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Interrelationships among Magnesium Deficiency, Ketogenic Diet, and Fasting on Seizure Susceptibility

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INTERRELATIONSHIPS AMONG MAGNESIUM DEFICIENCY, KETOGENIC DIET, AND FASTING ON SEIZURE SUSCEPTIBILITY

by

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DEDICATION

TO

Martyr Yassin Al-Hamdani

&

Martyr Aoumar Al-Hamdani

For their defense for the freedom of Iraq; against its enemy, Iran.

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Hamdia Al-Hamdani

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ABSTRACT

Interrelationships among Magnesium Deficiency, Ketogenic Diet, and Fasting on Seizure Susceptibility

by

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Fasting and ketogenic diet prevent seizures in epileptic children, magnesium-deficient rats and other animal models of seizure disorders. This effect has been attributed to increased levels of circulating ketone bodies. The purpose of this study was to determine the role of serum ketone bodies, measured as beta-hydroxybutyrate (BHB), in preventing audiogenically-induced seizures in weanling rats fed a magnesium-deficient diet for 17 days.

The effect on seizure susceptibility was investigated by feeding a magnesium-deficient diet to weanling rats for 17 days. Fasting and ketogenic diet (dietary medium chain triglycerides, MCT) markedly decreased seizure incidence that was associated with increased serum BHB level. Also, rats fasted for 24h or fed 28 percent dietary MCT had decreased seizure incidence as compared with rats fed 3 percent dietary MCT or rats fasted for 6h. These effects were not caused by differences in caloric density or percentage of calories from fat in the diets.

Gavaging 2 mmoles of BHB resulted in lower seizure incidence; as compared with rats gavaged with 0.5 mmoles BHB when measured 30 min after dosing. In contrast, gavaging 5.6 mmoles of glucose resulted in increased seizure incidence in 24-h-fasted rats.

Gavaging 5.6 mmoles of glucose with 0.5 mmole of BHB simultaneously resulted in higher seizure incidence than gavaging with 2.0 mmole BHB and 1.4 mmole glucose

simultaneuosly. In addition, gavaging 5.6 mmoles of glucose with 2 mmoles of BHB resulted in higher seizure incidence than gavaging 2 mmoles of BHB alone, which markedly reduced seizure incidence in fasted animals.

Fasting, ketogenic diet (MCT) and gavaging BHB increase serum BHB and decrease serum glucose concentrations. Gavaging glucose reduced serum BHB and increased serum glucose concentration. There was an inverse relationship between serum BHB and glucose in all treatments of this study. Although some treatments affected serum minerals, these effects were not consistent among experiments. Therefore, fasting, ketogenic diet (MCT) and gavaging BHB or glucose does not affect serum minerals markedly or consistently; and modifications in serum minerals caused by these treatments do not account for this effect on seizure incidence and severity. Finally, increases in serum BHB and decreases in serum glucose were consistently associated with dose-dependent reductions in seizure susceptibility of rats fed a magnesium-deficient diet for 17 days.

(164 pages)

CHAPTER I

INTRODUCTION

Generalized epilepsy is a disease of the central nervous system characterized by a massive neuronal discharge followed by tetanic muscle contraction; paralleled with an increase of cerebral blood flow and metabolism and followed by a loss of consciousness that may last for only a few seconds, as in petit mal attacks, or may be accompanied by convulsions, as in grand mal attacks. It occurs more frequently in children than in adults (Robinson, 1972). Epilepsy affects about 2.5 million Americans and is second only to stroke as the leading neurological disorder (Porter et al., 1985), with an incidence of 0.3-7.0/1000 among men and women of all ages (Anonymous, 1957). There are 20-40 million epileptic people worldwide (Delgado-Escueta et al., 1986).

There are three types of minor motor seizures: (1) Akinetic seizures are characterized by a sudden, momentary loss of muscle tone. This may be partial, in which case the attack consists of a sudden dropping forward of the head and neck, known as the "saluum seizure" (Menkes, 1976). (2) The term myoclonic seizures describes a variety of convulsive episodes characterized by single or repetitive contractures of a muscle or a group of muscles. Myoclonic seizures may be isolated, as in benign essential myoclonus, a condition in which evidence for other neurological abnormalities is lacking, or may occur in association with other seizure forms (Grosz-Selbeck and Doose, 1975). (3) Atypical petit mal seizures, unlike those of true petit mal, have a tendency to occur in cycles and to disappear for periods of time up to several days (Menkes, 1976).

Audiogenic seizures are convulsions triggered by exposure to intense auditory stimulation; these seizures have been known in rats and mice since 1924 (Krushinsky et al., 1970). Although uncommon in man (Bickford and Klass, 1968), they can be experimentally induced in house mice (Vicari, 1951), deer mice (Watson, 1939), rats (Maier and Glaser, 1940) and rabbits (Nellhause, 1958). The age of an animal

significantly influences the audiogenic seizure susceptibility; young animals are more susceptible to audiogenic seizures than old animals (Vicari, 1951). Later, it was discovered that auditory exposure or audiogenic priming induces audiogenic seizures (Iturrian and Fink, 1968). Genetic factors and biochemical mechanisms, such as deficiencies in ATPase activities, alterations in neurotransmitter metabolism and differences in endocrine function, have been found responsible for audiogenic seizure susceptibility (Seyfried, 1979).

There are three principal reasons why investigation of induced convulsions is of value: (1) The appearance of seizures may be influenced by a number of factors; such as conflict, electrical stimulation, injection with certain drugs and deprivation of any of several dietary elements. The pattern of behavior might be employed as an assay indicator of the presence and strength of such factors. (2) Convulsions may alter other behavioral and physiological characteristics of the organisms, e.g., changes in brain metabolism and neurotransmitter concentrations, diurnal activity, physiological condition and general activity. (3) The nature of the convulsion is of considerable interest for its own sake. Its outward resemblance to human epileptic convulsions has frequently been pointed out. Even if nothing can be contributed to the understanding of these human syndromes, at least the controlled observation of the rat seizure will expand our knowledge of basic neurophysiological processes.

