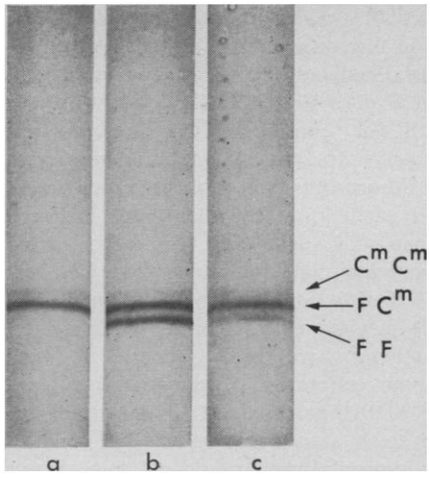

Fig. 1. Alcohol dehydrogenase zymograms of $Adh_1^F/Adh_1^{C(m)}$ kernel extracts. (a) Extract dialyzed against 0.01*M* carbonate-bicarbonate buffer, *p*H 10.0; (b) control, untreated extract; (c) extract incubated with root factor (see text). Origin is at the bottom and the anodal pole at the top.

the active SS, FF, and C^tC^t enzyme forms are irreversibly inactivated at the high *p*H (buffered with carbonate-bicarbonate or glycine-NaOH).

Our recent studies have established that a hybrid enzyme dimer which contains one C^m subunit is both active and stable at *p*H 10.0, as if the stable C^m subunit confers stability to the active subunit in the dimer. This is clearly shown by the following experiment. $Adh_1^F/Adh_1^{C(m)}$ heterozygous kernels were ground in a Wiley mill and the enzyme was extracted by steeping the meal in 0.005*M* sodium phosphate buffer, *p*H 8.0, for 15 minutes at room temperature, with occasional stirring (approximately 1 volume of meal to 2 volumes of buffer). The slurry was centrifuged at 40,000*g* for 10 minutes, and the supernatant was dialyzed overnight at 4°C against 500 volumes of 0.01*M* carbonate-bicarbonate buffer, *p*H 10.0. The dialyzed extract was subjected to starch gel electrophoresis at *p*H 8.3 and the gels were stained for alcohol dehydrogenase at *p*H 8.0 (Fig. 1a) as described in a previous publication (*1*). A control zymogram of an aliquot of the extract which had not been dialyzed at *p*H 10.0 is shown in Fig. 1b. After exposure to high *p*H the FC^m isozyme retains its activity, whereas the FF isozyme is almost completely inactivated. Both the FF and FC^m isozymes remain active when the dialysis is at *p*H 8.0. Similar results are obtained with the $Adh_1^S/Adh_1^{C(m)}$ heterozygotes. The FS, FC^t, and SC^t allodimers do not show this increased stability at high *p*H.

The FC^m dimer is not as stable to high *p*H as the C^mC^m isozyme. After overnight dialysis against the *p*H 10.0 buffer at a higher molarity (0.02*M*) both the FF and FC^m isozymes are completely inactivated while the C^mC^m isozyme retains its low level of activity.

This type of polypeptide interaction whereby an unstable subunit is stabilized by association in a dimer with a stable subunit can be shown to occur in vivo as well. Alcohol dehydrogenase activity in the seedling declines quite rapidly during germination (*3*). Zymograms of extracts from $Adh_1^F/Adh_1^{C(m)}$ seedlings show that the decay of the FF isozyme is considerably more pronounced than the FC^m isozyme as judged from the relative intensities of the two isozyme bands.

This difference in relative intensities of the FF and FC^m bands could also result if the FF and FC^m isozymes decay in vivo at the same rate, but in the seedling the $Adh_1^{C(m)}$ allele is more active in enzyme synthesis than Adh_1^F. If more C^m than F protomers are produced the ratio of FC^m to FF dimers formed would increase. To test this alternative explanation, an enzyme extract from $Adh_1^F/Adh_1^{C(m)}$ mature kernels, in which the FF and FC^m isozyme bands are of equal intensity, was incubated for ½ hour at room temperature with an equal volume of root extract which contains the factor which is responsible for the in vivo inactivation (*3*). Comparison of zymograms of control and treated extracts clearly shows the striking difference in retention of activity between the FF and FC^m isozymes (Fig. 1, b and c).

