Pharmacology Biochemistry & Behavior, Vol. 5, pp. 529–534.

Effect of Magnesium Deficiency on Nonspecific Excitability Level (NEL) and Audiogenic Seizure Susceptibility[1]

DOUGLAS R. BUCK, ARTHUR W. MAHONEY AND DELOY G. HENDRICKS

Department of Nutrition and Food Sciences – 87, Colleges of Family Life and Agriculture, Utah State University, Logan, UT 84322

(Received 28 May 1976)

BUCK, D. R., A. W. MAHONEY AND D. G. HENDRICKS. *Effect of magnesium deficiency on nonspecific excitability level (NEL) and audiogenic seizure susceptibility.* PHARMAC. BIOCHEM. BEHAV. 5(5) 529–534, 1976. – Magnesium deficiency in weanling rats caused an increase in NEL and in audiogenic seizure susceptibility. These behavioral effects were apparent after eight days of magnesium restriction and could be reversed by dietary rehabilitation. Serum magnesium declined rapidly from 1.87 ± 0.10 mEq/L to 0.91 ± 0.24 mEq/L in two days. Cerebrospinal fluid (CSF) magnesium decreased gradually from 1.86 mEq/L, becoming significantly lower (1.44 ± 0.23 mEq/L) after eight days. When deficient rats were injected IP with $MgCl_2$, raising the serum magnesium concentration to 6.6 mEq/L, NEL decreased to normal while audiogenic seizure susceptibility remained. Both NEL and audiogenic seizure susceptibility in rats reflect central nervous system magnesium concentration, except when serum magnesium concentration is very high. Very high serum magnesium concentration lowers NEL but does not reduce audiogenic seizure suseceptibility if CSF magnesium is low.

Magnesium deficiency Brain Behavior Audiogenic seizures Cerebro-spinal fluid

IN A recent review and case report [42], it has been surmised and reported that marginal magnesium deficit, not severe enough to elicit seizures, may, in fact be responsible for a number of complaints mistaken for psychoneurosis. Other reports [24,41] reflect the concern. They recognize that magnesium deficiency may be a practical problem for human beings.

Well-known and nearly universal symptoms of magnesium depletion are seizure susceptibility and hyperexcitability. Literature acknowledging the relationship between the mineral deficiency and these symptoms has been reviewed [29, 42, 46]. Those suffering malnutrition from gastrointestinal problems [15,31] and alcoholics [10,43] seem especially susceptible. The symptoms have been observed in rodents [1, 5, 13, 23, 36, 40], rabbits [25], dogs [33,44], ruminants [4,12], fowl [3], primates [45], and man [11, 15, 19, 20, 30, 39]. Assessment of seizure susceptibility has often been done by exposing magnesium deficient animals, usually rats, to a loud noise and counting the number thrown into seizure [2, 6, 13, 18, 35]. Assessment of hyperexcitability apart from seizure susceptibility in animals has usually been done by describing how they react in their cages to routine laboratory activity or to the presence of the researcher. In man, it has been done by self-report or clinical observation.

Although existing literature provides considerable descriptive information about heightened excitability due to magnesium deficiency and some quantitative information about seizure susceptibility, little has been done to quantitate the relationship between magnesium deficiency and other behavioral measures of excitability. It was our purpose to do this.

Lát [27] and Lát and Gollová-Hémon [28] have identified a phenomenon they call Nonspecific Excitability Level (NEL). NEL refers to the level of activity of an organism as measured by large vertical and horizontal movements such as rearing and locomotion. It bears a relationship to hippocampal slow wave (theta) activity [28]. Estimating NEL is done by (a) counting certain spontaneous movements such as rearing and locomotion an organism makes during an initial period in a novel environment; or (b) measuring the rate of habituation of these responses. Although NEL may be altered by drug [22], hormonal [27,28] and dietary [16, 27, 28] treatments, the effect of magnesium deficiency has not hitherto been assessed.

To assess the effect of magnesium on NEL, and to ascertain and clarify the relationships between NEL and audoigenic seizure susceptibility, we set the following objectives: (1) to see if magnesium deficiency causes an increase in NEL which is reversible; (2) to compare NEL with audiogenic seizure susceptibility; and, (3) to define the relationships between the two behavioral measures and serum and CSF magnesium. To attain these objectives,

[1] Published with the approval of the Director of the Utah State Agricultural Experiment Station as journal article number 2087. Funded by the Utah State Agricultural Experiment Station, College of Family Life, and Sigma Xi.

three experiments were designed. The first was to assess the effects that length of time on a magnesium deficient diet had on NEL. The second was to see if these effects could be reversed by dietary rehabilitation. The third was to assess the effects of injecting deficient animals with magnesium.

