Exploring the Magnesium-deficient Weanling Rat as an Animal Model for the Sudden Infant Death Syndrome: Physical, Biochemical, Electrocardiographic, and Gross Pathologic Changes

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Summary

An hypothesis has been proposed that a relative or absolute deficiency of magnesium in the rapidly growing infant might be a major cause of the sudden infant death syndrome (SIDS, crib death). We studied the magnesium-deficient weanling rat as an animal model for the human infant, exploring physical, biochemical, and gross pathologic changes during the sudden seizure episode of magnesium deficiency, as compared with during fatal strychnine seizures. In addition, the electrocardiographic changes were examined during this episode of magnesium deficiency.

No audiogenic seizures could be elicited on day 7 in any of five rats with acute magnesium deficiency (0-Mg) that fasted for 24 hr. The plasma magnesium concentration of 0-Mg rats with no known seizures was 0.36 ± 0.02 (27); the plasma magnesium value increased during fasting to 0.96 ± 0.06 ($P < 0.001$).

Apparently new findings in the blood chemistries associated with the seizure-shock episode of magnesium deficiency include a sharp increase in plasma osmolality, hematocrit, blood sugar, blood urea nitrogen, uric acid, and the activity of SGOT, SGPT, LDH, and creatine kinase.

Electrocardiograms were taken in rats that weighed 28-32 g at the onset of the study. During the seizure-shock episode, there was sinus arrest, and the ventricular rate precipitously dropped to about one-third of the normal rate. The ventricular complexes were sometimes initiated from two electrical foci. Apnea persisted and bradycardia was rapidly progressive in rats that died. Other rats made rapid, spontaneous recoveries; the heart rate and rhythm improving as animals resumed respirations. Spiked electrical complexes sometimes developed during the early recovery period. The tracings normalized within 18 hr.

Pronounced intrathoracic pathologic changes were found in rats after a fatal tetanic and apneic episode. In these animals there was some variation in the severity of pathologic change. The most severe pathology was usually found in the smallest and youngest animals and usually included scattered petechiae on the pleura, the thymus, and the pericardium; expanded lungs that were plum-colored in areas extending from the hilus and in scattered patches throughout; edema and congestion on the cut surface; and foamy fluid, sometimes blood-tinged, in the bronchi. The right atrium and the right ventricle were usually distended with dark, fluid blood. The urinary bladder was always contracted and empty.

When weighed with fluid contents, both the heart and lungs of the 0-Mg rats that died of seizures weighed more than those of 0-Mg rats with no known seizures. The weights in grams of four rats from each of these two groups were, respectively: body weights, 53.9 ± 0.05 and 53.8 ± 0.08 ; lungs, 0.831 ± 0.038 and 0.585 ± 0.05 ($P < 0.001$); and hearts, 0.636 ± 0.042 and 0.40 ± 0.03 ($P < 0.005$).

In contrast to rats dying of magnesium deficiency, rats that died of seizures due to strychnine were only mildly cyanotic. Their lungs were only slightly inflamed, with occasional petechiae. Their body weights and the weights of their lungs and hearts were not significantly different than the weights of four controls.

Speculation

The sudden, potentially fatal episode occurring in the very young magnesium-deficient rat and in SIDS victims appears to be a form of shock similar to anaphylactic shock, but without a sensitizing agent. Pathologic changes are not due to the violence of seizures alone, but could well be caused by biochemical events associated with the shock state.

INTRODUCTION

Sudden unexpected death in human infants (SIDS, crib death) is the largest single cause of postneonatal infant mortality (8). It has an incidence of 2 to 3 per 1000 live births (8), and involves approximately one-third of all babies dying after the first week and before the end of the first year of life in the United States (64). The cause remains unknown, although much new information concerning SIDS has been learned through recent research, as summarized in Hasselmeier's report from the National Institute of Child Health and Human Development (47). A hypothesis (19) has been proposed that a relative or absolute deficiency of magnesium (possibly with an associated mineral imbalance) (29) in the rapidly growing infant might be a major cause of SIDS.

Magnesium deficiency is a relatively important problem in the human infant (24, 63, 71, 73, 85). In one series from Edinburgh, Scotland (14), of the 12 per 1000 neonates with convulsions, about 7 per 1000 were due to primary, uncomplicated hypocalcemia/hypomagnesemia. Magnesium deficiency has been diagnosed in infants and young children who had metabolic and nutritional disorders and presented with nonspecific findings including hyperirritability, failure to thrive, apathy, anorexia, sudden onset of apnea or respiratory distress, and tonic and clonic seizures (7, 16, 21, 26, 28, 45, 63, 71, 73). Because magnesium deficiency occurs in conjunction with other medical problems, it is best to investigate the syndrome through study of animal models without complicating illness.

Magnesium deficiency causes sudden, unexpected death in animals. There are species differences and intraspecies variations. Young cows sometimes died quietly (57,68) or violently (70). Normal appearing magnesium deficient mice died almost immediately after a single violent spasm (4), while young rats (20, 49, 62) and dogs (62) usually developed vasodilatation, hyperirritability, and a sudden episode that involved apnea, tonic or clonic seizures, and often death. The lower the weight and age of the animal at the onset of magnesium deprivation, the shorter the life and the more abrupt the death (49,62). Early death may follow a period of rapid growth, particularly if the diet is limited in magnesium but rich in protein, calcium, and phosphorus, three nutrients that increase the metabolic requirement for magnesium, as previously reviewed (15, 24). One would expect the period of most rapid growth in early infancy to be the most vulnerable age in man (19).

It is not known whether magnesium deficiency may provoke a sudden, fatal episode in the human infant, but because of its potential importance, we have studied this event in detail in the magnesium deficient weanling rat as an animal model for the human infant. We explored physical, biochemical, and gross pathological changes related to the sudden seizure episode of magnesium deficiency, as compared to these after fatal strychnine seizures. The electrocardiographic changes were studied during this episode of magnesium deficiency. Data from magnesium deficient weanling rats were compared with reported findings in various types of shock and in human infants dying from SIDS. Finally, we have reviewed studies that have tested the hypothesis directly and indirectly, in the light of findings from recent SIDS research (47).

MATERIALS AND METHODS

Animals

Male weanling Sprague-Dawley rats weighing 28-40 g (Hilltop Lab Animals, Scottsdale, Pa.) were individually housed in raised bottom stainless steel cages and provided with deionized water *ad libitum*. Unless otherwise stated, they were fed *ad libitum* and were in the fed state when studied. All rats were weighed daily at 8 A.M. on a top loading balance (Model P 11 N, Mettler Instrument Corp., Princeton, N.J.), and their growth and physical behavior were monitored.

Animal Diets

To achieve acute magnesium deficiency that often resulted in spontaneous seizures and death at one week (a model for SIDS infants that died before six months of age), weanling rats were fed a diet that provided only 0.3 mg of magnesium/100 g of diet and no added magnesium (0-Mg), 20 % casein, and other nutrients listed in Table 1. Their controls were fed the same diet, but with 100 mg of magnesium/100 g (100-Mg).

To achieve attenuated magnesium deficiency that often resulted in spontaneous seizures and death at two weeks (a model for SIDS infants that died during the second six months of life), weanling rats were fed a diet that provided 5 mg of added magnesium (5-Mg), 40 % casein, and other nutrients listed in Table 1. Their controls were fed the same diet, but with 150 mg of magnesium/100 g (150-Mg), the higher magnesium to accommodate the higher protein, calcium, and phosphorus in the additional casein (27).

Natural controls were fed laboratory chow (LC) (No. 5001, Ralston Purina Co., St. Louis, Mo.) for one or two weeks. This contained 23.4 % crude vegetable protein and 210 mg of magnesium/100 g.

STUDY DESIGNS AND PROCEDURES

A rat was studied once for one procedure (blood sampling, pathology, etc.) at one point in the experiment (during seizures, $\frac{1}{2}$ hr post seizures, etc.) and was then destroyed. There was one exception: the same rats in the electrocardiograph study were photographed at autopsy. There were 6 to 14 samples in each final experimental group, representing 8 to 15 rats per group initially raised for study. Some died and could not be studied. Hemolyzed samples were discarded. Rarely, plasma from two similar animals was combined as one sample. All chemical determinations in one experiment could not be conducted simultaneously; 60 rats were raised for study at one time; controlled studies were run serially in close sequence. The combined purified controls were therefore the largest groups.

