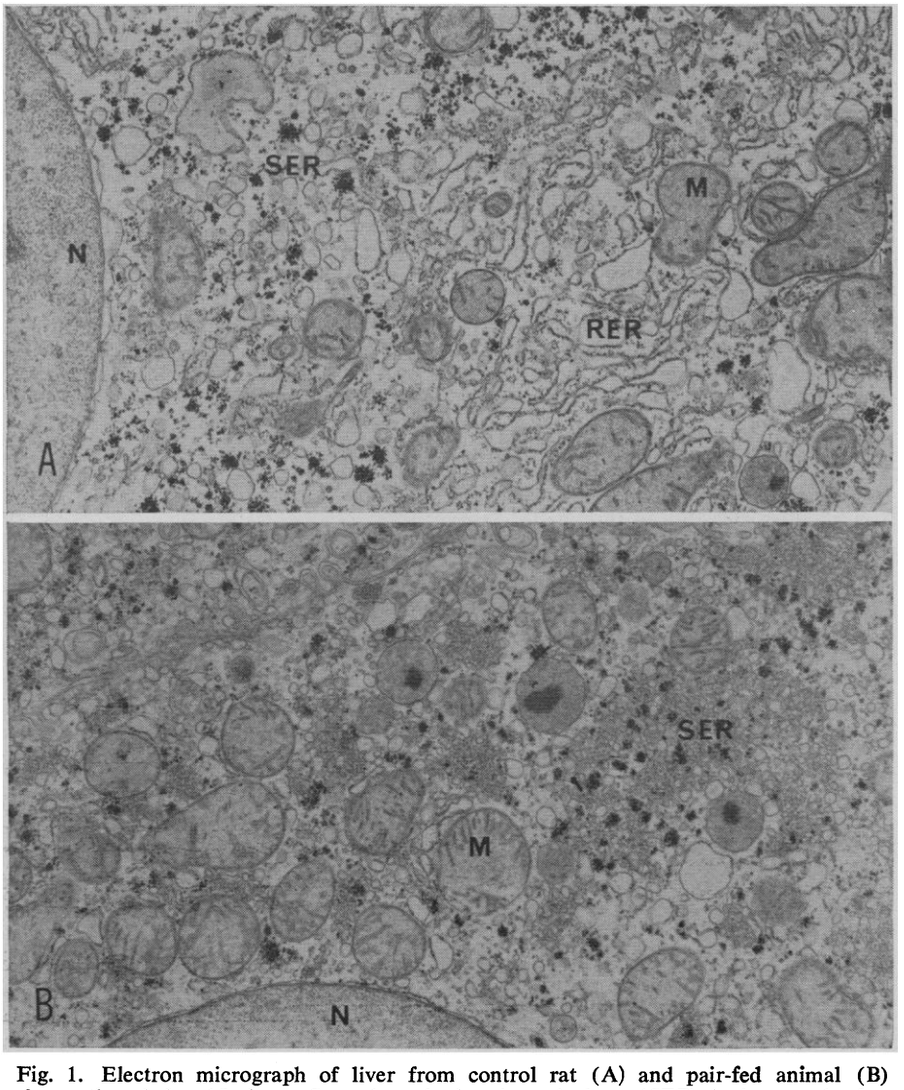


Fig. 1. Electron micrograph of liver from control rat (A) and pair-fed animal (B) given ethanol for 15 days. Smooth endoplasmic reticulum (*SER*) is increased after ethanol ingestion. Nucleus (*N*), mitochondrion (*M*), rough endoplasmic reticulum (*RER*) (× 11,200) (*11*).