METHOD

Male weanling Sprague-Dawley rats were fed control diet for at least a day and allowed to become acclimated before experiments were commenced. They were housed in stainless steel cages with wire fronts and bottoms. Diet was provided in glass, and deionized water was provided in polyethylene containers having rubber stoppers and stainless steel lick spouts. Temperature of the animal room was maintained between 20 and 23°C. Lights were on daily from 7:00 a.m. to 7:00 p.m. Diet consisted of 20% casein, 5% sucrose, 53% corn starch, 10% corn oil, 2% vitamin mix and suggested minerals for growth [32] with and without magnesium. Cellulose (7.2%) was added to make up to 100%. The vitamin fortification mixture (ICN Pharmaceuticals) consisted of, per kg, the following: 4.5 g vit. A, 0.25 g vit D, 5 g αtocopherol, 45 g ascorbic acid, 5 g inositol, 75 g choline chloride, 2.25 g menadione, 5 g p-aminobenzoic acid, 4.5 g niacin, 1 g riboflavin, 1 g pyridoxine-HCl, 1 g thiamine-HCl, 3 g Ca pantothenate, 20 mg biotin, 90 mg folic acid and 1.35 mg vit. B-12. The magnesium deficient diet was found by analysis to contain 55 mg of Mg per kg diet. Control diet was prepared by adding 400 mg Mg per kg as $MgCO_3$ to the deficient diet.

Nonspecific excitability level (NEL) was determined by a modification of Lát and Gollová- Hémon's procedure which was by counting the number of responses made by each rat during the first two minutes after it was placed in a chamber consisting of a glass enclosure 11 cm wide × 22 cm long × 23 cm high with a wire mesh bottom. The responses, counted by two observers, were: the number of times the rat moved from one corner to another, the number of times its nose was raised above the body, the number of rearing responses (counted also as nose-raise responses), the number of grooming responses, the number of fecal pellets excreted, and whether or not it urinated. These responses were respectively weighted: 1, 1, 1, 2, 1 and 2, and added to obtain an activity total for each.

Audiogenic seizures were induced in susceptible animals by the method of Patton [34]. Our device utilized two school bells producing 115 dB inside a metal chamber. Rats were exposed to the noise for 1½ minutes or until tonus occurred. Death from seizures was usually prevented by chest massage until normal respiration was regained. CSF was collected from the cisterna magna in Yale 26 gage ½ in needles with the points slightly bent to prevent clogging during insertion. Blood was collected by inserting heparinized capillary tubes into the retro-ocular capillary bed.

In Experiment 1, 54 rats were randomly assigned to nine groups of six and housed two per cage. Group one was terminated at the onset. Groups 2 through 8 were fed the magnesium deficient diet; one of these groups was selected to be terminated every other day, the last being terminated on the 14th day of the experiment. Group 9 was fed control diet throughout and was terminated on the 14th day. NEL was measured between 10:30 and 11:00 a.m. on only the group of rats being terminated. These were then tested for audiogenic seizure susceptibility. CSF and serum were collected just before killing.

In Experiment 2, 20 rats were paired according to initial NEL and were individually housed. Both groups were fed magnesium deficient diet for eight days. Blood was taken and serum magnesium level determined. Final adjustments were made in group matchings so that between group magnesium levels were identical. One group was then fed control diet four days while the other remained on deficient diet. Rats were pair fed during this period. Terminal NEL for each rat was compared with initial NEL. This was done because blood sampling (or giving IP injections) increased within group variability. Subtracting initial from final NEL reduced this and made it possible to obtain statistical significance in some cases where it was not otherwise obtainable. CSF and serum were collected just before termination.

In Experiment 3, 24 rats were matched according to initial NEL and assigned to three groups of eight. They were individually housed. Two groups were fed magnesium deficient diets. The third group was fed control diet. All were fed ad lib for five days, and pair fed thereafter. On the 12th day, the first group was injected with $MgCl_2$ (0.1 ml of 5% $MgCl_2.6H_20$ per 20 g rat weight). The second and third groups were injected with equiosmolar NaCl. NEL was determined beginning three minutes postinjection and compared with initial NEL. Assessment of audiogenic seizure susceptibility was made beginning 5½ min postinjection. CSF and blood were collected at about 8 min postinjection.

