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Alcohol Withdrawal and Magnesium Deficiency in Mice[1]

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BELKNAP, J. K., J. H. BERG AND R. R. COLEMAN. *Alcohol withdrawal and magnesium deficiency in mice.* PHARMAC. BIOCHEM. BEHAV. 9(1) 1–6, 1978.—DBA/2J mice exposed to chronic alcohol (ethanol) intoxication were found to have lower whole brain magnesium (Mg) concentrations than control animals. The symptoms of alcohol withdrawal were found to be strikingly similar to those seen in Mg deficient mice exposed to a low Mg diet without alcohol exposure. These findings suggest that CNS Mg deficits produced by alcohol exposure could contribute to the observed alcohol withdrawal syndrome. Serum Mg concentrations were also determined, and low correlations (≤ 0.3) were found with brain Mg concentrations.

Alcohol withdrawal Magnesium deficiency DBA/2J mice

THE SYMPTOMS of alcohol (ethanol) withdrawal in physically dependent persons include coarse tremors, spasms, convulsions, delirium (confusion, disorientation), hallucinations, delusions, shock, hyperreflexia, and hyperreactivity. The symptoms of magnesium deficiency in man (not involving alcohol) are strikingly similar, which has led to the suggestion that Mg deficiency may be involved in producing many of the symptoms of alcohol withdrawal [5].

Magnesium deficiency is usually assessed clinically by the blood serum Mg concentrations. Normal serum levels typically fall in the relatively narrow range of 1.5–2.0 mEq/ℓ in man. However, about a dozen studies reviewed by Flink [5] have indicated a very high incidence of hypomagnesemia (levels below 1.5 mEq/ℓ) among alcoholic patients, most of whom were studied during withdrawal. While Mg deficiencies do not occur in all of the alcoholic patients studied, the very high incidence of hypomagnesemia is quite remarkable across the over 300 cases reported in the literature. In addition, several studies have shown that the lowest serum Mg levels are found in those patients who eventually manifest the most severe withdrawal symptoms [17, 18, 19, 20, 22, 23].

Hypomagnesemia in alcoholics would be expected for several reasons. Inadequate food intake is well known among alcoholics, and this could lead to inadequate ingestion of minerals, including Mg. Vomiting and diarrhea would greatly increase the rate of Mg loss [5]. Thirdly, the ingestion of alcohol has been shown to lead to a marked increase in urinary magnesium loss in both alcoholic and nonalcoholic subjects [12, 13, 15, 16, 17].

While most investigators agree that Mg deficiency is a common occurrence in chronic alcoholics, it is not clear what role this deficiency plays in the manifestations of the alcohol withdrawal syndrome [6]. This problem is complicated by the fact that some alcoholics have been observed to show delirium tremens or convulsions with essentially normal serum Mg levels. Conversely, abnormally low levels have been observed in some patients showing minimal withdrawal symptoms [21]. These findings can be interpreted to mean either that (1) magnesium deficiency contributes little to the symptoms of alcohol withdrawal, or that (2) magnesium deficiency contributes importantly, but serum Mg levels are inadequate for assessing Mg deficiency in the central nervous system. A major obstacle in resolving this issue is the paucity of Mg determinations in the CNS of alcoholic patients. Glickman [7] found no significant differences in cerebrospinal fluid (CSF) Mg levels between alcoholics and nonalcoholics, although the difference was in the direction of reduced levels in the alcoholic patients. However, Mg is primarily an intracellular cation, so CSF levels would be expected to represent only a small percentage of the total brain Mg content (<5%) or the total brain Mg concentration (<15%).

One reason for the lack of a perfect correlation between serum Mg levels and alcohol withdrawal severity may be that serum Mg levels may not be a sufficiently accurate predictor of Mg deficiency. Several investigators have reported a low correlation between serum Mg levels and intracellular red blood cell or whole muscle Mg levels [14, 20, 21]. One reason for this is that only a small fraction (less than 3%) of the total body Mg exists in the extracellular fluid space, and the exchange of Mg between intracellular and extracellular compartments is very slow. This is especially true in the brain, where the levels of brain Mg are highly buffered relative to the rest of the body. It is widely believed that an energy dependent process is involved at the blood-brain interface

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TABLE 1

MEAN (± SD) BRAIN AND SERUM MG CONCENTRATIONS IN EXPERIMENT 1 (VAPOR INHALATION METHOD)