## Chronic Administration of Ethanol and Drug and Ethanol Metabolism

The levels of microsomal enzymes in the liver are not necessarily the rate-limiting factor in metabolism of drugs under all conditions. We therefore performed a series of experiments in rats to determine whether or not chronic ingestion of ethanol increases the rate of drug metabolism at various levels (*23*). At the organelle level, hepatic microsomes (supernatant resulting from centrifugation of liver homogenate at 9000*g*) from rats chronically fed ethanol metabolized meprobamate about twice as fast as those from pair-fed controls. To investigate drug metabolism at the cellular level, liver slices obtained from chronically intoxicated rats and from pair-fed controls were incubated with [$^{14}$C]meprobamate. Again, tissue from the rats fed ethanol metabolized meprobamate twice as fast as that from the controls. Finally, in the whole animal, the mean half-life in the plasma of intraperitoneally administered meprobamate was halved by chronic administration of ethanol: 138 minutes in the chronically intoxicated rats, compared to 254 minutes in the control rats. It is interesting to note that the degree of acceleration of meprobamate metabolism was comparable at all levels studied. This suggests that the activity of hepatic drug-metabolizing enzymes may have been rate-limiting under these conditions.

Rats metabolize drugs considerably faster than man does, a fact which makes it hazardous to extrapolate murine data to man. For this reason, we used volunteers in investigating the effect of chronic ethanol consumption on the disappearance of meprobamate and pentobarbital in the plasma of man. The volunteers were maintained on a metabolic unit under controlled dietary conditions (*23*). In alcoholic volunteers, the mean half-life of orally administered meprobamate in the plasma was decreased by half after 4 weeks of ethanol consumption, from a mean control value of 16.7 to 8.1 hours (Fig. 3). In the normal volunteers, ethanol consumption for 3 to 4 weeks similarly reduced the mean half-life of meprobamate in the plasma, from 13.7 hours during the control period to 8.2 hours. Excretion of meprobamate in the urine was unchanged by ethanol consumption. In each of four normal subjects, the half-life of ingested pentobarbital was also decreased.

Since chronic administration of a specific drug usually induces metabolism of that drug, it would be reasonable to expect the same for ethanol. This proved to be the case. The disappearance of ethanol from the blood was accelerated by chronic ethanol consumption, in both the alcoholic and the normal volunteers (*23*). In alcoholic volunteers, the mean rate of ethanol metabolism increased from a control value of 13.6 to 18.5 milligrams per 100 milliliters of plasma per hour. In normal subjects, it increased from a control value of 13.8 to 23.8 milligrams per 100 milliliters. These data are consistent with increased production of $^{14}CO_2$ in alcoholics after administration of ethanol (*24*).

It should be emphasized that the acceleration of drug metabolism by chronic administration of ethanol was produced in subjects who were given a diet that was more than adequate with respect to protein, vitamins, and minerals. Moreover, no other drugs were permitted during the control period or during the period in which ethanol was being consumed. It is therefore likely that the increased rate of drug metabolism found in patients who are hospitalized for treatment of alcoholism (*25*) is also a direct result of alcohol ingestion.

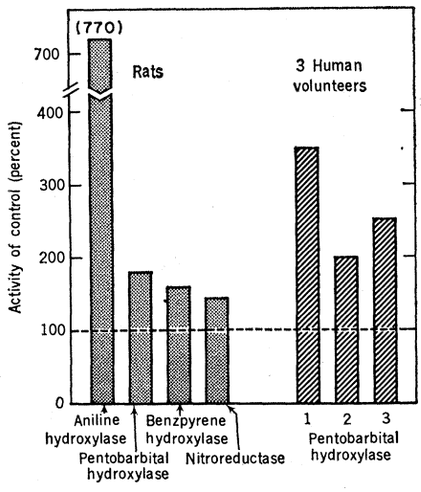


Fig. 2. Effect of chronic administration of ethanol on activities of hepatic drug-metabolizing enzymes in rats and human volunteers. The activity of every enzyme studied was increased by chronic ingestion of ethanol.