Any CSF containing cells, and blood were centrifuged for 10 min and the clear CSF or serum collected. All samples were diluted with 0.1 N HCl (70:1 dilutions for CSF, 50:1 dilutions for serum) and analyzed by atomic absorption spectroscopy for magnesium.

Comparing the number of rats susceptible to audiogenic seizures in one treatment group with the number susceptible in another was made by Chi Square analysis. Other data comparsions were made against least significant differences computed by analysis of variance, or by *t*-tests.

RESULTS

The effect of duration of magnesium deficiency on CSF and serum magnesium concentration is shown in Fig. 1. Serum magnesium concentration decreased rapidly from 1.87 mEq/L to 0.91 mEq/L by Day 2, and to 0.63 mEq/L by Day 6 where it essentially remained for the remainder of the experiment. CSF magnesium concentration decreased from 1.86 mEq/L initially to 0.94 mEq/L by Day 14. The rate of this decrease was more gradual than for serum magnesium concentration. When CSF magnesium concentration was 1.44 mEq/L or less, namely on Days 8, 12 and 14, NEL was significantly greater, and susceptibility of some rats to audiogenic seizures was observed (Figs. 2 and 3).

The effect of duration of magnesium deficiency on NEL is shown in Fig. 2. Significant ($p<0.05$) increases in NEL from day 0 were observed by Day 8. On Days 12 and 14, significant increases in NEL of these deficient groups were also demonstrated when compared with the NEL of a control group terminated on Day 14. The classic magnesium deficiency symptoms of vasal dilation appeared after 5 days. From Day 8 on, deficient animals seemed more irritable than controls. When placed in the activity chamber, they would remain very active for the full two minutes. Control animals tended to be active at first while exploring

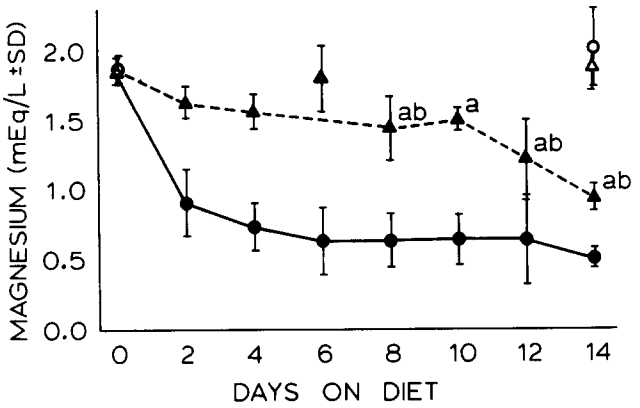

FIG. 1. CSF and Serum Magnesium Concentration vs. Length of Time Rats were Fed a Magnesium Deficient Diet. ▲, CSF Mg of rats fed magnesium deficient diet. ●, serum Mg of rats fed deficient diet. △, CSF Mg of rats fed control diet. ○, serum Mg of rats fed control diet. LSD 0.01 for serum Mg = 0.51 mEq/L. N = 6, all groups. (a) Significantly lower ($p<0.05$) than CSF Mg concentration of rats terminated on Day 0. (b) Significantly lower than CSF Mg of rats fed control diet terminated on Day 14. (T-tests were performed on the CSF data using the pooled variance (0.0225). The number of rats in each group from which clear CSF was successfully collected were: 5, 5, 6, 3, 3, 4, 4 and 5 for Days 0, 2, . . . , 14; and, 4 for rats fed control diet terminated on Day 14.)

