ply intense intraspecific producer competition and also a relatively low tendency for exploiters to become hungry or satiated and to modify their behavior accordingly. In such a system there is no tendency for extinction regardless of productivity.

However, if $(a + g)$ is less than 1, there is a positive V^* and

$$\frac{\partial R}{\partial V^*} = \frac{aQ(1-g)\ (V^*)^{a-1}}{(1-a-g)} \quad (3c)$$

Clearly Eq. 3c is always positive if V^* is biologically real. Hence, in model 3, if there is any threat of system extinction, it is increased by enrichment.

Models 1 and 6 are similar and most complex. It turns out that Eq. 6b is satisfied by two values of V. One is V^*. Another is a very small value of V that occurs over a trough in the V isocline. Thus, there is ambiguity in the following:

$$\frac{\partial \ln K}{\partial V^*} = \frac{(e^{cV^*} - 1)\,(e^{cV^*} - 1 - cV^* - c^2V^{*2})}{(e^{cV^*} - cV^* - 1)^2} \quad (6c)$$

This equation, set to zero, holds for both V^* and the V under the trough. The unstable equilibrium values of V are those between V (trough) and V^*. Model 6c is positive for V^* and negative for V (trough) (*9*). Hence, as enrichment proceeds, the range of unstable V is increasing at both ends. Therefore again, enrichment unambiguously tends to weaken the steady state. Model 1 has the same characteristics (*9*).

Until we are confident that the conclusions based on these systems do not apply to natural ecosystems, we must remain aware of the danger in setting enrichment as a human goal.

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11. I thank Drs. E. Leigh, III, S. Levin, D. McNaught, L. Segel, and M. Slatkin for valuable comments; and J. Riebesell, whose careful work was helpful in making mathematical errors scarce, if not entirely absent. Supported by the National Science Foundation and the Research Foundation of the State University of New York.

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Induction of Liver Acetaldehyde Dehydrogenase: Possible Role in Ethanol Tolerance after Exposure to Barbiturates

Abstract. *Mice were injected twice a day for 4 days with saline or phenobarbital or ethanol. Treatment with phenobarbital, but not ethanol, increased the amount of liver acetaldehyde dehydrogenase activity. More rapid removal of acetaldehyde, which is a toxic metabolic intermediate of ethanol, may contribute to the alcohol tolerance exhibited by persons who use barbiturates regularly.*

Acetaldehyde, a pharmacologically active metabolite of ethanol, appears to contribute to the actions of ingested ethanol (*1*). Acetaldehyde can induce nausea, vomiting, and sweating; it causes release of catecholamines and depression of oxidative phosphorylation in isolated brain tissue. Recently it was shown that acetaldehyde condenses with catecholamines (*2*) or potentiates a similar condensation by certain endogenous aldehydes (*3*) to form isoquinoline alkaloids that may possess biologic activity. Because the pharmacologic effects of acetaldehyde are generally subjectively unpleasant, it has even been suggested that "while ethanol actions may be the reason that people drink alcohol, the actions of acetaldehyde may be more related to why they stop" (*1*).

The purpose of our study was to see if phenobarbital, an inducer of many liver enzymes (*4*), would increase the levels of acetaldehyde dehydrogenase (AcDH), which is believed to be the enzyme primarily responsible for detoxication of acetaldehyde. We were interested in the tolerance to the effects of ingested alcoholic beverages exhibited by persons who use barbiturates regularly. If AcDH activity were elevated after exposure to barbiturates, the consequent more rapid removal of acetaldehyde could contribute to ethanol tolerance. Our results with mice support this hypothesis.

Male and female Paris R-III or C-57BL mice were injected intraperitoneally twice a day (at approximately 9:30 a.m. and 4:30 p.m.) for 4 days with either isotonic saline (buffered at *p*H 7.4 with 0.01*M* sodium phosphate) or with sodium phenobarbital (75 mg/kg) or with ethanol (2.4 g/kg, administered as a 30 percent by volume solution), each dissolved in the isotonic buffer. On day 5, the animals were killed by cervical dislocation, and the liver was homogenized (ten strokes in a Teflon and glass homogenizer) in 20 volumes of ice-cold water. The homogenate was kept at about 0°C for the duration of the experiment. Cell debris was removed by centrifugation at 700*g* for 5 minutes. Enzyme assays were completed within 2 hours after death.

