EFFECTS OF MAGNESIUM/MINERAL SALT DEPRIVED/ REPLETED DIETS ON THE SEIZURE FREQUENCY AND BIOCHEMISTRY OF MONGOLIAN GERBILS

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CHAPTER I

INTRODUCTION

The enigma of epilepsy still engenders in the lay person a certain amount of fear and an unwillingness to associate with known epileptics. For the scientist, however, the nature and etiology of epilepsy is a source of divergent and interesting research. One of the major stumbling blocks for the understanding of the machinations of epilepsy has been the difficulty in finding an experimental model that could serve as a substitute for the naturally occurring phenomenon. Attempts to induce seizures in experimental animals using techniques such as electric shock, photogenic methods and chemical agents, cortical freezing, and intracerebral injections of tetanus toxin have not fully matched the spontaneous, chronically, recurrent character manifested in human epilepsies (Behavioral Research, Epilepsy, p. 246).

The Mongolian gerbil (Meriones unguiculatus), a small rodent introduced into the United States as a laboratory animal in 1954 (Schwentker, 1963), accorded researchers an opportunity to study an animal that was prone to chronically recurrent, spontaneous seizures that were described as epileptiform in character (Thiessen, Lindzey, and Friend, 1968). Specifically, Loskota and Lomax (1975) observed a correlation of EEG abnormalities with the motor manifestations of seizures in gerbils. They also recorded paroxysmal bursting in the parietal cortex of seizure sensitive animals in which no concomitant peripheral motor activity was

evident. These observations of motor seizure manifestations together with indication of possible genetic predisposition have led many researchers to suggest the gerbil as a suitable animal model for the study of epilepsies (Kaplan and Miezejeski, 1972; Loskota, Lomax, and Rich, 1972).

Attempts to relate the seizing activity of the gerbil with central nervous system disorders and vitamin deficiencies have been unsuccessful (Robinson, 1968). Nonetheless, descriptions of the tonic-clonic convulsions in the Mongolian gerbil resemble the local manifestations of magnesium (Mg) tetany in other experimental animals (Kruse, Orent, and McCollum, 1932). Of particular importance is Harriman's (1974) study in which the seizure activity of nonsusceptible gerbils was significantly increased when maintained on a magnesium deficient diet.

Magnesium tetany is distinguishable from other forms of tetany by a normal blood calcium level and an absence of alkalosis. Furthermore, magnesium is known to chemically interact with other mineral salts, especially calcium and potassium.

The purpose of the present study was to extend the research relating "spontaneous" seizing in Mongolian gerbils to magnesium tetany.

Nature of Epilepsy

The term epilepsy, derived from the Greek word for "seizure", identifies a variety of chronic convulsive disorders that have in common brief episodes of seizures that are associated with loss or alteration of consciousness. These seizures are usually accompanied by abnormal EEG patterns and characterized by rapidly alternating muscular contraction and relaxation (Wright, 1975).

Many famous individuals suffered from epilepsy including Julius Caesar, Mohammed, Napoleon, Lord Byron and Van Gogh. In 95 B.C. the following vivid description was given by Lucretius of a major seizure:

> Oft, too, some wretch before our startled sight
> Struck as with lightning by some keen disease
> Drops sudden by the dread attack o'erpowered.
> He foams, he trembles and he faints.
> Now rigid, now convulsed, his laboring lungs
> Heave quick, and quivers each exhausted limb,
> Spread through the frame, so deep and dire disease
> Perturbs his spirit; as the briny main
> Foams through each wave beneath the tempest's ire
> But when, at length, the morbid cause declines,
> And the fermenting humors from the heart
> Flow back--with staggering foot the man first treads
> Led gradually on to intellect and strength (Scott, 1973, p. 5).

Presently, the cause of epilepsy is still an enigma, but a seizure is now known to be an abnormal and excessive discharge of neurons in the brain. Penry (1975) states that:

> This excessive abnormal discharge influences and physiologically inhibits or excites other neurons. The manifestations of the abnormal and excessive discharges are determined by the number and location of the malfunctioning neurons. Consequently, the manifestations of seizures may vary tremendously (p. 267).

This may account for the fact that more than half of the individuals suffering from epilepsy have more than one type of seizure, thus adding to the severity of their affliction.

According to the Epilepsy Foundation of America (1974), there are approximately four million sufferers in the United States alone. The disease is equally prevalent among males and females, with a higher incidence reported for children.

As mentioned previously, epilepsy is a blanket term; therefore it is often difficult to differentiate between epilepsy as a symptom of organic brain disease (including trauma), and epilepsy which is due

exclusively to the faulty operations of a normal brain. While organic brain disease and damage, and genetic or familial factors account for more than half of the persons who suffer from epilepsy (Penry, 1972), the etiology of a great many cases is labeled idiopathic, or "cause unknown". It is with this group that no apparent pathology, such as scar tissue, tumor or vascular abnormalities, is discernible.

Predisposition to a disorder seems to be the ultimate factor in many bodily diseases. The inheritance of tissue weakness, rather than the inheritance of the disease itself, predisposes certain people to develop the disease as they progress through life (Scott, 1973). Given certain chemical or mechanical irritations to the brain, it is the low-convulsive-threshold person who is likely to become a seizure patient while the high-convulsive-seizure person will not. With the idiopathic group, where no thermal, mechanical, or chemical damage to the brain is detected, other triggering factors such as emotional upset may play a part. Emotional upset is not the sole cause of seizure, but merely precipitates a convulsion by acting on an already disordered part of the brain, or on an idiopathic, low-convulsive-threshold person. Table I presents a classification of seizures based on the origin of the epileptic discharge.

Classification of Seizures

Focal Epilepsy

In focal epilepsy (see Table I), an abnormal neural discharge begins at the site of an abnormal focus ("lesion") within the CNS. The classic Jacksonian seizure, associated with organic brain damage in the motor

TABLE I

SEIZURE CLASSIFICATION*

Type of Seizure	Origin of Epileptic Discharge	Example
Focal cerebral seizure (lesions)	Hemishperical gray matter, usually cerebral cortex	Jacksonian seizures
Centrencephalic seizures "highest level" seizures, idiopathic	Central integrating system of higher brain stem	Grand mal, petit mal
Cerebral seizures, not yet classified	Undiscovered	

*After Penfield and Jasper, 1954.

area of the frontal lobe, is characterized by local movements of some part of the body. In the "Jacksonian March" there is a spread of movement from one part of the body to another.

The psychomotor, or temporal lobe, seizure is characterized by two distinguishing factors: Automatic behavior and amnesia for the duration of the seizure. There is often an automatic continuation, or onset, of apparently purposeful behavior, although the individual may have no recall of the events. The most common automatic movements are sucking and chewing movements of the mouth; others are running, jumping over objects, throwing objects around or breaking them, tearing up paper, pushing or striking people. This seizure occurs in approximately one third of adult epileptics (Penfield and Jasper, 1954).

Centrencephalic Seizures

Seizures classified under this heading in Table I are thought to originate in the central integrating system of the brain stem, thus involving both cerebral hemispheres. The grand mal ("big sickness") attack is the most common seizure in adults. Fifty per cent of the patients have a sensory warning (aura) of the oncoming convulsion. The grand mal seizure is characterized by a major symmetrical convulsion and is divided into two phases. The tonic phase involves an abrupt loss of consciousness and rigidity or spasm (extensor muscles dominating the flexor muscles) of all skeletal muscles. As the second, or clonic phase, sets in, the skeletal muscles begin to alternately relax and contract. After the seizure there is a postseizure depression referred to as a "postictal automatism" or "postictal twilight state". Status epilepticus is the term given to a rapid series of grand mal attacks

so close together that the person has no intervening periods of consciousness.

The petit mal ("little sickness") is almost always associated with childhood. It is characterized by brief periods of loss of consciousness. There is no aura, no cry, and most often no falling. If there is no clonic movement such as eyelid blinking, it is called a pure petit mal. If clonic movements are present, it is referred to as myoclonic petit mal. One of every three patients with petit mal later develop grand mal seizures, although more than half outgrow the affliction (Penfield and Jasper, 1954).

Cerebral Seizures

Convulsive attacks in which the site of neural discharge cannot be determined are put in this classification (see Table I). The seizure may be the result of a diffuse abnormality of the brain or it may be the result of some general systemic abnormality, such as hypoglycemia (Penfield and Jasper, 1954).

The treatment for today's epileptics involves a combination of anticonvulsants, nerve sedatives, and/or tranquilizers. Eighty per cent of all epileptics are kept seizure-free with this regimen. Nevertheless, the side-effects of a strict drug schedule, ranging anywhere from a mild skin rash to a psychotic-reaction, dictate a need for a continuing search into the causes of epilepsy (Wright, 1975).

The Mongolian Gerbil

Initial research into the causes of epilepsy required the artificial inducement of seizures in the experimental animal. Procedures such

as metal powder implants in the motor cortex of monkeys (Chusid and Kopeloff, 1967) were employed. However, with the introduction to the U.S. of the Mongolian gerbil (Meriones unguiculatus Milne-Edwards, 1867) researchers were accorded a rare and unique opportunity.

In 1935, Kasuga captured twenty pairs of Mongolian gerbils from the region of the Amur River basin in eastern Mongolia and Manchuria. The animals were maintained in a closed, random-bred colony, and, in 1954, Schwentker imported eleven pairs of gerbils from a sub-colony of this stock. Of these, only five females and four males could be induced to breed. Schwentker's random-bred, closed colony at the West Foundation, Brant Lake, New York, was the source of all U.S. colonies of gerbils.

Meriones unguiculatus is classifed as follows: order Rodentia; sub-order Myomorpha; family Cricetidae; sub-family Gerbillinae (Marston and Chang, 1965). They are the commonest of the five Gerbillinae listed for China and Mongolia and are perhaps the most abundant small mammal in that region. The gerbil is a burrowing desert animal. Field reports suggest that the gerbil is diurnal with a tendency toward the crepuscular habit. Concomitantly, the gerbil is also fossorial and must spend a good deal of time and activity underground, in total darkness (Marston, 1972).

Mongolian gerbils also have a great capacity for heat regulation. They have a critical temperature (i.e., the lowest ambient temperature at which the animal remains in a basal or resting metabolic condition) of 30°C and their range of thermal neutrality extends to 40°C. Individually caged animals can tolerate temperatures of 40°C for five hours without apparent discomfort according to Robinson (1959) who concluded that the Mongolian gerbil could spend much of its time in activity outside the burrow during the hottest parts of the day.

Rich (1968) gives the following description of the physical appearance of the gerbil:

> They weigh 50 to 100 grams and are extremely modified for desert life; there is a tendency for the hind limbs to be lengthened; they stand upright readily; and some species jump as a means of locomotion. The tail is usually fully-haired and about the same length as the body. The eyes are fairly large, slightly protruding, dark, and sparkling. The ears are medium sized, motile, and hairy. The color of the dorsum is generally a brownish-grey tipped with black, paling at the sides, with pale buff underparts. The shape of the skull is characteristic of saltatorial rodent forms, with broadening of the brain case and enlargement of the mastoids and auditory bullae (p. 235).

In their natural environment, Mongolian gerbils live in large groups but nothing is known about social organization within a group. The behavior of foot "drumming" or "stomping" may be a significant communicating or alerting mechanism since the gerbil does not vocalize (Marston, 1972).