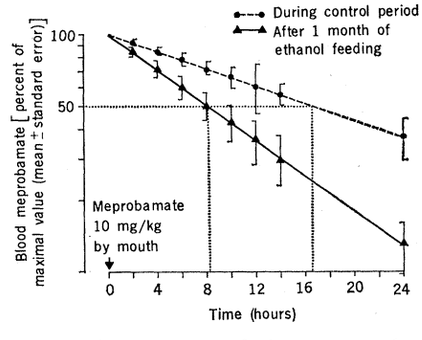


Fig. 3. Disappearance of plasma meprobamate in human volunteers before and after chronic administration of ethanol (*23*). The half-life of meprobamate in the blood was halved by chronic intoxication.

## Inhibition of Drug Metabolism by Ethanol

Since ethanol appears to act as a drug, it should inhibit the activities of other microsomal drug-metabolizing enzymes in vitro. When given simultaneously with another drug, it should interfere with the metabolism of that drug. As in previous studies of induction of drug metablism, we investigated the inhibition of drug metabolism at the subcellular and cellular levels, and in the intact animal. Our first experiments were studies in vitro of the effects of ethanol on drug-metabolizing enzymes in the hepatic microsomes. The addition of ethanol, in concentrations that may be found in the blood of inebriated individuals, to suspensions of hepatic microsomes inhibited the hydroxylation of aniline, pentobarbital, and benzopyrene, and the demethylation of aminopyrene and ethylmorphine (*11, 16, 26*) (Fig. 4). Analysis of the kinetics of these reactions showed the inhibitions of aniline hydroxylase and aminopyrene demethylase to be competitive, while those of pentobarbital hydroxylase and ethylmorphine demethylase were of a mixed type. At the cellular level, the addition of ethanol to rat liver slices incubated with meprobamate produced a conspicuous inhibition of meprobamate metabolism. As in the previous studies, we considered the fact that the activities of hepatic drug-metabolizing enzymes might not be rate-limiting under all conditions and, therefore, determined the effects of ethanol in vivo on drug metabolism in rats and man. In rats, ethanol (5 grams per kilogram, given orally) doubled the half-life of pentobarbital in the plasma. This was not caused by altered compartmentalization of the drug or by changes in urinary excretion, since the half-life of total body pentobarbital was comparably prolonged.

Because of possible differences in human and murine metabolism, normal volunteers were given meprobamate

