Table 1. Scoring system for graded withdrawal reaction. "Tail lift" (column 1) was defined as raising the tail over the back, one of the abnormal postures used in Freund's grading of withdrawal signs in mice (*5*). "Startle to noise" was elicited by a sharp tap on the cage.

Sign	Score	Minimum degree	Maximum degree
Lethargy	0– 4	Slow movement, dragging limbs	Coma
Tremor	0– 4	Twitching, occasional trembling	Continuous tremor
Tail lift	0– 4	One per hour	Four per hour
Startle to noise	0– 4	Twitch	Convulsion
Convulsion on handling	0– 4	Mild, tonic	Severe, clonic
Spontaneous convulsion	0–10	Mild, tonic; jumping	Three or more generalized tonic-clonic convulsions per hour
Death	10		Death

scoring each group of mice. Two observers sometimes record mean scores that differ by one point; usually they agree more closely.

The two experiments shown in Figs. 1 and 2 illustrate the uses of this technique. Swiss-Webster male mice (34 to 44 g) were used. With air alcohol concentrations of 12 mg/liter (and daily pyrazole at 1 mmole/kg), the blood alcohol stayed at 1.5 to 1.8 mg/ml for 4 days (Fig. 1, curve A). It took 24 hours to reach this level. Vapor concentrations of 17 to 18 mg/liter, with the same pyrazole dose, resulted in a rise of blood alcohol levels to 3.6 mg/ml after 2 days (Fig. 1, curve B). During the alcohol exposure, the mice were lethargic and ataxic, and they were limp when handled. They did not show convulsions (spontaneous or elicited by handling), abnormal posture, or startle responses, as were seen during the withdrawal reaction.

Figure 2 shows the withdrawal reaction in the same mice. The maximum intensity of the syndrome occurred approximately at the time that alcohol disappeared from the blood. This was at about 4.5 and 9 hours in experiments A and B, respectively, with an alcohol elimination rate of 0.40 mg/ml in both. The syndrome regressed slowly over the next 24 hours. The overall intensity of the reaction is represented by the area under the curve (score × hours). Areas under the curves for individual mice were calculated from the scores and time intervals. The mean areas and standard errors were computed for each experiment: 34.0 ± 4.7 for experiment A; 66.4 ± 13.5 for experiment B. The difference is significant ($P < .05$). The higher blood alcohol level achieved in experiment B thus led to a more severe withdrawal reaction, although the duration of that experiment was shorter and the total alcohol intake was less than in experiment A. (The total alcohol exposure was computed as the area under the blood alcohol curves for individual mice, in [milligrams per milliliter] × hours. The mean area was 148.9 ± 5.7 in experiment A and 118.9 ± 5.4 in experiment B.)

Because pyrazole is toxic (*8*), we are concerned about its contribution to the withdrawal reaction. Mice lose weight during the alcohol-pyrazole treatment. There was a 10 percent loss of body weight in experiment A and a 14 percent loss in experiment B. Either alcohol or pyrazole alone at these doses caused some weight loss. To assess the role of pyrazole, we subjected two groups of mice to comparable amounts of alcohol (blood levels of 1.8 mg/ml for 3 days) with and without pyrazole, using different vapor concentrations. Typical withdrawal signs were seen in both groups. After administration of pyrazole alone for several days, no such signs were seen.

The vast literature on voluntary consumption of alcohol by laboratory animals shows that animals do not consume enough ethanol in the drinking water to produce physical dependence. Indeed, they rarely achieve intoxicating blood alcohol levels at all. Our simple technique allows sufficient alcohol intake for production of physical dependence. It is much more convenient and less stressful than intravenous infusion, and it can be used for large numbers of animals. We have here shown that physical dependence to ethanol in mice can develop in as short a time as 2 days. The time scale is thus similar to that of known physiological regulatory processes, which we postulate to be the mechanism of physical dependence. The techniques described here have been used in a study of the possible relationship between alcohol dependence and opiate dependence in mice (*9*).