The first progress in the study of convulsive disorders began with classification of the causes of convulsions in children. This made possible the diagnosis of epilepsy. The next advance was made in the study of the metabolic changes that precipitate convulsions in the potentially epileptic child. The beneficial effects of fasting and purging have been known since antiquity, but their mechanism has never been satisfactorily established. The favorable effect of dietary ketosis has been well-studied and effectively demonstrated (Peterman, 1946). It is well known that a ketogenic diet decreases seizures in epileptic individuals (Huttenlocher et al., 1971; Mike, 1965; Wilder, 1921).

Induction and characteristics

Several factors can cause susceptibility to audiogenic seizure: conflict, electrical stimulation, drugs and deficiency of some dietary nutrients such as vitamin B6, vitamin D, magnesium and calcium (Smolen et al., 1982). Many sound-generating systems, such as electric door bells and high-frequency sounds, can induce audiogenic seizures in mice (Dice, 1935). Sound between 90 and 120 decibels at frequencies between 10-20 KHZ is most effective for inducing audiogenic seizures (Alexander and Alexander, 1976; Schreiber, 1977).

Audiogenic seizures are characterized by the onset of an explosive burst of wild running, which develops into a clonic seizure where the rat falls onto its side. After that, the tonic seizure appears as all four legs are rigid and extended to the rear. Death will occur unless the animal is resuscitated by a relaxation of the pinnae (Buck et al., 1978). Seizures can be given a score based on the severity of the animal's response to the sound: 0= no seizure, 1= wild running, 2= clonic seizure and 3= tonic seizure (Schreiber and Schlesinger, 1972). Death is scored higher than tonic seizure (Willott and Henry, 1976). Buck et al. (1978) used the following scoring system for magnesium-deficient rats: 0= no seizure, 1= rapid running, 2= clonic, characterized by full body convulsions but with the animal usually on its feet, 3= tonic flexion, characterized by the animal on its side, rigid andkicking, 4= tonic extension, characterized by rigidity and extended legs and 5= lethal seizure.

Biochemical studies

Although there is a considerable evidence of biochemical differences involved with the manifestation of audiogenic seizures, the underlying mechanism responsible for audiogenic seizures has not been conclusively reported. Rosenblatt et al. (1976) and Davies and Peterson (1989) found that cation (Ca^{+2}, Mg^{+2}, K^{+}, and Na^{+}) imbalance induced convulsive disorders. The (Na^{+},K^{+})-ATPase activity was decreased in the brains

of audiogenic seizure-susceptible mouse strains (Fromby, 1975). Coleman (1960) found a positive relationship between audiogenic disorder and the deficiency of phenylalanine hydroxylase; decreasing the activity of this enzyme decreased the levels of serotonin and norepinephrine and enhanced seizure susceptibility. Schlesinger et al. (1975) also observed that a decreased concentration of brain serotonin and norepinephrine was associated with the induction of audiogenic seizures in genetically susceptible mice.

Epilepsy is a disease of cell excitability. The intracellular concentrations of Na^+ and K^+ ions are important in regulating cell-membrane excitability in nerve, muscle and secretory cells. A direct correlation between brain excitability and intracellular Na^+ ion has been known since 1955 (Woodbury, 1955). The inward current of Na^+ ions is overpowered by the outward current of K^+ ions. Seizures cause an exchange of Na^+ for K^+ in the brain-cells (Woodbury, 1955). The (Na+,K+)-ATPase enzyme is responsible for maintaining the inward sodium electrochemical gradient across cell membranes (Flatman, 1984), the resting potential of the all cell membranes. The (Na^+,K^+)-ATPase is activated by Na+ on the inside and by K^+ on the outside of the cell; consequently, insufficient activation of both glycolysis and cell-membrane ATPase by K^+ leads to seizures (Viukari, 1970). The three biochemical defects found in epileptic tissue are low potassium, acetylcholine and glutamate levels. Therefore, any defect of the (Na^+,K^+)-ATPase enzyme system leads to seizure susceptibility (Viukari, 1970).

It is now generally thought that many hormonal effects are mediated through the activation of membrane (Na^+,K^+)-ATPase (Robinson, 1972). In the central nervous system, (Na^+,K^+)-ATPase is known to be concentrated in synaptic membranes (Coan and Collingridge, 1985) where neurohormones are also found. Yoshimura (1973) showed that catecholamine (epinephrine, dopamine or serotonin) increases brain (Na^+,K^+)-ATPase activity, which transforms ATP to cyclic AMP; then c-AMP as well as potassium, promotes glycolysis; which activates phosphofructokinase. Low K+ levels have been

found in epileptic individuals; therefore, insufficient activation of both (Na^+,K^+)-ATPase and glycolysis may play a part in epilepsy (Viukari, 1970).

Norepinephrine and serotonin play important roles in the regulation of seizures. Neurotransmitter abnormalities have been implicated as the cause of seizure susceptibility in rats and humans (Jobe and Laird, 1981). Neurotransmitter defects associated with cholinergic, catecholaminoergic, serotonergic and amino-acid systems have also been reported in humans with epilepsy (Jobe and Laird, 1981). Jobe and Laird (1981) showed that an increase in norepinephrine levels had a marked anticonvulsant action; whereas elevation of dopamine did not. Ko et al. (1982) and Shaywitz et al. (1978) reported that some drugs increased brain norepinephrine and dopamine content; which was associated with reduced seizure intensity. Pharmacologic studies showed that elevation of catecholamine levels, especially dopamine, lower seizure intensity and susceptibility (Jobe and Laird, 1981). In contrast, reduction in brain serotonin by p-chlorophenylalanine caused an inhibition of audiogenic seizure in mice (Alexander and Kopeloff, 1977).