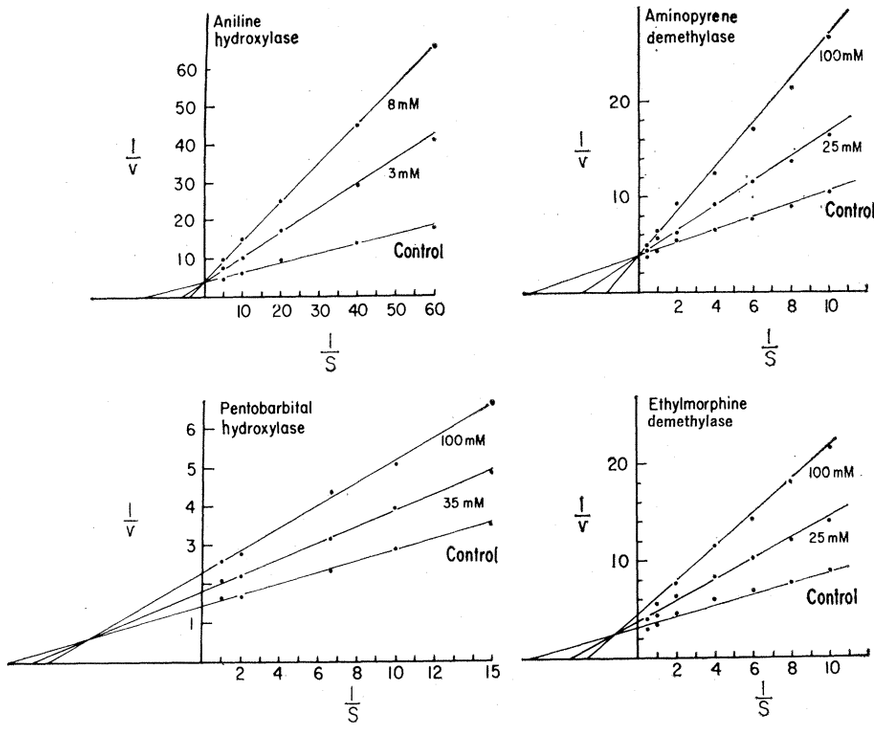


Fig. 4. Lineweaver-Burk plots showing inhibition of various hepatic drug-metabolizing enzymes by ethanol (*26*). Intersection of the lines on the ordinate, in the cases of aniline hydroxylase and aminopyrene demethylase, indicate competitive inhibition. Intersection of the lines between the ordinate and the abscissa, in the cases of pentobarbital hydroxylase and ethylmorphine demethylase, indicate a mixed type of inhibition. The concentrations of ethanol are shown in each graph. (*S* indicates millimolar concentration of substrate; *V* indicates velocity of the reaction.)

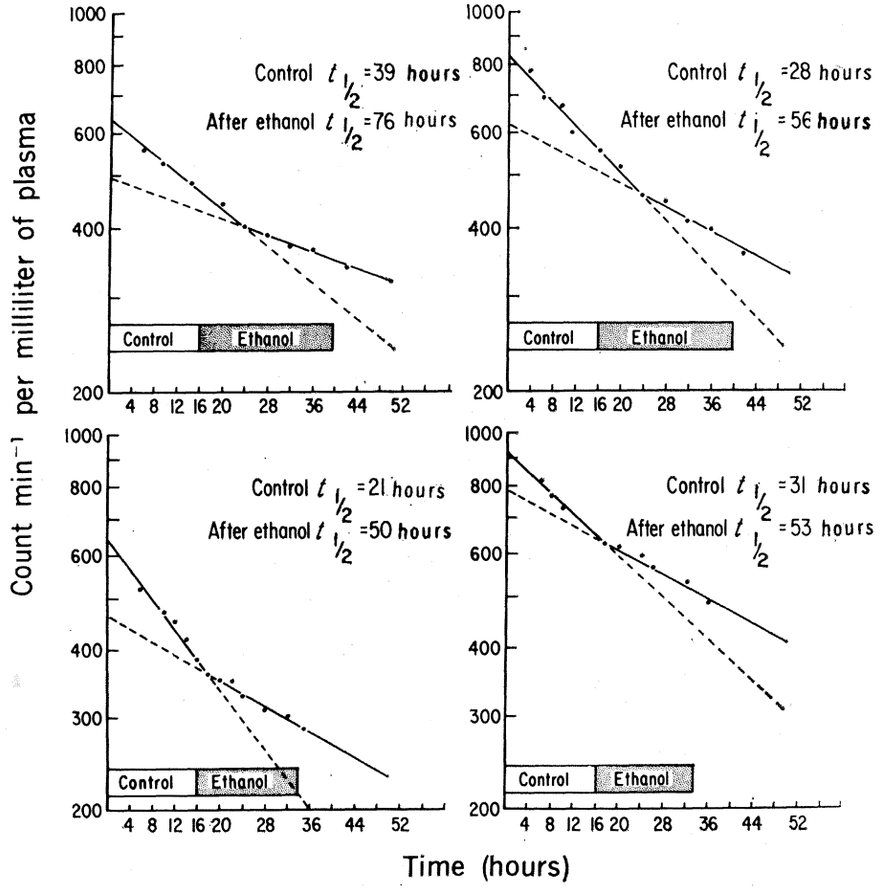


Fig. 5. Effect of acute ethanol intoxication on the disappearance of pentobarbital from the blood of four human volunteers (*26*). After ethanol administration (1 gram per kilogram, followed by 30 grams every 2 hours), the half-life of pentobarbital in the blood was approximately doubled. Solid lines are plotted from data points. Dashed lines are extrapolated.

and pentobarbital orally, after which serial blood measurements were made for 16 hours (*26*). At that time, 1 gram per kilogram of ethanol was given orally, followed by 30 grams every 2 hours. After administration of the ethanol, the half-life of pentobarbital was doubled (Fig. 5) and that of meprobamate was two to five times longer. We were thus able to demonstrate that, in the presence of ethanol, drug metabolism is inhibited in hepatic microsomes at the subcellular level; in liver slices at the cellular level; and in the plasma of both rats and man.