	Run No. 1	Run No. 2	Run No. 3
Brain Mg (mEq/kg)			
Control, ad lib	—	13.31 ± .61 (9)	13.14 ± .31 (4)
Control, pair fed	13.90 ± 1.85 (8)	13.59 ± 1.06 (9)	13.74 ± .62 (8)
Alcohol-intox	12.30 ± 1.42 (9)	—	—
Alcohol-withdrawn	12.64 ± 1.89 (10)	12.82 ± .52 (10)	13.08 ± .69 (7)
Serum Mg (mEq/l)			
Control, ad lib	—	1.67 ± .37 (9)	1.80 ± .33 (4)
Control, pair fed	1.63 ± .13 (8)	1.67 ± .39 (9)	1.76 ± .49 (8)
Alcohol-intox	1.57 ± .13 (9)	—	—
Alcohol-withdrawn	1.56 ± .21 (10)	1.50 ± .25 (10)	1.55 ± .57 (7)

Sample Sizes (N) are in Parentheses.

which is responsible for the relatively stable Mg levels in brain despite wildly fluctuating plasma or serum levels [3,14]. Thus, it becomes extremely important to measure brain Mg concentrations in order to assess properly the extent of behaviorally relevant Mg deficiency and the role this may play in the symptoms of alcohol withdrawal.

The purpose of the present report was to utilize animal models of alcohol physical dependence to answer basic questions which would be extremely difficult or impossible to pursue in human alcoholics. These questions are: (1) is there a Mg deficiency in brain tissue resulting from chronic alcohol intoxication, and (2) to what extent can serum Mg concentrations be used to predict those in brain.

METHOD

In Experiment 1, physical dependence on alcohol was produced by the vapor inhalation method of Goldstein and Pal [9]. DBA/2J male mice (Jackson Laboratories, Bar Harbor, ME), 12–17 weeks of age, were chronically exposed to an intoxicating alcohol vapor for 4 days at 24 ± 1°C. Four 17 liter jars with tight fitting lids (Eco-Jar, Nalge Co.) served as living chambers with a maximum of 8 mice housed per jar. Because of the limited capacity of the chambers, it was necessary to conduct three separate runs under identical conditions. The groups and N's involved are shown in Table 1.

The flow rate was 35 to 50 ml/min of air per mouse delivered from a regulated compressed air cylinder. For the alcohol-exposed groups, this air flow was divided into two parts: the first (1/3 of the total) passed through the head space of a 1000 ml flask containing 250 ml of 95% alcohol, and the second (pure air) passed directly to the chambers. The ratio of these two air flows into the chambers (controlled by two Gilmont flowmeters with needle valves) determined the alcohol vapor concentrations. Each day an exhaust sample from each chamber was collected by a gas sampling valve in a Carle Model 211 gas chromatograph (FID, Porapak Q column at 130°C) to monitor the alcohol concentrations in each of the alcohol vapor chambers. This concentration was always maintained between 9 and 10 mg/ℓ. Tail vein blood samples (10 μl) were assayed for alcohol by gas chromatography [1]. Five mice were sampled at random from the alcohol chambers on Days 2 and 4 during Runs 1 and 2.

Two control groups were run concurrently with the alcohol-exposed mice; one was allowed ad lib access to food (Purina Lab Chow), while the other (pair fed control) was given the same amount of food (as a group) as was consumed ad lib by the alcohol-exposed groups. The control groups were housed and treated identically to the alcohol-exposed groups except for the introduction of alcohol vapor into the chambers. Water was available ad lib for all groups. The Mg content of the diet was 125 mEq per kg as determined by atomic absorption spectroscopy (see below). At the same time each day (3–4 hr after light onset on a 12L:12D cycle), all of the animals and uneaten food were weighed, and new food (preweighed), bedding (pine shavings), and fresh water provided. All groups also received daily injections (IP) of pyrazole (68 mg/kg), an alcohol dehydrogenase inhibitor, to stabilize the blood alcohol levels as recommended by Goldstein [8]. The first injection was given just prior to placing the mice in the chambers; the last (fourth) was given 1 day prior to withdrawal. For the alcohol-exposed groups, the initial pyrazole injection was given along with a 2 g/kg priming dose of alcohol. After four days of alcohol exposure, withdrawal was accomplished by removing the mice from the chambers at 3 hr after light onset.

