known phenolic alkaloid as the hydrochloride were unsuccessful, so we obtained nuclear magnetic resonance (NMR) spectra with the free base (*21*). Mass spectrometry revealed a large molecular ion peak at *m/e* 225.136 ($C_{12}H_{19}O_3N$) and fragmentation (substituted tropylium ion at *m/e* 167.072) consistent with a proposal from the NMR data that the unknown was *N,N*-dimethyl-3-hydroxy-4,5-dimethoxy-β-phenethylamine (3-demethyltrichocereine). This compound was synthesized (*22*) and crystallized as the oxalate. The melting point and mixed melting point of the isolated [yield, 10.0 mg (0.000018 percent)] and synthetic oxalates were identical (155° to 156°C), and the NMR spectra of the synthetic base were essentially the same as those of the isolated base.

Lundstrom has recently detected 3-demethyltrichocereine in extracts of *L. williamsii* (*2*), and, with the exception of *N*-methyl-3,4-dimethoxy-β-phenethylamine, all of the eight alkaloids we have identified in *P. aselliformis* have been previously detected in *L. williamsii*. This is the first report of the presence of mescaline in any North American cactus species other than peyote, although mescaline has been found in several South American cacti (*11*). The presence of these alkaloids would seem to verify the reported native uses of the plant as a drug. However, the fact that the content of mescaline detected in *P. aselliformis* is so small (< 0.00002 percent versus > 1 percent in dried peyote) (*1*) casts doubt on this speculation (*23*). Peyote alkaloids are definitely present in this cactus, but it is questionable whether these alkaloids are present in concentrations sufficient to cause physiological effects upon ingestion.

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20. Mass spectrometry was performed with an AEI model MS 9 mass spectrometer, direct inlet at 300°C and 70 ev [see Creveling *et al.* (*18*)].
21. Nuclear magnetic resonance spectra were obtained with a Varian T-60 spectrometer with the use of deuteriochloroform in a cylindrical microtube. Exchange with deuterium oxide revealed a phenolic proton.
22. We thank Drs. S. Teitel and A. Brossi of Hoffmann–La Roche, Inc., for synthesizing this compound.
23. Extraction of freshly uprooted *P. aselliformis* yielded similarly small quantities of the alkaloids, thus discounting the possibility that a depressed physiological condition of the original plants might have caused a lower alkaloid content. The possibilities still exist that geographical, seasonal, and strain variations might affect the amount of alkaloid accumulated.

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Ethanol Oxidation: Effect on the Redox State of Brain in Mouse

Abstract. *Administration of a single large dose of ethanol to mice results in increases, for concentrations in the brain, of ratios of lactate to pyruvate, of α-glycerophosphate to dihydroxyacetone phosphate, of malate to oxaloacetate, and of glutamate to the product of α-ketoglutarate and ammonium ion. These changes are noticed as early as 5 minutes after the single dose is given. Ethanol administration for 30 days also produces these changes in metabolite concentrations in the brain. However, in contrast to the single alcohol dose, long-term alcohol administration results in a marked decrease in the concentration of adenosine triphosphate in brain and increases in those of adenosine diphosphate and adenosine monophosphate. Pyrazole, an inhibitor of alcohol dehydrogenase, prevents the effects of ethanol on the concentration of brain metabolites. These results may provide new insight into the biochemical and pharmacological effects of alcohol on brain metabolism and the importance of alcohol dehydrogenase activity in the brain.*

The dominant role of the liver in the metabolism of ethanol is documented (*1*). However, by the use of sensitive techniques (*2*) it has been demonstrated (*3*) that the brain also oxidizes small amounts of ethanol. Alcohol dehydrogenase from brain has been isolated and its kinetics studied (*3*). In liver, when ethanol is being metabolized the "redox state" is shifted toward greater reduction. Oxidation of ethanol in liver, catalyzed by alcohol dehydrogenase, results in an increased ratio of reduced nicotinamide adenine dinucleotide (NADH) to the oxidized form (NAD). The value of this ratio at the site of oxidoreductions is important because it bears on the metabolism of oxidizable and reducible substrates. We investigated the effect of ethanol oxidation on the redox state of brain as a means of understanding the most dramatic biochemical and pharmacological actions of ethanol on the central nervous system.