Baseline studies were made on day 6 in acutely deficient 0-Mg animals with no known previous seizures (Fig. 1), and on day 13 the 5-Mg rats with attenuated magnesium deficiency. One day after baseline studies, similar rats were individually subjected to an auditory stimulus, ringing metal keys that had a sound pressure level of 80 decibels, concentrated between 4 and 32 kilohertz. The keys were rung until the rats convulsed, but not in excess of 60 seconds. A group of five 0-Mg rats with no known seizures were fasted for 24 h between days 6 and 7 and challenged with auditory stimuli.

There were three separate investigations concerning blood chemistries that included whole venous blood hematocrit, plasma magnesium, and a clinical chemical profile performed on an autoanalyzer.

1. Comparisons during seizures in acute magnesium deficiency with those due to strychnine.

100-Mg controls were fed *ad libitum* or pair-fed with 0-Mg rats. Some pair-fed 100-Mg rats were studied as controls, and some were treated with strychnine in the dose of 1.9 mg/kg of body weight intraperitoneally, a dose found to produce fatal seizures that clinically best simulated those of acute magnesium deficiency. The strychnine-treated rats were studied at the end of severe seizures, at the point of death. Groups of 0-Mg rats with acute magnesium deficiency were studied in the pre-seizure period on day 6, and on day 7 at the onset of seizures and immediately after prolonged seizures.

2. Further studies during the seizure episode in acute magnesium deficiency.

Baseline studies were made in 0-Mg rats with no known seizures and in 100-Mg and LC controls on day 6. Other 0-Mg rats were studied on day 7 during seizures, or during the recovery period, 18 h after seizures.

Additional chemical studies were made in 0-Mg and 100-Mg rats raised in close sequence to the other rats studied in Experiment 2. Plasma osmolality was found by freezing point determination of lithium-heparinized plasma on an osmometer (Fiske Assoc., Uxbridge, Mass.), and plasma specific gravity was measured on a Ts meter (AO Instrument Co., Buffalo, N.Y.). Acetoacetate was tested in plasma and urine using Ketotix (Ames Co., Elkhart, Ind.); the urine was obtained from the urinary bladder under direct visualization using a tuberculin syringe. Plasma lactic acid was measured on a Calbiometer (Calbiochem Co., La Jolla, Cal.); and pCO_2 on a pH blood gas analyzer (Model 213, Instrumentation Lab., Inc., Lexington, Mass.). When available, blood from the animals raised for these additional chemical studies was sometimes analyzed for whole venous blood pH, hematocrit, or plasma magnesium at appropriate intervals between days 6 and 8.

3. Studies during the seizure episode in attenuated magnesium deficiency.

Baseline studies were made on 5-Mg rats with no known seizures and in 150-Mg and LC controls on day 13. Other 5-Mg rats were studied on day 14 post repeated seizures or after death, or on day 15, 18 h after seizures.

Animals were anesthetized with sodium pentobarbital (Abbott, N. Chicago, Ill.) in an intraperitoneal dose of 5 mg/100 g of body weight for all painful procedures, and at the end of such procedures they were killed with an overdose of this drug. About 1 ml of blood was drawn percutaneously from the right ventricle in plastic syringes that had been prepared with lithium heparin (Sigma, St. Louis, Mo.). Whole blood pH readings were made on a digital pH meter (Model 3550, Beckman Instruments, Irvine, Cal.). Duplicate microhematocrit determinations were made using a microhematocrit centrifuge (Damon/IEC Divisions, Needham Hts., Mass.), and read manually. The blood was centrifuged at 39° (Damon/IEC/PRJ), and the plasma was separated and stored at 39° until analyzed within 24 h. Duplicate plasma samples were diluted 1:50 in 0.1 % lanthanum solution and read for magnesium on an atomic absorption spectrophotometer (Model 303, Perkin-Elmer Corp., Norwalk, Conn.). The chemical profile was made on the SMAC autoanalyzer (Technicon Instrument Corp., Tarrytown, N.Y.), for which 0.5 ml of plasma was required. The mean coefficient of variation for the SMAC system was 3.2 (81).

Electrocardiograms.

Electrocardiograms were taken on a model VS4 Cambridge instrument (Ossining, N.Y.) on unconscious or sedated animals, using needle electrodes inserted in the dorsum of the feet of supine animals. This study was limited to rats that weighed 28-32 g on day 0 of the experiment. On day 6, electrocardiograms were taken in five 100-Mg controls and five 0-Mg rats that had not convulsed. On day 7, five 0-Mg rats that became unconscious and tetanic on gentle handling were studied without any sedation.

Gross pathological examinations.

Gross pathological examinations were conducted immediately after death. No blood samples were taken; the intact hearts and intact lungs were weighed on a model H 20 Mettler balance without disturbing their fluid contents.

Statistical analyses.

Statistical analyses were made using standard tables for two-tailed Student's *t* distribution or the analysis of variance. The statistics throughout the study were reported as the mean, the standard error of the mean, the number of samples in brackets, and the probability.

RESULTS

GROWTH AND PHYSICAL BEHAVIOR

Controls.

Analysis of variance testing showed no difference in the increment of weight gain between days 13 and 14 in the three groups of 10 control rats that were studied for growth; their initial weights approximately doubled at one week and tripled at two weeks. All controls remained calm throughout the study and ignored the auditory stimuli; none developed erythema and none died spontaneously.

Acute magnesium deficiency.

For the first three days, the 0-Mg rats in this study grew as well as the controls, but they then became anorexic and between days 3 and 4 their weight gain was reduced ($P < 0.05$). They began to develop hyperemia, which was fading by day 6. Between days 6 and 7, the difference in weight gain between the control and acute groups was highly significant ($P < 0.001$), and the animals were tremorous, hyperirritable, with exaggerated startle reactions. On day 7, rats that initially weighed 35-40 g usually developed apnea and tonic seizures. (Rats that did not convulse on day 7 often had physical changes suggesting recent seizures, and they were not studied). Rats that survived the seizure episode began to breathe spontaneously and began to exhibit variable activity that included hyperventilation, clonic seizures, or lassitude, followed by a return to normal activity. They usually resisted further audiogenic seizures for at least 24 h.

No audiogenic seizures could be elicited on day 7 in any of the 0-Mg rats that had fasted for 24 h.

Attenuated magnesium deficiency.

Between days 2 and 3, the 5-Mg rats showed significantly less weight gain than those fed 150-Mg control diet, ($P < 0.05$), a trend that persisted throughout this study. Erythema usually developed by day 7 and faded by day 12, when the rats were extremely tremulous and hyperirritable. When challenged with auditory stimuli on day 14, these rats usually had violent and prolonged convulsions and became progressively cyanotic, limp, exhausted, with cold paws and often with bloody froth at the mouth or nostrils. Some died; surviving animals recovered more slowly than surviving 0-Mg rats with acute magnesium deficiency.

CHEMICAL CHANGES IN THE BLOOD.

Experiment 1.: Comparisons during seizures in acute magnesium deficiency with those due to strychnine.

Examination of the clinical chemical profile (Tables 2 A,B,C) showed some differences between 100-Mg controls fed ad libitum and those pair-fed. The expected findings due to magnesium deficiency, reduced plasma protein and reduced activity of alkaline phosphatase (EC 3.1.3.1.) were found. During the seizure-shock episode, the blood pH rapidly decreased, while highly significant increases ($P < 0.001$) were found in blood hematocrit, plasma magnesium, calcium, potassium, inorganic phosphorus, uric acid, iron, total protein, total bilirubin, and the plasma activity of Glutamic-oxaloacetic transaminase (SGOT) (EC 2.6.1.1.), Lactate dehydrogenase (LDH) (EC 1.1.1.27), and ATP:Creatine N-phosphotransferase (Creatine kinase) (EC 2.7.3.2.), and a smaller increase ($P < 0.01$) was found in plasma activity of Glutamic-pyruvic transaminase (SGPT) (EC 2.6.1.2.).