## Microsomal Metabolism of Ethanol

We have shown that ethanol resembles many other drugs that are metabolized by hepatic microsomes, both in its ability, when administered chronically, to induce proliferation of SER and microsomal drug-metabolizing enzymes, and in its inhibition of the metabolism of other drugs when given acutely. It therefore appeared likely that ethanol itself might also be metabolized by a microsomal system, particularly in view of the fact that the demonstrated acceleration of ethanol metabolism by chronic consumption occurs without an increase in the activity of hepatic alcohol dehydrogenase (ADH) (*27*) (Fig. 6), an enzyme located exclusively in the soluble fraction of the cell.

That this might indeed be the case was strongly suggested by Orme-Johnson and Ziegler, who first demonstrated a microsomal system capable of oxidizing methanol and, to a small extent, ethanol (*28*). Lieber and DeCarli later described (*29*) a microsomal system that metabolizes ethanol at ten times the rate of the system described by Orme-Johnson and Ziegler. While both systems require NADPH and oxygen, the Lieber and DeCarli system differs in being sensitive to CO; its *p*H optimum is in the physiologic range. Moreover, chronic ethanol feeding in rats doubled the activity of this microsomal ethanol-oxidizing system (MEOS) (Fig. 6). The activity of MEOS can be

differentiated from that of ADH, which may contaminate microsomes, in a number of ways. Washed microsomes display no ADH activity, even when 3-acetylpyridine-NAD is used as a cofactor in place of NAD (nicotinamide adenine dinucleotide) (*30*). By contrast, ADH activity in the cytosol is quadrupled by 3-acetylpyridine-NAD (*31*). Whereas ADH operates at a *p*H optimum of 10 to 11 and requires NAD as a cofactor, MEOS has a *p*H optimum of 7.4 and requires NADPH (*29*). Activity of ADH in the cytosol is minimal or absent using NADP (nicotinamide adenine dinucleotide phosphate) or NADPH as a cofactor (*31*). Conversely, little microsomal oxidation of ethanol occurs in the presence of NAD (*29*). Furthermore, ADH activity can be inhibited completely by 2 millimolar pyrazole, a concentration that has little effect on MEOS activity (*32*).

In the presence of NADPH and oxygen, NADPH oxidase can generate $H_2O_2$; the addition of catalase allows the oxidation of ethanol (*33*). A number of investigators (*31, 34*), therefore, have suggested that MEOS activity actually represents a combination of catalase contamination of microsomes and NADPH oxidase activity. However, experiments using several inhibitors have shown this not to be true. The administration to rats in vivo of 4.4 millimoles of pyrazole per kilogram reduced catalase activity of washed microsomes by 90 percent; ethanol oxidation by an $H_2O_2$-generating system was decreased 80 to 90 percent (*29*). At the same time, MEOS activity was practically unchanged. The addition of the catalase inhibitor azide to microsomes from rats treated in vivo with pyrazole almost completely abolished catalase activity. Under these conditions, 66 percent of MEOS activity remained (*29*). Although Isselbacher and Carter (*31*) were able to reduce MEOS activity by using an inhibitor of NADPH oxidase, such as cholate, it should be noted that cholate also inhibits other microsomal enzymes and converts cytochrome P450 to inactive P420 (*30*). For these reasons, which are treated in greater detail elsewhere (*35*), the microsomal ethanol-oxidizing system appears to be akin to other drug-metabolizing systems and distinct from ADH, catalase, and NADPH oxidase, although ethanol feeding enhances the activity of NADPH oxidase (*36*) (Fig. 6).