In the study reported here, the effects of a single large dose of ethanol and of long-term ethanol administration on the concentrations of certain dehydrogenase-linked redox pairs has been investigated. Albino Swiss mice (25 to 28 g) were used. In experiments where the effects of a single alcohol dose was studied, mice were divided into two groups. One group received ethanol (3 g/kg, intraperitoneally) as a 25 percent solution in water, and the other group received equal

Table 1. Effect of ethanol, administered once or for 4 weeks, on the redox state of brain. Metabolite concentrations in the brain (in micromoles per gram of brain) were measured enzymatically; the concentrations were averaged and the ratio calculated. Results are given as the mean ± standard error of the mean, the values being from 12 animals in each case.

Mice given one large dose			Mice treated for 4 weeks	
Control	Alcohol	Pyrazole and alcohol	Control	Alcohol
Lactate/pyruvate (μmole/μmole)				
15.1 ± 0.5	18.9 ± 0.4	15.8 ± 0.6	15.3 ± 0.5	19.1 ± 0.6
α-Glycerophosphate/dihydroxyacetone phosphate (μmole/μmole)				
5.2 ± 0.10	5.9 ± 0.05	5.2 ± 0.09	5.3 ± 0.1	5.9 ± 0.07
Malate/oxaloacetate (μmole/μmole)				
50.0 ± 1.9	51.6 ± 1.5	50.3 ± 1.9	50.1 ± 1.9	51.4 ± 1.1
Glutamate/(2-oxoglutarate × NH_4^+) (μmole/μmole²)				
40.8 ± 1.8	46.9 ± 1.6	41.0 ± 2.0	41.0 ± 1.0	46.5 ± 1.0

volumes of physiological saline. For th study of the effect of long-term ethanol administration the mice were pair-fed on liquid diet for 4 weeks. The liquid diet consisted of 61 percent Metrecal and 6 percent ethyl alcohol or isocaloric sucrose (values are weight by volume). Mice were killed by total immersion in liquid nitrogen (*4*). Unless otherwise stated a piece of skin above the skull was removed 48 hours before animals were killed. With this technique (*5*) a fast drop in the cortical temperature is obtained when animals are immersed in liquid nitrogen. Brain samples were powdered in a cold mortar with added liquid nitrogen. The powdered brain was extracted in a glass homogenizer containing cold 6 percent $HClO_4$. The homogenized tissue was centrifuged at 0° to 4°C and the sediment was reextracted with 2 percent $HClO_4$ and centrifuged again. The two supernatants were pooled and neutralized with K_2HCO_3. The neutralized supernatant was used for the enzymatic estimation of various metabolites (*6*). The effect of pyrazole on ethanol metabolism in the brain was studied in some experiments. Pyrazole (90 mg/kg) was given intraperitoneally, and animals were killed after 20 hours (*7*).

Intraperitonal injection of ethanol (3 g/kg) in mice resulted in increases in the concentrations of lactate, α-glycerophosphate, malate, and glutamate in the brain as early as 5 minutes after the infection. Concentrations of pyruvate, dihydroxyacetone phosphate, oxaloacetate, and 2-oxoglutarate in the brain decreased as compared to those of control animals. The ammonia concentration in the brain, however, remained unchanged. Thus the ratios of the concentrations of lactate to pyruvate, of α-glycerophosphate to dihydroxyacetone phosphate, of malate to oxaloacetate, and of glutamate to the product of 2-oxoglutarate and NH_4^+ showed small but significant ($P < .002$) increases (Table 1) in brains of mice treated with single ethanol doses as compared with brains of corresponding controls. Changes observed in metabolite concentrations in the brain 10 and 15 minutes after ethanol injection were similar to those observed 5 minutes after ethanol was given.