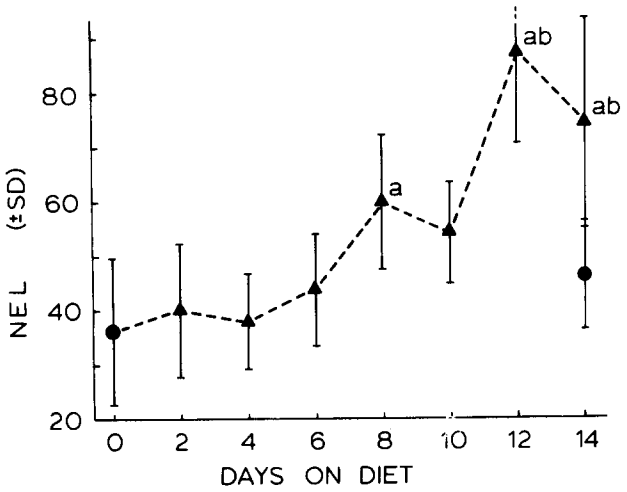

Fig 2. Nonspecific Excitability Level (NEL) of Rats Fed a Magnesium Deficient Diet. ▲, rats fed magnesium deficient diet. ●, rats fed control diet. N = 6, all groups. LSD 0.05 = 13.3. (a) Significantly greater than NEL of rats terminated on Day 0. (b) Significantly greater than NEL of rats fed control diet terminated on Day 14. R^2 for the relationship between NEL and CSF Mg concentration = 0.72. R^2 for the relationship between NEL and serum Mg concentration = 0.28.

their new environment. Then they would settle down and be more content to pursue a single mode of behavior such as sniffing, grooming or resting. One rat in the deficient group terminated on Day 12, and two in the group terminated on Day 14 went into mild spontaneous seizures while in the chamber.

The effect of duration of magnesium deficiency on audiogenic seizures susceptibility is shown in Fig. 3. Although younger rats sometimes ran widly about the cage when subjected to a 115 dB bell, none developed tonus until Day 8. By Day 14, all deficient rats developed tonic or

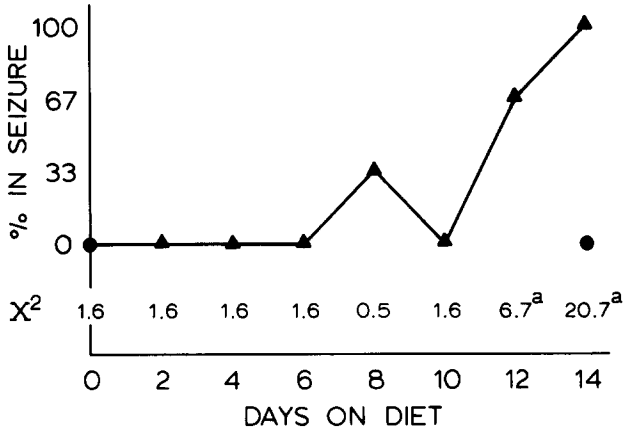

FIG. 3. Percent of Rats Fed a Magnesium Deficient Diet that Seized when Exposed to a 115 dB Bell. ▲, rats fed magnesium deficient diet. ●, rats fed control diet. N = 6, all groups. Bell was on for 1½ min or until tonus occurred. (a) Chi Square is significant ($p<0.05$). R^2 for the relationship between percent susceptible to audiogenic seizure and CSF Mg concentration = 0.83. R^2 for the relationship between percent susceptible to seizure and serum Mg concentration = 0.17.