Greater changes were found in blood measurements such as the hematocrit and the plasma enzyme activity in the convulsing 0-Mg rats compared with pair-fed rats dying of strychnine seizures ($P < 0.001$). The mean pH value of five 0-Mg rats that died of seizures, 6.94 ± 0.01 , was significantly different from that of four rats that died of strychnine seizures, 7.09 ± 0.01 ($P < 0.05$).

Experiment 2.: Further studies during the seizure episode in acute magnesium deficiency.

Blood chemistries were studied before, during, and $\frac{1}{2}$ h after a single seizure episode. During the seizure episode, the whole venous blood pH decreased (Fig. 2), while the hematocrit and plasma magnesium values sharply increased (Fig. 3 and 4), returning to pre-seizure levels during the recovery period.

The SMAC clinical chemical profile was conducted in the following groups of rats; the numbers in brackets indicate the final number of samples analyzed: purified controls (100-Mg) (22), laboratory chow-fed controls (4) and at the following times in magnesium deficient 0-20 rats: on day 6 in animals with no known seizures (9), during a single seizure episode (20), $\frac{1}{2}$ h after a seizure episode (5), and 18 h post seizures (6). The same plasma constituents reported in Table 2 were studied. No significant differences were found in the blood chemistries of the 100-Mg and the laboratory chow controls. The values of the 100-Mg controls and the pattern of change in the 0-Mg rats during seizures in this study were essentially the same as corresponding data in Experiment 1 (Table 2).

One noteworthy change was seen $\frac{1}{2}$ h after a single seizure episode: the mean plasma glucose value increased from the pre-seizure level of 148.4 ± 7.6 to 195.2 ± 11.8 mg/dl ($P < 0.01$).

Within 18 h of a single seizure episode, all blood chemistries had returned to pre-seizure levels.

Additional studies in acute magnesium deficiency on days 6 and 7.

Plasma osmolality. No difference was found in the plasma osmolality of 100-Mg controls, 306.0 ± 1.54 (4) and 0-Mg rats with no known seizures 306.6 ± 2.07 (4), but after the onset of tetany, the osmolality was increased, 337.7 ± 3.6 (4) ($P < 0.001$), and it further increased after prolonged seizures to 353.2 ± 4.6 (4) ($P < 0.001$), and after fatal seizures to 407.0 ± 5.6 (2).

The mean osmolality of five 0-Mg rats that fasted for 24 h, 319.8 ± 2.26 , was greater than that of the fed 100-Mg controls, ($P < 0.005$) and of the fed 0-Mg rats ($P < 0.01$).

Plasma specific gravity. The mean plasma specific gravity for two 100-Mg controls was 1.03. Mean values found in two 0-Mg rats were 1.027 in rats with no known seizures, 1.03 at the onset of seizures, 1.035 after prolonged seizures, and greater than 1.035 after fatal seizures. These data follow the trend of the plasma osmolality.

Plasma lactic acid. Plasma lactic acid, determined in three rats in each group, was, in mg/dl: 49.3 ± 12.2 in sedated 100-Mg controls, 60.7 ± 6.3 in tremulous 0-Mg rats, and 117.2 ± 13.1 in rats with severe convulsions.

Right ventricular pCO_2 . The right ventricular blood pCO_2 of 100-Mg controls was 41.75 ± 1.01 (4) and for hyperirritable 0-Mg rats was 37.0 ± 2.16 (3). Following prolonged apnea and tonic-clonic seizures, the value in 0-Mg rats was 58.1 ± 3.29 (4).

Plasma and urinary ketones. No acetoacetic acid was found in plasma or in available samples of urine except for urine from five fasted 0-Mg rats, which was a mean value of 1.2 on a 0 to 4+ scale. No urine was found in the bladder of rats that had had a seizure episode.

Experiment 3.: Studies during the seizure episode in attenuated magnesium deficiency.

Blood chemistries were studied before, during, 18 h after seizures, and at death. The 5-Mg rats in the two-week study of attenuated magnesium deficiency showed a pattern of change similar to that of 0-Mg rats with acute magnesium deficiency: values were distorted during or soon after seizures and usually returned to the pre-seizure level within 18 h post seizures.

The venous blood pH of 150-Mg controls and 100-Mg controls was the same, and the 5-Mg rats closely followed the pattern of the 0-Mg rats shown in Fig. 2. The mean hematocrit of the 150-Mg rats was, in percent: 34.5 ± 0.68 , significantly higher than that of the 100-Mg rats (Fig. 3) ($P < 0.001$). The pre-seizure 5-Mg animals had a mean hematocrit of 38.7 ± 0.63 (13), which increased to 44.6 ± 1.21 (5) after seizures ($P < 0.001$), and to 49.4 ± 2.16 (5) in rats

that died ($P < 0.001$). The mean plasma magnesium value of 5-Mg rats followed the pattern of change shown in the 0-Mg rats in Fig. 4. Prior to known seizures, the value was, in mEq/ liter, 0.43 ± 0.02 (10), which increased to 1.39 ± 0.12 (6) post repeated seizures ($P < 0.001$), and returned to the pre-seizure level, 0.40 ± 0.02 (10) 18 h after seizures.

The SMAC clinical chemical profile was conducted in 150-Mg controls (12), laboratory chow-fed controls (11), post repeated seizures (7), at the point of death (7), and 18 h post seizures (7). The 150-Mg control plasma protein value, 4.83 ± 0.05 g/dl (12) and urea nitrogen value, 30.16 ± 1.80 mg/dl (12) were both higher than corresponding values found in 100-Mg controls (Table 2) ($P < 0.001$). Highest values for blood urea nitrogen were found after seizures in moribund 5-Mg animals, 36.75 ± 1.43 mg/dl (7), which was significantly higher than the 5-Mg pre-seizure value, 30.41 ± 0.67 mg/dl (12) ($P < 0.001$). Similarly, the uric acid values at the point of death were 3.50 ± 1.50 mg/dl (7), increased compared to pre-seizure values, 1.82 ± 0.26 mg/dl (12) ($P < 0.001$). As in the first two experiments, nearly all of the blood components tested, including the plasma enzyme activity, increased significantly during seizures, but then quickly returned to pre-seizure levels; only three plasma values were still significantly elevated above the pre-seizure level 18 h post seizures: calcium ($P < 0.025$); protein, ($P < 0.05$), and urea nitrogen, ($P < 0.05$).

ELECTROCARDIOGRAPHIC CHANGES

Changes in the electrocardiograms are shown in Fig. 5, and are described in the legend.

GROSS PATHOLOGICAL CHANGES

Findings after fatal seizures due to magnesium deficiency.

External features. Control rats fed the purified diets and those fed laboratory chow were indistinguishable. Rats dying of acute magnesium deficiency appeared to be well hydrated and in good physical condition, although their body weights were lower than the mean weights of magnesium-fed controls. Rats dying of attenuated magnesium deficiency had coarser and thinner coats and weighed less than controls, but they appeared to be well hydrated and adequately nourished.

Rats that died quickly in apnea and tetany rarely showed more than mild cyanosis. Those dying after prolonged seizures, whether from acute or attenuated magnesium deficiency, were deeply cyanotic, and they often had froth in the nostrils. They often wedged the muzzle area of their faces into a corner, sometimes securing a wire of the cage in their mouths. Their forepaws were tightly flexed.

Internal features. The 100-Mg controls had light salmon-colored lungs that filled about two-thirds of the thoracic cavity. There were no areas of inflammation and no petechiae (Fig. 6). About 0.5 ml of urine could usually be obtained from the urinary bladder.

The lungs of 0-Mg rats that had had no known seizures were slightly inflamed, and their hearts were not remarkable.