Acetaldehyde dehydrogenase was assayed at room temperature by a modification of the method of Maxwell and Topper (*5*). Assay tubes contained 6 ml of buffer (0.1*M* glycine at *p*H 9.5, containing 4.0 m*M* mercaptoethanol and 2.0 m*M* ethylenediaminetetraacetic acid), 0.4 ml of nicotinamide adenine dinucleotide (NAD) solution (Calbiochem, 10 mg/ml), and 0.1 ml of acetaldehyde solution (Eastman, 1 percent by volume in water). A blank containing all of the above except acetaldehyde was used. The reaction was started by the addition of 0.2 ml of liver extract. Reduced NAD (NADH) concentration was measured by the optical density at 340 nm in a Gilford model 300 spectrophotometer after 3.5 and at 7.5 minutes; enzyme activity was given by the increase in optical density during the 4-minute interval, with the blank substracted.

In three separate experiments, increases in liver AcDH activity were observed after 4 days of treatment with phenobarbital, but AcDH activity was not increased after treatment with ethanol. Pooled results of the three experiments (Fig. 1) indicate that

AcDH activity was doubled ($P < .001$) after phenobarbital treatment. In order to express the enzyme activity in terms of its potential action in the whole animal, data were calculated as total liver AcDH activity (measured by the NADH increase) divided by the body weight. When the results were expressed as NADH increase per gram of liver, a 60 percent increment in AcDH activity ($P < .001$) was evident; thus the apparent increase in AcDH activity was not due to changes in liver size or body weight.

We were concerned about the difficulty in obtaining absolute estimates of AcDH activity because alcohol dehydrogenase in liver homogenates can remove NADH by catalyzing the reduction of acetaldehyde. Apparent changes in AcDH activity between experimental groups might reflect changes in alcohol dehydrogenase activity. We verified our result in several ways.

Separate analyses of alcohol dehydrogenase, performed with a modification of the method of Rodgers *et al.* (*6*), showed no significant difference between control animals (average, 0.108 optical density unit) and phenobarbital-treated animals (average, 0.104 optical density unit). This indicated that the apparent increase in AcDH activity was not due to a decrease in alcohol dehydrogenase activity. Another experiment was performed with a group of 18 female mice of both species. The mice were equally divided between control and phenobarbital-treated groups, and their livers were assayed for enzyme activity. However, the reaction mixture for the enzyme assay in this experiment also contained 1 m*M* pyrazole (Eastman), an inhibitor of alcohol dehydrogenase (*7*, *8*). Results confirmed the original observations; the observed AcDH activity levels were increased 140 percent after phenobarbital treatment ($P < .001$).

The potential contribution of NADH oxidase activity was evaluated separately. When acetaldehyde was omitted from the reaction system and a small amount of NADH (about 0.1 optical density unit) was added, little or no loss in NADH was observed; the amount of NADH added was in the range of the upper limit of NADH generation during the AcDH assay. When both acetaldehyde and NAD were omitted and larger amounts of NADH (about 0.8 optical density unit) were added, loss in NADH was observed. However, the activity was randomly distributed among the animals and did not show an inverse relation to the AcDH activity; that is, increased AcDH activity could not be attributed to a decreased rate of removal of NADH from the system. Moreover, the NADH oxidase activity was not decreased in the phenobarbital-treated group but appeared, in fact, to be slightly increased (average, 0.095 optical density unit) over the controls (average, 0.077 optical density unit). We conclude that interference by NADH oxidase could not have caused the apparent increase in AcDH activity.

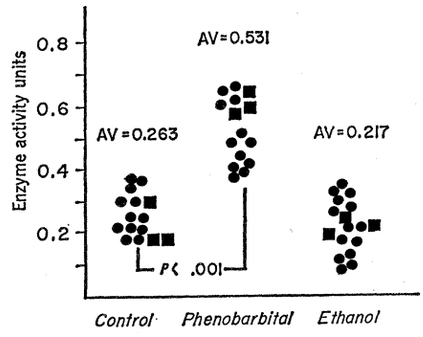

Fig. 1. Liver acetaldehyde dehydrogenase activity in control, phenobarbital-treated, and ethanol-treated mice. Squares are C-57BL mice, and circles are Paris R-III mice. Data are NADH (optical density) produced in 4 minutes, corrected for the total liver weight and divided by the body weight (activity per gram body weight). Although subgroups were small, increased enzymatic activity after phenobarbital treatment was evident when the data were organized by sex and species.