Coleman (1960) found a positive relationship between audiogenic seizures and deficient phenylalanine hydroxylase activity; a decrease in this enzyme could enhance seizure activity by lowering the concentration of the neurotransmitters serotonin and norepinephrine. Schlesinger et al. (1975) found a genetic relationship between decreased levels of serotonin and norepinephrine and increased seizure susceptibility in mice.

Other studies showed that taurine, gama-aminobutyric acid (GABA), glycine and aminoisobutyric acid cause a dose-dependent reduction in audiogenic seizure activity in genetically epilepsy-prone rats (Jobe and Laird, 1981; Laird et al., 1984). Laird and Huxtable (1976) found that injection of taurine directly into the coliculi (auditory nuclei) increased seizures in genetically epilepsy-prone rats but not in normal rats. Bonhaus et al. (1982) also found that taurine, with pyridoxine deficiency, induced seizures in rats; which they attributed to increased brain glutamate decarboxylase activity. Harriman (1988)

reported that seizure attacks in magnesium-deficiency states resembled those occurring in pyridoxine-deficiency.

Thus, there are many animal models of seizure disorder (Buck et al., 1978; Burnham, 1985; Chutkow, 1974; Fisher, 1989; Gaito, 1976), but until recently, magnesium-deficiency has received very little attention as a causative agent. However, it is unique as a metabolic model in that seizure susceptibility is completely eliminated by magnesium therapy (Anderson et al., 1986; Buck et al., 1978; Harriman, 1988; Jones and Heinemann, 1988; Leaver et al., 1987).

Objectives

The purpose of this study was to investigate the mechanism of fasting and ketogenic diet on reducing audiogenic seizure susceptibility of magnesium-deficient rats. The study design included the use of acute oral doses of ß-hydroxybutyrate (BHB), a ketone body and/or glucose, and varying the level of dietary medium-chain triglycerides, a ketogenic semi-synthetic fat, to determine their effect on serum BHB and glucose levels and audiogenic seizure susceptibility of magnesium-deficient rats.

CHAPTER II

LITERATURE REVIEW

Magnesium biochemistry

Magnesium is the fourth most abundant cation in the human body, exceeded only by potassium, sodium and calcium in extracellular fluid and by potassium in intracellular fluid (Agarwal and Agarwal, 1976; Rude and Singer, 1981; Wacker and Parisi, 1968). Magnesium is one of the most vital elements in the body and is involved in numerous biochemical reactions, activating about 300 enzymes involving cAMP, ATP and ADP. Moreover, alkaline phosphatase is an activator of alkaline phosphatase, acid phosphatase, ATPases and phosphatase (Aikawa, 1976; Pedemonte and Beauge, 1986). Magnesium plays a role in all ATP-requiring reactions and in stabilization of the structure of DNA, RNA and the ribosomal translation in gene expression (Aikawa, 1976). A lack of magnesium inhibits at least seven enzymatic reactions essential to the glycolytic pathway for carbohydrate metabolism (Aikawa, 1973). Magnesium is involved in the regulation of cellular permeability and neuromuscular excitability (Ebel and Gunther, 1980).

Magnesium is important in the sodium-dependent ADP-ATP exchange catalyzed by (Na^+, K^+)-ATPase; as well as for the overall hydrolysis of ATP in the presence of Na^+ and K^+ (Ghijsen et al., 1984; Pedemonte and Beauge, 1986). It has been suggested that the enzyme (Na^+, K^+)-ATPase increase in magnesium-deficient rats may reflect cellular action to increase excitability (due to the deficiency) by increasing activity of this enzyme (Buck et al., 1980). Also, (Na^+, K^+)-ATPase activity increases in experimental brain injury (Lim and Jacob, 1972), hypoxia (Stastny et al., 1971), electric shock and injection with pentylenetetrazol (Bignami et al., 1966). All of these lead to seizure susceptibility. In contrast, decreased (Na^+, K^+)-ATPase activity has been noted in uremia (Minkoff et al., 1972); which inhibits excitability. In addition, magnesium inhibits the release of the

brain synaptosomal fractions (Madeira and Antunes-Madeira, 1973; Rodrigues et al., 1988).

Distribution of magnesium in the organisms

Magnesium is the fourth most abundant cation in the body; second to potassium in its intracellular concentration; and participates in various biological reactions (Aikawa, 1971; Kantak, 1988). The human male, with a weight of 70 kg, contains 21-28 g of magnesium (Widdowson et al., 1951). Approximately one-half of the total body magnesium is in the bones, the remainder being almost equally distributed between nonmuscular soft tissues and the muscle (Flink, 1985). Normal serum magnesium values are 1.5-2.0 mEq/L (Rude and Singer, 1981). Walser (1967) showed that 20-30 percent of magnesium in blood is bound to protein; and the rest is diffusible as free ionized magnesium. The kidney has an important role in maintaining blood magnesium within the normal range by changing the tubular magnesium reabsorption through glomerular filtration of blood magnesium (Chesly and Tepper, 1958). Three to five percent of filtered magnesium is excreted in the urine, whereas the rest is reabsorbed (Dirks and Quamme, 1978), although in the magnesium-deficient state, the renal conservation of magnesium is increased (Shils, 1969).

Magnesium deficiency in man

Magnesium deficiency is defined as a reduction in total body magnesium content despite the absence of clinical symptoms of magnesium deficiency. There are many criteria to establish the subclinical deficient state: magnesium balance, muscle analysis, exchangeable magnesium determined by radioisotopes, retention of magnesium after acute parenteral administration, serum magnesium concentration and magnesium level in erythrocytes (Agarwal and Agarwal, 1976). Clinical identification of magnesium deficiency is dependent on the recognition of hypomagnesemia (Whang, 1984).