The alcohol-exposed groups were either sacrificed for Mg assay on the last day of intoxication (Run 1 only, 16 hr prior to withdrawal) or 24 hr later at the approximate time of maximal withdrawal severity (7–9 hr after withdrawal). This was done so that all animals could be sacrificed at about the same time of day (just before light offset). The controls were sacrificed concurrently.

Magnesium levels were determined by atomic absorption spectroscopy (Perkin-Elmer Model 303). The mice were decapitated and the whole brain removed, weighed, and digested in 15 volumes of a mixture of 80% concentrated nitric, 15% concentrated sulfuric, and 5% perchloric acid (70%). Blood samples (.2 ml) were also taken and diluted 6 fold in 0.9% saline, allowed to stand for about 60 min, and centrifuged at 3000×g for 10 min to remove cells and coagulum. Following appropriate dilutions, the brain and blood samples were aspirated into the atomic absorption spectrophotome-

TABLE 2
MEAN (± SD) BRAIN WT., % BRAIN WATER, BODY WEIGHTS AND FOOD CONSUMPTION DATA FROM EXPERIMENT 1 (VAPOR INHALATION METHOD)

	Alcohol	Pair Fed	Ad Lib	Runs
Brain Wt (mg) (at sacrifice)	365 ± 17 (36)	369 ± 18 (25)	371 ± 15 (13)	1-3
Brain % water	78.6 ± .8 (10)	78.4 ± .5 (9)	78.3 ± .5 (9)	2
Body Wt (initial)	28.6 ± 2.0 (36)	27.8 ± 1.9 (25)	28.2 ± 1.7 (13)	1-3
Body Wt (at sacrifice)	25.7 ± 2.6 (36)	25.6 ± 1.7 (25)	27.6 ± 1.4 (13)	1-3
Food Consumption (g/day/mouse)	1.7	1.6	3.0	1-3
Blood Alcohol	2.36 ± .40 (20)	—	—	1-2

Sample Sizes (N) are in Parentheses.

ter according to the Perkin-Elmer procedures manual. Lanthanum oxide (.5% final concentration) was present in all samples. All Mg determinations were done on a blind basis. Brain water content was determined in some of the animals by comparing wet weight (fresh) with dry weight (after 20 hr at 150°C). The percentage water content was virtually identical in all groups (Table 2).

The mortality rate during intoxication (Runs 1–3) averaged 11%: these appeared to be due to overdoses. These mice were excluded from the data analysis.

A fourth run was conducted as described above, except that these mice (N=19) were allowed to undergo withdrawal for the purposes of monitoring the withdrawal symptomatology following four days of alcohol exposure. At 2 hr intervals, each animal was suspended by the tail for 10 sec, returned to its home cage, and observed for the next 1 min. Six symptom categories were chosen on the basis of (1) ease of objective evaluation and (2) distinctness from any behavior seen in the control mice. These symptom categories were: convulsions on handling (CH)—myoclonic spasms of the face, usually extending to the rest of the body; tremor (TR)—marked tremulousness of the entire body; Straub tail (ST)—arching of the tail over the back, a sign of muscular rigidity; hyperreactivity (HR)—pronounced jumpiness when touched resulting in leaps in excess of the animal's body length; seizures (SZ)—clonic or tonic-clonic seizures (nonlethal) involving most of the body; lethal seizures (LSZ)—tonic extensor seizures resulting in respiratory paralysis. Convulsions on handling was scored while the mouse was suspended by the tail; all of the other symptom categories were scored while the animal was in its home cage. Convulsions on handling have been further described by Goldstein [8,10].

In Experiment 2, nine DBA/2J male mice, 11 weeks old, were rendered physically dependent on alcohol by the liquid diet method of Goldstein and Arnold [11]. This experiment was considered desirable because it involves a very different method of physical dependence production compared to the vapor inhalation method, and pyrazole injections were not employed.

In the pretreatment phase, the alcohol-exposed mice were given a 3.9% (w/v) alcohol solution in water as their sole fluid source for 5 days. This was done to minimize the development of a conditioned aversion to the alcohol solutions during the subsequent treatment phase. Purina lab chow was available ad lib. On Days 6 through 12, the treatment phase consisted of the ad lib presentation of a liquid diet (Carnation Slender, chocolate flavor) containing 3.9% w/v alcohol (final concentration) as the sole fluid and food source. This was accomplished by adding 17 ml of 95% alcohol and 30 ml of water to each can (300 ml) of liquid diet. A control group (N=9) was treated identically to the alcohol-exposed group except that isocalorically equivalent amounts of sucrose were substituted for the alcohol during the pretreatment and treatment phases. The control group was pair-fed (as a group) to insure that both groups received equivalent calorie and nutritional intake throughout the experiment. Blood alcohol determinations were made at 2 hr after light onset on Days 3 and 6 of the treatment phase as described in Experiment 1. Venipuncture was also done on the controls, but no assays were performed.