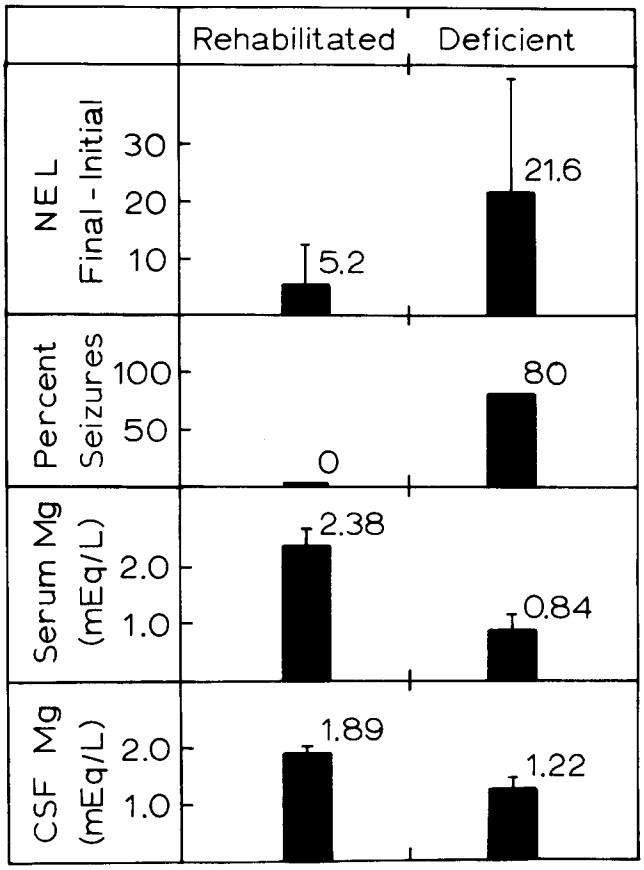

FIG. 4. Effect of Magnesium Rehabilitation on NEL and Audiogenic Seizure Susceptibility. All comparisons are significant ($p<0.05$). Rats were assigned to two groups according to initial NEL and were fed Mg deficient diet 8 days. N = 10, both groups. One group was then rehabilitated four days. Seizures included animals running wildly about the cage. Respective t-values for NEL, serum and CSF Mg concentrations = 2.45, 12.08 and 7.06. Chi Square for comparing the percent susceptible to audiogenic seizure = 7.80.

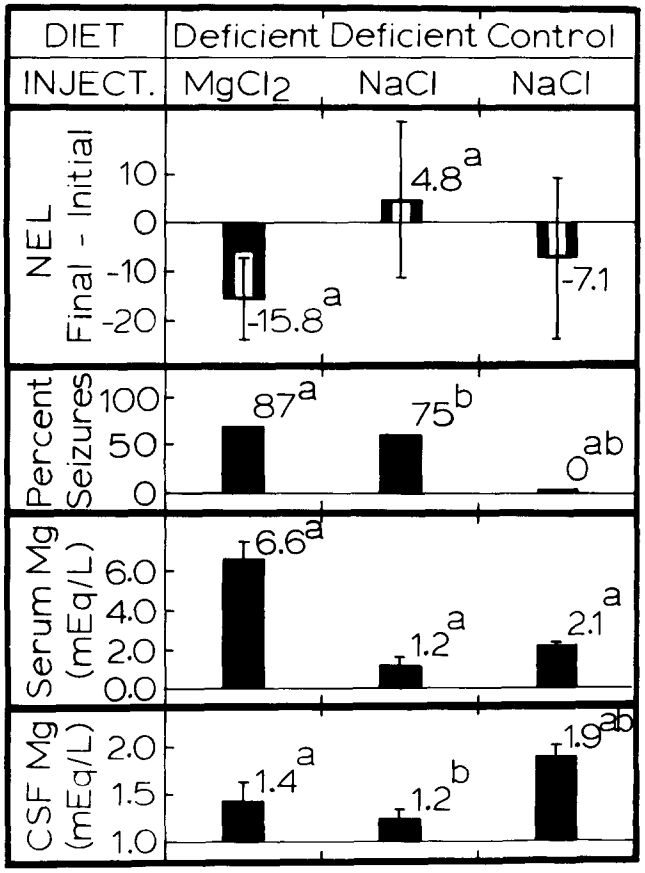

FIG. 5. Comparison of Several Parameters among Three Treatment Groups in Experiment 3. Two groups of rats were fed Mg deficient diet 12 days. A third group was fed control diet 12 days. N = 8, all groups. One deficient group was injected with $MgCl_2$ (0.1 ml of 5% $MgCl_2.6H_20$ per 20 gm rat weight). The other deficient group and the control group were injected with equiosmolar NaCl. NEL was determined at 3 min postinjection; audiogenic seizure susceptibility was determined at 5½ min postinjection; and CSF and blood were collected at about 8 min postinjection. (a) Values indicated by similar letters differed significantly ($p<0.05$). Respective LSD 0.05 for NEL, CSF and serum Mg concentrations = 14.2, 0.18 mEq/L and 0.65 mEq/L. Respective Chi Square values for comparisons between the percent susceptible to audiogenic seizure of two deficient groups and the control group = 5.86 and 4.88.

tonic-clonic seizures in response to the bell. The relationship between seizure susceptibility and NEL from a group by group comparison was strong ($R^2 = 0.76; p<0.001$).

The effects of dietary rehabilitation are shown in Fig. 4. Rats rehabilitated for four days with control diet had terminal CSF and serum magnesium concentrations of 1.89 and 2.38 mEq/L, respectively, as compared with 1.22 and 0.84 mEq/L for the deficient group. NEL was significantly reduced among the rehabilitated rats. None of the rehabilitated rats was susceptible to audiogenic seizures.