Phenobarbital is known to induce a variety of enzymes associated with the smooth endoplasmic reticulum (*4*, *9*). We looked for acetaldehyde-oxidizing activity of the type associated with the endoplasmic reticulum (microsomes) in our liver homogenates. The microsomal mixed-function oxidases require oxygen and reduced nicotinamide adenine dinucleotide phosphate (NADPH) as cofactor. We did observe an acetaldehyde-dependent oxidation of NADPH in neutral solution (*p*H 7.4) in our crude liver homogenates. However, this activity could not be blocked by carbon monoxide, an inhibitor of the microsomal oxidases; but it was blocked by *o*-phenanthroline, an inhibitor of alcohol dehydrogenase. Similar results were obtained with crystalline horse-liver alcohol dehydrogenase (Worthington). These observations are consistent with the back reaction for alcohol dehydrogenase with NADPH as cofactor (*10*).

Persons who regularly take barbiturates or ethanol develop a tolerance to the drug that they take (*11*). In addition, barbiturate users become cross-tolerant to ethanol while alcoholics become cross-tolerant to barbiturates. (Cross-tolerance is observed when one drug is taken alone after earlier exposure to the other drug; when both drugs are taken together, the combined effects are dangerous and can be lethal.) There appear to be two mechanisms for tolerance. One is increased metabolism of the drug, and the other is diminished response in the nervous system (*11*). Because phenobarbital and other barbiturates induce those enzymes that are responsible for their own metabolism, it is believed than a more rapid removal of the drug contributes to the tolerance of barbiturate users toward barbiturates (*4*, *9*, *11*).

We have shown that phenobarbital increases the activity of AcDH in the livers of mice. The formation of acetaldehyde from ethanol, mediated by alcohol dehydrogenase, is generally considered to be the slowest or rate-limiting step in ethanol metabolism (*12*). Therefore, increased AcDH activity would not be expected to materially affect the overall rate of ethanol disappearance. However, it should result in lower steady-state levels of the pharmacologically active acetaldehyde in tissues. This, in turn, could be a contributory factor to the cross-tolerance to the effects of ingested ethanol exhibited by barbiturate users.

Rubin and Lieber (*13*) have shown both in the rat and in man that ethanol induces the liver enzymes that metabolize barbiturates and suggest that this contributes to the cross-tolerance of alcoholics for barbiturates. These observations and our finding of increased AcDH activity after phenobarbital treatment indicate that enzyme induction may underlie both sides of cross-tolerance.

A recent report (*14*) has confirmed and extended our observation (*15*) of increased AcDH activity after phenobarbital administration.

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Aggregations of 35-Nanometer Particles Associated with Neuronal Cytopathic Changes in Natural Scrapie

Abstract. *Neuronal vacuolation and intravacuolar budding of vesicles and cytoplasmic processes appear to be the most characteristic cellular lesion in natural scrapie, a chronic degenerative disease of the central nervous system of sheep which is transmissible by injection. Membrane-bound accumulations of the 35-nm particles are found in the cytoplasmic processes that project inside the vacuoles of the neuronal perikaryon. Such particles are present only in a small number of vacuolated neurons.*

Scrapie is a progressive degenerative disease of the central nervous system of sheep. It has been experimentally transmitted by injection to sheep (*1*), goats (*2*), and, more recently, to small laboratory animals (*3*) after long incubation periods. Vacuolation of neurons is a characteristic of the disease (*4*). In the present study, five sheep affected with scrapie and seven normal sheep were satisfactorily perfused with glutaraldehyde for electron microscopy.

Large membrane-bound vacuoles were found within neuronal cell bodies, dendrites, and axonal terminals in the medulla, pons, cerebellar cortex, supraoptic nucleus of the hypothalamus, paramammillary nucleus, and pyramidal layer of the hippocampus. Vacuolation of glial cells was not observed. Small invaginations indicative of pinocytosis were found along the vacuolar membrane, which suggests that it possessed some of the features of a plasma membrane.