DORA B. GOLDSTEIN
NANDITA PAL
Department of Pharmacology, Stanford University School of Medicine, Stanford, California 94305

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Alcohol Dependence and Opiate Dependence: Lack of Relationship in Mice

Abstract. *According to a recently proposed hypothesis, physical dependence upon alcohol is due to the formation of an endogenous opiate. We tested the hypothesis by determining whether or not ethanol-dependent mice would show typical opiate-dependent behavior (withdrawal jumping syndrome) when challenged with the opiate antagonist naloxone. Our results do not support the hypothesis.*

It has been known since 1963 (*1*) that mammalian liver converts dopamine to tetrahydropapaveroline (THP), a benzylisoquinoline alkaloid that is an intermediate in morphine biosynthesis in the opium poppy (*2*). The further

conversion of THP to an opiate related to morphine has not yet been demonstrated in any mammalian tissue. Davis and Walsh (*3*) showed that THP is formed from dopamine in rat brain homogenate and that this conversion is somewhat enhanced by ethanol or acetaldehyde. They advanced the hypothesis that the formation of an opiate might be responsible for the addictive properties of ethanol in vivo.

The improbability of this hypothesis has already been pointed out (*4*). Moreover, the increase in THP synthesis from dopamine demonstrated by Davis and Walsh (*3*) was very modest indeed. In the presence of 100 m*M* ethanol, a concentration that would be in the lethal range in man, the increase was only 20 percent. And it was only 25 percent in the presence of 0.5 m*M* acetaldehyde, a concentration 20 times higher than attained in human blood after a large intake of ethanol (*5*); however, the acetaldehyde concentration in liver, where it is formed, could possibly be much higher than it is in blood.

A direct test of the hypothesis can be carried out in mice. D. B. Goldstein (*6*) has described a reliable method for making mice physically dependent upon ethanol by housing them in a constant atmosphere of ethanol vapor for a number of days. Mice can be made physically dependent upon opiates, either by frequent injections or by subcutaneous implantation of a morphine pellet (*7*). When the opiate is withdrawn, such animals display various signs, the most remarkable of which is jumping activity, best elicited with the narcotic antagonist, naloxone (*8*). The median effective dose (ED_{50}) for naloxone-induced jumping is a good graded measure of the degree of dependence: the more dependent the animals, the lower the necessary dose of naloxone. According to the Davis-Walsh hypothesis, therefore, mice that have been made dependent upon ethanol—and that are supposed to be dependent upon the putative opiate derived from dopamine—should display the jumping behavior that is typical of opiate withdrawal if they are challenged with naloxone.

Two experiments were carried out, each with 20 male Swiss-Webster mice (33 to 42 g) housed in the ethanol chamber (*6*). In experiment A the airflow and ethanol vaporizer setting were adjusted to produce a constant mild

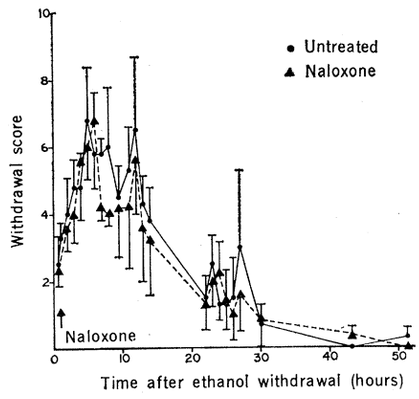

Fig. 1. Alcohol withdrawal syndrome in mice after 6 days in the ethanol chamber. Mean withdrawal scores and standard errors are shown for five mice that received no treatment after withdrawal from ethanol, and four mice treated with naloxone (55 mg/kg, intraperitoneal) 1 hour after withdrawal from ethanol (experiment B). Scoring is based on intensity of the following signs: lethargy, tremor, raising of the tail, startle to noise, convulsive behavior and facial grimace on handling, spontaneous convulsions, and death (*6*).

degree of intoxication, as evidenced by mild ataxia and sluggish righting reflexes. In experiment B periodic measurements were made of the air and blood alcohol concentrations by using the yeast alcohol dehydrogenase method with photometric assay of NADH (reduced nicotinamide adenine dinucleotide) (*9*). The air alcohol concentration was maintained at about 14 mg/liter and the blood alcohol levels varied within the range of 1.5 to 3.0 mg/ml. Every morning for 6 days the animals were removed from the chamber, weighed, injected intraperitoneally with pyrazole (1 mmole/kg), and duplicate 10-μl blood samples were drawn from the tail tips. Blood samples were also drawn every afternoon. In the course of the experiments the animals lost, on the average, 16 percent of body weight. In experiment B the mice were kept in a more intoxicated state than in experiment A, and 11 of them died. Findings relative to alcohol withdrawal are therefore based upon 20 and 9 mice, respectively, in the two experiments.