NEL was significantly lower for magnesium deficient rats injected with $MgCl_2$ than for deficient rats injected with saline (Fig. 5), but they were still susceptible to audiogenic seizure. Serum magnesium was greatly elevated as a result of the $MgCl_2$ injection, while CSF magnesium increased only slightly. Compared with a control group, those injected with magnesium exhibited significantly more seizure susceptibility, higher serum magnesium concentration, and lower CSF magnesium concentration.

DISCUSSION

Under the conditions of our experiments, magnesium deficiency in weanling rats causes an increase in NEL which is reversible. Differences in NEL appear after 8 days on a deficient diet and are clearly evident by 12 days (Fig. 2). Extreme deficiency, however, tends to reduce NEL by inducing spontaneous seizures in the activity chamber; while seizing, rats are unable to move about. Elevated NEL, as a result of magnesium deficiency, is reduced by dietary rehabilitation (Fig. 4).

There is a strong relationship between NEL and audiogenic seizure susceptibility when magensium intake is controlled by diet ($R^2=0.76$; Fig. 2 and 3); increased NEL occurs concurrently with increased audiogenic seizure susceptibility. Deficient animals, rehabilitated on a control diet, show both reduced NEL and audiogenic seizure susceptibility (Fig. 4). Changes in NEL do not coincide with changes in audiogenic seizure susceptibility, however, when deficient rats are injected IP with magnesium raising the serum magnesium concentration to 6.6 mEq/L, provided CSF magnesium concentration remains low (1.4 mEq/L) (Fig. 5). Under these conditions those injected with magnesium show reduced NEL while maintaining seizure susceptibility.

NEL appears to be a more sensitive estimate of generalized CNS excitability than audiogenic seizure susceptibility during the earlier stages of magnesium deficiency. Significant increases in the index were observed by Day 8, while increases in seizure susceptibility were not evident before Day 12 (Figs. 2 and 3). This opens the possibility that some kind of activity measure might be devised which could assess the effects of marginal magnesium deficit before the more serious symptoms of hallucinations, delerium tremens and convulsions develop.

Discomforture from retro-ocular puncture or IP injection tends to reduce the effectiveness of NEL as an excitability index by increasing within group variability. Some rats, normally highly excitable, just sit. Others move about more. The adverse effects of this discomforture may be minimized by comparing final NEL values with the initial.

With dietary manipulation of magnesium intake, NEL and audiogenic seizure susceptibility appear to reflect changes in CSF magnesium concentration (Figs. 1, 2 and 3). Differences in serum magnesium concentration in rats fed magnesium deficient diet are evident in two days (Fig. 1) [7,8]. A decrease in CSF magnesium concentration occurs more slowly. We demonstrated this decrease after eight days and found a concurrent increase in NEL. These observations and those of others [6, 7, 9, 14, 37] show that hyperexcitability relates to CSF magnesium but not to serum magnesium concentration. Lát and Gollová-Hémon [28] found that NEL is a good indicator of generalized CNS activity as reflected in hippocampal slow wave activity. Our data suggest that NEL may be a good indicator of increased CNS activity due to magnesium deficiency.

Very high serum magnesium (6.57 mEq/L) lowers NEL, even though audiogenic seizure susceptibility of magnesium-deficient rats may remain high due to low CSF magnesium concentration (Fig. 5). Under these conditions NEL appears to be a poor indicator of CNS activity. It has

been suggested [8, 21, 42, 46] that a block due to elevated serum magnesium concentration may occur at the neuromuscular junction. This would reduce the effects of efferent impulses upon the musculature. Thus, even though CNS activity may remain high, a behavioral activity index would yield low values.

Possible neurological mechanisms [17, 26, 38] leading to the behavioral effects of magnesium deficiency are: (1) increased ease of transmission across synapses of the CNS; and, (2) axonal effects resulting in lowered depolarization thresholds. Both mechanisms serve to make nerve cells more sensitive to stimulation from whatever source and more susceptible to premature firing. That the nervous system becomes overly responsive to stimulation as a result of magnesium deficiency has been amply demonstrated, although the precise connection between the nutritional insult, CNS excitability and behavior has yet to be elucidated. Our data, showing that NEL increases as a result of magnesium deficiency, suggest, along with the observations of other researchers [16, 27, 28], that altered intake of some nutrients plays an important role in CNS excitability. A note of caution is urged in assuming that an activity index reflects CNS excitability in all cases, however, since some treatments (Experiment 3) may dampen the activity response while considerable CNS excitability remains.