Although there are no observations of aggression under natural conditions, such behavior may become a problem when gerbils are mismanaged in the laboratory. Because gerbils are monogamous, females often fight and kill males introduced into their cages after the death of their mate. Pre-pubertal gerbils can be successfully caged together in groups numbering 50-100 individuals, although it is advisable to separate the sexes (Marston, 1972).

Gerbils have a gestation period of 24 days, plus 8-30 hours. The litter size is from 1 to 12, and the average is 4.1. Sexual maturity in the gerbil is not reached until 10-12 weeks (Schwentker, 1963).

The Mongolian gerbil is aptly suited for experimental study. They require less care than most research animals. Because of their unique metabolism, only small quantities of water are required (Schwentker,

1963). They adapt to a wide range of nutritional schedules; commercially prepared pelleted mouse or rat diets provide adequate nutrition. Gerbils excrete only a few drops of urine per day, and have small, dry fecal pellets, therefore it is only infrequently necessary to change the litter (Rich, 1968). The gerbil has no critical environmental requisites (Schwentker, 1963). Approximately 200 square inches of floor space is ample for a breeding pair and their litter. In the laboratory a temperature of 15°-21°C is most suitable. Controlled lighting, giving a 12- to 14-hour day is recommended (Marston, 1972).

The easy maintenance and agreeable disposition of the "gentle gerbil" are merely supplemental. The real value of this experimental animal lies in its broad spectrum of susceptibilities. M. unguiculatus has proved useful as an experimental animal in kidney and heart research, and in studies of plague, leptospirosis, brucellosis, salmonellosis, tuberculosis, rabies, poliomyelitis, anthrax, ornithosis, schistosomiasis, helminthiasis, filariasis, and bartonellosis (Rich, 1968).

Gerbil: Experimental Model for the Study of Epilepsy

In addition to the above mentioned utility of the gerbil in experimental research, some scientists now feel that the gerbil may become an efficacious tool in studies of epilepsy. The Mongolian gerbil is unique in that it is prone to chronically recurrent, spontaneous seizures, described as epileptiform in character (Thiessen, Lindsey, and Friend, 1968). The "spontaneous" seizures actually appear to be related to novel or trivial stimuli, and/or environmental changes. Nonetheless, seizures have even occurred when no apparent stimulus was present

(Robinson, 1968; Thiessen, Lindzey, and Friend, 1968). In an attempt to determine the nature of the stimulus that triggers seizures in gerbils, Kaplan (1976) tested gerbils for seizure activity in one of three experimental conditions: Novelty (a strange environment), Extra Stimulation (swinging by the tail), and Extra Stimulation plus Novelty. Kaplan found that novelty, in the form of a strange environment was most effective in triggering seizures in gerbils. She further suggested that the effectiveness of novelty might be related to its stress-inducing properties since a strange environs is not the only stimulus that triggers seizures (Robinson, 1968). Seizures have occurred after handling and following the onset of bright light (Thiessen, Lindzey, and Friend, 1968), after rapid change of temperatures or lighting, and after exposure to testing devices, and confinement in small areas (Robinson, 1968).

Stress may indeed be an important factor when one considers that age is also a significant factor in the seizure susceptibility of the gerbil. Loskota, Lomax, and Rich (1972) found that incidence of seizures in a colony depended on age: "The mean age of onset of seizures in young animals tested from day 30 was 57 $\pm$ 3 days in males and 47 $\pm$ 3 days in females" (p. 190). Further, Kaplan and Miezejeski (1972) reported that no seizures occurred in animals under the age of 2 months and only eight per cent of the two-month old gerbils seized. At 3 months, the number of seizures rose to over 44 per cent, and then stabilized at 38 per cent between 4-10 months. This may account for the reported incidence of seizures ranging anywhere from 20 per cent (Thiessen, Lindzey, and Friend, 1968) to about 80 per cent (Goldblatt, Konow, Shoulson, and MacMath, 1971).

Kaplan and Miezejeski (1972) also reported that the seizure response was nearly suppressed when animals were tested once a week from the age of seven days. Of 62 animals tested, only one displayed a typical epileptiform seizure. The authors attributed this suppression to the following: (1) one week between tests may not have been sufficient to prevent the animals from becoming habituated to the test situation; (2) early stimulation of the young gerbils had a permanent effect, i.e., irreversible changes in arousal level, affecting the development of the nervous system, and ultimately, the gerbils' readiness to seize. Furthermore, the gerbils' predisposition for seizing depends on both external "triggers" and internal conditions underlying "seizure proneness" (Thiessen, Lindzey and Friend, 1968). Kaplan and Miezejeski (1972) concluded that readiness to seize is determined by "(a) immediate conditions such as the buildup of ions, chemicals, or membrane changes, and (b) the chronic or general level of CNS excitability" (p. 272). Consistent with this statement are Thiessen, Lindzey, and Friend's (1968) findings that seizure frequency and intensity increased during the night when behavioral activity of the gerbil colony was maximal. Loskota, Lomax, and Rich (1974) also report that the observable behavior of their gerbils prior to a seizure (e.g., foot thumping), is indicative of high arousal. Stress (e.g., novel stimuli), nevertheless, remains the triggering agent, and its effects are exacerbated by a lowered threshold for CNS excitability.

Although the data are inconclusive, they seem to indicate that some relationship exists between chronic level of automatic and CNS functioning (i.e., arousal level, emotionality, or excitability) and seizure susceptibility or proneness. Additionally, the data support

the opinion that seizure susceptibility in the Mongolian gerbil is under genetic control. Selective breeding by a closed-colony technique of mating yields highly reliable seizure sensitive strains and seizure-resistent strains (Loskota, Lomax, and Rich, 1972; 1974). The authors further report that the ". . . severity of seizures in the colony increased with continued selective breeding" (1974, p. 114), and that the incidence of seizures increased to 97 per cent from the previously reported 80 per cent in non-selectively bred colonies (Goldblatt, Konow, Shouldson, and MacMath, 1971). More recently, Robbins (1976) reports that of 72 animals, 41.5 per cent of agouti gerbils seized when placed in the center of a white box, while only 16.1 per cent of their albino litter mates seized. Although there is no conclusive evidence for the low seizure incidence in the albino gerbils, Robbins suggests that it is probably a pleitropic effect of the albino gene.

Seizures in Mongolian gerbils may exhibit numerous behavioral characteristics, but the pattern constituted by these characteristics in individual gerbils is distinctly stereotyped. Loskota, Lomax, and Rich (1972) provide the following description:

> At onset, ongoing motor activity abruptly ceases, vibrissae, pinnae, and eyelids spasmodically twitch and the body and head are lowered to the substratum (20-25 sec duration). The seizure may then apparently cease and the animal continues locomotion. ['Type I']. . . .
>
> 'Type II' seizures. . . progress rapidly after VT [vibrissae twitching] onset. Myoclonic jerks, followed by body rollover with contraposto twisting, develop into various patterns of tonic limb extensor rigidity and opisthotonus (50 sec duration). The quiescent tonic animal slowly regains preictal motor coordination during a long recovery period (200 sec) characterized by copious, sometimes bloody, salivation, coughing and choking, foot thumping, Straub tail, repetitive pawing at the nose, and inhibited movement followed by continuous circling which ceases abruptly (p. 190).

Latency of seizure onset, seizure duration, and recovery from the seizure vary as a function of seizure severity and number of behavioral characteristics involved. Generally, the more severe the seizure the longer the latency and duration of the seizure, the longer the recovery from the seizure, and the greater the number of motor manifestations. Thiessen, Lindzey, and Friend (1968) report the following times: Latency, 48.8 $\pm$ 46.9 sec; duration, 29.6 $\pm$ 23.0 sec; and recovery, 108.0 $\pm$ 101.8 sec. Additionally, Loskota, Lomax, and Rich (1972) state that on a seven-grade scale (0 = no seizure to 6 = seizure progressing to death), latencies for Grades 1 to 3 ranged from 17 to 23 sec while Grades 4 and 5 ranged from 30 to 35 sec. The duration of less severe seizures ranged from 10 to 15 sec, compared to 279 to 309 sec for more severe seizures. No sex-related difference has been found for incidence (Thiessen, Lindzey, and Friend, 1968; Kaplan and Miezejeski, 1972; Harriman, 1974), latency, duration or severity of seizures (Loskota, Lomax, and Rich, 1974).

If indeed the gerbil is to be the experimental model for epilepsy, then not only must the precipitating environmental stimuli be determined, but the possibility of self-initiated, self-sustained seizures must be explored. EEG recordings of seizure-sensitive animals may shed some light (Loskota and Lomax, 1975). The authors were able to correlate the observed motor manifestations with EEG recordings from various brain areas. Of greater consequence, however, was their finding that interictal spiking (paroxysmal bursting) was recorded in the parietal cortex of the gerbils even when no concomitant peripheral motor activity was present [i.e., genuine spontaneity].

Although the behavioral characteristics of seizures in the Mongolian gerbil have been documented, and the "triggering" role of stress explored, the internal conditions (metabolic, chemical, electrical) responsible for, or as an adjunct to, the seizure activity of the gerbil remain unspecified.

Magnesium Tetany

Seizure activity, both in man and rodent, may be contingent upon metabolic dysfunctions such as hypomagnesaemia, hypocalcemia, hyponatremia, and several types of avitaminosis (Finger, 1947; Patton, 1947; Bevan, 1955; Hirschfelder and Haury, 1938; Patton, Karn, and Longenecker, 1943). Initial efforts to relate seizing in gerbils to metabolic dysfunctions were unproductive. Schwentker [as reported by Robinson, 1968] found no relationship between vitamin B deficiency and seizure frequency; massive doses of supplementary vitamins failed to allay seizures. Additionally, "dietary restriction for calcium, sodium, and pyridoxine hydrochloride in different depletion tests did not promote seizure activity . . ." (Harriman, 1974, p. 227). Of particular interest, however, was the observation that the seizing activity of the Mongolian gerbil approximated hypomagnesaemia or magnesium tetany in rodents (Orent, Kruse, and McCollum, 1932), and grass tetany in calves (Todd and Horvath, 1970). In addition, magnesium tetany is distinguishable from calcium tetany by normal blood calcium, an absence of alkalosis (Greenberg and Tufts, 1938), and an absence of carpopedal spasm and laryngospasm (Kruse, Orent, and McCollum, 1933).

In the study done by Orent, Kruse and McCollum (1932), laboratory rats were fed a diet deficient in magnesium (Mg) (i.e., 1.8 ppm of Mg)

but with adequate amounts of other dietary subtances. The magnesium tetany was characterized by vasodilation, hyperirritability, trophic disturbances and tonic-clonic convulsions.

> Out of a comparative study [between dogs and rats] of the syndrome came the view that Mg deficiency manifests itself locally by increased irritibility of the nervous system and constitutionally by nutritive failure (Kruse, Orent, and McCollum, 1933, p. 603).

The onset of the hyperirritibility phase is contingent upon the Mg level (Greenberg and Tufts, 1938), but the average onset is around 10 to 14 days. Further, when the Mg is low, ". . . the hyperexcitability is quite pronounced, the growth curve shows little rise, and the life span is short" (Orent, Kruse, and McCollum, 1932, p. 457). In the second phase of Mg deficiency, symptoms of malnutrition became apparent. These included: Anorexia, inanition, weight loss, hair loss, a rough and sticky coat, edema, and kidney failure. In milder forms of Mg deficiency, the effects of malnutrition are prolonged and overshadow the convulsive phase. Death may be precipitated either by exhaustion from the tonic-clonic convulsion, and/or dietary deficiency (Tufts and Greenberg, 1937).

