Seizure episodes during open-field tests among Mongolian gerbils fed different combinations of minerals

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Tonic-clonic convulsions characteristic of hypomagnesemic tetany were shown with greater frequency during open-field tests among gerbils undergoing simple dietary Mg deficiency than among controls deprived of all minerals. The greater effect from simple Mg deficiency may result from antagonistic activity of other mineral ions. Because seizures in acute hypomagnesemia resemble "spontaneous seizures" of apparently normal gerbils, there is a possibility that the latter animals may be afflicted with chronic hypomagnesemia of the "latent tetany" type owing to an impairment in Mg metabolism.

Apparently, the first indication that susceptibility to an endogenous disorder had emerged within American colonies of Mongolian gerbils was contained in a report by Marston and Chang (1965). These investigators found that handling provoked transient spasticity in the musculature of certain animals. Somewhat earlier, Schwentker (1963) had been able to conclude that gerbils in this country were free from any signs of spontaneous disease. Nonetheless, Marston and Chang's observation was confirmed in reports which described a more advanced disturbance involving tonic-clonic convulsions of varying severity (Robinson, 1968; Thiessen, Lindzey, & Friend, 1968; Zeman, 1967). These investigators found the affected gerbils to be healthy and free from any identifiable dietary need. Even so, some form of metabolic dysfunction may have underlain what were labeled "spontaneous seizures" by Thiessen et al. (1968). Noting the resemblance of so-called "spontaneous seizures" to hypomagnesemic tetany, Harriman (1974, 1978) provoked seizing among previously non-susceptible gerbils by creating a simple dietary magnesium (Mg) deficiency. The symptom picture for seizing gerbils fed low-Mg diets resembles that seen in the "latent tetany" form of hypomagnesemia that results from conditions of feeding or impairment in Mg absorption (Fontenot, 1979; Littledike & Cox, 1979). Consequently, so-called "spontaneous seizures" may result from Mg deficiency produced either through some metabolic dysfunction or from selective dietary restriction. Nonetheless, even if hypomagnesemia were a predisposing condition, readiness to seize might easily result from disturbance of other ionic levels, particularly for cations Na, Ca, and K, which, together with Mg, importantly affect the irritability level of tissue (Loeb, 1905). The present study was undertaken to explore the relations be-

tween Mg and other mineral ions in inciting disposition to seize among gerbils. For the purpose, gerbils were fed diets which held or lacked Mg and, appositionally, which held or lacked a salt mix containing all other essential minerals.

METHOD

Subjects

The subjects were 60 female and 60 male gerbils, aged 140 to 160 days and born to stock ["Tum: (MON)"] acquired from Tumblebrook Farm, Inc. Because gerbils in the desired age range were limited in number at a given time, four repetitions of the experimental procedure were needed. From the time of weaning until the start of the experiment, the gerbils, separated by sex, were reared in group cages and were fed Purina Laboratory Chow and distilled water ad lib. At the beginning of the experiment, the gerbils were placed individually in suspended Wahmann LC-75 cages, which were moved to a Percival environmental chamber which measured 6.1 m long $\times$ 3.0 m wide $\times$ 2.4 m high. The ambient temperature was kept at 24°C, the relative humidity was 50%, and the cages were lighted uniformly from 0800 to 2000 h daily.

Apparatus and Procedure

The experimental design for the work was a 2 by 2 factorial arrangement in which the variables were as follows: (1) Mg-repletion (Mg:R) vs. Mg-depletion (Mg:D) and (2) repletion for minerals other than Mg (MS:R) vs. depletion for all minerals (MS:D). Pursuant to the arrangement, the gerbils were distributed randomly among four groups, subject to the restriction that there be 15 females and 15 males in every group. Thereafter, for a period of 28 days, the groups were each fed either a different combination of mineral salts or no salts at all in an otherwise complete ration which was provided in the form of a mash (Harriman, 1974). The food and distilled water were supplied ad lib. Both of the Mg:R groups were fed 1.0% (wt/wt) MgSO_4 (FW 120.39) in the mash, while the two Mg:D groups were fed an equal quantity of nonnutritive fiber (General Biochemicals, Inc.). Furthermore, each of the Mg:R and Mg:D groups assigned the MS:R treatment was given a commercial low-Mg salt mix (ICN Nutritional Biochemicals) which was entered into the food at the rate of 4.8% (wt/wt). The low-Mg salt mix contained the other six principal elements (Ca, Cl, K, Na, P, and S) together with five essential trace

elements (Cu, Fe, I, Mn, and Zn). Both the Mg:R group and the Mg:D group assigned to the MS:D condition were fed 4.8% non-nutritive bulk (wt/wt) in lieu of the low-Mg salt mix. Analyses of the maintenance rations were performed through use of an atomic absorption spectrophotometer (Perkin-Elmer, Model 403). Where Mg was withheld from the food, whether or not the low-Mg salt mix was added, there was <1 ppm Mg.