The internal pathological changes were chiefly intrathoracic in magnesium deficient rats that died spontaneously after a sudden apneic and tetanic episode. The animals represented in Fig. 6 weighed 28-32 g initially, and they were shipped in December, 1977, a cold month. In these 0-Mg rats, scattered petechiae were usually found on the pleura, the thymus, and the pericardium. The lungs were fully expanded and did not collapse with time; plum-colored areas extended from the hilus and in scattered patches throughout. Edema and congestion were seen on the cut surface, and foamy fluid, sometimes blood-tinged, was found in the bronchi. The right atrium and the right ventricle were often distended with dark, fluid blood. The livers were darker than those of the 100-Mg controls. The urinary bladders were contracted and empty. These features describe all magnesium deficient weanling rats dying spontaneously throughout this study, with some variation in the number and distribution of petechiae, lung expansion, severity of hemorrhage, and other pathological findings. The most severe changes were usually found in the smallest and youngest animals.

Both the hearts and lungs of the 0-Mg rats that died of seizures weighed more than those of 0-Mg rats with no known seizures. The weights in grams of four rats from each of these two groups were, respectively: body weights, 53.9 ± 0.5 and 53.8 ± 0.8 ; lungs, 0.831 ± 0.038 and 0.585 ± 0.05 ($P < 0.001$); and hearts, 0.636 ± 0.042 and 0.40 ± 0.03 ($P < 0.005$).

Findings after fatal seizures due to strychnine poisoning.

The 100-Mg rats that died of seizures due to strychnine poisoning were mildly cyanotic, and their bodies and hind legs were in rigid extension. Their lungs were slightly inflamed and, of four rats examined, two each had two small petechiae on the pleurae. Their body weights and the weights of their lungs and hearts were not significantly different from the weights of four 100-Mg controls. These respective weights in grams were: body weights, 67.0 ± 2.8 and 72.8 ± 2.5 ; lungs, 0.68 ± 0.02 and 0.69 ± 0.02 ; and hearts, 0.47 ± 0.02 and 0.50 ± 0.07 .

DISCUSSION

PART I. TESTING THE HYPOTHESIS IN AN ANIMAL MODEL (THE PRESENT STUDY)

The present study examined the sudden, potentially fatal seizure episode in the magnesium deficiency syndrome in weanling rats serving as models for the human infant of several months to one year of age. The model for the infant during the first six months of life was studied in more detail, because the greatest number of SIDS deaths occur before six months of age (87), with a peak incidence between two and four months (8, 87). Since the newborn rat has many characteristics of the premature human infant (37), an early-weaned rat is an approximate model for the full term human infant. If three years in the life of a rat is approximately equal to 90 years of human life (37), six rat-days would correspond to about six months of human life. The rate of growth in the control rats is similar to that of the full term human infant that usually doubles his birth weight in five months and triples it in one year (89).

An attenuated magnesium deficiency (27) was produced by feeding an amount of magnesium known to support life under normal dietary conditions (20), combined with high dietary protein, which increases the metabolic requirement for magnesium and aggravates the magnesium deficiency syndrome (15, 20, 57). The

higher protein and non-protein nitrogen products in the blood of animals fed 40 % casein suggest that these young rats, particularly those deficient in magnesium, were unable to completely utilize this large load of dietary protein.

COMPARISON OF CHANGES FOLLOWING FATAL SEIZURES DUE TO ACUTE MAGNESIUM DEFICIENCY WITH THOSE DUE TO STRYCHNINE

The data in Experiment 1 showed that the biochemical changes in 0-Mg rats that survived seizures were more severe than the changes after fatal strychnine seizures. The magnesium deficient rats had evidence of greater hemoconcentration than the strychnine-treated rats, and only the magnesium deficient animals had evidence of significant increases of fluid in the lungs. The increase in activity of SGOT, SGPT, LDH, and creatine kinase following the seizure episode of magnesium deficiency was greater than the increase that followed fatal strychnine seizures. This suggests a greater increase in membrane permeability in the magnesium deficient animals at that time. Kulka (50) reported temporary plasma leakage from dilated vessels in the ears of magnesium deficient weanling rats.

COMPARISON OF REPORTED FINDINGS IN SHOCK WITH THOSE OF THE SEIZURE-SHOCK EPISODE OF MAGNESIUM DEFICIENCY

Physical findings. The classic symptom complex common to all forms of shock may include apathy, pallor, hypothermia, hypotonia, and tremors (92). Particularly in anaphylactic shock, the onset may be sudden and unexpected, and the condition may be rapidly fatal (6, 93), or self limiting (93), followed by rapid and complete recovery (33).

The rats in the present study appeared to be in shock during the seizure episode. The unpredictability of their courses, ranging from precipitous death to spontaneous, rapid recovery, was like that of anaphylactic shock.

Electrocardiographic changes. Variable changes in rate, rhythm, and the configuration of the ST and T complexes of the electrocardiogram have been reported in anaphylactic shock (11, 13, 43), with reversal to the preshock pattern within 3½ hours in one patient (13).

The electrocardiographic changes in 0-Mg rats in the present experiment were nonspecific. They also involved changes in rate, rhythm, and what appeared to be the ST-T complexes, and rapidly normalized in recovering animals.

Chemical changes in the blood. Many conditions such as hemorrhage, trauma, and anaphylaxis can cause a shock state (10, 75) which initiates a chain of biochemical events that tends to maintain the shock (10). This may include involvement of neurohumoral transmitters and vasoactive substances, and possibly various proteases, lysosomal enzymes, and kinins (10). In anaphylaxis, the clinical manifestations are entirely due to the action of diverse chemical mediators that are rapidly released. Histamine, slow reacting substance of anaphylaxis, and eosinophilic chemotactic factor act to increase vascular permeability, contract smooth muscle, and attract inflammatory cells (6).

Changes in the blood chemistries common to all forms of shock may include the following, with modifications depending on the form of shock: decreased pH; increased hematocrit; increased plasma potassium, calcium, magnesium, and phosphorus; an early rise and subsequent decline in plasma glucose; elevation of plasma non-protein nitrogen components; and increased activity of several enzymes in plasma, including SGOT, SGPT, and LDH (44,59, 75).

During and immediately after the seizure episode, the rats in the present study had chemical changes in the blood similar to those of shock. Terminal findings included elevation of both plasma pCO_2 and plasma lactate, associated with a decreased blood pH. As Moore pointed out (59), this combination of chemical changes suggests that the parenchymatous pulmonary disease may have superimposed respiratory acidosis upon the already mounting lactic acidosis of the low flow state of shock. The rats were apparently in shock during the seizure episode which might well be called seizure-shock.

Pathological changes. In anaphylactic shock as it occurs in man (51) and cattle (3), findings include frothy fluids in the bronchi and generally inflated lungs that fill both pleural cavities. These lungs weigh more than usual and show areas of pink emphysema and plum-colored atelectasis and scattered petechiae on the visceral pleurae. The heart may show right ventricular dilatation (51), and the urinary bladder is usually empty (33).

The pathological changes of animals dying suddenly of magnesium deficiency closely resembled the above description. As noted above, these findings were not seen in rats dying of strychnine seizures, although tetany, apnea, and sometimes clonic seizure activity was common to both syndromes. The cause of death in strychnine poisoning is generally considered to be hypoxia (36). The pathological changes of magnesium deficiency were not due to the physical activity during seizures, but could have been related to the biochemical events initiated during the seizure-shock episode.

COMPARISON OF REPORTED FINDINGS IN SIDS WITH THE SEIZURE-SHOCK EPISODE OF MAGNESIUM DEFICIENCY

Neonatal features and growth. Prospective studies have shown that the SIDS infant was often born prematurely (8, 40), or was underweight for gestational age (61), had lower Apgar scores (61), was in greater perinatal distress, was more irritable (61, 65), and had some impairment of growth either throughout the first six months (65), or just before death (61).

Unlike the SIDS babies, the rats in the present study were normal until fed the magnesium-restricted diets. The acutely deficient rats had impaired growth terminally, and rats with attenuated magnesium deficiency had suboptimal growth throughout the study. Thus both types of growth retardation found in SIDS (61, 65) were represented. A better model for SIDS might have been pups born to magnesium deficient dams; in this situation the dams look well, but the pups may be stillborn (90), of low birth weight (34, 90), and have high perinatal morbidity (34,90) and mortality (90).