Magnesium deficiency symptoms in humans include neuromuscular dysfunction, behavioral disturbance and cardiovascular abnormalities, particularly muscular twitching and tremor of varying of any or all muscles; including the tongue. Convulsions occur in severe depletion and are the signal of magnesium deficiency in infants and young children (Bronner and Coburn, 1981; Flink, 1976; Rude and Singer, 1981).

Symptoms and biochemical features of magnesium deficiency have been studied experimentally by producing magnesium deficiency in humans (Shils, 1969) and in animal models (Buck et al., 1978). Magnesium deficiency in humans leads to neuromuscular hyperexcitability and disturbances (Flink, 1985). Patients may present with tetany, leading to generalized tonic-clonic seizures as well as focal seizures. Also, ataxia, muscular weakness, depression, irritability and psychotic behavior have been found. These symptoms are reversed by treating with magnesium (Goodman and Knobil, 1961). Tetany is the most distinctive symptom in hypomagnesemic patients who have normal serum electrolyte concentrations and normal acid-base balance (Vallee et al., 1973).

Most patients with hypomagnesemia have serum magnesium concentrations less than 1 mEq/L. Biochemical changes; in addition to hypomagnesemia; found in magnesium-depleted patients are hypokalemia, hypocalcemia and hyperphosphatemia due to either defective release of parathyroid hormone, resistance to the peripheral effects of the hormone or a combination of the two (Nesbakken and Reinlie, 1985; Whang, 1984; Whang et al., 1985). In contrast, Graber and Schulman (1986) reported that hypocalcemia caused by hypomagnesemia is independent of the effects of parathyroid hormone in patients. Clinical etiology of magnesium deficiency as reported by Flink (1985) include the following descriptions. **Gastrointestinal disorder.** Magnesium deficiency may occur with malabsorption syndrome (Agarwal and Agarwal, 1976; Whang, 1984), prolonged diarrhea, pancreatitis (Edmondson et al., 1952) and ulcerative colitis (Booth et al., 1963). **Alcoholism.** Magnesium deficiency may occur in chronic alcoholic patients. It was found that significant hypomagnesemia, positive magnesium balance during recovery,

significant decrease in muscle magnesium, depletion of total exchangeable magnesium and diuresis of magnesium were produced by ingestion of alcohol (Flink, 1985). **Cirrhosis of the liver.** Hypomagnesemia is found in cirrhotic patients due to malnutrition, damage to the liver or constant magnesium loss due to alcohol ingestion (Sullivan et al., 1969). **Fluid loss.** Magnesium deficiency developed due to prolonged and severe loss of body fluid during surgery with multiple drainage of patients (Barnes, 1969). **Diabetes.** A urinary magnesium excretion of more than 35 mEq/day in diabetes mellitus patient leads to hypomagnesemia (Martin, 1969). **Bone diseases.** Hypomagnesemia was seen in osteolytic bone diseases and also in hypoparathyroidism due to the transfer of magnesium into soft tissues or bone since urinary excretion did not change (Eliel et al., 1969; Ford et al., 1989). **Kidney disfunction.** Hypomagnesemia occurs in certain kidney diseases (Smith and Hammersten, 1958). Hanna and ManIntyre (1957) found that aldosterone increases urinary and fecal magnesium excretion. **Drugs.** Gentamycin, viomycin, capreomycin and sodium sulfate infusion causes hypomagnesemia (Bar et al., 1975). Diuretics, especially mercurial and thiazide; appear to cause both potassium and magnesium depletion by increasing urinary excretion (Hollifield, 1984; Lim and Jacob, 1972; Whang, 1984).

Magnesium deficiency in animals

Magnesium deficiency has been observed in sheep and cattle in which the syndrome develops spontaneously. Two types of magnesium deficiency occur in cattle: (1) It develops in animals raised exclusively on whole milk (Allcroft, 1956) and (2) it develops in an endemic disease known as grass staggers or grass tetany (Aikawa, 1971; Sjollema, 1932; Wacker and Parisi, 1968). Magnesium defeciency has been induced in weanling rats on a diet containing .09 mEq Mg^{++}/kg; leading to hyperemia with vasodilatation and a subsequent heightening of nervous irritability and then to generalized and sometimes fatal seizures (Kruse et al., 1932). Rats fed a low diet level (1.8 ppm magnesium) develop

acute hyperemia of the skin, loss of hair and convulsions; fatalities may occur within 10-12 days. The first phase of deficiency, lasting about 2 weeks, is characterized by vasodilation (reddening of the ears and feet), skin lesions, hyperemia and hyperexcitability. The second phase is marked by signs of malnutrition and renal damage (Aikawa, 1973). During the final stage of acute magnesium deficiency, a massive quantitative release of three catecholamine, epinephrine, norepinephrine, and serotonin occurs in rats which causes an extremely rapid onset and development of pulmonary edema and leads to the pathogenesis of the seizure-shock syndrome (Caddell et al., 1986; Weiner, 1985). These symptoms almost invariably culminate in death caused by a tonic seizure. A very similar effect was observed in dogs (Orent et al., 1932), and rabbits (Bradbury et al., 1968) fed low-magnesium diets.

In hypomagnesemia, magnesium concentration in the cerebrospinal fluid (CSF) is greater than magnesium in plasma ultrafiltrate (Chutkow, 1971). Moreover, magnesium infused intravenously increases plasma magnesium level, but CSF magnesium tends to remain stable (Buck et al., 1978; Leaver et al., 1987). Oppelt et al. (1963) found that magnesium is transported from blood to CSF by an active transport mechanism, and the removal of magnesium from CSF to blood occurs by diffusion and bulk filtration. However, there is a direct relation between serum magnesium and CSF magnesium level (Oppelt et al., 1963). Magnesium deficiency affects losses of magnesium from the central nervous system in rats shortly after severe hypomagnesemia (Buck et al., 1976; Chutkow, 1974). There is a high correlation between plasma and cerebrospinal-fluid calcium and magnesium concentrations; indicating that convulsions occurred when cerebrospinal fluid magnesium and plasma calcium concentrations decreased (Leaver et al., 1987).