At the end of 6 days the mice were removed from the ethanol chamber and were randomly assigned to two groups: control and naloxone. That the mice were in fact dependent upon ethanol was clear from the results of scored evaluation of withdrawal signs, carried out at frequent intervals for 52 hours (*6*). Characteristic withdrawal signs occurred in all mice that had been in the ethanol chamber but never in untreated or pyrazole-treated controls. Withdrawal signs were not seen during the 6-day period of intoxication, nor immediately upon removal from the ethanol chamber after 6 days. They first appeared an hour or two later, persisted for about 16 to 24 hours, and then subsided. In experiment A there were no spontaneous convulsions and no deaths during the withdrawal period. In experiment B one of the nine mice withdrawn from alcohol had spontaneous convulsions and died. The withdrawal scores for experiment B are shown in Fig. 1.

Mice assigned to the naloxone group were placed on a circular platform (30 cm in diameter, 70 cm off the floor) at 2 hours (experiment A) or at 1 hour (experiment B) after removal from the ethanol chamber, when incipient alcohol withdrawal signs were just evident. They were given naloxone hydrochloride intraperitoneally at 10-minute intervals. The naloxone dosage (as free base) was 1, then 10, then 100 mg/kg in experiment A; and 5, then 50 mg/kg in experiment B. The dose of 100 mg/kg produced convulsions in 8 of the 20 mice; previously the ED_{50} for naloxone convulsions in untreated controls had been found to be 150 ± 13 mg/kg (*10*). No jumping occurred in any of the mice. After the naloxone test all the animals were observed for signs of ethanol withdrawal; the ethanol-naloxone group was indistinguishable from the ethanol controls (Fig. 1).

Untreated mice do not exhibit jumping at naloxone doses up to lethality. In mice with a morphine pellet implanted subcutaneously, the ED_{50} for naloxone jumping falls to 0.75 mg/kg at 86 hours. Even a single moderate dose (20 mg/kg) of levorphanol, a morphine congener, initiates a sufficient degree of dependence so that the ED_{50} for naloxone-induced jumping becomes measurable in 1 hour and falls to 84 mg/kg at 8 hours. Without naloxone such animals display no opiate withdrawal signs whatsoever. Thus, the naloxone method is capable of detecting a degree of dependence too insignificant to be manifested as a spontaneous withdrawal syndrome. Yet the alcohol-dependent mice displayed no naloxone-precipitated jumping.

In summary, mice were made dependent upon ethanol, as judged by

the occurrence of a typical alcohol withdrawal syndrome. Naloxone, at doses up to the convulsive range, caused no opiate-withdrawal jumping in any of the animals; nor did naloxone modify the course of the alcohol withdrawal syndrome. Since even a mild degree of opiate dependence can be detected by the naloxone test, we conclude that alcohol dependence is not a manifestation of dependence upon any endogenous opiate, as proposed by Davis and Walsh (*3*).

AVRAM GOLDSTEIN
BARBARA A. JUDSON
Department of Pharmacology,
Stanford University,
Stanford, California 94305

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Lunar "Anorthosite"?

The origin of terrestrial anorthosite is highly controversial (*1*), and the discovery of anorthosite on the moon (*2–4*) could help to answer many questions about the origin of many large terrestrial anorthosite bodies and the early history of the earth, such as the following: Does the existence of "anorthosite" on the surface of the moon lead to the conclusion that the upper parts of the early crust of the earth comprised large masses of anorthosite? Does the observation that all major terrestrial anorthosite bodies are Precambrian in age support this view and the view that anorthosite is a "primitive" rock formed only in the early stages of the earth's development as a planet? Is it correct to conclude that no anorthosite is now forming at great depth below the surface of the earth?