It is not uncommon for the exhibited seizing behavior in rodents to occur merely as a consequence of a Mg deficient diet (i.e., spontaneously), although environmental stimuli such as handling (Greenberg and Tufts, 1938) and sound (Finger, 1947; Chutkow and Grabow, 1972) may expedite the convulsive behavior.

The relationship between "spontaneous" seizures in gerbils and Mg tetany in other mammals is further supported by Wong and Teh's (1968) finding that 13 cases of convulsive children had low serum Mg. Recovery occurred after administration of Mg and when serum levels returned to normal.

Harriman (1974), elaborating on the above findings, examined the effect of Mg depletion upon seizure activity in Mongolian gerbils tested in an open-field arena. The combination of Mg depletion and open field testing produced a 34-fold increase in seizure activity. Animals fed Mg repleted diets and tested, and animals fed Mg depleted diets but not tested, presented 1 and 0 seizures respectively.

Testing further the assumption that "spontaneous" seizing is generated by a dysfunction in Mg metabolism, Harriman (in press) studied gerbils (shown to be nonsusceptible to seizures during pre-tests) in open field tests. The various groups of animals were given different loadings of Mg in their diets (1, 62.5, 125, 250, 500, 1000, and 2000 ppm).

As the Mg loadings in the diet decreased, survival rate decreased and seizure frequency increased in animals subjected to environmental stress. Harriman (in press) concluded that the results may have significance for studies of essential epilepsy in man, but ". . . the problem of what may constitute other sufficient organismic conditions for these seizures remains open for investigation" (p. 9).

Magnesium Metabolism

Electrolytes are chemical compounds that dissociate in water, breaking up into separate particles called ions; the process is referred to as ionization. Magnesium (Mg^{++}) is the most abundant intracellular divalent cation (positive ion), varying in concentration from 5 to 30 milliequivalents (mEq) per kilogram wet weight, depending upon the organism or tissue (Wacker and Parisi, 1968). In terms of the amounts of each cation present in the body, Mg is fourth, being surpassed only

by calcium (Ca+), sodium (Na+), and potassium (K+). Mg is present in all tissue, but about 70 per cent of the total body Mg is in the skeleton. Approximately one-third of this is available for mobilization to soft tissue when dietary intake is inadequate (Kaneko and Cornelius, 1971).

The minimal daily requirement of Mg is 6 mg. per kg. per day. For a 140 pound woman, this comes to 385 mg. of Mg daily; for a 185 pound man, at least 500 mg. Diets rich in protein, Ca and vitamin D, however, may require an intake of 7 to 10 mg. per kg. per day for adequate metabolism of the mineral (Seelig, 1964).

The intracellular function of Mg is as an activator of numerous enzymes such as phosphatases and the enzymes catalyzing reactions involving adenosine triphosphate (ATP). Since ATP is required in a variety of functions, the action of magnesium extends to all the major anabolic and catabolic processes involving the main metabolites. In the extracellular fluid, magnesium plays a role in the production and the destruction of acetylcholine, the substance necessary for the transmission of impulses at the neuromuscular junction. Low concentrations of Mg potentiate the release of acetylcholine. Conversely, direct application of Mg to central nervous tissue blocks synaptic transmission and may cause general anesthesia (Wacher and Parisi, 1968; Kaneko and Cornelius, 1970).

Absorption of Mg is generally thought to occur in the small intestines. Since a decrease in dietary Ca enhances the absorption of Mg, Mg and Ca may share a common transport pathway. Additionally, observations suggest that protein and parathyroid hormone also increase Mg absorption. MacIntyre, Boss, and Troughton (1963) hypothesized that the mechanism underlying the homeostasis of plasma Mg is the parathyroid

hormone. Presumably, a rise in plasma Mg inhibits secretion of parathyroid hormone producing an increased excretion of Mg in the urine and a return to normal plasma level. Any surfeit of the bodily requirement for Mg is excreted via the kidneys. Therefore, hypermagnaesmia is evidenced in renal failure (Haury, 1942; Kaneko and Cornelius, 1970; David, 1977).

Of importance are some of the common interrelations between the major cations. The intracellular content of K is high; whereas, the intracellular content of Na is low. In extracellular fluids these ratios are inverted. Intracellular and extracellular concentrations of Mg and Ca have a similar relation. Also, an increase in intracellular Mg and Ca is accompanied by a concomitant increase in cellular P, while the cellular K and Na content remained unchanged.

Table II presents serum reference values in experimental animals and man for the major cations and inorganic phosphorous.

Biochemistry of Magnesium Tetany

The seminal work regarding the biochemistry of Mg tetany in rodents was performed by Kruse, Orent, and McCollum (1933). They reported that in animals receiving Mg-deficient diets, there was:

> . . . an early and progressive decrease in Mg; several weeks prior to death a persistent rise in total cholesterol due to an increase in cholesterol esters; shortly thereafter a fall in the volume percentage of erythrocytes; terminally a slight elevation in sugar, and a rise in non-protein nitrogen due largely but not entirely to an increase in creatine. The other blood constituents remain unchanged (p. 639).

Inconsistent with these findings was Smith and Nisbet's (1968) report that Mg deficiency is associated with transient hypercalcaemia. The discrepency may be due in part to the time blood samples were taken.

TABLE II

SERUM ELECTROLYTES: NORMAL MAMMALS AND HUMANS

Species[1]	Electrolytes (Range)*				
	Calcium (mg/dl)	Magnesium (mg/dl)	Phosphorous (mg/dl)	Potassium (mEq/l)	Sodium (mEq/l)
Albino Mouse	3.20- 8.50	0.80-3.90	2.30- 9.20	4.85-5.85	128.-145.
Rat (Fisher Inbred)	9.60-12.2	1.60-4.35	6.72-10.3	4.10-7.70	125.-148.
Mongolian Gerbil[2]	---	---	4.70- 7.00 (mg/100 ml)	3.30-6.30	141.-171.5
Golden Hamster	7.40-12.0	1.90-3.50	3.40- 8.24	4.00-5.90	106.-146.
Humans	8.50-10.7	1.80-2.90	2.50- 4.80	3.60-5.50	135.-155.

*See Appendix B for conversion of measurements.

[1]Source: Mitruka and Rawnsley (1977).

[2]Source: Mays (1969).

The former authors sampled blood 16 hours after the animals were completely food deprived. The latter authors, however, made no time reference in their study.

Subsequently, Kruse, Schmidt, and McCollum (1934) described the changes in mineral metabolism in Mg-deficient dogs. Mg, although steadfastly retained, was still diminished throughout the survival period. The rate at which the plasma Mg drops depends largely upon the degree of the deficiency of the diet (Tufts and Greenberg, 1937). Ca, also initially retained, was then excreted in progressively increasing amounts. The authors suggested that the early Ca retention was due to the antagonism between Ca and Mg. The subsequent excretion of Ca, as well as nitrogen, was attributed to nutritive failure. The elimination of P was inconstant, but never excessively excreted. This was in disagreement with Chutkow (1965), who reported a significant hypophosphatemia in Mg deficient rats. The difference may be species-specific. As regards the soft tissue, Mg deficiency increases the Ca level in the kidneys, and decreases the Mg level; renal lesions have also been noted (Wook, Fellers, and Craig, 1962; Goldsmith, 1967; Smith and Nisbet, 1968).

Additional research has centered on manipulations of various mineral salts in conjunction with Mg deficiency. When rats are fed diets deficient in both Mg and K, the resultant symptomatology and pathology more closely resembles potassium deficiency, i.e., loss in weight, cyanosis, abdominal distention and lethargy leading to coma and death. However, the rats also exhibited the early hyperirritability of Mg deficiency (Schrader, Prickett, and Salmon, 1937). Colby and Frye (1951b) indicated a definite interrelationship between protein, calcium and magnesium. Mg

deficiency increases in severity when rats are fed a diet high in Ca and K, and normal in protein; high protein levels alone also have a similar effect. Similarly, Toothill (1963) reported that high levels of dietary Ca and phosphate levels significantly reduced the absorption of Mg. A further reduction occurred when both were simultaneously increased. Guinea-pigs given a diet inadequate in Mg and high in Ca and P gained less weight and died sooner than animals given diets containing less Ca and P.

Of further interest, are the findings that serum Mg rises just prior to and during the full tonic phase of the seizure. The increased serum magnesium concentration may be partly attributed to the transfer of Mg from the intracellular fluid to the extracellular fluid, and from the mobilization of Mg in the bone to the serum (Orent, Kruse, and McCollum, 1933; Tufts and Greenberg, 1937; Palfreyman, 1971).

Table III presents serum cation and phosphorous levels in Mg deprived mammals.

Statement of the Problem

It is apparent that the introduction of a suitable animal model would enhance research into the causes of epilepsy. This, in turn, could ultimately benefit the population of epileptics.

Because of their "spontaneous" seizing behavior (Thiessen, Lindzey, and Friend, 1968), Mongolian gerbils have been suggested as suitable experimental models for the study of human epilepsies (Kaplan and Miezejeski, 1972; Loskota, Lomax and Rich, 1972).

The relationship between Mg deficiency and seizure activity was demonstrated early by Kruse, Orent, and McCollum (1932). However, 20

TABLE III

SERUM ELECTROLYTES: MAGNESIUM DEPRIVED MAMMALS AND HUMANS

Electrolytes*	Species			
	Dog[1]	Rat[2]	Rat[3]	Humans[4]
Calcium	10.2mg/100cc serum	10.5mg/100cc serum	5.4mEq/1	4.39mEq/1
Magnesium	1.1mg/100cc serum	1.07mg/100cc serum	0.34mEq/1	0.92mEq/1
Phosphorous	6.4mg/100cc serum	8.1mg/100cc serum	4.8mg/100ml	---
Potassium	---	---	3.7mEq/1	---
Sodium	---	---	145mEq/1	---

*See Appendix B for conversion of measurements.

[1]Source: Kruse, Schmidt, and McCollum (1934).

[2]Source: Orent, Kruse, and McCollum (1934).

[3]Source: Whany and Welt (1962).

[4]Source: Wong and Teh (1968).

years elapsed between the introduction of the gerbil to the United States (Schwentker, 1963) and the study by Harriman (1974) which indicated a possible dysfunction of Mg metabolism in the seizing behavior of the gerbil. Further, Mg is known to have intricate functions and interrelationships with other mineral salts, making its role in seizing behavior more complicated and elusive.

Therefore, the present study was undertaken in an attempt to elaborate the clinical and biochemical aspects of Mg deprivation, in conjunction with other mineral salts, on the seizing behavior of Mongolian gerbils. In addition, normal Mg and Ca levels for the Mongolian gerbil, heretofore not recorded, will be presented. Finally, it is hoped that the present study will shed some light on the nature and etiology of epilepsy.

CHAPTER II

METHOD

Behavioral Experiment

Subjects

The subjects (Ss) were 48 naive female Mongolian gerbils (Meriones unguiculatus) [Tum - (MON) strain], aged 64 to 69 days. The mean weight of the gerbils was 54 grams with a range of 34-61 grams. The Ss, when acquired from Tumblebrook Farm, Inc., were aged 42 to 47 days and were maintained on a commercial rat chow during adjustment to the laboratory. The gerbils were housed in individual cages, each of which contained a steel food cup, and a class or plastic drinker. Food and deionized water were provided ad libitum. The mean ambient temperature and humidity were 24°C and 60 per cent respectively. The 10 feet by 20 feet room was lighted from 6:30 a.m. to 6:30 p.m. daily.