At the start of the 28-day feeding program, all of the gerbils were tested for disposition to seize by being placed separately in an open field for 4 min. An episode of seizing was recorded for each gerbil that showed disruption of ongoing motor behavior to the point where (1) myoclonic jerks of the forelimbs were shown and (2) there was loss of normal posture throughout a period of no less than 20 sec. Gerbils that seized during screening were discarded from the experiment and were replaced with other gerbils of the same parentage. On Day 4 of the feeding program, the gerbils in each group were entered into a series of seven further open-field tests that were separated, one from the next, by 4-day intervals. On test days, each session of open-field tests was begun at 2100 h because gerbils seize more readily at night than during the day (Thiessen et al., 1968). To reduce inequality in influences among groups related to the hour of testing, the groups were tested in changed order from test day to test day. Also, the order in which members of a given group were tested was reversed from one session to the next.

During the series of seven sessions, each subject was tested separately. The animal was carried in its home cage to a quiet, darkened room next to the chamber that housed the animals. Then the gerbil was put in the center of an open field that was 51 cm in diameter and 12 cm in height. The field was uniformly lighted by two 15-W fluorescent lamps placed at a height of 32 cm above a 3/8-in. wire-mesh screen that blocked escape from the field. The gerbil was watched for 4 min. If no seizure occurred, the animal was returned to its home cage and was observed for 2 min in a dimly lit area of the testing room. A seizure was counted for a gerbil on a particular test day if the gerbil seized with sufficient severity to meet the criteria described above in connection with selection of the subjects. Furthermore, seizure activity during the time of observation, ranging from a single event to the analogue of *status epilepticus*, was treated as a single episode.

RESULTS

At the start of the 28-day experiment, the 60 males weighed a mean of 62.0 ± 10.7 g, and the 60 females weighed a mean of 59.5 ± 7.2 g. Differences in respect to body weights among groups [$F(3,116) = 1.50$] and between sexes [$F(1,118) = .64$] were insignificant ($p > .20$). Gains in body weight during the period of the experiment were confined to members of the

Mg:R/MS:R group. At the end of the experiment, the gerbils in the Mg:R/MS:R group, all of which survived, had gained significantly in weight [$F(1,28) = 10.81$, $p < .01$] and weighed a mean of 67.0 ± 2.1 g. The sex difference factor was insignificant [$F(1,28) = .18$, $p > .20$]. Both deaths and weight losses among the survivors occurred in all three groups fed other forms of the mash. Table 1 indicates that, by the end of the experiment, there had been one death in the Mg:R/MS:D group, two deaths in the Mg:D/MS:D group, and 29 deaths in the Mg:D/MS:R group. The 15 males in the Mg:D/MS:R group lived a mean of 15.3 ± 4.4 days, and the 14 nonsurvivors among the females lived a mean of 16.5 ± 4.2 days. Nine of the deaths in the Mg:D/MS:R group took place either during open-field tests or during the 2-min observation periods that immediately followed the tests. By the end of the 28th day of the experiment, body weight losses, as mean percentages of starting body weights, were severe among the survivors in both the Mg:R/MS:D group (18.2%) and the Mg:D/MS:D group (21.0%). The severity of these weight losses may be measured against the finding (personal observation) that adult gerbils generally fail to outlast body weight losses exceeding 25% of initial weights. Many of the deaths in the Mg:D/MS:R group occurred so early in the experiment that no obvious relation of feeding plan to weight loss could be drawn.

Among the 120 subjects, 15 males and 11 females contributed one or more seizures to the total of 50 seizures listed in Table 1. The proportions of seizing males and seizing females differed insignificantly ($z = .93$, $p > .20$). Of the 26 affected animals, 18 belonged to the Mg:D/MS:R group, 6 were in the Mg:D/MS:D group, and 2 were in the Mg:R/MS:D group. A chi-square test which incorporated correction for low cell frequencies (Pirie & Hamdan, 1972) showed that the inequality in numbers of seizing gerbils among groups was significant [$\chi^2(1) = 4.93$, $p < .05$]. Furthermore, 46 of the 50 seizures took place among gerbils fed one or the other of the Mg-deficient diets. Presence of the low-Mg mineral mixture in the food aggravated effects of Mg-deprivation,

Table 1
Seizure Ratios Among Four Groups of Mongolian Gerbils Variouslly Repleted and Depleted (D) for Magnesium (Mg) and/or Other Mineral Salts (MS)

Mineral Combinations in Diets	Days on Which Open-Field Tests Were Given During 28-Day Period								Total Seizures	Total Subjects Seizing
	0	4	8	12	16	20	24	28		
Groups Fed Magnesium (Mg:R)										
Mg:R/MS:D	0/30	0/30	0/30	1/30	1/30	0/30	0/29	0/29	2	2
Mg:R/MS:R	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0	0
Groups Not Fed Magnesium (Mg:D)										
Mg:D/MS:D	0/30	0/30	2/30	1/30	1/30	2/29	1/29	3/28	10	6
Mg:D/MS:R	0/30	4/30	9/29	7/26	10/20	5/8	2/2	1/1	38	18

Note—Numerator = N of gerbils seizing; denominator = N of survivors tested.

for the Mg:D/MS:R group seized at 3.8 times the rate for the Mg:D/MS:D group.