The implication of these findings to the problem of heterosis is obvious. In general, in order to function effectively in the growth and development of an organism an enzyme must be both active and stable. Through selection, either natural or artificial, alleles which specify such enzyme forms are fixed in the population. However, in the case of some enzymes, and perhaps even many, high stability and activity may be mutually exclusive (that is, no single primary structure can confer both high activity and high stability to the polypeptide). We propose that hybrid vigor may in part result from combining in heterozygotes alleles for active but relatively unstable enzyme forms with alleles which specify stable but inactive enzymes, and that the gene products interact to confer both stability and activity to the hybrid enzyme. It has recently been reported for nitrate reductase in maize that the F_1 hybrid (B 14 × Oh 43) resembles one parent in rate of enzyme synthesis and the other parent in the rate of in vivo decay (*4*).

This advantage of heterozygosity would of course be insured by gene duplication where both alleles are inherited as a unit and transmitted in single gametes. It is of interest in this regard that the only Adh_1 duplication which has been found combines the $Adh_1^{C(m)}$ allele which specifies the stable protomer, and an Adh_1^F allele which specifies a relatively unstable but active protomer. The two genes are very closely linked (*1*).

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Alcohol Metabolism: Role of Microsomal Oxidation in vivo

Abstract. *A role for microsomal alcohol oxidation cannot be demonstrated in vivo. Factors affecting microsomal drug hydroxylations had no effect on alcohol metabolism in the rat in vivo. The compound SKF 525-A which inhibits, and chronic phenobarbital treatment which enhances microsomal drug hydroxylations did not alter alcohol oxidation.*

Chronic ethanol administration produces a proliferation of the smooth endoplasmic reticulum (*1*) and an induction of microsomal mixed-function oxidase reactions (*1, 2*). Also, microsomal oxidations of alcohols such as ethanol and methanol have been described (*3, 4*). Ethanol oxidation in microsomes depends upon the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and oxygen, is inhibited by carbon monoxide, and is enhanced by certain inducing agents (*3, 5*). No reversal of carbon monoxide inhibition by light has yet been observed.

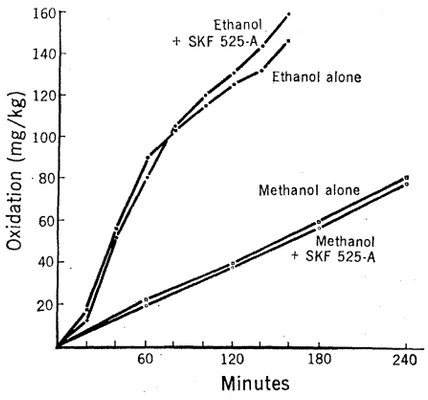

Fig. 1. Effect of SKF 525-A on ethanol-1-^{14}C and methanol-^{14}C oxidation in the rat in vivo. The SKF 525-A (130 μmole/kg) was administered intraperitoneally 15 minutes before intraperitoneal administration of ethanol (250 mg/kg) or methanol (1 g/kg). Expired ^{14}C-CO_2 was collected at timed intervals as described by Tephly *et al.* (*7*). Radioactivity was determined by standard liquid-scintillation techniques. Each point represents the mean value of four animals.

We have reviewed the process of alcohol metabolism with respect to the significance of this microsomal ethanol-oxidizing system in the oxidation of alcohols in vivo. Velocities for ethanol oxidation reactions have been reported to be 7.70 nmole per milligram of microsomal protein per minute in the untreated male rat, while male rats treated with ethanol provide about 9.95 nmole per milligram of microsomal protein per minute (*3*). Our microsomal preparations usually yield about 20 mg of microsomal protein per gram of liver (wet weight). Therefore, about 200 nmole of ethanol should be oxidized