Both the alcohol-exposed and control groups were sacrificed just before light offset at the end of the sixth day of the treatment phase. The procedure for magnesium assay was the same as in Experiment 1.

In Experiment 3, an additional group of mice (N=9) were placed on distilled water and a low Mg diet (Nutritional Biochemicals) containing 0.08 mEq/kg diet for 21 days (no alcohol). Beginning on Day 13, when the symptoms of Mg deficiency were evident in all of the mice, a 1.5 g/kg IP dose of alcohol was given to three of the animals chosen at random. Another three mice received 0.75 g/kg of alcohol and the last three received only saline. The injection volume was 10 ml/kg in 0.9% saline. The symptoms of Mg deficiency were then monitored as described above (Experiment 1) at 15 min intervals for 75 min. There were thus a total of five observations made per mouse after each injection. This procedure was repeated on Days 15, 17, 19 and 21, with each animal assigned to each of the three treatment groups in a counterbalanced fashion.

RESULTS

The Mg assay data for all three runs in Experiment 1 (vapor inhalation) are shown in Table 1. Combining brain Mg concentration data for all alcohol-exposed (N=36), pair fed

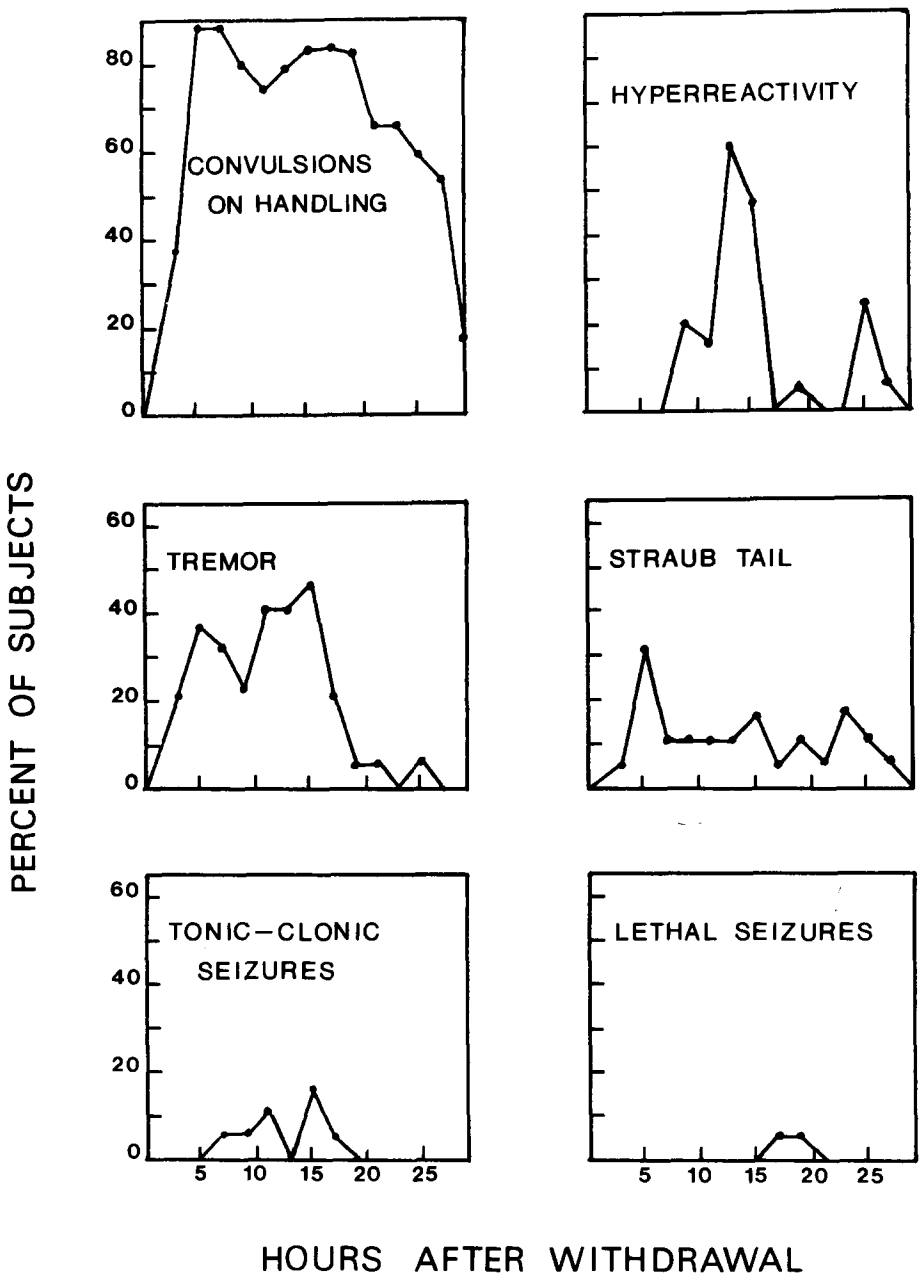

FIG. 1. The relative frequencies for the six symptom categories used to assess alcohol withdrawal in DBA/2J male mice (N=19) as a function of time (hours) after withdrawal (Experiment 1).

(N=25) and ad lib control animals (N=13) over all three runs resulted in an F ratio of 6.2 ($p<0.003$, $df=2/72$). Comparisons of these three groups combined across all three runs showed that the alcohol-exposed mice had significantly lower brain Mg concentrations than either pair fed ($t=3.2$, $p<0.002$) or ad lib controls ($t=2.2$, $p<0.03$). An unexpected tendency for pair fed mice to have higher brain Mg concentrations than ad lib controls was also observed ($t=1.6$, $p<0.10$). A similar analysis was performed on serum Mg concentrations. While the mean serum Mg concentrations followed the same pattern as the brain Mg concentrations, the F ratio was nonsignificant (F=1.68, $p<.20$, $df=2/72$). When pair fed and ad lib controls, which did not differ ($t=0.21$, $p<0.6$), were pooled and compared with the alcohol-exposed animals, significance was nearly achieved (F=3.36, $p<0.07$, $df=1/73$).