However, in mice that had been treated with pyrazole this effect of ethanol on metabolite concentrations in the brain was not observed (Table 1). Pyrazole inhibits alcohol dehydrogenase activity both in liver (*8*) and brain (*3*).

Table 2. Effect of ethanol, administered once or for 4 weeks, on adenine nucleotide concentrations in brain. Concentrations of adenine nucleotides were determined fluorometrically and are expressed as micromoles per gram of brain tissue. Results are given as the mean ± standard error of the mean and are for six animals in each case.

Adenine nucleotide	Mice given one large dose		Mice treated for 4 weeks	
	Control (μmole/g)	Alcohol (μmole/g)	Control (μmole/g)	Alcohol (μmole/g)
ATP	2.2 ± 0.08	2.1 ± 0.07	2.3 ± 0.09	1.85 ± 0.05
ADP	0.40 ± 0.02	0.43 ± 0.02	0.40 ± 0.02	0.78 ± 0.03
AMP	0.27 ± 0.03	0.28 ± 0.02	0.27 ± 0.02	0.42 ± 0.02

Feeding ethanol to mice for 4 weeks also results in increases, for concentrations in brain, of ratios of lactate to pyruvate ($P < .001$), of α-glycerophosphate to dihydroxyacetone phosphate ($P < .05$), of malate to oxaloacetate ($P < .05$), and of glutamate to the product of 2-oxoglutarate and NH_4^+ ($P < .001$). The changes observed in metabolite concentrations in the brains of animals after long-term alcohol treatment are not significantly different than those observed after animals were injected with alcohol. The decrease in the concentration of adenosine triphosphate (ATP) ($P < .02$) and the increases in concentrations of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) ($P < .01$) observed in brains of animals fed ethanol for 4 weeks (Table 2) can be probably attributed to the ethanol-induced increase in activity of adenosine triphosphatase in brain (*9*). The suppressive effect of long-term ethanol treatment on hepatic ATP concentrations has been observed (*10*).

The changes caused by ethanol in the concentration ratios of various dehydrogenase-linked substrates in brain are significantly smaller than those observed in liver in vivo (*6*). However, this is in good agreement with the low rates of ethanol oxidation by the brain and the low activity of alcohol dehydrogenase in brain, about 1/4000 of that observed in liver (enzyme activity expressed per weight of wet tissue) (*3*). These data indicate that ethanol, administered in a single dose or for many weeks, results in an increase in the ratio of reduced to oxidized metabolites in the brain. The fact that pyrazole, an inhibitor of alcohol dehydrogenase, prevented the effect of ethanol on the redox state of brain, suggests that the ethanol-induced changes in the redox state are mediated by ethanol metabolism catalyzed by this enzyme in brain. Our observations are at variance with a report (*11*) that ethanol had no effect on the ratio of NADH to NAD in rat brain. However, those results can not be compared with ours, since the brains were removed from rats after cervical dislocation rather than after rapid total immersion in liquid nitrogen. We emphasize that rapid freezing of the brain in situ should be done when the labile metabolites in the brain are measured. The concentrations of metabolites in the brain are subject to alteration during the period between removal of tissue and freezing, which halts metabolism.

After ethanol administration, the changes observed in the steady-state concentrations of metabolites may significantly influence regulatory processes in brain. Such alterations may be the cause of the acute pharmacological action of alcohol on the central nervous system and may be associated with the neural disorders observed during chronic alcohol consumption.

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Age Changes in the Neuronal Microenvironment

Abstract. *The extracellular space of the rat brain was visualized by electron microscopy in sections of cerebral cortex fixed by freeze-substitution. The volume of tissue occupied by the extracellular space was estimated stereometrically, and was found to decrease from 20.8 percent in 3-month-old rats to 9.6 percent in senescent animals, 26 months of age. This decrease in extracellular space indicates an age-associated change in the microenvironment of nerve cells.*

The extracellular tissue compartment of the brain and spinal cord (extracellular space) is composed primarily of submicroscopic channels between cellular elements (*1*). It is believed to be the site of physiologically important reactions, involving metabolites and ions, upon which neuronal metabolism depends; it thus constitutes the microenvironment of neurons (*2*). Its submicroscopic distribution appears to be more adequately preserved by freeze-substitution than by more traditional methods of chemical fixation (*1*, *3*), and its volume is readily estimated by the stereometric assessment of electron micrographs of brain specimens preserved by freeze-substitution (*1*). In the normal adult rat the extracellular space occupies about 22 percent of the volume of superficial cerebral cortical tissue (*1*).