Magnesium deficiency in animals restricted to a diet containing only 2 ppm of magnesium manifests itself with vasodilation and a subsequent heightening of nervous irritability; which terminates invariably in a convulsive seizure (Kruse et al., 1932; Buck et al., 1978). Other phenomena, including nutritive failure and chemical changes in the

blood, bone and mineral excretion, likewise ensue (Kruse et al., 1934; Orent et al., 1932), but it is the involvement of the nervous system, the so-called local effect, that gives a certain specifity to magnesium deficiency. Cockburn et al. (1973) reported that neonatal convulsions may result from a variety of causes; including primary disturbances of mineral metabolism. A well-established relationship exists between magnesium status and excitability in mammals (Allsop and Pauli 1985; Leaver et al., 1987; Wacker and Parisi, 1968). Buck et al. (1976), working with young rats, reported that they became hyperactive within a few days when fed a low-magnesium diet and, shortly thereafter, became highly susceptible to audiogenic seizures.

Magnesium deficiency also affects other electrolytes and metabolites. Magnesium depletion caused hypocalcemia by direct action on bone due to its effect on calcium absorption (Graber and Schulman, 1986). Dietary magnesium depletion in rats is accompanied by muscle potassium depletion (Whang et al., 1985). Concurrent hypomagnesemia among hypokalemic patients has been estimated to be between 38 percent and 42 percent (Boyd et al., 1983; Whang, 1984; Whang et al., 1985). A similar result was found between the magnesium and calcium in the extracellular and intracellular fluids. Wacker and Parisi (1968) also found that greater intracellular $(Mg^{++})/(Ca^{++})$ and $(K^{+})/(Na^{+})$ ratios accompany a higher metabolic activity of cells. Calcium, sodium, potassium and total and ultrafiltrable magnesium were decreased, while brain, muscle and CSF retained normal concentrations of magnesium; slight changes occurred in brain Na and K. Magnesium depletion in animals results in a decrease of potassium and increased sodium levels in muscle and heart (Whang et al., 1985).

Magnesium homeostasis

The homeostasis of magnesium is dependent on intake, absorption and excretion. Daily intakes among individuals varied greatly from 132 to 350 mg for women and from 157 to 595 mg for men (Lakshmanan et al., 1984). Absorption of magnesium from the gut

and its excretion by the kidneys are the main factors determining serum magnesium levels (Massry and Coburn, 1973).

Magnesium reabsorption in the nephron is affected by a number of physiologic and metabolic factors; as well as by drugs and disease states. Hypermagnesemia, hypercalciuria, hyperthyroidism, alcoholism, diuresis, nephrotoxic drugs and acidosis increase excretion of magnesium (Hulter and Paterson, 1984; Meyer, 1989). In contrast, hypomagnesemia, hypocalcemia and alkalosis decrease magnesium excretion (Ford et al., 1989; Graber and Schulman, 1986). In addition, a number of hormones affect magnesium reabsorption by the kidney. Parathyroid hormone (PTH) participates in the regulation of magnesium homeostasis. Excesses of parathyroid hormone cause negative magnesium balance by increasing urinary excretion and decreasing gastrointestinal absorption. Serum magnesium levels decrease; but cellular magnesium concentration is unchanged due to ancillary protective mechanisms (Hulter and Paterson, 1984). Nonetheless, hypomagnesemia leads to inhibition of thyroid hormone synthesis by reducing the efficiency of the iodine-trapping mechanism. Hence, excess production of thyroid hormone is moderated, causing in turn better magnesium balance and less hypomagnesemia (King and Stanbury, 1970). Calcitonin can reduce magnesium excretion by increasing loop reabsorption (Marone and Sutton, 1983). However, the administration of calcitonin in humans results in increased or an unchanged urinary magnesium excretion (Cockburn et al., 1973). Also, thyroid hormone and aldosterone are involved in magnesium homeostasis; with an increase in these hormones, intestinal magnesium absorption is decreased and urinary magnesium excretion is increased (Wacker and Parisi, 1968; Wallach, 1976). Magnesium metabolism is also affected by adrenocorticoids due to their ability to expand fluid volume; causing hypermagnesemia. Serum magnesium decreases, leading to impaired adenylate cyclase activation by acetylcholine due to decreased adrenocortical secretion rates which in turn improves magnesium balance (Wallach, 1976). Growth hormone, insulin and glucagon in hypoglycemia usually cause hypomagnesemia

by stimulating cellular influx of magnesium. Increased cell-magnesium concentration in islet beta cells leads to displacement of cell calcium and a reduction of calcium secretion. The consequent decrease in insulin secretion leads to normal serum and cell magnesium concentrations (Wallach, 1976).

Magnesium and calcium share the same transport systems in the gut and kidneys (Massry and Coburn, 1973) and are affected by parathyroid hormone (PTH) and vitamin D. Since magnesium and calcium share a common transport system in the kidney, the increased filtered load of calcium inhibits the tubular reabsorption of magnesium, which, in turn, overcomes any direct effect of parathyroid hormone on the tubular handling of magnesium (Wilkinson, 1976).

The intestinal and renal conservation and excretion mechanisms in normal subjects permit homeostasis over a wide range of dietary magnesium intake. When magnesium intake is low in normal humans, magnesium output becomes very small (Shils, 1969). Supplementing the normal magnesium intake increases urinary excretion without changing normal serum levels (Heaton, 1969).