Apparatus

The apparatus included 48 individual Wahmann LC-79 stainless steel cages. They measured 10.2 cm X 17.8 cm X 12.7 cm and were accomodated in a Wahmann LC-79/RP cage stand. The circular testing field measured 51.0 cm in diameter with a 12.7 cm high metal wall. It was uniformly lighted by two F15T8 Cool White bulbs, centered approximately 38 cm above the field. Observation times were measured with a stopwatch.

Procedure

The 48 Ss were randomly assigned to one of eight experimental groups produced by the factorial combination of Mg-Deprived (Mg-D)/Mg-Repleted (Mg-R) diets, Mineral Salt-Deprived (MS-D)/Mineral Salt-Repleted (MS-R) diets, and Test/No Test Groups, with six gerbils in each group. The general mash diet (see Table IV for composition of test diets and salt mix) for the experimental groups was based on Harriman's (1969) study of self-selection of diet in the Mongolian gerbil. The Mg-D/MS-D diet (negative control) contained 250 parts per million (ppm) Mg plus non-nutritive fiber substituted for the salt mix. The Mg level for the deprived diets (250 ppm) was chosen so as to be low enough to induce seizing but high enough to prolong the onset of deaths from nutritive failure or seizure exhaustion (Harriman, in press). The Mg-D/MS-R diet contained 250 ppm Mg plus salt mix. The Mg-R/MS-D diet contained 2000 ppm Mg plus non-nutritive fiber. The Mg-R/MS-R diet (positive control) contained 2000 ppm Mg plus salt mix.

At five-day intervals, the Test Group animals were observed for seizure susceptibility in six open-field tests, while the remaining animals were observed in their home cages. Only one diet group was tested or observed per night, so that one full experimental session (involving all 48 gerbils) took place over four consecutive nights.

Because the gerbil tends toward the crepuscular time (Marston, 1972), and seize more frequently at night (Thiessen, Lindzey, and Friend, 1968), all testing sessions were begun at 7:00 p.m., a half hour after light offset.

TABLE IV

CONSTITUENTS OF THE TEST DIET FED TO THE GERBILS

Composition of Test Diet	%(wt/wt)	Items in Low-Mg Salt Mix[1]	%(wt/wt)
Casein	25.5	Calcium carbonate	31.39
DL methionine	0.3	Copper sulfate	31.87
$MgSO_4$ (anhydrous)	1.0*	Dipotassium phosphate	7.86
Low-Mg salt mix	4.8*	Ferric citrate	2.93
Sucrose	51.4	Manganous sulfate	0.42
Vitamin Diet Fortification Mixture	2.0	Mono calcium phosphate	7.86
Wesson oil	15.0	Potassium iodide	0.09
		Sodium chloride	17.56
Total	100.0	Zinc carbonate	0.02
		Total	100.00

*Where removed from the food of a particular group, item was replaced with non-nutritive fiber (cellulose).

[1]All chemicals were of reagent grade.

The Test Group animals were carried in their home cages to an adjacent darkened room and were placed individually in the center of the open arena for four minutes where each was observed for any seizure activity. Each animal was then returned to the home chamber and observed for an additional two minutes. Any animals seizing during the test session were watched until normal activity resumed (i.e., end of recovery period). The No Test animals from the same diet group were then observed for six minutes in their home cages.

Seizures were recorded according to Harriman's (1974) classification: severe, moderate or mild. Severe seizures were signalled by a vigorous fore-period at least one clonic episode, and a subsequent passive phase. Moderate seizures did not have appreciable fore-periods, and only a single clonic episode from which footing was regained within six minutes. Mild seizures were transcient motor dysfunctions involving spasmodic movements of the body. Full recovery usually took place within a few seconds. Furthermore, additionally significant behaviors of the gerbils during test sessions were noted. Observations regarding physical appearance were also recorded. Finally, times for seizure latency, seizure duration, and recovery from a seizure were recorded.

Visual evaluations of all animals were made on a daily basis. Following the fourth test session, more frequent checks (two to three daily) were made on moribund gerbils. Litter was changed daily, but cages, food containers and water bottles were cleaned during each groups' test session. All animals were weighed on test days 0, 15 and 30.

Biochemical Experiment

Subjects

The Ss were all the surviving animals from the original 48 gerbils. The mean weight was 40 grams with a range of 28-62 grams. There were five gerbils in the Mg-D/MS-D/Test Group, four gerbils in the Mg-D/MS-D/No Test Group; five gerbils each in the Mg-D/MS-R/Test Group and Mg-D/MS-R/No Test Group; four gerbils in the Mg-R/MS-D/Test Group, two gerbils in the Mg-R/MS-D/No Test Group; six gerbils in the Mg-R/MS-R/Test Group, and finally six gerbils in the Mg-R/MS-R/No Test Group, for a total of 37 animals. Serum analysis was also performed on an additional seven gerbils, similar in age to the experimental animals. (See Procedure.)

Apparatus

Cardiac puncture was done with a 23 gauge needle. Whole blood was centrifuged on a Servall Superspeed RC-2 automatic refrigerated centrifuge. Analysis of the cations was performed on a Perkin-Elmer atomic absorption spectrophotometer, instrument series X03, model 403, Inorganic P was analyzed on a photometer.

Procedure

Following the sixth and final test session, blood was obtained from all surviving animals for the purpose of analyzing the serum cations and inorganic P levels. Serum was chosen for analysis because it is the plasma and serum levels of these substances which generally change in disease (Bush, 1975).

The gerbils were anaesthetized with 1/4 - 1/2 ml (depending on weight of the animal) of 5 mg/ml Ketaset (ketamine hydrochloride) (Crowie, 1976). The injection was given intramuscularly in the leg, and the anaesthesia lasted from five to 10 minutes. Approximately 1/4 ml of blood was exsanguinated by cardiac puncture. The blood was transferred to a non-heparinized test tube with special care so as to prevent hemolysis of the blood. The blood was then allowed to stand for 15 minutes and centrifuged at 2000 RPMs for 20 minutes. The serum was pipetted off and pooled within groups so as to provide a sufficient quantity of serum for the analysis (Orent, Kruse, and McCollum, 1934). Finally, the serum was refrigerated until analysis the following morning (approximately 10 hours later) (Bush, 1975).

The serum analysis for Mg, Ca, K, and Na was done according to the procedure outlined in the Perkin-Elmer Manual (see Appendix C). The inorganic P was determined according to the Fiske and Subbarow (1925) method (see Appendix D).

Because of the obtained results, additional serum analyses were done on three gerbils maintained on a completely Mg-D diet, i.e. less than one ppm Mg. Comparisons of the serum levels were made between this group and the Mg-D/MS-R Group. Further, six gerbils maintained on a regular commercial diet were bled and their serum analyzed and compared to the Mg-R/MS-R Group. Procedures were the same as those outlined for the experimental animals.

CHAPTER III

RESULTS

Behavioral Experiment

Body Weights

Weight was recorded for each gerbil on the 0, 15, and 30th day of the experiment. Mean weights of the animals were computed for main effects (Table V). A 2x2x2x3 analysis of variance (ANOVA) indicated significant decrease in weight, across groups as a function of weighing day (Table VI).

The weight of the gerbils significantly decreased when fed either the Mg-D or the MS-D diet. Figure 1 shows the mean weight for these groups. There was also a statistically significant interaction between the Mg and the day factors, and between the MS and the day factors.

Although there was no significant interaction between Mg, MS, and Day, a comparison between Figure 1 and Figure 2 (mean weight for diet groups) indicates that MS depletion was the important factor in reducing the gerbils weight in both the Mg-R and Mg-D diets. In fact, when the weight of animals is collapsed across days, there is only a statistically significant effect for MS ($F = 28.03$, $df = 1/44$, $p < .001$).

A t test for unequal group numbers yielded a significant effect for weight and number of seizing animals on the third weighing day ($t = -2.44$, $df = 19$, $p < .05$). Figure 3 presents the mean weights for seizing and

TABLE V

MEAN BODY WEIGHT (GRAMS) FOR THE EXPERIMENTAL DIET GROUPS ON THREE WEIGHING DAYS

Experimental Diet Groups	Weighing Days 1	2	3
Mg-D/MS-D			
Test	56.0	46.2	36.3
No Test	52.5	41.4	29.5
Mg-D/MS-R			
Test	51.7	52.0	44.0
No Test	54.7	50.3	49.0
Mg-R/MS-D			
Test	56.0	41.9	32.8
No Test	48.9	39.0	28.3
Mg-R/MS-R			
Test	56.3	46.5	49.8
No Test	53.2	47.3	53.0

TABLE VI

WITHIN SUBJECTS' SUMMARY OF ANALYSIS OF VARIANCE FOR WEIGHT BY GROUP AND TEST DAY*

Source	Degrees of Freedom	SS	MS	F	P
Day	2	4293.85	2146.93	101.71	.001
Mg x Day	2	167.35	83.68	3.96	.05
MS x Day	2	1707.68	853.84	40.45	.001
Mg x MS x Day	2	106.29	53.15	2.52	
Subject x Day (Mg x MS)	88	1857.5	21.10		
Total	96	8132.67			

*See Appendix E for complete ANOVA summary.

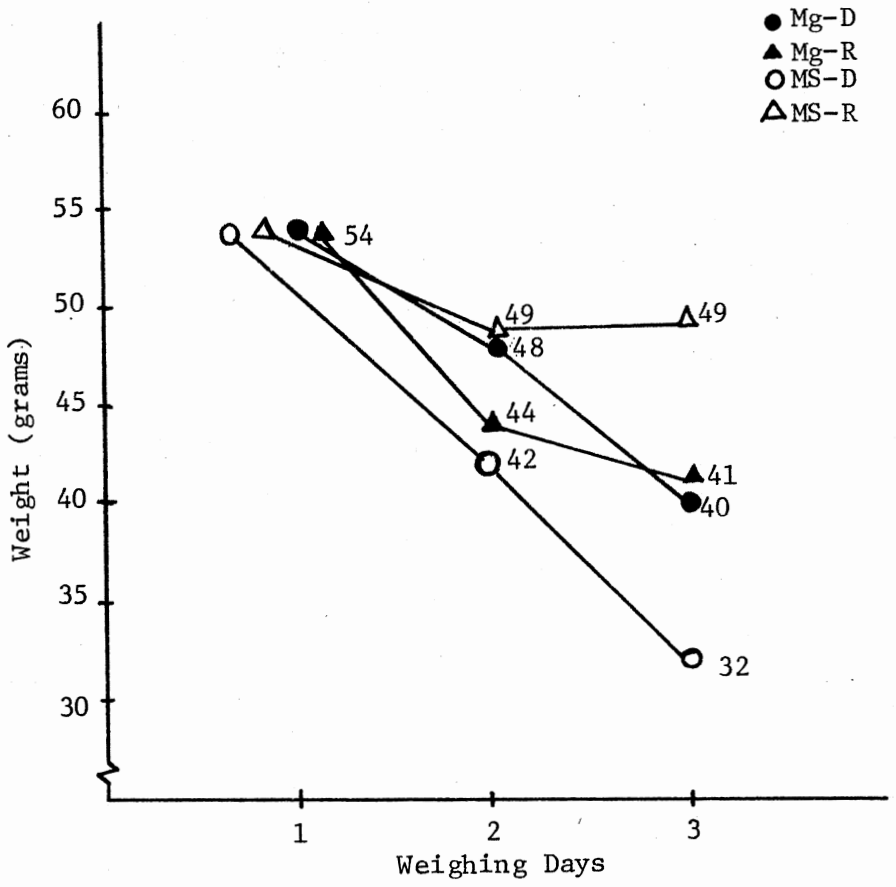

Figure 1. Mean Weight of Mongolian Gerbils as a Function of Day, and Mg and MS Levels