The most striking feature was that of vesicles 100 to 150 nm in diameter, and of multiple cytoplasmic projections budding inside the vacuole (Fig. 1). In some vacuolated neurons these projections contained membrane-bound accumulations of round particles of uniform size, 35 nm in diameter (Fig. 2). Some of the particles displayed a dense central core. Others had an electron-lucent center (Fig. 2, inset). Particles of similar size have been observed in large amounts within the distended neuronal cell processes of the cerebral cortex of mice injected with experimental scrapie material (*5*). These particles lay free in the cytoplasm and had electron-lucent centers. They were not thought to represent the scrapie agent (*5*), mainly because of the discrepancy between the size of the particles and the size of the scrapie infectious agent as determined by radiation experiments. The scrapie

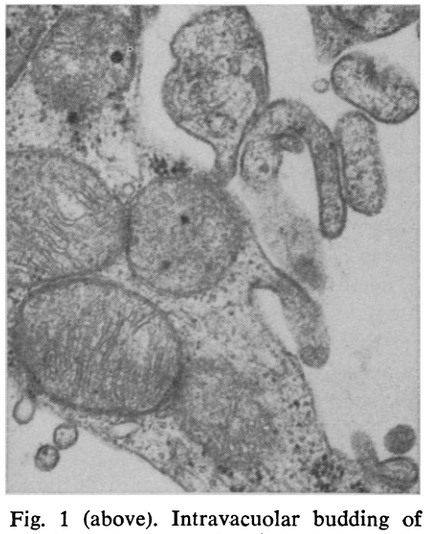

Fig. 1 (above). Intravacuolar budding of vesicles and of cytoplasmic processes in a pontine neuron (× 31,000).

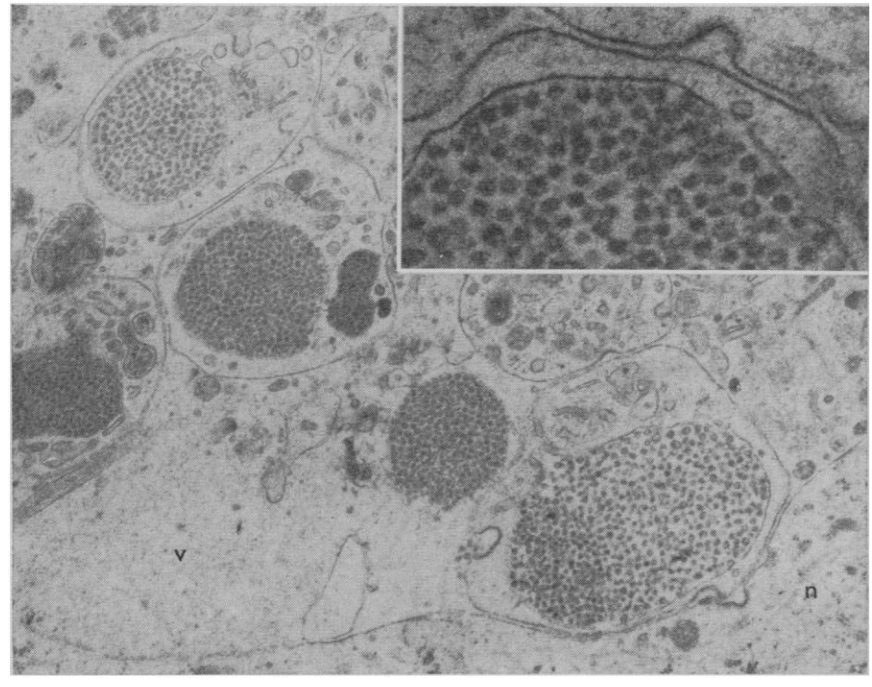

Fig. 2 (right). Membrane-bound collections of 35-nm particles within a vacuolated neuron (*n*) in the supraoptic nucleus of the hypothalamus. These collections are contained in cytoplasmic processes extending inside the vacuole (*v*) (× 21,000). (Inset) An electron dense core is recognizable in some of the particles (× 53,000).