To summarize: Magnesium deficiency in weanling rats causes an increase in NEL. This effect is reversible by diet. There is a strong relationship between NEL and audiogenic seizure susceptibility except when serum magnesium concentration is very high. Both NEL and audiogenic seizure susceptibility relate inversely to CSF magnesium concentration except in the case of very high serum magnesium. In this instance, NEL is depressed while seizure susceptibility remains. Possible mechanisms explaining the effects of magnesium on NEL and audiogenic seizure susceptibility have been briefly discussed.

REFERENCES

1. Alcock, N. W. and M. E. Shils. Comparison of magnesium deficiency in the rat and mouse. *Proc. Soc. exp. Biol. Med.* **146**: 137–141, 1974.
2. Bevan, W. Sound-precipitated convulsions. *Psychol. Bull.* **52**: 473–504, 1955.
3. Bird, F. G. Magnesium deficiency in the chick. 1. Clinical and neuropathologic findings. *J. Nutr.* **39**: 13–30, 1949.
4. Blaxter, K. L. and B. A. M. Sharman. Hypomagnesemia tetany in beef cattle. *Vet. Rec.* **67**: 108, 1955.
5. Brookfield, R. W. Magnesium deficiency in the rat. *Br. Med. J.* 1: 848–849, 1934.
6. Chutkow, J. G. Clinical-chemical correlations in the encephalopathy of magnesium deficiency. *Mayo Clin. Proc.* **49**: 244–247, 1974.
7. Chutkow, J. G. Metabolism of magnesium in central nervous system *Neurology* **24**: 780–787, 1974.
8. Chutkow, J. G. Role of magnesium in neuromuscular physiology, In: *1st International Symposium on Magnesium Deficit in Human Pathology,* edited by J. Durlach. Brionne, France: Imprimerie Amelot, 1971, pp. 113–134.
9. Chutkow, J. G. and S. Meyers. Chemical changes in the cerebrospinal fluid and brain in magnesium deficiency. *Neurology* **18**: 963–974, 1968.
10. Cline, W. B. and J. V. Colemen. The treatment of delirium tremens. *J. Am. med. Ass.* **107**: 404–409, 1936.
11. Davis, J. A., D. R. Harvey and J. S. Yu. Neonatal fits associated with hypomagnesaemia. *Archs Dis. Child.* **40**: 286–290, 1965.
12. Duncan, C. W., C. F. Huffman and C. S. Robinson. Magnesium studies in calves. I. Tetany produced by a ration of milk or milk with various supplements. *J. biol. Chem.* **108**: 35–44, 1935.
13. Finger, F. W. Convulsive behavior in the rat. *Psychl. Bull.* **44**: 201–248, 1947.
14. Flink, E. B., F. L. Stutzman, A. R. Anderson, T. Konig and R. Fraser. Magnesium deficiency after prolonged parenteral fluid administration and after chronic alcoholism complicated by delirium tremens. *J. Lab. clin. Med.* **43**: 169–183, 1954.
15. Fourman, P. and D. B. Morgan. Chronic magnesium deficiency. *Proc. Nutr. Soc.* **21**: 34–41, 1962.
16. Frankova, S. Relationship between dietary fat intake and higher nervous activity in rats. *Acta Nerv. Suppl* 4: 471–475, 1962.
17. Gordon, H. T. and J. H. Welsh. The role of ions in axon surface reactions to toxic organic compounds. *J. cell. comp. Physiol.* **31**: 395–419, 1948.
18. Griffiths, W. J., Jr. Audiogenic fits produced by magnesium deficiency in tame domestic Norway rats and wild Norway and Alexandrine rats. *Am. J. Physiol.* **149**: 135–141, 1947.
19. Hanna, S. Plasma magnesium in health and disease. *J. clin. Pathol.* **14**: 410–414, 1961.
20. Hirschfelder, A. D. Clinical manifestations of high and low plasma magnesium. *J. Am. med. Ass.* **102**: 1138–1141, 1934.
21. Hoff, H. E., P. K. Smith and A. W. Winkler. Effects of magnesium on the nervous system in relation to its concentration in serum. *Am. J. Physiol.* **130**: 292–297, 1940.
22. Holland, H. C. and B. D. Gupta. Effects of drugs on the rearing response in emotionally reactive and non-reactive rats. *Acta. Nerv. Suppl.* **9**: 134–136, 1967.
23. Ko, K. W., F. W. Fellers, and J. M. Craig. Observations on magnesium deficiency in the rat. *Lab. Invest.* **11**: 294–305, 1962.
24. Krehl, W. A. Magnesium. *Nutr. Today* **2**: 16–20, 1967.
25. Kunkel, H. O. and P. B. Pearson. Magnesium in the nutrition of the rabbit. *J. Nutr.* **36**: 657–666, 1948.
26. Kuruyama, H. Effect of calcium and magnesium on neuromuscular transmission in the hypogastric nerve-vas deferens preparation of the guinea pig. *J. Physiol.* **175**: 211–230, 1964.
27. Lát, J. Nutrition, learning and adaptive capacity. In: *The Chemical Senses and Nutrition,* edited by M. R. Kare and Owen Mallers. Baltimore: The John Hopkins Press, p. 169–180, 1967.
28. Lát J. and E. Gollová-Hémon. Permanent effects of nutritional and endocrinological intervention in early ontogeny on the level of nonspecific excitability and on lability (emotionality). *Ann. N.Y. Acad. Sci.* **159**: 710–719, 1969.
29. Magnesium Deficiency. (Leading Article) *Br.. Med. J.* (Apr) 1967, p. 195.
30. Miller, J. F. Tetany due to deficiency in magnesium. *Am. J. Dis. Child* **67**: 117–119, 1944.
31. Muldowney, F. P., T. J. McKenna, L. H. Kyle, R. Freaney and M. Swan. Parathormone-like effect of magnesium replenishment in steatorrhea. *New Engl. J. Med.* **282**: 61–68, 1970.
32. Nutrient requirements of laboratory animals, 2nd revised ed., In Nutrient Requirements of Domestic Animals, No. 10. Washington, D. C. National Academy of Sciences, 1972.
33. Orient, E. R., Kruse, H. D. and E. V. McCollum. Studies on magnesium deficiency in animals. II. Species variation in symptomatology of magnesium deprivation. *Am. J. Physiol.* **101**: 454–461, 1932.
34. Patton, R. A. The effect of vitamins on convulsive seizures in rats subjected to auditory stimulation. *J. comp. Psychol.* **31**: 215–221, 1941.
35. Patton, R. A. Sound induced convulsions in hamsters associated with magnesium deficiency. *J. comp. physiol. Psychol.* **40**: 283–289, 1947.