Physical and electrocardiographic changes during apparent life-threatening episodes. Severe, apparently life-threatening episodes suggested to Bergman (9) that there might be a continuum of severity in the sudden infant death syndrome. Harris (46) described an infant who had syncopal episodes of sudden onset during which the electrocardiogram revealed ventricular rates of 30 to 40 / min., but was normal between attacks. The infant died suddenly during such an episode; petechiae, presumably intrathoracic, were found on postmortem examination (46). Froggatt et al. (38) emphasized the history of previous fainting, cyanotic or apneic episodes, or periodic breathing. Stein-

schneider (78) studied five infants who developed apnea and cyanotic episodes of undetermined origin; two died of SIDS. In one baby he studied, the bradycardia improved when the cardiac electrical activity was initiated from an ectopic focus (77). Stevens (79) traced the progression in four infants from the sudden onset of apnea, pallor, and bradycardia to labored and deep inspiratory efforts, and finally death with over-inflated lungs. In a study of cardiac responsiveness to an auditory stimulus, Salk et al. (72) noted that the future SIDS victim showed more lability in cardiac rate and poorer stabilization of rate than other infants that survived. A number of these findings have been attributed to instability of the autonomic nervous system (72, 77, 78).

Geertinger emphasized sudden, brief fits of rigidity associated with pallor or cyanosis, abrupt onset of difficult breathing, and a strange look or glassy stare in infants that subsequently died of SIDS and suggested that such infants may have had an electrolyte imbalance (40).

In the week before death, and particularly in the 24 to 48 h period prior to death, symptoms commonly reported in SIDS infants included irritability, loss of appetite, restlessness, dyspnea, and protracted crying, sometimes of abnormal pitch (2, 31,38,61,69,86).

During the last few days of life, the rats fed magnesium deficient diets were anorexic and apathetic, but when disturbed they were jittery, tremulous, and had exaggerated startle reactions, sometimes with brief tetanic spasms and sudden pallor (blanching of the ears). During the terminal episode, some rats emitted shrill squeaks.

Magnesium deficient rats appeared to have instability of the autonomic nervous system. They showed hypersensitivity to movement (handling) or to auditory stimuli and developed sudden loss of consciousness, prolonged apnea, and electrocardiographic changes (bradycardia of about one-third of the normal cardiac rate, sinus arrest with ventricular complexes initiating from one or two foci, and sudden shifts of cardiac rate and rhythm). The normal appearance of the electrocardiogram one day after near-fatal seizures corresponded to the normal electrocardiograms that Harris found between episodes of vasovagal syncope in a future SIDS infant (46).

The most remarkable similarity between the sudden episode of magnesium deficiency and SIDS was the ability of the magnesium deficient baby rat and the human infant to suddenly collapse and either die or spontaneously recover, appear normal for a time, and then suddenly die.

Blood chemistries before, during, and following life-threatening episodes. Some of the changes in blood chemistries that have been found in SIDS include severe acidosis, hyperkalemia, inconstant hypercalcemia, variable abnormalities in blood sugar values, very high blood urea levels, markedly increased activity of serum LDH and SGOT, and high plasma osmolality (53,55,86). As the investigators have pointed out, findings from blood samples taken postmortem must be evaluated in controlled studies. Changes like those described above were found in a future SIDS infant who was stricken at home and studied during life at the Massachusetts General Hospital (30). His initial findings included severe metabolic acidosis; elevation of serum potassium, urea nitrogen, glucose, osmolality, and SGOT activity; and high normal CPK plasma activity. The T waves of the electrocardiogram were peaked, possibly due to hyperkalemia. The osmolality was unexplained; Brady (12) pointed out that there was no dehydration, diarrhea, or hyperpyrexia.

In the present study, the blood sampled in living 0-Mg and 5-Mg animals after seizures showed the nonspecific changes found in shock in man (44,75), as well as the changes found in the SIDS victim during life (30) and in SIDS infants after death (53,55,86). Apparently new findings in the blood chemistries associated with the seizure-shock episode of magnesium deficiency include a sharp increase in plasma osmolality, hematocrit, blood sugar, blood urea nitrogen, uric acid, and the activity of SGOT, SGPT, LDH, and creatine kinase. The increases in plasma potassium and the increased plasma enzyme activity suggest increased membrane permeability (44). This study demonstrated the lability of plasma magnesium and the unreliability of plasma as a diagnostic tissue.

Few studies have been made during the seizure episode of magnesium deficiency; no chemical studies could be found in the very young mammal. In a study of adult magnesium deficient rats, Martindale and Heaton (54) noted increased plasma calcium, inorganic phosphorus, and magnesium after convulsions, with no change in plasma potassium or specific gravity, suggesting mobilization of skeletal rather than intracellular electrolytes and no appreciable hemoconcentration. In view of the higher mortality in the very young, one would expect greater distortions of plasma chemistries in weanling rats than in mature animals.

Prolonged fasting (in cattle) has previously been associated with a fall in serum magnesium, frequently within the hypomagnesemic range (70). The young magnesium deficient rats responded initially to fasting with elevated plasma magnesium that apparently protected them from audiogenic seizures.

Gross pathological changes following sudden death: External features. Although he may have had suboptimal growth (61, 65, 67), the typical SIDS baby usually appears normally nourished and hydrated, cyanotic (8, 12, 87, 88), often with frothy fluids in the mouth and nostrils (1,2,9,12,53,82,86). The fists may be clenched (82,86), with threads caught tightly in the fingers, suggesting antemortem struggle (8, 12,69). The face may be covered with bedding (2, 69), or may be straight down in the bedding, with pressure pallor involving the center of the face (8). Smothering can usually be ruled out (2, 8, 78). Convulsions (91), gasping respirations (91), and other activity have been observed in some infants (32); others have been observed to die quietly in a matter of minutes (46, 82, 91).

In the animal model, some rats died after severe convulsions. Others died peacefully after circling once, when they fell over unconscious, with tetany, apnea, and bradycardia (Fig. 5). The usual findings of the weanling rat dying of the magnesium deficiency syndrome included cyanosis, frothy fluid at the mouth and nostrils, wedging of the nose and mouth, tight flexion of the forepaws, and rigid extension of the hind legs (49).

Gross pathological changes following sudden death: Internal features. The most striking gross pathological changes in SIDS are intrathoracic (1, 2, 8, 31, 40, 53, 82, 86-88). The lungs are often so expanded that they completely fill the thoracic cavity (12, 88); in other cases (53), the lungs are normally expanded. Variable numbers of petechiae may be present in the pleura, thymus, and epicardium (1, 8, 12, 82, 86-88): they have been attributed to hypoxia prior to cardiac arrest and to vigorous respiratory movement (42). Most of the lobe of a lung may be purple, poorly aerated, with varying amounts of

edema and congestion seen on cut surface (1,8,31,82,86-88, 91). The blood in the heart is fluid (8, 82,88), and the right heart may be distended (8,82). The urinary bladder is usually empty (8,88).

In this study, young 0-Mg rats dying after sudden seizure-shock episodes had gross intrathoracic pathology (Fig. 6) that was remarkably similar to that found in SIDS (Fig. 7); young magnesium deficient rats examined at other times in the magnesium deficiency syndrome in the present experiments and in other studies (50,76) showed no such changes. All of the conditions influencing the pathological changes are not understood. Postmortem findings in lambs and calves dying of seizures due to magnesium deficiency have been reported as extensive hemorrhages coupled with acute congestion of internal organs, which were attributed to the violence of the convulsions (70).

PART II. TESTING THE HYPOTHESIS IN HUMAN SUBJECTS

(A REVIEW OF PREVIOUSLY REPORTED STUDIES)

A. TESTING THE HYPOTHESIS DIRECTLY.

Studies in SIDS cases and controls.

To learn whether or not there is an absolute or relative deficiency of magnesium in SIDS, one would analyze appropriate diagnostic tissues in SIDS and control infants for magnesium and interrelated minerals and compare the results. Accordingly, several investigators have analyzed blood plasma, liver, and vitreous humor and have found no abnormality in magnesium concentration in SIDS, as recently reviewed (8, 27, 88).