Serum Mg concentrations proved to be a rather poor predictor of brain Mg concentrations. Combining over all runs, the observed correlations between brain and serum levels were, for the alcohol-exposed animals, r=.17; for pair fed controls, r=.02; and for ad lib controls, r=.06. Combining over all groups within each run yielded correlations of .30 (Run 1), .09 (Run 2) and .16 (Run 3).

Figure 1 displays the time course for the six symptom categories used to monitor withdrawal in Experiment 1. The symptoms began at about 3 hr after withdrawal and were

essentially ended at about 30 hr. The vapor inhalation technique produced a marked degree of physical dependence as evidenced by the withdrawal syndrome.

The data for Experiment 2 (liquid diet method) are shown in Table 3. Once again, the alcohol-exposed group exhibited significantly lower brain Mg concentrations than did the pair fed controls ($t=4.30$, $p<0.001$). The serum Mg concentrations were lower in the alcohol-exposed group, but significance was not achieved ($t=1.26$, $p<0.25$). The correlation coefficient between brain and serum Mg concentrations was .23 (N=18). In our experience, the withdrawal severity produced by this method is equal to or slightly greater than that produced by the vapor inhalation method. The withdrawal symptomatology and time course are very similar for both methods of physical dependence production (unpublished). The alcohol consumption for individual mice can be readily calculated with the liquid diet method. The correlation coefficient between total alcohol consumed (corrected for body weight) in the treatment phase and brain Mg concentrations was −.85 (N=9). The corresponding correlations for serum Mg concentrations was −.20 (N=9). Thus, animals consuming the most alcohol tended to show the lowest Mg concentrations.

TABLE 3

MEAN (± SD) VALUES IN EXPERIMENT 2 (LIQUID DIET METHOD) DURING THE TREATMENT PHASE

	Alcohol	Control
Brain Mg (mEq/kg)	13.10 ± .43	13.86 ± .31
Serum Mg (mEq/l)	1.53 ± .26	1.66 ± .17
Body Wt (initial)	23.0 ± 3.4	22.9 ± 3.6
Body Wt (at sacrifice)	19.6 ± 2.7	19.8 ± 2.6
Diet Consumption (ml/day/mouse)	9.9 ± 1.2	10.4 ± .2
Alcohol Consumption (g/kg/day)	19.0 ± 4.3	—
Blood Alcohol (mg/ml), days 3 and 6	2.02 ± 1.0	—

N=9 per group.