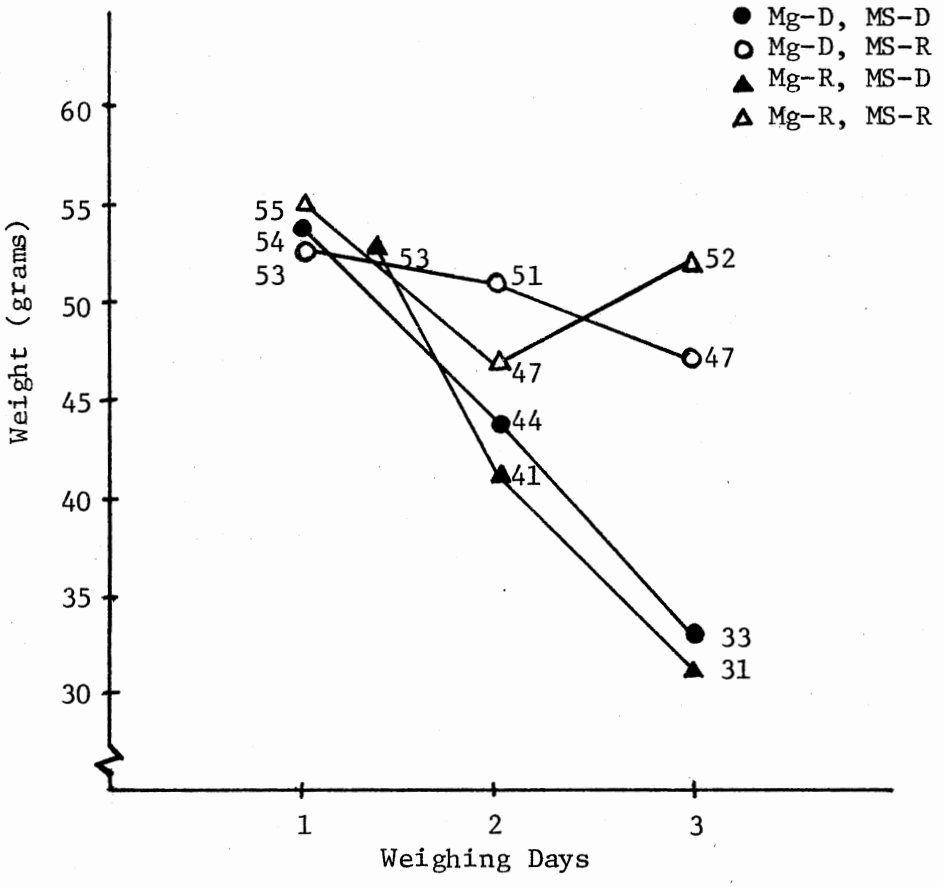

Figure 2. Mean Weight of Mongolian Gerbils as a Function of Day and Diet Group

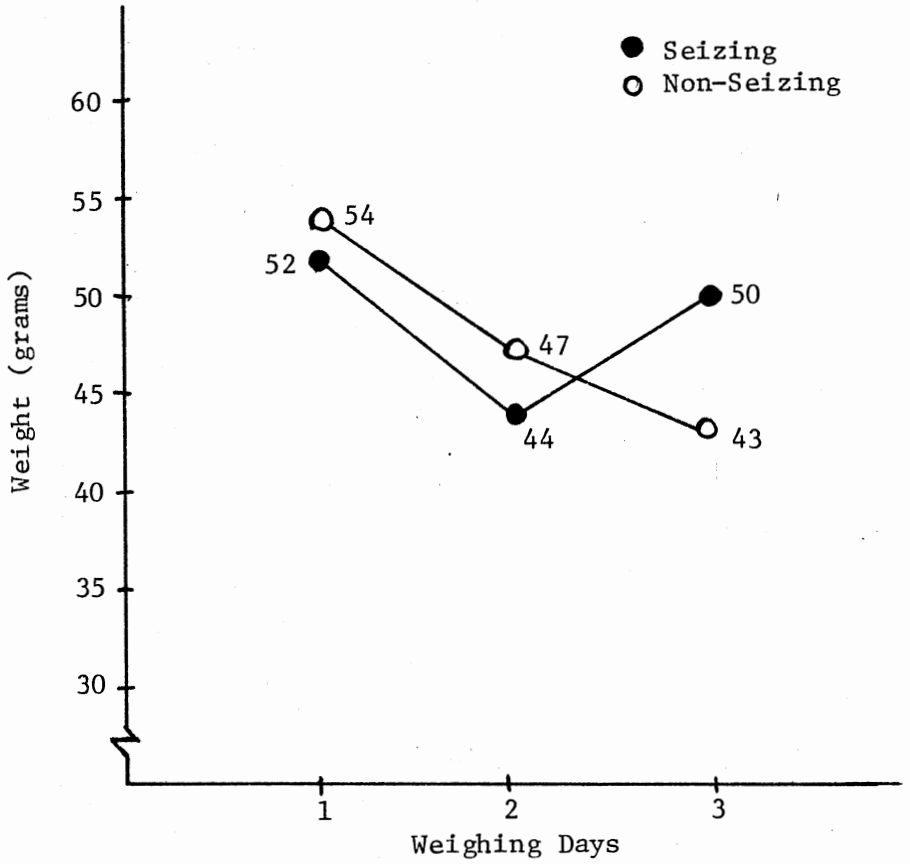

Figure 3. Mean Weight of Seizing and Non-Seizing Gerbils as a Function of Weighing Day

non-seizing gerbils on each weighing day. From the figure it can be noted that only on the third weighing day did the weight of gerbils increase, and this increase was ostensibly related to seizing gerbils.

Seizures

Table VII shows that a total of 32 seizures occurred among the eight groups of gerbils over a 30 day period. Of the 32 seizures only two occurred on non-weighing days, and, only in the Mg-R/MS-R Group did seizures occur on the first day of testing. There was no statistically significant main effects or interaction found for seizure frequency. Diet deficiency and/or testing (stress factor) the gerbils did not significantly increase the incidence of seizing behavior.

Of the six seizures in the Mg-D/MS-D Group, five were rated mild and one moderate. In the Mg-D/MS-R Group seven were mild and two were moderate. All the seizures in the Mg-R/MS-D Group were mild. Finally, in the Mg-R/MS-R Group, seven were mild and three were moderate. There were no severe seizures throughout the 30 day experiment.

As mentioned previously, the majority of the seizures occurred on weighing days. In addition, all but three of the seizures occurred while actually weighing the animals.

In the Test Groups, the median latency for the seizures was 270 sec. All seizures in the No Test Groups occurred while the animals were being weighed (i.e., immediately upon being handled).

Although not significant, the data indicated a trend for the Mg-D/MS-R diets to increase seizure duration. Mean seizure durations and ranges for each diet group are presented in Table VIII.

TABLE VII

RATIO OF SEIZING SUBJECTS AMONG GROUPS OF MONGOLIAN GERBILS FED DIFFERENT DIETS, AND TESTED IN AN OPEN FIELD ARENA OR OBSERVED IN HOME CAGES

	Test Days							
Diet Group	0*	5	10	15*	20	25	30*	Total Seizures
Mg-D/MS-D								
Test	0/6	0/6	0/6	1/6	0/6	0/6	2/5	3
No Test	0/6	0/6	0/6	3/6	0/6	0/5	0/4	3
Mg-D/MS-R								
Test	0/6	0/6	1/6	3/6	0/6	0/6	2/4	6
No Test	0/6	0/6	0/6	2/6	0/6	0/5	1/5	3
Mg-R/MS-D								
Test	0/6	0/6	0/6	4/6	1/6	0/6	0/4	5
No Test	0/6	0/6	0/6	2/6	0/6	0/3	0/2	2
Mg-R/MS-R								
Test	2/6	0/6	0/6	1/6	0/6	0/6	0/6	3
No Test	2/6	0/6	0/6	2/6	0/6	0/6	3/6	7

*Weighing days.

Note: Numerator = N of seizing gerbils; denominator = N of survivors.

TABLE VIII

CHARACTERISTICS OF SEIZURE DURATIONS FOR THE FOUR EXPERIMENTAL DIET GROUPS

Diet Group	Mean (secs)	Range (secs)
Mg-D/MS-D	15.20	5-60
Mg-D/MS-R	51.67	2-140
Mg-R/MS-D	8.25	2-10
Mg-R/MS-R	13.29	2-40

Of importance, is the finding that only the Mg-D Groups had seizure durations longer than 60 secs. The longest seizure lasted for 140 sec, and occurred in the Mg-D/MS-R Group. Concomitantly, recovery from the seizures was immediate for all of the gerbils except the Mg-D Groups. The mean recovery rate for these three gerbils was approximately three minutes.

Physical and Behavioral Observations

Although the following observations are not statistically significant, they warrant mention as sources for subsequent inquiry and research. As mentioned diet did not affect seizure frequency. However, there was a trend for the Mg-D gerbils to exhibit more hyperirritibility, as well as hopping and circling behavior when placed in the test arena. Further, the Mg-D Group displayed an increased urine output along with a change in fecal appearance (the fecal pellets were more elongated). This group also had a larger percentage of gerbils with erythema.

All groups showed slight-severe alopecia by the fourth test session. Of importance is the fact that the Mg-R/MS-D Group exhibited alopecia and wet coats by the second test session. Also, alopecia was most severe for the MS-D Groups. The Mg-R/MS-R Group, although initially developing alopecia, exhibited regrowth of hair by the sixth test session.

Besides the behavior elements covered by Harriman's (1974) classification, all mild seizures were noted as consisting of myoclonic jerks and of vibrissae and pinnae twitching. Moderate seizures also had identifiable rear and frontal ataxia. Some moderate seizures had tonic extensor rigidity. Finally, death occurred most often as the weight of the gerbil dropped to about 30 grams.

Deaths

Table VII shows that there was a total of 11 deaths during the 30-day study. Three deaths occurred in the Mg-D/MS-D Group, two deaths in the Mg-D/MS-R Group, six deaths in the Mg-R/MS-D Group, and no deaths occurred in the Mg-R/MS-R Group. A 2 x 2 ANOVA revealed a statistically significant effect for MS and death (Table IX). Death happened more frequently in the MS deficient groups.

Biochemical Experiment

Table X presents the cation and inorganic P serum levels for the eight experimental groups, for the group given no Mg in their diet (N-Mg), and for the group receiving commercially prepared pellets for their diet (P-Diet). A 2 x 2 x 2 ANOVA was performed for each cation on the pooled serum in each diet group. Summaries of nonsignificant

analysis of variances are presented in Appendix E.