Plasma, liver, and vitreous humor are inappropriate tissues to establish the diagnosis of magnesium deficiency post mortem. Plasma magnesium is extremely labile, sharply increasing during seizures and after death in children and animals (27). The concentration of magnesium in liver does not decrease during acute magnesium deficiency (20,27). Swift and associates (80) found that the magnesium level of vitreous humor in human infants are age-related, with highest levels in the neonate, and with a wide range of values for each age group.

Studies in an animal model for the human infant of SIDS age.

We studied an animal model to determine the optimal tissue and conditions needed to assess the magnesium status of a young mammal after death (27). The literature regarding changes in mammalian tissues in magnesium deficiency reveals conflicting results. No studies of the vitreous humor in any mammal with biochemically established magnesium depletion could be found.

Vitreous magnesium. We (27) measured vitreous magnesium in 0-Mg and in 100-Mg rats (the latter represents 2.5 times the recommended daily allowance for growing rats). The values all fell within a narrow range, and there was considerable overlapping of individual values among the three groups. Grouped data showed a significant difference between 0-Mg experimental animals and 100-Mg controls, but not laboratory chow controls. The vitreous magnesium did not reflect the known degree of magnesium deficiency.

The optimal tissue and conditions found. Bone was found to be the best tissue to determine whether or not a young mammal has adequate stores. Since different bones from the same animal did not all have the same mineral composition, one bone must be selected for analysis. The irregular distribution of magnesium within a single bone warrants the study of one entire bone (27).

B. TESTING THE HYPOTHESIS INDIRECTLY.

The results of some studies of magnesium metabolism in young mothers, infants, and children can be matched against known facts in the sudden infant death syndrome; this constitutes an indirect test for the hypothesis. The plasma and urinary magnesium values and the results of the parenteral magnesium load test were determined.

The parenteral magnesium load test. This test has been used extensively to assess the magnesium status of individuals during life (22, 23, 25, 45, 83). It is essentially a test of tissue saturation of magnesium, based on the fact that the normal kidney regulates the body stores of magnesium, promptly excreting an excess, but avidly retaining the mineral during a deficiency state (45, 83). It was introduced because although low plasma magnesium values usually indicate magnesium deficiency, normal plasma magnesium values are unreliable (21, 22, 58).

A preload collection of urine is made; 0.5 mEq of magnesium/ kg of body weight is given parenterally; postload urine is collected (the time depending on the age and maturity of the patient; it is usually 24 h for children); urinary magnesium is measured; and the percent retention is calculated. Retention of up to 40 % of the load is usually considered normal for children over one month of age (26,45, 83), and for adults (83); higher retention suggests deficiency.

For example, in a study of malnourished 25-month-old Thai children weighing about 7 kg, 15 with known mineral losses through prolonged severe antecedent diarrhea retained 73 % of the load, while 15 with little or no diarrhea retained only 27 % initially. On day 70 when clinically well, the mean retention of the entire group was 17 %. The test appeared to be a reliable guide to the expected magnesium stores in the children (22).

1. STUDIES IN HUMAN MOTHERS

A study in well nourished Thai mothers.

A study was made to assess the magnesium stores of postpartum Thai women whose typically Oriental diets included an abundance of magnesium-rich foods, such as nuts, legumes and other green vegetables, and shrimp (23). Their magnesium status was evaluated by plasma and urinary preload magnesium values and by the parenteral magnesium load test, in which the dose of 0.4 mEq of magnesium/ kg of estimated lean body weight was given.

Nulliparous controls retained $22.6 \pm 6\%$ (9), and postpartum women, $36.7 \pm 4.7\%$ (24) of the magnesium load; the numbers represent the mean, SEM (number in sample). Despite high parity and prolonged lactation in some, there was no obligatory retention; over one-third of the patients retained a mean of only 9 % of the load.

A study of low income urban American mothers.

A subsequent study was made to assess the magnesium status in 185 postpartum women in St. Louis City and St. Louis County Hospitals. The patients were tested in the same manner as the Thai women and they retained a mean of 51 % of the parenteral load, with a range of 0 to 100 %.

Patients with high retention of magnesium reported a high intake of refined starches and fats, a magnesium-poor diet that was fairly typical of the diets of the low income young urban women. Patients with high retention had lower plasma magnesium and lower preload urinary magnesium values than patients who retained less than 40 % of the load.

The strongest evidence for magnesium deficiency, mean retention exceeding 90 % of the parenteral magnesium load, was found in young mothers of twins and in multiparas less than 17 years of age; this appears to reflect the high magnesium requirement of adolescent growth and multiple or repeated pregnancies.

Multiparous mothers over 30 years of age had the longest interval since the previous pregnancy and the lowest retention value, $32.3 \pm 5.8\%$ (18).

Maternal factors in SIDS.

In the light of the foregoing, it is useful to consider some observations in SIDS:

1. SIDS has been reported predominantly in Western cultures (8). In California (48), Oriental people had the lowest incidence of SIDS.
2. The incidence of SIDS is high among economically depressed urban dwellers, and lower in predominantly agricultural areas (8, 39).
3. There is a significantly increased risk for young mothers of increased parity (39).
4. The incidence is high if mothers are less than 20 years of age, lower in older mothers (48).
5. Twins have a higher risk than singleton births (8, 40, 48, 88), and the risk further increases for triplets (48).
6. There is a significant recurrence rate in families; it is not thought to involve any specific inheritance mechanisms (38).

A possible interpretation of maternal factors in SIDS. The Thai mothers represent the Oriental whose diet is rich in magnesium and other minerals. They serve as a control for the low income urban American women, whose diet is often poor in magnesium. The high requirements during adolescent growth and repeated pregnancies may not be met from such a diet. The heaviest demands for magnesium would be during twin or multiple pregnancies in young women, or in closely spaced, successive pregnancies that do not permit time for maternal repletion of essential nutrients. This might explain the high incidence in infants born to young multiparas, in twins, and the recurrence of SIDS in families.

2. STUDIES IN HUMAN INFANTS AND CHILDREN

Studies in malnourished young children with enteric disorders.

Profound losses of magnesium occur during diarrhea and during catabolism associated with starvation (45,70,83). In some areas of the tropics and subtropics, severe malnutrition with antecedent enteritis constitutes a major pediatric problem in 1 to 4-year-old children who often weigh only 6 to 9 kg (15-17, 84).

For many years (84), the therapy of severely malnourished children included gavage-fed formulas that contained high concentrations of lactose-free cow's milk protein hydrolysates, high in protein, calcium, and phosphorus, but poor in magnesium. Vitamins and minerals other than magnesium were added to the therapy. During the phase of accelerated protein anabolism, when the children appeared to be recovering, some died suddenly and without apparent reason. This occurred throughout the tropics, as previously reviewed (84). Findings at autopsy included a little fluid in the lungs; no cause of death was found (84).

Subsequent studies of malnourished children in the tropics have demonstrated magnesium deficiency and other problems (5, 74). Low concentration of magnesium in plasma (7, 16, 21), urine (7, 21, 52, 58) and muscle (16, 21, 56,58); high retention of parenterally administered magnesium (22), and long-continued positive magnesium balance during recovery from malnutrition (52) together indicate a true deficit of magnesium (5, 16, 21, 58, 85).

Signs and symptoms linked with hypomagnesemia in young infants and children. These include: anorexia (16, 21, 45); sudden onset of pallor or cyanosis (16, 45); sudden loss of consciousness (7); apathy (45); appearing to be out of touch with surroundings (63); extreme irritability (63); jumping at the slightest noise (63); poor sleep (63); apnea while sucking a bottle (21); fixed staring (21) or rolling the eyes upward (7, 16, 21, 63); tremors or twitching (7, 16, 21, 63); marked hypertonia (7, 16, 21) or hypotonia (63); and tonic or clonic, localized or generalized, seizures (7, 16, 21, 45, 63, 73).

Results of magnesium therapy. For magnesium deficient children already receiving otherwise balanced therapy, magnesium was rapidly beneficial (15-17, 45, 52, 73) and sometimes appeared to be lifesaving (5, 15-17, 63, 83, 85).

An animal model to study the phenomenon of sudden death during the period of growth and apparent recovery in the malnourished child.