DISCUSSION

These findings agree with previous work (Harriman, 1974, 1978) in demonstrating that withholding Mg from the food significantly increases the chances gerbils will seize upon exposure to an open-field arena. Again, there appears to be no sex difference with respect to susceptibility to seizures of this sort. In addition to confirming the earlier reports, the present findings show that the physiological and behavioral effects are exacerbated if other nutritionally important minerals are entered into the diet in normally suitable amounts during a period of Mg deprivation. Specifically, only one gerbil in the Mg:D/MS:R group survived the 28-day period of MG deprivation. In contrast, Table 1 shows that 28 gerbils in the second Mg-deprived group (Mg:D/MS:D group), which was fed no minerals whatever in the food, were alive after the same lapse of time. Furthermore, both the number of gerbils subject to seizing and the frequency with which the susceptible animals seized were sharply greater in the Mg-deprived group fed the salt mix (Mg:D/MS:R group) than in the Mg:D/MS:D group.

Consequently, the present results may, on first inspection, appear inconsistent by showing, in effect, that selective depletion of Mg in gerbils can produce more severe effects than follow from deprivation of all essential minerals. That there is here no more than an apparent paradox may be seen in the context of investigations into relations between equilibrium of ionic potential and irritability of living tissue. Loeb was the first to show (Kruse, Orient, & McCollum, 1932) that constancy in the irritability of normal tissue depends upon stability in the proportions of various ions, particularly Na, Ca, K, and Mg. Changes from normal ionic levels that were induced for any one of the four ions imparted properties to the tissue involved that it did not possess ordinarily. Thus, increase in either Na or K, as well as decrease in either Ca or Mg, led to elevated irritability of isolated muscle. Later work has shown that when selective ionic shifts are attempted, other ions are also affected. The hyperesthesia induced directly by reducing the Mg level may result also indirectly through feeding or withholding other metal ions from the food (Littledike & Cox, 1979).

In an early investigation of simple dietary Mg deficiency, Kruse et al. (1932) described a syndrome of fatal course in rats which was characterized by an advancing hyperesthesia and, ultimately, by convulsive tetany. The great importance of the Mg ion in the physiological economy of animals is indicated by findings that tetany generally does not occur in the

absence of a state of hypomagnesemia (Wilkinson & Stuedemann, 1979). Because other metal ions influence Mg homeostasis (Fontenot, 1979), effects of these ions upon the character of hypomagnesemic tetany merit attention. Unlike previous work on hypomagnesemia, the current investigation made use of a null mineral diet group so that contrasts could be drawn between effects of total mineral deprivation and effects of selective Mg deprivation. The high survival rate plus the low seizure rate in the Mg:D/MS:D group vis-à-vis the Mg:D/MS:R group may perhaps be comprehended in Loeb's terms as due to differences in the extent to which proportions of the usual ions found in muscle and its blood supply were changed by the dietary manipulations. Conceivably, there was less effect in the Mg:D/MS:D group because antagonism among cations was better maintained while the group underwent global mineral depletion than was the case for the Mg:D/MS:R group, which was deprived of a single mineral but which was intensively fed the other essential dietary minerals.

Results from the current study may be helpful in comprehending the nature of so-called "spontaneous seizures" shown by certain gerbils within colonies fed commercial chows exclusively or these chows plus supplements (Robinson, 1968; Thiessen et al., 1968). At issue is the possibility that some degree of disturbance in Mg homeostasis underlies both seizing incited by Mg deprivation and "spontaneous seizures" exhibited by apparently normal gerbils. In the latter category, seizing may reveal effects of age or genetic factors upon efficiency in Mg utilization (Fontenot, 1979; Littledike & Cox, 1979). Because of individual difference factors, assertions that gerbils subject to "spontaneous seizures" are free from dietary need (Robinson, 1968; Thiessen et al., 1968; Zeman, 1967) may be brought into question. Selective absorptive capacity for Mg may be insufficient in some gerbils within a colony to maintain an adequate Mg balance if the food is a commercial ration. In comment on the composition of such diets, Finger (1947) concluded that they tend to keep animal colonies in an intermediate state of nutritional balance because of variations in assimilative efficiencies. Even when the diets are fortified with important dietary elements, some fraction of the animals so fed may be presumed to suffer from nutritional lack owing to metabolic deficiency. From this analysis, gerbils said to seize "spontaneously," no less than gerbils undergoing Mg deprivation, are likely to be afflicted with hypomagnesemia of the type called "latent tetany" (Littledike & Cox, 1979). This form of tetany is a condition of hyperesthesia which is punctuated by tonic-clonic convulsions provoked by arousal no more severe than that provided by trivial stimuli. It may be that gerbils fed low-Mg

food develop "latent tetany" as a transitional stage in an acute metabolic deficiency for Mg which ends in death (Littledike & Cox, 1979). "Latent tetany" may also be chronic in nature as a result of an innate impairment in capacity to use sufficiently the Mg available in a commercial chow and so account for "spontaneous seizures." As an outcome of either overlapping eventuality, a seizure could be triggered, once "latent tetany" was created, by placing the gerbil in a suitably stressful surround, such as an open field.

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