TABLE IX

SUMMARY OF ANALYSIS OF VARIANCE FOR DEATHS

Source	Degrees of Freedom	SS	MS	F	P
Mg	1	0.02	0.02	0.13	0.718
MS	1	1.02	1.02	6.49	0.014
Mg x MS	1	0.50	0.50	3.31	0.076
Error	44	6.92	0.16		
Total	47	8.46			

Calcium

Table XI reveals a statistically significant effect for the Mg diets, and the MS diets on calcium level. A statistically significant interaction was also found between the Mg and the MS diets.

Figure 4 shows that serum Ca was significantly lower in gerbils fed either a Mg-D or a MS-D diet, with the lowest serum level in the Mg-D/MS-D Group. However, only in the Mg-D/MS-D Group were the serum values within normal limits (compare Table II with Table X). When the Experimental Diet Groups were compared to the P-Diet Group, the Ca serum level was significantly higher only in the Mg-R/MS-R Test and No Test Groups ($t=37.24$, $df=1$, $p < .02$, $t=31.13$, $df=1$, $p < .05$, respectively).

TABLE X

SERUM MINERAL SALT LEVELS IN THE MONGOLIAN GERBIL UNDER VARYING EXPERIMENTAL CONDITIONS

Mineral Salts (ppm)	Diet Groups									
	Mg-D,MS-D		Mg-D,MS-R		Mg-R,MS-D		Mg-R,MS-R			
	Test	No Test	Test	No Test	Test	No Test	Test	No Test	P-Diet	N-Mg
Magnesium	40.0	28.0	43.0	35.5	76.0^{+}	73.0^{+}	65.0^{+}	48.5^{+}	39.0	28.5
Calcium	90.2	113.0	132.0^{+}	135.5^{+}	155.0^{+}	113.0^{+}	290.0*	240.0*	103.0	112.0
Potassium	220.0	245.0	308.0*	276.0*	273.0^{+}	240.0	350.0*	250.0	225.0	211.0
Sodium	1340.0^{-}	1202.0^{-}	3341.0	3229.0	1230.0^{-}	1250.0^{-}	2176.0^{-}	1732.0^{-}	3516.0	2619.0^{-}
Phosphorous	--	--	69.0^{+}	77.0^{+}	136.0^{+}	124.0^{+}	76.0^{+}	63.0	10.0^{+}	96.0^{+}

*Values obtained are significantly higher than the normal range.

$^{+}$Values obtained are above the normal range.

$^{-}$Values obtained are below the normal range.

Note: See Appendix B for conversion of measurements; see Appendix E for ANOVA summaries.

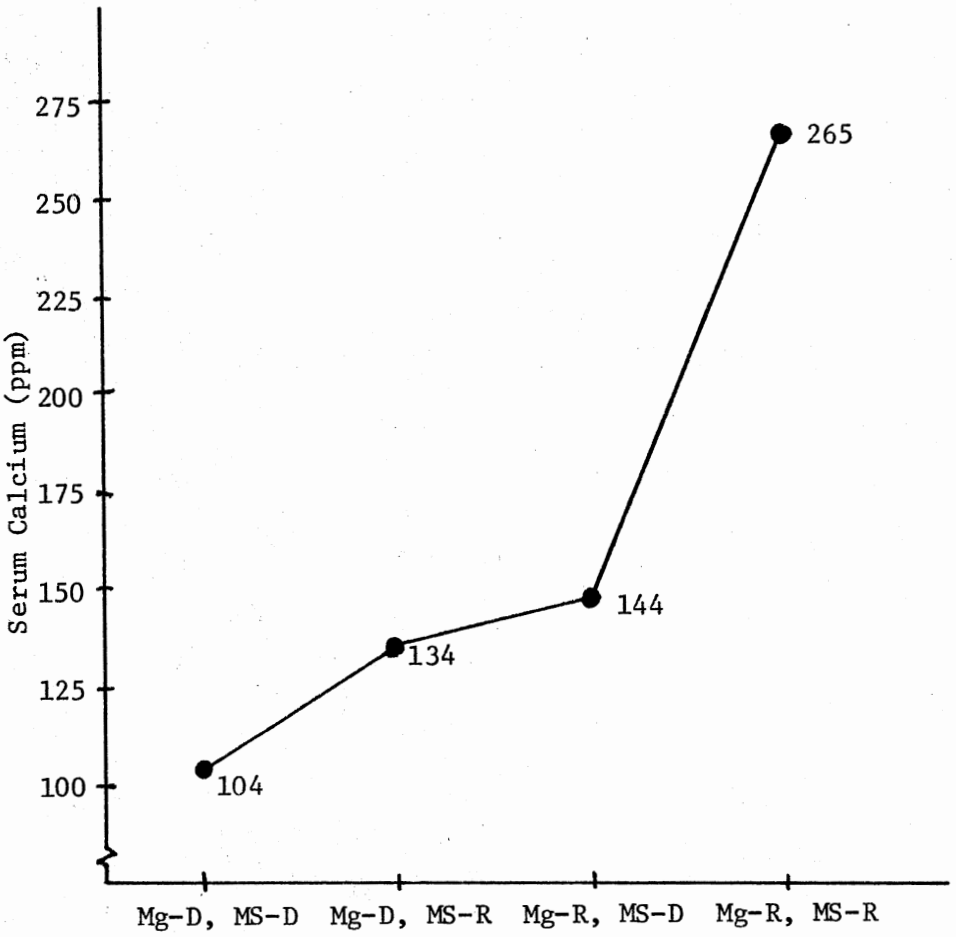

Figure 4. Serum Calcium Level as a Function of Diet Group

Potassium

A significant effect was found for the MS factor and for the Test/No Test factor on the serum K levels (Table XII). Serum K levels increased in animals that were tested in the arena and also increased in animals which were fed the MS-R diet (see Figure 5). However, only the animals which were both tested and received MS-R diet had serum K levels higher than normal (see Table X). A comparison between the Mg-R/MS-R Group and the P-Diet Group showed a significantly higher K serum in the Mg-R/MS-R Test Group ($t = 25$, $df = 1$, $P < .05$). In addition, when the serum K levels were compared between the Mg-D/MS-R Group and the N-Mg Group, the serum K again was significantly higher in the Test Group only ($t = 16.6$, $df = 1$, $P < .05$).

Magnesium

Although not statistically significant, the Mg-R diet groups had Mg serum levels higher than the normal range (Table X). Ms-D diets and Testing appeared to produce a cumulative effect; the highest Mg serum level was in the Mg-R/MS-D Test Group.

Sodium

No statistical significance was found for the Na serum levels. Nevertheless, only the Mg-D/MS-R Group and the P-Diet Group had serum Na values within the normal range. All other groups had Na serum levels below normal. The lowered Na serum level was also the only abnormal serum value in the N-Mg Group.

TABLE XI

SUMMARY OF THE ANALYSIS OF VARIANCE
FOR CALCIUM SERUM LEVELS

Source	Degrees of Freedom	SS	MS	F	P
Mg	1	15077.16	15077.16	1593.57	.05
MS	1	11727.46	11727.46	1239.53	.05
Test/No Test	1	261.06	261.06	27.59	
Mg x MS	1	3947.16	3947.16	417.19	.05
Mg x Test/No Test	1	1207.86	1207.86	127.66	
MS x Test/No Test	1	279.66	279.66	29.56	
Error*	1	9.46	9.46		
Total	7	32509.83	32509.83		

*Three-way interaction.

TABLE XII

SUMMARY OF ANALYSIS OF VARIANCE FOR
POTASSIUM SERUM LEVELS

Source	Degrees of Freedom	SS	MS	F	P
Mg	1	512.00	512.00	40.96	
MS	1	5304.50	5304.50	424.36	.05
Test/No Test	1	2450.00	2450.00	196.00	.05
Mg x MS	1	128.00	128.00	10.24	
Mg x Test/No Test	1	1984.50	1984.50	158.76	
MS x Test/No Test	1	1922.00	1922.00	153.76	
Error*	1	12.50	12.50		
Total	7	12313.50	12313.50		

*Three-way interaction.

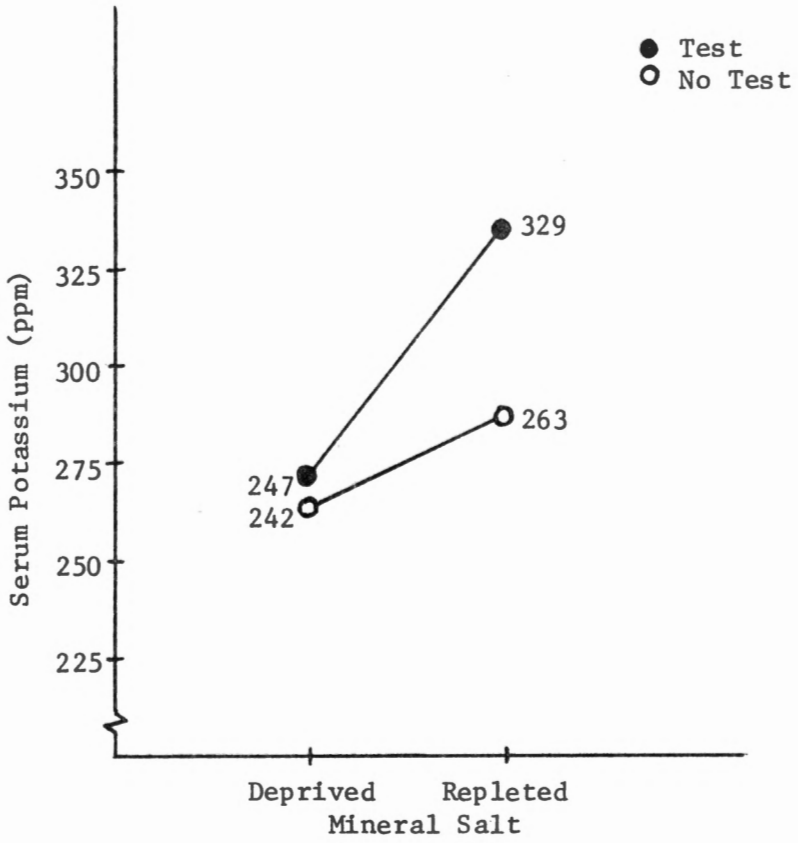

Figure 5. Serum Potassium Level as a Function of Mineral Salt and Test/No Test Group

Phosphorous

Because of procedural problems, no P serum levels were obtained for the Mg-D/MS-R Group. Therefore, it was felt that no statistical comparisons should be made. However, it should be noted, that of the groups whose serum was analyzed, only the Mg-R/MS-R Test Group was within normal limits. All other values were above normal, with the highest values in the MS-R Group.

CHAPTER IV

DISCUSSION

Experimentally-Induced Magnesium Tetany

The present study assessed the effectiveness of Mg deprivation, in conjunction with other mineral salts, in effecting seizures in stressed and non-stressed Mongolian gerbils. It is apparent from the results presented in Chapter III that the 250 ppm loading of Mg in the diet did not significantly affect seizure frequency. In fact it was the Mg-R/MS-R Diet Group which manifested the greatest number of seizures.