Weanling rats were fed low protein diets to serve as models for malnourished children. They were then refed high dietary casein without added magnesium. They were gaining weight and appeared to be recovering well when they began to die suddenly and unexpectedly. Similar rats refed high dietary casein with added magnesium grew at accelerated rates and recovered uneventfully (20).

Comparison of the above magnesium deficient infants and children to infants that subsequently die of SIDS.

The age difference. The magnesium deficiency syndrome would be expected to be more acute and more lethal in young infants of SIDS age than in 1- to 4-

year-old children. And it is evident that the nonspecific signs and symptoms linked with hypomagnesemia in young infants and children are essentially the same as the signs and symptoms linked with SIDS.

Depletion resulting from growth in a subject with poor magnesium reserves. Premature or low birth weight infants, over-represented in SIDS, have low magnesium requirements to support "catch-up growth" (24). Sudden infant death is more common in bottle-fed than breast-fed infants (66, 88). Porter (66) believes that SIDS infants receive excess protein from reconstituted dried cow's milk and infant cereal preparations. Such foods are also rich in calcium and phosphorus. Artificially fed infants might develop a critical imbalance resulting in relative magnesium deficiency (19). Breast feeding would not protect the infant if the mother were malnourished, as in Templeman's series of destitute, multiparous mothers (82). Maternal magnesium deficiency is reflected in magnesium-poor milk (41, 90), which may lead to magnesium deficiency in nursing offspring (90).

Studies in American infants 1-6 months of age with possible near miss episodes

We have been utilizing the parenteral magnesium load test to assess the magnesium stores of infants referred to the Cardinal Glennon Memorial Hospital for Children, St. Louis, Mo. Of these, some were referred because of recent episodes similar to near-miss episodes of SIDS.

In the first series (26), a group of seven infants averaging 8 weeks of age presented with one to three life-threatening episodes. Signs and symptoms included: apnea; sudden loss of consciousness; bradycardia; tonic rigidity, followed by flaccidity; dyspnea; a dazed look; flushing; drooling; and sweating. This group retained a mean of 88 % of the parenteral magnesium load, despite pre-load magnesium treatment during the acute episode in two.

In a larger series since December, 1974, 25 infants over one month of age who were studied because of episodes similar to near miss episodes retained 60 to 100 % of the load, even though some of them had received magnesium therapy prior to the load test. However, some other infants with similar episodes had lower retention of the magnesium loads.

Comment. These magnesium load studies were made in infants 1 to 6-months of age, the age at which SIDS usually occurs. The symptoms exhibited were nonspecific, but were typical of those that may occur during the near miss episode. Some infants had repeated episodes. Although the similarity of the two syndromes, acute magnesium deficiency and SIDS, is striking, we believe that some factor other than magnesium deficiency may be acting in conjunction with magnesium deficiency in SIDS, if indeed SIDS is caused by a mineral imbalance (28).

CONCLUSIONS.

The cause of SIDS remains unknown. We believe that further, more appropriate testing of SIDS infants and of carefully selected controls is indicated to determine whether or not magnesium deficiency is a major contributing cause of SIDS. If so, since magnesium operates in conjunction with other minerals in the body, we would expect to find an associated mineral imbalance.

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Rats Studied	0 - Mg, 100 - Mg, LC									5 - Mg, 150 - Mg, LC						
Days on Study	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Experimental Event	No Known Seizure ↓ Audogenic Seizure - Shock ↓ 18 h Post - Seizure									No Known Seizure ↓ Audogenic Seizure - Shock ↓ 18 h Post - Seizure						
Condition of 0 - Mg Rats	Normal Growth and Behavior				Slower Growth Erythematous Sudden Death				Hyperirritable Erythema Faded Sudden Death							
Condition of 5 - Mg Rats	Suboptimal Growth				Erythema				Erythema Faded				Tremorous Hyperirritable Sudden Death			

Fig. 1. The plan to study the seizure-shock episode in magnesium deficient weanling rats. The acute syndrome was studied at one week in rats fed 0 added magnesium and 20 % casein (0-Mg), and their controls fed a similar diet with 100 mg of added magnesium / 100 g dry diet (100-Mg), or laboratory chow (LC). The attenuated deficiency was studied at two weeks in rats fed 5 mg of added magnesium and 40 % casein (5-Mg), and in controls fed a similar diet with 150 mg of added magnesium (150-Mg), or LC.

Fig. 2 A. Venous blood pH in acute magnesium deficiency. Mean values for 100-Mg controls, 7.44 ± 0.01 and LC controls, 7.50 ± 0.01 , are shown by horizontal lines. Numbers in parentheses indicate the number of samples making up the mean. Values for acutely deficient (0-Mg) rats with no known seizures were obtained on day 6, and were not significantly different from the 100-Mg values. There was a significant difference after seizures and death on day 7 ($P < 0.001$), normalizing at the pre-seizure level 18 h post seizures.

Fig. 2 B. Venous blood pH in 0-Mg rats before and after a 24 h fast, showing no difference from control values.

Fig. 3 A. Venous blood hematocrit in percent in acute magnesium deficiency. Mean values for 100-Mg controls, 30.7 ± 0.34 and LC controls, 33.9 ± 0.59 , were significantly higher than in 100-Mg controls ($P < 0.001$) and after death ($P < 0.001$).

Fig. 3 B. The hematocrit did not change during the 24 h fast.

Fig. 5. Electrocardiograms were taken in rats that weighed 28-32 g at the onset of the study. Tracings were taken at twice normal standard (20 mm/mv) and at twice normal paper speed (50 mm/sec) in supine rats that were sedated in A) and B), but studied with no sedation in tetanic, apneic, unconscious 0-Mg animals during the seizure-shock episode (C through F). The tracings of 100-Mg controls in A) and the 0-Mg rats with no known seizures in B) had rates of 575 and 480/min., respectively, and the electrical patterns were indistinguishable. In C) and D), during the seizure-shock episode, there was sinus arrest, and the ventricular rate precipitously dropped to about one-third of the normal rate. Apnea persisted, bradycardia was progressive, and the rats died. In D) and E), the ventricular complexes were initiated from two electrical foci. In D) and F), during the early recovery period, the heart rate and rhythm improved as both animals spontaneously resumed respirations. Spiked electrical complexes developed in F). The tracings of 0-Mg rats were as in B) 18 h post-seizures (not shown).

Fig. 4 A. Plasma magnesium concentrations in mEq/liter in acute magnesium deficiency. Mean values for 100-Mg controls, 1.75 ± 0.03 and LC controls, 1.62 ± 0.03 , were not significantly different. The magnesium concentration of 0-Mg rats with no known seizures, 0.36 ± 0.02 (27) was significantly different from 100-Mg controls ($P < 0.001$), but during extended seizures or immediately after fatal seizures, the deficient animals had normal magnesium values. The concentration of magnesium was at the pre-seizure level 18 h after seizures.

Fig. 4 B. The plasma magnesium value increased during fasting to 0.96 ± 0.06 mEq/liter ($P < 0.001$).

Fig. 6. From left to right: the first, second, and fourth lungs were from 0-Mg rats that died after a tetanic-apneic episode that was provoked by gentle handling only, and the third lungs were from a 100-Mg control.

Fig. 7. Lungs of an 11-week-old white male infant who was found blue and apneic in his crib two hours after his mother had last seen him and thought that he was normal. He failed to respond to resuscitation. Autopsy (A75-365) was performed within 6 h of discovery by Dr. George E. Gantner, M.D., Chief Medical Examiner, St. Louis County. The lungs weighed 75 g, had a mottled appearance, external petechiae, and were congested throughout, with posteriorly located atelectasis. The diagnosis was the sudden infant death syndrome.