In Experiment 3 (low Mg diet), the saline injected mice exhibited the following relative frequencies (in percent of total observations)—CH, 67%; TR, 27%; ST, 13%; HR, 15%; and SZ, 15%. The 1.5 g/kg alcohol injected mice showed no symptoms whatsoever ($p<0.05$ for each symptom category, Fisher Exact Probability Test), while the 0.75 g/kg injected mice showed no symptoms except CH, 40% and HR, 11%. This suppression of Mg deficiency symptoms occurred at doses of alcohol which produced no overt ataxia or other signs of gross intoxication. The Mg deficiency symptoms essentially returned to control (saline) levels at about 3 hr after injection.

DISCUSSION

The results represent the first demonstration that chronic alcohol exposure can produce a state of Mg deficiency in brain tissue. The alcohol-exposed mice averaged 7.7% lower Mg concentrations than the pair fed controls and 4.3% lower than the ad lib controls (Experiment 1). In Experiment 2, the alcohol-exposed group averaged 5.5% lower brain Mg concentrations vs. pair fed controls. But is this Mg deficit large enough to be behaviorally significant? Some insight can be gained from studies where a Mg deficiency was induced by means of a low Mg diet (no alcohol). Belknap, *et al.* [2] exposed DBA/2J mice to a low Mg diet for 14 days, at which time a 10.8% reduction in brain Mg concentration was observed. Unfortunately, brain Mg determinations were made at only one point in time (Day 14) while the symptoms began much earlier on the fifth day of diet exposure. Using a low Mg diet with weanling rats, Chutkow and Grabow [4] first observed audiogenic seizures in over half of the subjects at a time (Day 4) when the brain Mg concentrations had declined by only 4.2% compared to pair fed controls. In a similar study, Chutkow [3] reported spontaneous and audiogenic tonic-clonic seizures in the majority of rats after 8 days of low Mg diet exposure. At that time, an 8.2% decline in brain Mg concentrations was noted vs. pair fed controls. Thus, it is possible that the brain Mg deficiency seen in the alcohol-exposed mice might be large enough to contribute to the withdrawal symptomatology; however, more work is needed to determine the extent of the contribution.

One drawback of the vapor inhalation method is that it calls for the use of pyrazole to stabilize the blood alcohol levels [11]. However, the results from Experiment 2 (liquid diet method) are closely similar to those seen in Experiment 1 (vapor inhalation method). This strongly suggests that the use of pyrazole in both the control and alcohol-exposed groups in Experiment 1 did not appreciably affect the overall pattern of the results.

The factors responsible for the Mg loss in brain tissue are not known, but it could be due to either a greater rate of renal loss relative to dietary intake, or a redistribution of Mg among body pools. The latter possibility has been suggested by some studies with human alcoholics [17, 22, 23]. During withdrawal, serum Mg levels are closely correlated with (respiratory) alkalosis, and these pH imbalances have been hypothesized to produce shifts in Mg pools [17, 22, 23]. Definitive evidence is lacking, however, and more work is needed to determine the mechanisms responsible for the low Mg concentrations in brain and serum.

The results from Experiment 3 clearly demonstrate that moderate doses of alcohol can suppress the symptoms of Mg deficiency produced by a low Mg diet. This finding probably explains why the intoxicated animals from Experiment 1 (3 1/3 days of alcohol intoxication) did not exhibit any symptomatology in spite of the low brain Mg concentrations found in these animals. These results are consistent with the hypothesis that alcohol masks the latent hyperexcitability due to CNS Mg deficiency: when the alcohol is withdrawn, the Mg deficiency may contribute to the manifest hyperexcitability characteristic of the alcohol withdrawal syndrome.

The low correlations between serum and brain Mg concentrations are consistent with the low correlations reported by others between serum and intracellular fluids such as blood cells or whole muscle in man [14, 20, 21]. The use of serum concentrations to assess CNS Mg deficiency is open to considerable error, and this consideration is very important in interpreting the data from human alcoholics where serum values are typically the only ones available.

Finally, the symptoms of Mg deficiency in mice (Experiment 3, low Mg diet) were seen to be strikingly similar to those seen in alcohol withdrawal (Experiments 1 and 2). Thus, the present findings suggest that alcohol-induced Mg deficiency may contribute, along with other factors, to the etiology of the withdrawal syndrome.

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