36. Patton, R. A. and A. D. Lazovik. Sensory pre-conditioning and the convulsions associated with magnesium deficiency in the rat. *J. comp. Psychol.* **39**: 265–273, 1946.
37. Randall, R. E., Jr., E. C. Roosmeisl, K. H. Bleifer. Magnesium depletion in man. *Ann. Intern. Med.* **50**: 257–287, 1959.
38. Richards, C. D. and R. Sercombe. Calcium, magnesium and the electrical activity in guinea-pig olfactory cortex in vitro. *J. Physiol.* **211**: 571–584, 1970.
39. Roselle, N. and K. Doneker. La tetanie magnisiprive chez l'homme. Etude biochemique et electromyographique. *Path. Bact.* **7**: 17–18, 1835–1847, 1959.
40. Schrader, C. A., C. O. Prickett and W. D. Salmon. Symptomatology and pathology of potassium and magnesium deficiencies in the rat. *J. Nutr.* **14**: 85–104, 1937.
41. Seelig, M. S. The requirement of magnesium by the normal adult. Summary and analysis of published data. *Am. J. clin. Nutr.* **14**: 342–390, 1964.
42. Sellig, M. S., A. R. Berger and N. Spielholz. Latent tetany and anxiety, marginal magnesium deficit and normocalcemia. *Dis. Nerv. System* **36**: 461–465, 1975.
43. Sullivan, J. F., P. W. Wolpert, R. Williams and J. D. Egan. Serum magnesium in chronic alcoholism. *Trans Acad. Sci.* **162**: 947–962, 1969.
44. Vitale, J. J., E. E. Hellerstein, M. Nakamura and B. Lown. Effects of magnesium deficient diet upon puppies. *Circulation Res.* **9**: 387–394, 1961.
45. Vitale, J. J., H. Velez, C. Guzman and P. Corres. Magnesium deficiency in the Cebus monkey. *Circulation Res.* **12**: 642–650, 1963.
46. Wacker, W. E. C. and A. F. Parisi. Magnesium metabolism. *New Engl. J. Med.* **278**: 658–663, 712–717, 772–776, 1968.