The quantitative contributions of ADH and MEOS to ethanol metabolism in vivo are uncertain because extrapolation of in vitro rates to the in vivo situation is hazardous. When allowance is made for loss of microsomes during fractionation procedures, activity of MEOS in vitro could account for about 20 percent of the total ethanol oxidation in vivo (*35*). After addition of 2 millimolar pyrazole, a concentration that completely inhibits ADH activity, to liver slices in vitro (*29*) or to the medium during liver perfusion (*37*), 25 percent of ethanol oxidation remains. Thus in this case, the in vitro and in vivo data agree. The enhancement of ethanol oxidation in vivo by prior chronic treatment of rats with ethanol is probably a result of the induction of MEOS activity, since ADH activity is not induced (*27*). The relative contributions of each system to ethanol oxidation may also be a function of the concentration of ethanol; the Michaelis constant of ADH oxidation of ethanol is 2 millimolar, while that of MEOS is about 8 millimolar. Thus, the role of MEOS may be more important at high alcohol concentrations than at low. It should be noted that, while the quantitative contribution of MEOS to in vivo metabolism of ethanol is not settled, the mere fact that ethanol is oxidized by microsomes can explain the effects of ethanol on drug metabolism.

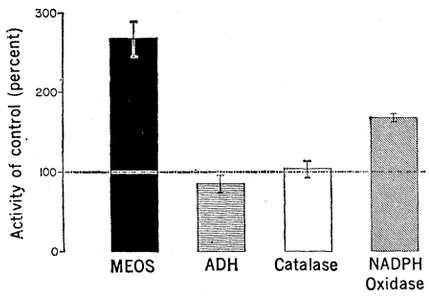


Fig. 6. Effect of chronic ethanol ingestion on the activities of hepatic microsomal ethanol-oxidizing system (MEOS), alcohol dehydrogenase (ADH), catalase, and NADPH oxadise (*36*) in rats. Activity of MEOS is more than doubled, while that of ADH is slightly decreased, and that of catalase is unchanged. Activity of NADPH oxidase is moderately increased.

## Interaction of Ethanol and Microsomal Hemoprotein

If ethanol is indeed to be considered to be a drug metabolized by hepatic microsomes, it, like phenobarbital and other drugs, should interact with microsomal hemoprotein (cytochrome P450). Drugs that are metabolized by hepatic microsomes bind to microsomal hemoprotein, yielding two types of difference spectra in the Soret band, depending upon the molecular structure of the drug. The spectrum of type 1 has a peak at 390 nanometers and a trough at 420 nanometers, whereas the spectrum of type 2 is characterized by a trough at about 390 nanometers and a peak at about 430 nanometers (*8*). The addition of ethanol to hepatic micro-