Carbohydrate metabolism and magnesium

Insulin plays an important role in the development of the hypertriglyceridemia associated with magnesium deficiency (Bennett et al., 1969). Magnesium affects insulin secretion by changing the sensitivity of the beta cells of the pancreatic islets to glucose (Curry et al., 1977). McNeill et al. (1982) confirmed these results; showing that increased phosphoenolpyruvate carboxykinase activity, due to magnesium deficiency, leads to increased hepatic glucose production so blood glucose level also increases and insulin levels are low.

Legrand et al. (1987) found a complex interplay between serum magnesium and glucose metabolism; chronic magnesium deficiency alters several parameters of glucose homeostasis in the rat by exhibiting a poor insulin response to glucose. Glucose

metabolism may affect magnesium status of the body; diabetes mellitus is frequently associated with hypomagnesemia (Mather et al., 1979). Decreased serum magnesium concentration is inversely related to the quality of blood glucose control in individuals with diabetes (Mather et al., 1979; Yajnik et al., 1984). Hypomagnesemia mostly results from increasing excretion of magnesium in urine and a substantial depletion of body magnesium stores. Lostroh and Krahl (1972) found that an insulin dose causes a net increase in the accumulation of magnesium and potassium in the retrogressed uterus and that a functioning ion pump is one requisite for this action of insulin.

On the other hand, magnesium may also influence glucose homeostasis. Moles and McMullen (1982) reported that hypomagnesemia could cause insulin resistance during treatment of diabetic ketoacidosis. In in vitro studies, magnesium plays an important role in the action of insulin, and magnesium was also involved in the regulation of the beta-cell functions due to the inhibition of insulin release and biosynthesis (Lostroh and Krahl, 1972). Lowering extracellular magnesium inhibits insulin secretion and stimulates insulin biosynthesis by isolated rat islets (Lin and Haist, 1973). Curry et al. (1977) reported that Ca^{++}/Mg^{++} ratio in the serum is a primary regulator of the insulin secretory process, since a relatively slight alteration of the physiologic ratio of Ca^{++}/Mg^{++} (approximately 2.5) results in a marked inhibition of total insulin secretion. This dependency on magnesium may reflect the fact that magnesium is required for many processes, including enzyme reactions catalyzed by ATP (Rubin, 1975; Wacker and Parisi, 1968). Magnesium dependency may also be related to the importance of magnesium for increasing hormone-receptor affinity, as in posterior pituitary hormones and vascular smooth muscle (Somlyo et al., 1966).

Cerebrospinal fluid-magnesium

Rat brain mitochondria phosphotransferases, such as creatine kinase and adenylate kinase, are activated by magnesium (Sugano et al., 1972). Magnesium and potassium exert a direct stimulatory effect on the penetration of substrates into brain mitochondria in

various metabolic states (Sugano et al., 1972). Magnesium is essential for membrane integrity, ATPase functions and the synthesis of co-factors such as coenzyme-A (CoA) and thiamine pyrophosphate (Aikawa, 1981). Magnesium also plays an important regulatory role in the gating of excitatory amino-acid neurotransmitter channels within the brain (Nowak et al., 1984). Excitatory amino-acid neurotransmitters; such as glutamate and aspartate; participate in tissue damage caused by cerebral ischemia and brain injury (Choi, 1987; Rothman and Olney, 1986). Altura et al. (1984) reported that magnesium ion plays an important role in the regulation of cerebral vascular tone by inducing a rapid; spontaneous vascular tone in cerebral arteries and veins of different species (rats, rabbits, dogs, pigs and human) with the lowering magnesium content of cerebral blood vessels.

Magnesium concentration in cerebrospinal fluid (CSF) is higher than in serum, and this level remains stable in case of changing plasma concentration (Cohen, 1971). Magnesium moves rapidly from plasma into the ventricular fluid against an electrochemical gradient by active transport across the epithelial cell of the choroid plexus (Flatman, 1984; Oppelt et al., 1963). Direct influx of magnesium into a normal brain through the cerebral capillary endothelial blood-brain barrier is slow (Ginsburg, 1973). However, magnesium may be transported actively across the blood-brain barrier into the cerebral cortex to maintain a higher magnesium concentration in the cortical interstitial fluid than in the ventricular fluid (Bito, 1969). The choroid plexus is involved in maintaining the constancy of the cerebrospinal fluid magnesium concentration by sensing changes in the normal cerebrospinal fluid magnesium concentration and altering its rate of active secretion of magnesium (Bradbury and Sarna, 1977; Reed and Yen, 1978). Magnesium deficiency due to a low-magnesium diet appears first in serum, followed by a decrease in CSF concentration (Allsop and Pauli, 1985; Buck et al., 1976; Chutkow, 1974; Fisher et al., 1985). Tufts and Greenberg (1938) found a reduction of about two-thirds in whole-body magnesium in weanling rats, whereas only 18 percent reduction in CSF magnesium occurred due to a magnesium-deficient diet. Other investigators showed a sharp decrease

in serum magnesium, while little change occurred in the CSF or whole brain due to a low-magnesium diet (Bradbury et al., 1968). Chutkow (1974) observed that the maximal degree of magnesium loss is 10 percent smaller in whole-brain than in muscle, plasma or serum due to a magnesium-deficient diet. This loss was accompanied by an increase in nervous system hyperexcitability and seizures in rats. Buck, et al. (1976 and 1979) confirmed these observations.