Windley (*5*) has suggested that the lunar "anorthosite" has certain similarities to what he calls "Group III' terrestrial anorthosites, as opposed to the commonly described massif-type anorthosite (typified by the Adirondack, Labrador, and southern Norwegian anorthosite bodies) and stratiform-type anorthosite (typified by the Bushveld and Stillwater complexes) (*1*, *6*). The main similarities Windley points out between Group III terrestrial anorthosite and lunar "anorthosite" are that both have calcic plagioclase, a low Ti content, high Ca and Al contents, and a relatively high Cr content. He points out that the Group III terrestrial anorthosites, like the lunar "anorthosites," are "fine-grained," but he does not give actual grain-size measurements for the Group III anorthosites to allow proper comparison with the lunar material (*5*, *7*).

An examination of the reports of Wakita and Schmitt (*2*) and Wood *et al.* (*3*) on lunar "anorthosite," and many data on terrestrial anorthosite bodies (*1*, *5–8*), left me unconvinced that these lunar rocks are anorthosite at all. The main differences between terrestrial anorthosite and lunar "anorthosite" involve grain size and anorthite content. Lunar "anorthosite" is very fine grained. Although Wood *et al.* (*3*) do not give grain-size measurements, the rock fragments they studied ranged in size from 1 to 5 mm. Fragments were all polycrystalline, and the photomicrograph they provide shows that the size of an individual grain is 0.1 mm or less. The anorthite content of the plagioclase in the lunar "anorthosite" is mainly 96 to 98 percent, although some anorthite contents as low as 75 percent have been reported. Terrestrial anorthosite, on the other hand, is generally a coarse-grained, plutonic rock in which individual plagioclase crystals generally exceed the size of the complete rock fragments described by Wood *et al.*

As a field geologist who specializes in the study of anorthosite, I would not feel justified in identifying such small fragments as coming from an anorthosite body. Individual crystals in even the finer grained terrestrial anorthosite bodies are on the order of 10 to 100 times larger than those in the lunar rocks. The anorthite content of terrestrial anorthosite ranges from about 30 percent to between 85 and 90 percent, and, except for Windley's Group III anorthosite (in which the anorthite content may be as high as 90 percent), averages in the range from 55 to 70 percent anorthite for stratiform anorthosite bodies and from 45 to 55 percent anorthite for massif-type bodies (*1*).

The term "anorthosite" has always been restricted to plutonic rocks. When fine-grained rocks of generally anorthositic chemical composition were found in northern Sweden, Von Eckermann invented a new rock name (kenningite) to characterize them (*8*). A fine-grained rock, like the lunar "anorthosite," consisting almost entirely of anorthite (96 to 98 percent) (*2*, *3*), does not resemble anorthosite, kenningite, or any other widely distributed terrestrial rock that I know of.

Turner (*9*) has commented on the compulsion felt by many petrologists to speculate on possible analogies between lunar and terrestrial rocks. To go too far is, in Turner's view, "to indulge in free speculation of the kind that is generally considered permissible in more serious types of science fiction." Many of the conclusions and predictions seem to imply, in Turner's words, "that the lunar and terrestrial systems of petrogenesis must be closely similar, and that common lunar rocks must resemble common rocks of our own planet." Turner urged petrologists to pay more attention to the possibility of the uniqueness of lunar rocks rather than to succumb to the tendency to overgeneralize on the basis of inadequate information.

An immense amount of analytical work has been done on the small samples of rock collected on the moon (*10*, *11*). However, I shall personally need to know of large fragments (at least several centimeters in diameter) of coarse-grained, lunar plagioclase rock before I am convinced by the descriptions of Wakita and Schmitt (*2*), Wood *et al.* (*3*), and others (*11*) that the rocks they have studied warrant the name "anorthosite" and can be logically compared with terrestrial anorthosites. The petrogenetic schemes they propose, based on models developed for terrestrial rocks unlike the lunar ones, must also be questioned. Detailed examination of the relationships of these rocks to each other in outcrop is needed before acceptable petrogenetic models can be established.