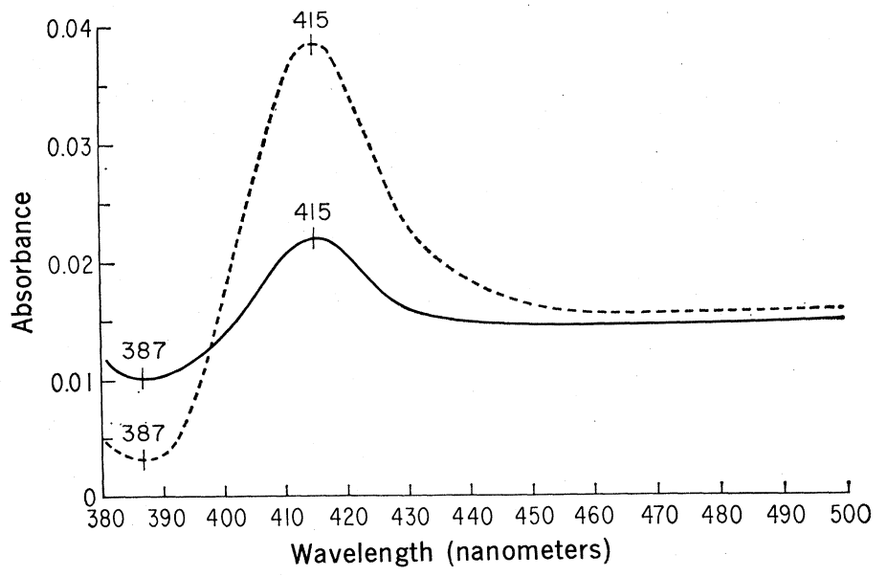


Fig. 7. Difference spectrum produced by the addition of 100 millimolar ethanol to hepatic microsomes of rats. Solid line represents spectrum from microsomes obtained from untreated rat, and dashed line represents spectrum from ethanol-treated rat (*14*). The magnitude of the peak in this modified type 2 spectrum is tripled by chronic consumption of ethanol.

somes results in a modified type 2 spectrum, with a trough at 390 to 394 nanometers and a peak at 415 to 420 nanometers, depending upon the buffer used (*14*) (Fig. 7). By measuring the magnitude of the changes in the spectra with varying concentrations of ethanol, we demonstrated that this binding conforms to saturation kinetics, the apparent dissociation constant being about 100 millimolar (*14*). Compounds such as aniline or pyrazole, which yield a type 2 binding spectrum, and which presumably bind to the heme iron (*8*), inhibit the binding of ethanol (*38*). In contrast, type 1 binders, such as hexobarbital, which are thought to bind to the protein moiety of the molecule (*8*), have no effect on the ethanol binding. Ethanol binding also inhibits the binding of aniline, but it has no effect on the binding of a type 1 binder. Thus, ethanol may compete with other type 2 binding compounds for sites on the hemoprotein.

Compounds which produce a change in the type 2 spectrum inhibit the rate of reduction of cytochrome P450 by NADPH, whereas type 1 binders facilitate this process (*39*). As might be expected from its binding spectrum, ethanol inhibits NADPH–cytochrome P450 reductase activity (*26, 38*).

The characteristics of ethanol-induced inhibition of drug-metabolizing enzymes also suggest that ethanol is closer to type 2 binders than to type 1. The inhibition constant for the hydroxylation of aniline, a type 2 binder, by ethanol is much smaller than that for the type 1 binders (*26*). In other words, ethanol is a far more potent inhibitor of the metabolism of compounds that produce a type 2 spectrum, than of those that produce a type 1 spectrum. Moreover, ethanol inhibits aminopyrene demethylase competitively and ethylmorphine demethylase in a mixed fashion, a pattern that is characteristic of inhibition by type 2 binders (*40*). By the same token, type 1 binders inhibit nitroreductase, but type 2 binders do not (*41*). Ethanol is similar to the latter in that it does not inhibit this enzyme activity.

## Conclusions

The induction of drug metabolism by the chronic consumption of ethanol may explain, in part, the accentuated tolerance of alcoholics, when sober, to drugs such as barbiturates. The inhibition of drug metabolism by acute administration of ethanol, in addition to effects of ethanol on the central nervous system, plays a role in the heightened sensitivity of inebriated persons to drugs.

Ethanol should be included in the list of drugs that are metabolized by hepatic microsomes. Chronic administration of ethanol and phenobarbital both produce the following: (i) increased SER, (ii) increased activities of drug-metabolizing enzymes in the hepatic microsomes, (iii) acceleration of its own metabolism, (iv) increased rate of metabolism of other drugs, and (v) increase in cholesterol biosynthesis (*42*).

Acute administration of ethanol or other compounds leads to inhibition of the metabolism of other drugs by hepatic microsomes and to interference with in vivo drug metabolism. Both ethanol and other drugs bind to microsomal cytochrome P450; simultaneous addition of certain drugs and ethanol to hepatic microsomes leads to mutual inhibition of microsomal binding.

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43. The original studies referred to in this paper were supported in part by U.S. Public Health Service grants MH 15558, AM 12511, and RR 71.