Magnesium Loading

As mentioned previously, Mg tetany manifests itself in two ways: (1) locally by increased irritibility of the nervous system, and (2) constitutionally by nutritive failure (Kruse, Orent, and McCollum, 1933). Since the present study was more interested in the local effect of Mg tetany (i.e., an increase in acetylcholine at the neuromuscular junction), an attempt was made to control for the effect of nutritive failure. This control was thought to be effected by raising the Mg loading in the diet to 250 ppm, instead of the more frequently used loading of 1.8 ppm. Harriman (in press) had reported 12 seizures over a 40-day period, with only three deaths out of 30 gerbils. However, the percentage of animals that seized in this study (i.e., 50 percent)

was comparable to rates reported for "spontaneous" seizing in other gerbil colonies (Thiessen, Lindzey, and Friend, 1968). The findings indicate, therefore, that the Mg loading chosen for this experiment was not low enough to effectively produce the local effects of Mg tetany. Instead, the obtained seizure rates may have been a consequence of novelty or the stress of being weighed. In that event the findings resemble those often reported by other experimenters. Kaplan (1976), for example, concurs that novelty, a triggering factor, increases arousal level and stress, and thus induces seizures in the gerbil. Further, the absence of severe seizures, and only a relatively few number of moderate seizures supports the hypothesis that Mg deficiency may not have been effectively induced. This concurs with Orent, Kruse and McCollum's (1932) finding that hyperexcitability is quite pronounced with low Mg diets (i.e., 1.8 ppm). Concomitantly, as the Mg loading increases the severity of the seizure would decrease. Subsequent studies, Tufts and Greenberg (1938) and Harriman (in press) indicate similar trends.

Nutritive Failure

Tufts and Greenberg (1938) report that in milder forms of Mg deficiency, the effects of nutritive failure are prolonged and overshadow the convulsive phase. Death is precipitated by exhaustion from tonic-clonic convulsions and/or dietary deficiency. A diet consisting of 1.8 ppm Mg is manifested by local Mg tetany; whereas, a diet containing 250 ppm initially manifests itself constitutionally. The symptoms of malnutrition (inanition, weight loss, alopecia, kidney failure, sticky and wet coats, dehydration, death) became evident in

many animals within a week. Some symptoms were more prevalent than others. Weight loss significantly affected death rate. The Mg-D/MS-D Group evidenced the least deaths, while nutritive failure was greatest in the Mg-R/MS-D Group.

Of interest is the observation that the groups present a picture of renal failure. In renal failure the animals are often dehydrated and have a variety of electrolyte disturbances (Kaneko and Cornelius, 1970). Serum sodium may be normal as in the Mg-D/MS-R Group, or abnormally low as in the other groups. The latter case indicates that the Na deficit is relatively greater than the water deficit. This is most often the result of both Na and water depletion followed by replacement of water alone (Kaneko and Cornelius, 1970). (Note: Water was given ad libitum in the present study.)

Hyperkalemia (increased K) also occurs in renal failure. It is frequently the result of a redistribution of the body K, i.e., the movement of intracellular K into the extracellular fluid. The P serum level may rise anywhere from 8 to 25 mg/100 ml (see Appendix B for measurement conversions) in renal failure. Since excess Mg is excreted via the kidneys hypermagnaesmia is evidenced in renal failure. And, finally, since the main pathway for the excretion of Ca is also the kidneys, hypercalcemia would also be evidenced in renal failure (Kaneko and Cornelius, 1970; 1971).

As already noted, the Mg-R/MS-R group initially showed signs of nutritive failure, but by the fifth week, the signs reversed themselves. The abnormal serum levels for this group may be reflective of the earlier symptoms. There may also have been a metabolic adjustment to the mash diet.

Magnesium Metabolism

The high Mg serum levels presented in Table X may indicate that the Mongolian gerbil has a greater need for magnesium, than do other mammals for which data are avilable (compare to Table XIII). The serum level for the N-Mg Group indicates that 28.5 ppm of Mg is reflective of a deprived state rather than a normal serum level as reported for other experimental animals. This may account for the "spontaneous" seizing in gerbils supposedly maintained on normal diets. Further, evidence that the Mongolian gerbil does indeed require more Mg can also be deduced from the high Mg serum levels in other groups (range 39.0 - 76.0 ppm Mg). Presently, it is known that the ability to absorb Mg is lost with increasing age, especially after one month of age (Kaneko and Cornelius, 1970). [This may also account for the low incidence of seizures in gerbils under one month of age.] However, what seems to be happening with gerbils is that they have developed a unique metabolism for Mg. Gerbils may be better adapted at absorbing Mg and utilizing their Mg reserve. The high Mg serum levels are apparently an artifact of the metabolic transfer of the Mg from the intracellular fluid to the extracellular fluid, and the mobilization of Mg in the bone to the serum (Orent, Kruse and McCollum, 1932). The results suggest that the Mongolian gerbil has a high need for Mg, and its metabolism has subsequently adapted so as to get the optimal usage out of what Mg is present in the body.

Finally, the values reported in Table X are thus far the first recorded serum values for both Mg and Ca in the Mongolian gerbil. Therefore, the values need to be replicated before they may be accepted or valid.

Electrolyte Interaction

The effects of normal or increased cation levels in conjunction with Mg deficiency have already been noted (Schrader, Prickett and Salmon, 1937; Toothill, 1963). Presently, no one has reported on the effects of decreased cation levels in conjunction with normal or high Mg levels. One purpose of the present study was to examine, both clinically and biochemically, the effect of the interactions of mineral salts on seizure activity. Specifically, this experimenter wanted to determine if it was the Mg deficiency which increased seizure activity or just a disruption in the electrolyte balance. The findings indicate it may be the latter. Specifically, the seizure durations reported in Table VIII indicate that higher cation levels exacerbate Mg tetany. The behavioral observations recorded by this experimenter support the conclusion. Kaneko and Cornelius (1970) also concur. They report that a disruption in the electrolyte balance may play a part in the pathogenesis of neuromuscular hyperirritability. Finally, the disruption in the electrolyte balance may present the gerbil with enough internal stress to precipitate a seizure. Further research would be most beneficial in this area of study.

Tyzzer's Disease

The cause of this disease is infection with Bacillus piliformis usually contracted from mice via infected food or bedding and is very contagious in gerbil colonies. The animals become lethargic, inappetant with some mild diarrhea, and excessive weight loss. In the acute form, there is a 70 percent mortality rate after the onset of symptoms.

In the milder form, all symptoms, except the diarrhea, occur. It runs a slower course and generally places no more than 10 percent of the animals at risk. In both forms the incubation period is 10 days. The milder form can also be exacerbated by stress to produce the fatal form of the disease (Marston, 1972; Cowie, 1976).

The above description aptly fits the behavioral observations of the experimental gerbils. It should be mentioned that the food for the rats and mice did become infected. Although the gerbils were housed separate from these animals, cages from all the animals were cleaned in the same dishwasher. Further, at the start of the experiment the heat regulator was not working, so that the water temperature was never excessively hot. Although this diagnosis was never confirmed, the possibility must be reported.

Summary

Although the present experiment failed to support the original hypothesis that Mg deficiency exacerbates seizures in Mongolian gerbils, it is felt that several reported findings are sufficiently noteworthy to merit further research. First, the Mg serum levels reported for the gerbil must be replicated since the implications of the levels are felt, by this experimenter, to be of tantamount importance to the understanding of the seizures exhibited by the Mongolian gerbil. If the results are indeed corroborated, then a possible factor in the etiology of epilepsy may have been discovered. Second, the function of electrolyte imbalance needs further exploration. The results indicate Mg-D/MS-R diet optimally induces seizures. However, a low Mg diet comparable to the 1.8 ppm Mg diet developed by Kruse, Orent

and McCollum (1932) should be utilized instead of the 250 pp Mg diet employed in this study. Additionally, the specific role of each electrolyte in conjunction with Mg deficiency needs further exploration.

The role of Mg deficiency in the inducement of seizures in Mongolian gerbils needs further clarification. It is hoped that the present experiment has offered some directional guidance for this future research.

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APPENDIXES

APPENDIX A

SELECTED GLOSSARY

alkali - any one of a class of compounds which forms salts with acids and soaps with fats.

alkalosis - increased alkali reserve in the blood and tissue.

alopecia - loss of hair from normally hairy areas of the body.

anhydremia - diminuition of the fluid content of the body.

anorexia - loss of appetite.

anoxia - lack of oxygen.

cachexia - malnutrition.

cataleptoid - like catalepsy (suspended animation, loss of voluntary muscles, limbs remain where placed).

clonic - spasm in which rigidity and relaxation succeed each other.

convulsion - involuntary spasm or contraction of muscles; spasm.

erythemia - redness of the skin due to congestion of the capillaries.

exsanguination - forcible expulsion of blood from a part.

hyperemia - excess of blood in a part.

hypersensitivity - exaggerated susceptibility to a substance or stimulus.

hypoxia - decreased supply of oxygen.

ichthyosis - dryness, roughness and scaliness of the skin.

ictus - a sudden attack.

inanition - exhaustion from lack of food or inability to assimilate it.

mineral - non-organic homogenous substance.

nephrosis - a disease or inflamation of the kidney.

opisthotonus - tetanic spasm which flexes the head and feet backward.

paroxysmal - episode of abrupt onset and termination.

salt - compound of metal with an acid radical.

tetany - sharp flexion of the wrist and ankle joints.

trophic - nutritional.

Source: Dorland's Pocket Medical Dictionary (1968).

APPENDIX B

CONVERSION OF MEASUREMENTS

From	To	Multiply By
mg%	µg%	1000
	mEg/l	(10/eq. wt.)
	µg/ml	10
µg%	mg%	0.001
	mEg/l	(0.01/eq. wt.)
	µg/ml	0.01
mEg/l	mg%	0.1 x eq. wt.
	µg%	100 x eq. wt.
	µg/ml	eq. wt.
g/l	oz/gal	0.1335
	mg/l	1000
	µg/ml	1000
	wt%	0.1
oz/gal	g/l	7.491
	µg/ml	7491
	wt%	(0.7491/ρ)
oz/ton*	ppm	30.61
	wt%	0.003061
	fine	0.03061
fine	oz/ton*	32.67
	wt%	0.1
	ppm	1000
molar	µg/ml	1000 x atomic wt.
	ppm	(1000 x atomic wt/ρ)

From	To	Multiply By
wt%	g/l	10
	oz/gal	1.335ρ
	oz/ton*	326.7
	fine	10
	ppm	10,000
	µg/ml	10,000ρ
ppm	g/l	0.001
	oz/gal	0.0001335
	oz/ton*	0.03267
	fine	0.001
	µg/ml	ρ
	wt%	0.0001
	molar	(ρ/1000 x atomic wt.)
µg/ml	mg%	0.1
	µg%	100
	mEq/l	(1/eq. wt.)
	g/l	0.001
	ppm	(1/ρ)
	wt%	(1/10,000ρ)
	oz/gal	0.0001335
	oz/ton*	(0.03267/ρ)
	fine	(1000/ρ)
	molar	(1/1000 x atomic wt.)

*troy oz/long ton
eq. wt. = atomic wt/valence
1 nm = 10^{-9} m = 1 mµ = 10 Angstroms
ρ = density or specific gravity of solution

Source: Perkin-Elmer Manual.

APPENDIX C

PROCEDURE FOR ANALYSIS OF SERUM CALCIUM, MAGNESIUM, SODIUM AND POTASSIUM

Scope

This method describes the determination of calcium, magnesium, sodium and potassium in blood serum and plasma. Normal levels in serum are 9.0-11.0 mg% calcium (4.5-5.5 meq/1); 1.8-3.1 mg% magnesium (1.5-2.5 meg/1); 135-155 meq/1 sodium; and 3.6-5.5 meq/1 potassium.