Nerve impulse conduction

In nerve conduction, magnesium and calcium stabilize the transmission of axonal impulses. Calcium and magnesium function in a parallel manner in this process of synaptic transmission. Calcium is required for synaptic transmission because it plays a permissive role in acetylcholine secretion at the neuromuscular junction. Magnesium antagonizes the Ca^{++}- dependent release of acetylcholine at the motor endplate. There is a competition between calcium stimulation and magnesium inhibition of acetylcholine release. A similar antagonism exists between calcium and magnesium in the mammalian brain. In the brain, a high magnesium concentration appears to promote acetylcholine synthesis and inhibit its release (Wacker, 1980). In contrast to the antagonistic roles in the conduction of nerve impulses and normal muscle contraction, the effect of decreases in magnesium and calcium concentrations leads to enhanced neural excitability by decreasing membrane surface charge screening and thereby to enhanced activation of inward currents (Mayer et al., 1984; Mody et al., 1987). Changes in the concentration of Ca/Mg influence release of certain neurotransmitters. Gama-amino-butyric acid (GABA) and acetylcholine (Ach) are released in the superfusate of retinas during spreading-depression (SD). Decreasing calcium concentration from 1 to 0.5 mM and simultaneously increasing magnesium concentration from 1 to 2 or 4 mM led to decreased GABA and Ach release during SD (Rodrigues et al., 1988).

Extracellular calcium decreases during epileptic activity in vivo caused by electrical convulsive shock. In neurones, a massive influx of calcium through either voltage-dependent L-channels or receptor-operated channels (NMDA channel) is associated with the paroxysmal depolarization. A large increase in soma calcium concentrations could have a spillover effect with increases in presynaptic calcium. Calcium influx would then bind with calmodulin; leading to exocytosis and recruitment of additional neurones. Excitatory neurotransmitters would facilitate calcium entry through receptor-operated channels and potentially propagate seizure discharge (Meyer, 1989). Therefore, a massive entry of calcium into neurons occurs during seizure activity is thought to have several deleterious concequences including: (1) Enhanced activation of phospholipase A_2 attacking cellular membranes and neurofilaments liberating free fatty acids and altering permeability and cell function, the free fatty acids can be the source of free radicals; (2) risied intracellular calcium, mitochondria will accumulate calcium which will uncouple oxidative phosphorylation at a time when ATP production is reduced due to anaerobic metabolism; (3) altered receptor function, binding to the receptor would directly gate an inward calcium current leading to either initiation and/or propagation of an epileptic discharge; (4) facilitate exocytosis of punitive neurotransmitters, glutamate and aspartate led to neuronal destruction through the influx of calcium.

Magnesium concentrations have often appeared to influence the transmission of impulses across nerve membranes, and magnesium has been implicated in the (Na^+,K^+)-ATP-ase enzyme system that contributes to changes in cation potential that occur in nerve membranes during depolarization (Abood, 1966). The (Na^+,K^+)-ATPase (Na^+,K^+ pump); an enzyme located in the plasma membrane of eukaryotic cells; actively transports Na^+ and K^+ across the membrane (Askari, 1982; Skou, 1965). Sodium and potassium are actively transported in a large number of cell types, including those of the nervous system (Albers, 1967). Sodium is transported from the cytoplasm to the interstitial fluid against an electrochemical gradient by active transport (Ussing, 1960). This active transport requires

energy; which comes from ATP (Hoffman, 1960). As the concentrations of either Na^+ or K^+ are increased (the other being held constant); ATPase activity increases (Buck et al., 1979; Pedemonte and Beauge, 1986). (Na^+, K^+)-ATPase has been characterized in rat brain microsomal fractions; and its activity is stimulated when sodium, potassium and magnesium ions are present but is inhibited by the presence of calcium ion (Kauppinen et al., 1986; Rohani et al., 1982). (Na^+,K^+)-ATPase is also localized in the somatosensory cortex; especially fibers (axons and dendrites) of the rat brain (Stahl and Broderson, 1976).

There is a high association between magnesium-deficiency and (Na^+,K^+)-ATPase activity. That (Na^+,K^+)-ATPase activity is decreased in magnesium-deficient rats may reflect a cellular action to counter the increased excitability due to the magnesium deficiency by increased (Na^+,K^+)-ATPase activity (Buck et al., 1976; Chutkow, 1974).

The (Na^+,K^+)-ATPase has been widely implicated as the enzyme involved in the active transport of cations across cell membranes that maintain resting potentials in nervous tissue (Skou, 1965) due to the conversion of chemical energy into electrical potential by ionic movement. A direct correlation has been reported between excitability and intracellular sodium; epileptic seizures result when intracellular sodium levels increase and when potassium levels in the cerebrospinal-fluid decease (Viukari, 1979). The activity of (Na^+,K^+)-ATPase, which maintains intracellular Na^+ at a low level, intracellular K^+ at high levels and resting membrane potentials in nervous tissue, depends on many factors; such as concentrations of ATP, magnesium, sodium, potassium, calcium and inorganic phosphate concentrations (Reuss et al., 1984; Whang, 1984). Wacker and Parisi (1968) found that magnesium depletion causes a slowing of the pumping mechanism that maintains normal intracellular sodium and potassium gradients. Therefore, an abnormal flux of monovalent cations may be related to the epileptogenic process in humans. Such an abnormality may be linked to an alteration in the activity of (Na^+,K^+)-ATPase (Rapport et al., 1975). A reduced (Na^+,K^+)-ATPase activity in human epilepsy has been reported (Rapport et al., 1975). Also, it was found that (Na^+,K^+)-ATPase activity decreased in experimental brain

injury (Lim and Jacob, 1972), from hypoxia (Stastny et al., 1971) and from injection with pentylenetetrazol (Bignami et al., 1966). Enzyme activity was completely inhibited by ouabain, which also blocks active sodium and potassium transport (Donaldson et al., 1971; Swanson and Stahl, 1972). It was found that Zn^{+2} and Cu^{+2} inhibit (Na^{+},K^{+})-ATPase activity and causes seizures in rats (Donaldson, et al., 1972). All of these conditions lead to increased seizure susceptibility.