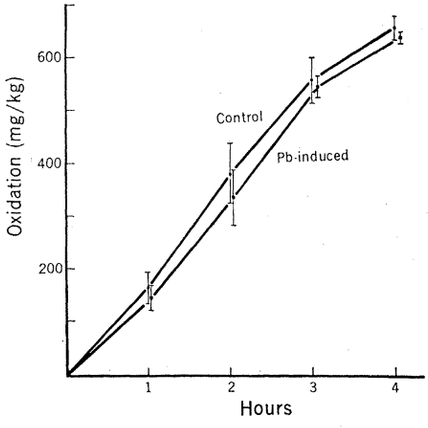

Fig. 2. Effect of phenobarbital (40 mg/kg) treatment (3 days) on subsequent oxidation of ethanol-1-^{14}C in the rat in vivo. Each point represents the average value of four animals ± standard error of the mean. The dose of ethanol was 1 g/kg injected intraperitoneally.

per gram of liver per minute or 12,000 nmole per gram of liver per hour. If we assume that, in the rat, the liver comprises about 4 percent of the body weight and convert to micromoles, we calculate that one may account for 480 μmole or 22 mg/kg per hour. Therefore, it would seem that, at best, alcohol oxidation by this microsomal system could account for about 7 percent of the total ethanol oxidized in the rat since the rate of ethanol oxidation in the rat is about 300 mg/kg per hour at high doses (*6*).

Ethanol oxidation in vivo in both the rat (*7*) and monkey can be inhibited (*8*) by 1-butanol, a fact which is consistent with the alternate substrate interaction of these alcohols with liver alcohol dehydrogenase (E.C. 1.1.1.1.) (LADH). Pyrazole, an inhibitor of LADH, decreases the metabolism of ethanol in vivo (*6, 9*). In the rat, where LADH is of lesser importance in methanol metabolism than in the monkey (*7, 8*) pyrazole exerts less inhibition (*9*).

The SKF 525-A does not affect the oxidation in vivo of ethanol-1-^{14}C and methanol-^{14}C in the male rat (Fig. 1) but at a dose of 130 μmole/kg SKF 525-A affects hexobarbital sleeping times and hexobarbital disappearance from the blood (*10*). Since SKF 525-A has a rather broad range of inhibitory actions on microsomal reactions (*11*), one would expect that if ethanol were oxidized significantly by a microsomal, mixed-function oxidase reaction in vivo, SKF 525-A would have exerted a measurable effect.

Treatment of rats for 3 days with phenobarbital sodium (40 mg/kg) has no effect on the subsequent oxidation of ethanol-1-^{14}C in vivo (Fig. 2). The *N*-dealkylation of ethylmorphine in microsomes from rats also treated with phenobarbital in this manner was increased threefold beyond that measured in control animals. If a microsomal, mixed-function oxidase reaction is involved in ethanol oxidation to a significant degree, oxidation of ethanol after phenobarbital treatment would have increased. This was not observed.

If ethanol is oxidized by a microsomal, mixed-function oxidase reaction similar to that described for many drugs, this system does not play a significant role in alcohol oxidation in vivo. This conclusion is based on the lack of effects of the phenobarbital treatment which usually enhances, and of the SKF 525-A treatment which usually inhibits, drug oxidation by hepatic microsomes. Since inhibitors and alternate substrates for LADH do drastically affect ethanol metabolism in vivo (*7–9*) the LADH system should still be considered the predominant catalyst for ethanol oxidation in vivo.

Microsomal alcohol oxidation may be carried out by a different reaction than that described for drugs. It is our view that the systems described for alcohol oxidation in microsomes (*3, 4*) in vitro represent a peroxidative reaction where H_2O_2 generated from some microsomal enzyme such as NADPH oxidase (*12*) is furnished to a small amount of catalase present in microsomal preparations. If this system is controlled by the rate of H_2O_2 generation, chronic treatment of rats with ethanol or phenobarbital may increase the amount of peroxide generated by the NADPH oxidase system and could explain microsomal alcohol oxidation in vitro. Enhancement of ethanol oxidation may represent enhancement of H_2O_2 generation. The view that hepatic microsomal alcohol oxidation depends on a catalase-H_2O_2 system is strengthened by recent studies by Roach *et al.* (*13*). They observed that the catalase inhibitor, 3-amino-1,2,4-triazole, inhibits microsomal ethanol oxidation and that NADPH can be replaced by a H_2O_2 generating system.

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