Reagents

Lanthanum diluent, 0.1% (w/v) La. Prepare by diluting 20.0 ml of the La stock solution, described under the Standard Conditions for lanthanum, to 1 liter with deionized water.

Potassium chloride, KCl.

Sodium chloride, NaCl.

Standard Solutions

Calcium standard solution, 10 mg% Ca (5.0 meq/1 Ca). Prepare by diluting 200 ml of the Ca stock solution, described under Standard Conditions for calcium, to 1 liter with deionized water.

Magnesium standard solution, 2.4 mg% Mg (1.97 meq/1 Mg). Prepare by diluting 24.0 ml of the Mg stock solution, described under the Standard Conditions for magnesium, to 1 liter with deionized water.

Sodium and potassium standard solution, 140 meq/1 Na and 5.0 meq/1 K. Prepare by dissolving 8.183 g of dried NaCl and 0.372 g of dried KCl in 500 ml of deionized water and diluting to 1 liter with deionized water.

Sample Preparation

For the determination of calcium, magnesium and potassium, dilute the sample 1:50 with the lanthanum dilutent solution. For the determination of sodium, an additional 1:50 dilution with deionized water is required.

Note: If calcium is not to be determined, the initial dilution may be made with deionized water. The La diluent solution is used only to remove a potential phosphate interference in the calcium determination.

Analysis

Determine the concentration of the element(s) of interest versus standards which have been similarly diluted and, where the La diluent has been used, versus a reagent blank. Use the Routine procedure described in the General Information section.

Calculations

With instruments or accessory readout devices capable of presenting a reading directly in concentration, set the diluted standards to read their undiluted concentration, and read the concentration of the element(s) of interest in the sample directly. For instruments not reading in concentration, prepare a working curve to determine the concentration of the element(s) of interest.

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Source: Perkin-Elmer Manual (1973).

APPENDIX D

PROCEDURE FOR ANALYSIS OF SERUM PHOSPHOROUS

Principle

A trichloroacetic acid filtrate of serum or urine is treated with molybdate reagent, which reacts with phosphate to form ammonium molybdophosphate (ammonium phosphomolybdate). This is thought to have the formula $(NH_4)_3[PO_4(MoO_3)_{12}]$. The addition of a suitable reducing agent such as aminoaphtholsulfonic acid produces a blue color of heteropolymolybdenum blue. A mild reducing agent is employed in order to avoid reduction of the excess of molybdate present. Other reducing agents, such as stannous chloride, ascorbic acid, Elon (ρ-methyl amino phenol) and N-phenyl-ρ-phenylenediamine (Semidine) have been utilized in this reaction, but aminonaphtholsulfonic acid is still widely used.

Reagents

1. Trichloroacetic acid, 5 g/100 ml. Place 50 g of trichloroacetic acid, AR, into a 1000 ml volumetric flask; dissolve in and fill to the mark with deionized water.

2. Sulfuric acid, 5 mol/l. Slowly add 300 ml of concentrated sulfuric acid, AR, to 750 ml of deionized water, mix well, and cool.

3. Molybdate reagent. Dissolve 25 g of ammonium molybdate, AR, in about 200 ml of deionized water. Into a 1 liter volumetric flask place 300 ml of 5 molar sulfuric acid, add the molybdate solution, dilute with washings to 1 liter with deionized water, and mix. Solution is stable indefinitely. Discard the reagent if blanks show a blue color.

4. Sodium bisulfite, 15 g/100 ml. Place 30 g of sodium bisulfite, AR, into a beaker, and dilute to 200 ml with deionized water from a graduated cylinder. Stir to dissolve; if the solution is turbid, allow

to stand well stoppered for several days and then filter. Keep reagent well stoppered.

5. Sodium sulfite, 20 g/100 ml. Dissolve 20 g of sodium sulfite (anhydrous), AR, in deionized water and dilute to the 100 ml mark. Filter if necessary. Keep well stoppered.

6. Aminonaphtholsulfonic acid reagent. Place 195 ml of sodium bisulfite solution (15 g/100 ml) into a glass-stoppered cylinder or other suitable container. Add 0.5 g of 1,2,4-aminonaphtholsulfonic acid and 5 ml of sodium sulfite solution, 20 g/100 ml. Stopper and shake until the powder is dissolved. If solution is not complete, add, with continuous shaking, 1 ml of sodium sulfite at a time, until solution is complete. Avoid excess of sodium sulfite. Transfer the solution to a brown glass bottle and store in the cold. The solution is stable for about 1 month.

7. Stock standard, 0.4 mg phosphorus in 5 ml (2.61 mmol P/l). Place exactly 0.351 g of dry potassium dihydrogen phosphate, AR, into a 1 liter volumetric flask, dissolve in deionized water, add 10 ml of 5 molar sulfuric acid, and dilute to the mark with deionized water.

8. Working standard, 0.004 mg P/ml. Place 5.00 ml of the stock phosphate standard into a 100 ml volumetric flask and make up to the volume with trichloroacetic acid (5 g/100 ml).

Procedure

1. Place 0.5 ml of serum into a 15 x 150 ml test tube or a 10 ml glass-stoppered cylinder.

2. Blow in 9.5 ml of trichloroacetic acid (5 g/100 ml), mix, and let stand for 5 min.

3. Centrifuge or filter through Whatman No. 42 filter paper.

4. Pipet 5 ml of clear filtrate into a test tube or glass-stoppered cylinder graduated at 10 ml. Prepare a blank by using 5 ml of trichloroacetic acid (5 g/100 ml), and prepare a standard by using 5 ml of working standard (5 x 0.004 = 0.02 mg P).

5. Add 1 ml of molybdate reagent to all test tubes.

6. Add 0.4 ml of aminonaphtholsulfonic acid reagent; mix.

7. Dilute to the 10 ml mark with deionized water, mix, and allow to stand for 5 min.

8. Set blank at 100 per cent T or zero A and read standard and unknowns at 40 min.

Calculations

Read results from a standard curve or calculate as follows:

$$\text{mg P/100 ml} = \frac{A_U}{A_S} \times 0.02 \times \frac{10}{5} \times \frac{100}{0.5}$$

$$= \frac{A_U}{A_S} \times 8$$

where

0.02 = mg P contained in 5 ml of working standard

10 = amount of filtrate prepared

5 = amount of filtrate used

100 = basis of expressing concentration (= 100 ml)

0.5 = amount of sample used

Source: Fiske and Subbarow (1925).

APPENDIX E

STATISTICAL TABLES

TABLE XIII

SUMMARY OF ANALYSIS OF VARIANCE FOR
MAGNESIUM SERUM LEVELS

Source	Degrees of Freedom	SS	MS	F	P
MG	1	1667.53	1667.53	38.98	0.1011
MS	1	81.28	81.28	1.90	0.3996
Test/No Test	1	195.03	195.03	4.56	0.2778
Mg x MS	1	270.28	270.28	6.32	0.2411
Mg x Test/No Test	1	0.03	0.03	0.00	0.9828
MS x Test/No Test	1	11.28	11.28	0.26	0.6980
Error*	1	42.78	42.78		
Total	7	2268.22			

*Three-way interaction.

TABLE XIV

SUMMARY OF ANALYSIS OF VARIANCE FOR
SODIUM SERUM LEVELS

Source	Degrees of Freedom	SS	MS	F	P
Mg	1	927522.0	927522.0	30.90	0.1133
MS	1	3720992.0	3720992.0	123.98	0.0570
Test/No Test	1	56784.5	56784.5	1.89	0.4002
Mg x MS	1	845000.0	845000.0	28.15	0.1186
Mg x Test/No Test	1	3784.5	3784.5	0.13	0.7828
MS x Test/No Test	1	23980.5	23980.5	0.80	0.5356
Error*	1	30012.5	30012.5		
Total	7	5608076.0			

*Three-way interaction.

TABLE XV

SUMMARY OF ANALYSIS OF VARIANCE FOR NUMBER OF SEIZURES IN MONGOLIAN GERBILS

Source	Degrees of Freedom	SS	MS	F	P
Among Subjects	7	3.667	0.524	0.84	0.563
Mg	1	0.083	0.083	0.13	0.717
MS	1	0.750	0.750	1.20	0.279
TNT	1	0.083	0.083	0.13	0.717
Mg x MS	1	0.000	0.000	0.00	1.000
Mg x TNT	1	0.333	0.333	0.53	0.469
MS x TNT	1	0.333	0.333	0.53	0.469
Mg x MS x TNT	1	2.083	2.083	3.33	0.075
Within Subjects	40	25.00	25.00		
Total	47	28.67			

TABLE XVI

SUMMARY OF ANALYSIS OF VARIANCE FOR NUMBER OF SEIZING MONGOLIAN GERBILS

Source	Degrees of Freedom	SS	MS	F	P
Among Subjects	7	1.000	0.143	0.52	0.814
Mg	1	0.083	0.083	0.30	0.585
MS	1	0.083	0.083	0.30	0.585
TNT	1	0.083	0.083	0.30	0.585
Mg x MS	1	0.000	0.000	0.00	1.000
Mg x TNT	1	0.000	0.000	0.00	1.000
MS x TNT	1	0.000	0.000	0.00	1.000
Mg x MS x TNT	1	0.750	0.750	2.73	0.107
Within Subjects	40				
Total	47	12.000			

TABLE XVII

SUMMARY OF ANALYSIS OF VARIANCE FOR SEIZURE DURATION

Source	Degrees of Freedom	SS	MS	F	P
Among Subjects	7	11000.64	1571.52	1.48	0.243
Mg	1	3469.10	3469.10	3.27	0.089
MS	1	2247.72	2247.72	2.12	0.165
TNT	1	3244.81	3244.81	3.06	0.099
Mg x MS	1	609.65	609.65	0.57	0.459
Mg x TNT	1	961.47	961.47	0.91	0.355
MS x TNT	1	340.29	340.29	0.32	0.579
Mg x MS x TNT	1	127.60	127.60	0.12	0.733
Within Subjects	16	16975.10	16975.10		
Total	23	27975.74			

TABLE XVIII

COMPLETE SUMMARY OF ANALYSIS OF VARIANCE FOR WEIGHT BY GROUP AND TEST DAY

Source	Degrees of Freedom	SS	MS	F	P
Among Subjects					
Mg	1	24.17	24.17	0.28	0.6020
MS	1	2458.51	2458.51	28.03	0.0001
Mg x MS	1	95.06	95.06	1.08	0.304
Subject (Mg x MS)	44	3859.25	3859.25		
Within Subjects					
Day	2	4293.85	2146.93	101.71	.001
Mg x Day	2	167.35	83.68	3.96	.05
MS x Day	2	1707.68	853.84	40.45	.001
Mg x MS x Day	2	106.29	53.15	2.52	
Subject x Day (Mg x MS)	88	1857.5	21.10		
Total	143	14569.66			

TABLE XIX

COMPLETE SUMMARY OF ANALYSIS OF VARIANCE FOR DEATHS

Source	Degrees of Freedom	SS	MS	F	P
Among Subjects	3	1.56	0.52	3.31	0.029
Mg	1	0.02	0.02	0.13	0.718
MS	1	1.02	1.02	6.49	0.014
Mg x MS	1	0.50	0.50	3.31	0.076
Within Subjects	44	6.92	0.16		
Total	47				

VITA 2

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