TABLE 1.
COMPOSITION OF THE PURIFIED DIETS

	Control 100-Mg	High protein control 150-Mg
g/100 g of diet		
Casein ¹	20.0	40.0
Corn oil ²	5.5	5.5
Glucose monohydrate ³	68.24	48.08
Mineral mix ⁴	4.26	4.42
Choline chloride ⁵	0.30	0.30
L-cystine ⁵	0.20	0.20
Water-soluble vitamins ⁶	1.00	1.00
Fat-soluble vitamins ⁷	0.50	0.50
Total	100.00	100.00

1. Vitamin-free test casein, Mogul Corp., Madison, Wis.
2. Mazola, Best Foods, Engelwood Cliffs, N.J.
3. Cerelease, Clinton Corn Processing Co., Clinton, Iowa.
4. Mineral mix provides (g/100 g of diet): Mg, 0, 0.005, 0.1, or 0.15; K, 0.49; Na, 0.17; Ca, 0.528; P, 0.455; Cl, 0.13. In ppm: Mn, 38.7; Cu, 5.7; Al, 0.20; Co, 1.6; Zn, 13.3; F, 0.016; I, 1.342; and Fe, 83.2.
5. Sigma Chemical Co., St. Louis, Mo.
6. Water soluble vitamins (mg/100 g of diet): Thiamin, 2.5; riboflavin, 1.0; calcium pantothenate, 7.0; pyridoxine, 2.2; nicotinamide, 5.0; folic acid, 0.4; biotin, 0.10; PABA, 2.5; inositol, 25.0; and B₁₂, 0.50.
7. Fat soluble vitamins (Units/100 g of diet): Retinyl palmitate, 1,000; irradiated ergosterol, 270 I.U. of vitamin D; DL-alpha-tocopheryl acetate, 10.0; and 3-phytylmenadione (vitamin K₁), 0.1 mg.

TABLE 2 A.
BLOOD VALUES IN WEANLING RATS: HEMATOCRIT AND PLASMA CATIONS

CATIONS IN MEQ/LITER (MEAN ± SEM).					
GROUPS OF RATS (Number)	VENOUS BLOOD HEMATOCRIT %	MAGNESIUM	CALCIUM	POTASSIUM	SODIUM
A. 100-Mg (24) Ad lib.*	28.4 ± 0.60 ²	1.68 ± 0.03 ²	5.15 ± 0.04	5.92 ± 0.21	136.6 ± 0.49
B. 100-Mg (20) Pair-fed	30.8 ± 0.78	1.58 ± 0.02	5.25 ± 0.06	5.55 ± 0.13	137.3 ± 0.56
C. 100-Mg (14) Pair-fed Strychnine seizures *	35.0 ± 0.94 ⁴	2.20 ± 0.09 ⁵	6.35 ± 0.10 ⁵	9.31 ± 0.26 ⁵	143.2 ± 0.59 ⁵
D. 0-Mg (9) No seizure*	33.6 ± 0.77 ¹	0.31 ± 0.01 ⁵	5.40 ± 0.08	8.04 ± 0.30 ⁵	134.4 ± 0.44 ⁴
E. 0-Mg (6) Early seizures **	37.7 ± 1.17 ³	0.38 ± 0.02 ¹	5.50 ± 0.12	8.55 ± 0.29	134.5 ± 0.69
F. 0-Mg (11) Prolonged seizures **	46.1 ± 1.18 ⁵	0.76 ± 0.07 ⁵	6.35 ± 0.15 ⁵	9.34 ± 0.58	141.3 ± 1.51 ²

(*) Compared with Group B, Pair-fed 100-Mg controls, using two-tailed Student's t test.

(**) Compared with Group D, 0-Mg rats with no known seizures, using two-tailed Student's t test.

1. P<0.05; 2. P<0.025; 3. P<0.01; 4. P<0.005; 5. P<0.001.

TABLE 2 B.
BLOOD PLASMA VALUES IN WEANLING RATS: PLASMA ENZYME ACTIVITY

UNITS/LITER (MEAN ± SEM).					
GROUPS OF RATS (Number)	ALKALINE PHOSPHATASE	SGOT	SGPT	LDH	CREATINE KINASE
A. 100-Mg (24) Ad lib.*	1264.2 ± 69.3 ⁵	118.2 ± 7.4	31.3 ± 2.7	365.2 ± 40.8	494.1 ± 50.8
B. 100-Mg (20) Pair-fed	907.1 ± 47.6	101.5 ± 3.2	27.2 ± 1.1	303.5 ± 37.1	384.5 ± 29.4
C. 100-Mg (14) Pair-fed Strychnine seizures*	1007.0 ± 99.2	130.5 ± 6.9 ⁵	35.7 ± 1.3	383.9 ± 32.4	525.8 ± 45.3 ²
D. 0-Mg (9) * No seizures	219.5 ± 20.6 ⁵	164.5 ± 15.6 ⁵	30.7 ± 1.6	437.5 ± 110.7	584.8 ± 150.2
E. 0-Mg (6) Early Seizures**	251.5 ± 13.2	141.0 ± 9.6	26.5 ± 1.6	399.3 ± 82.3	520.0 ± 70.7
F. 0-Mg (11) Prolonged Seizures**	294.0 ± 14.1 ³	377.3 ± 58.4 ³	58.9 ± 14.3	1448.7 ± 229.7 ²	1527.5 ± 239.5 ⁵

(*) Compared with Group B, Pair-fed 100-Mg controls, using two-tailed Student's t test.

(**) Compared with Group D, 0-Mg rats with no known seizures, using two-tailed Student's t test.

1. P<0.05; 2. P<0.025; 3. P<0.01; 4. P<0.005; 5. P<0.001.

TABLE 2 C.
BLOOD PLASMA VALUES IN WEANLING RATS: SELECTED ANIONS AND METABOLITES.

GROUPS OF RATS (Number)	CHLORIDES mEq/liter	INORG. PHOS. mg/dl	GLUCOSE mg/dl	BUN mg/dl	CREATININE mg/dl	TOTAL PROTEIN g/dl	URIC ACID mg/dl	IRON ug/dl	TOT. BILIRUBIN mg/dl
A. 100-Mg (24) Ad lib.*	102.5 ± 0.5 [#]	9.19 ± 0.14 ⁵	179.8 ± 4.6	18.4 ± 1.00	0.15 ± 0.01 ⁵	4.56 ± 0.02 ²	0.95 ± 0.08 ³	260.2 ± 24.1	0.03 ± 0.01
B. 100-Mg (20) Pair-fed	102.8 ± 0.6	7.90 ± 0.17	196.3 ± 30.5 ^x	18.1 ± 1.26	0.30 ± 0.03	4.42 ± 0.04	0.70 ± 0.04	244.6 ± 20.7	0.04 ± 0.01
C. 100-Mg (14) Pair-fed Strychnine seizures*	104.5 ± 0.5	10.25 ± 0.44 ²	197.4 ± 17.8 ^x	21.7 ± 1.04	0.30 ± 0.03	5.19 ± 0.06 ⁵	1.12 ± 0.09 ⁵	390.4 ± 40.8 ⁴	0.04 ± 0.01
D. 0-Mg (9) No seizures*	105.1 ± 0.8	8.55 ± 0.23 ¹	147.1 ± 3.4	21.5 ± 2.75	0.37 ± 0.05	3.81 ± 0.11 ⁵	1.02 ± 0.07 ⁵	359.6 ± 12.0 ⁴	0.10 ± 0.01 ²
E. 0-Mg (6) Early seizures**	102.8 ± 1.1	7.75 ± 0.15 ¹	153.8 ± 5.1	21.6 ± 5.15	0.43 ± 0.05	4.03 ± 0.06	0.85 ± 0.03	385.0 ± 18.5	0.13 ± 0.02
F. 0-Mg (11) Prolonged seizures **	105.0 ± 2.1	11.20 ± 0.59 ⁴	146.0 ± 5.2	18.2 ± 0.53	0.43 ± 0.01	4.52 ± 0.12 ⁵	3.62 ± 0.38 ⁵	458.2 ± 27.3 ³	0.12 ± 0.01

(*) Compared with Group B, Pair-fed 100-Mg controls, using two-tailed Student's t test.

(**) Compared with Group D, 0-Mg rats with no known seizures, using two-tailed Student's t test.

1. P<0.05; 2. P<0.025; 3. P<0.01; 4. P<0.005; 5. P<0.001.

(x) Rats consumed all of restricted ration 1-2 h before study

(#) Mean ± SEM