It has been demonstrated that the volume of the extracellular space in mammalian brain changes gradually during the course of early postnatal development (*1*). In the cerebral cortex of the rat, the volume of the extracellular space changes from about 40 percent during the first week after birth, to about 35 percent during the second week (*1*). During the third week, when electroencephalographic activity acquires adult-like characteristics (*4*), the volume of extracellular space was estimated to be about 26 percent, which closely approaches the volume in the adult (*1*). Under normal conditions, the volume of the extracellular space in the brain of the rat appears to remain relatively unchanged until 22 to 24 months, when senescent characteristics become evident (*5*).

The extracellular space of the brain appears to contain certain anionic substances that may be glycoproteins or mucopolysaccharides (*2*). In this regard, it resembles the ground substance of connective tissues (*6*). Because the ground substance of connective tissues condenses, becomes less hydrated, and is replaced by collagen as animals age (*7*), we asked if the extracellular space of brain also changed in the latter part of the life span. The apparent role of the extracellular space in neuronal nutrition further impelled our question.

Eleven male Sprague-Dawley rats were obtained commercially at 1 month of age, housed in the animal care facility of Northwestern University, and fed a standard chow diet with freely available water. At the time of this study, eight animals were 26 months old and three were 3 months old. They were killed by decapitation after which the calvaria and dural membranes were rapidly removed, and the brain was exposed. A thin slice of cerebral cortex, from the parieto-occipital region near the midline, was excised and frozen within 30 seconds in Freon-22 that had been previously chilled with liquid ni-

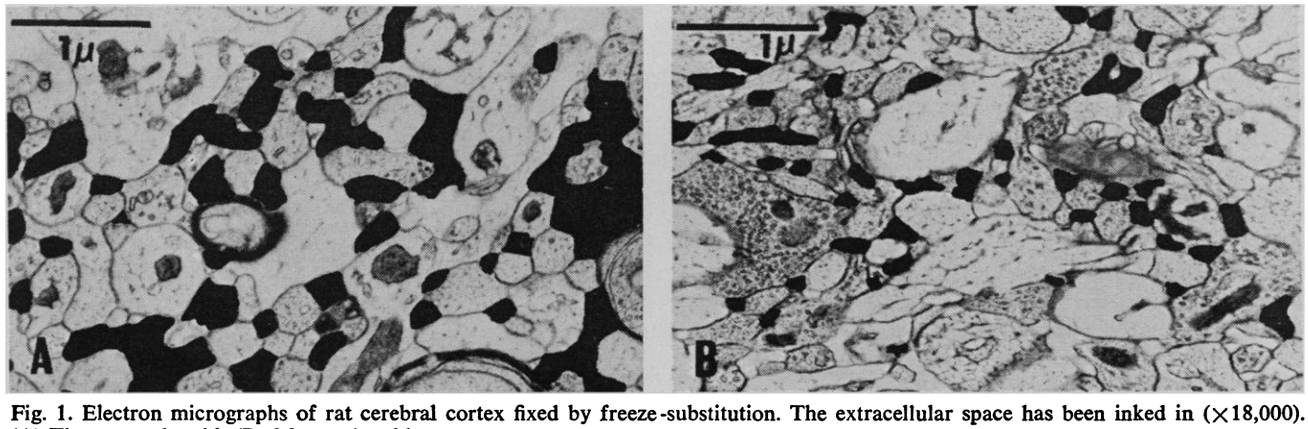

Fig. 1. Electron micrographs of rat cerebral cortex fixed by freeze-substitution. The extracellular space has been inked in (×18,000). (A) Three months old; (B) 26 months old.