N-methyl-D-aspartate (NMDA) is a synthetic analogue of aspartic acid that selectively activates a unique receptor on vertebrate neurones. The NMDA response is greatly potentiated by reducing the extracellular magnesium ion concentration below the physiological level (~1 mM) (Ault et al., 1980; Coan and Collingridge, 1985). In magnesium-free solutions, NMDA opens cation channels, especially those for Na+ and K+. Furthermore, the voltage dependence of the conductance does not result from a direct effect of the membrane potential on the receptor-channel complex but rather is the consequence of a voltage-dependent Mg^{++} blockade (Ascher and Nowak, 1988; Hegstad et al., 1989; Mody et al., 1987; Nowak et al., 1984; Stanton et al., 1986). Avoli et al. (1987) indicated that NMDA conductances are important for the genesis of seizure-like discharges in human epileptogenic neocortical slices. Lowering extracellular magnesium concentration to zero induced spontaneous epileptiform activity in the mature nervous system that consisted of bursts of low-amplitude isolated discharges lasting 50-90 sec, recurring every 90-300 sec. Application of the NMDA receptor antagonist DL-2-amino-7 phosphonoheptanoic acid caused a rapid, reversible suppression of epileptiform activity (Hegstad et al., 1989; Jones and Heinemann, 1988).

A well-established relationship exists between neurotransmitter abnormalities and seizure susceptibility (McNamara, 1984; Poenara et al., 1984). Several neurotransmitters contribute to this. One group of those neurotransmitters; secreted and stored in the adrenal medulla; is the catecholamines, which play an important role in increasing and decreasing

blood pressure, exciting the nervous system and increasing cardiac output (Classen, 1981; Gorbman et al., 1983; Kutsky, 1981).

Catecholamines, norepinephrine, epinephrine and dopamine, increase serum magnesium concentration in rats (Bronner and Coburn, 1981). Norepinephrine has been found to enhance brain (Na^+,K^+)-ATPase activity (Yoshimura, 1973) or have no effect on the activity of the enzyme (Logan and O'Donovan, 1976). Gorbman et al. (1983) reported that catecholamine retention in adrenal medulla requires ATP, magnesium and ATPase. Magnesium ion inhibits release of the catecholamines; which calcium ion enhances their release (Gorbman et al., 1983). Catecholamine depletion, especially norepinephrine; induces seizure susceptibility in rats (Burley and Ferrendelli, 1984; Woodbury, 1984).

Dopamine increases hyperexcitability in animals, perhaps by increasing (Na^+,K^+)-ATPase activity (Yoshimura, 1973). Dopamine concentration has been reported to increase in a magnesium-deficient rat brain (Poenaru et al., 1984). Gunther and Ising (1979) found that urinary excretion of catecholamine was increased when rats were exposed to a combination of magnesium-deficient diet and noise.

Serotonin (5-hydroxytryptamine) is a monoamine of the brain associated with convulsions and hyperexcitability when depleted (Pycock et al., 1978). Serotonin contributes to ATPase activation in the cerebral cortex by increasing (Na^+,K^+)-ATPase activity (Yoshimura, 1973). Decreasing serotonin synthesis increases seizure susceptibility (Browning, 1985; Laird et al., 1984). Serotonin concentration is higher in the brain of magnesium-deficient rats that are susceptible to audiogenic seizures (Buck et al., 1979). Chaistitwanich et al. (1987) reported that magnesium-deficient rats show increased serotonin and dopamine concentrations in the cerebral cortex, cerebellum, medulla oblongata and pons. However, Chutkow (1980) reported no change in brain serotonin, but the serum serotonin concentration increases in magnesium-deficient rats.

Fasting

Over 70 years ago, Benedict (1915) published data on the metabolism of fasting in humans in which he demonstrated that carbohydrate stores provide a small but significant component of the body's fuel for only the first few days. Thereafter, protein and fat are the sole sources of fuel, the former contributing 15 percent of the calories and the latter the balance. It was readily apparent that glucose has to be derived from protein to maintain cerebral metabolism during a prolonged fast. More recently, understanding of the fasted state has been further clarified by the demonstration that free fatty acids are both the major transport form of lipid-leaving adipose tissue (Dole, 1956) and the energy substrate that is readily utilized by liver, muscle and many other tissues (Gordon and Cherkes, 1956). McIlwain (1959) also found tissues preferentially use carbohydrate for energy over fat and protein. The quantity of carbohydrate stored can supply the required energy for the body to function for about one-half day; thereafter, protein and fat are the primary sources of fuel.

Fasting for five days caused a decrease in body weight, reduction in metabolic rate by 26 percent, quantitative reduction in energy expenditure due to energy conservation, a reduction in cardiac output and a decreased blood flow through the heart, portal-drained viscera, white adipose tissue, leg muscle, hindquarters and in the whole-body muscle (Ma and Foster, 1986). In response to food deprivation; protein synthesis in skeletal muscle decreases and the muscle protein degradation rate decreases (Li and Goldberg, 1976). Longer periods of fasting diminish the muscle RNA levels and inhibit protein synthesis (Li et al., 1979).

Starvation is characterized by an increased availability of free fatty acids (FFA) for cellular oxidative metabolism and a decrease in their esterification rate. Also, starvation blocks both the glucose-induced stimulation and inhibition of insulin and somatostatin but does not affect glucagon secretion (Rodriguez et al., 1984). Mobilization of fatty acids is increased by the action of the pituitary gland and the adrenal cortex hormones in fasting animals (Cahill et al., 1966; Fain, 1962; Goodman and Knobil, 1961).

Dumbauld et al. (1983) studied carbohydrate metabolism during a 72-h fast in 11 nondiabetic; endstage renal disease (ESRD) patients on chronic hemodialysis and six normal subjects. Serum potassium concentration was significantly higher in the ESRD patients; and two patients were removed before completion of the fast when severe hyperkalemia developed. Mean blood glucose, alanine, pyruvate, beta-hydroxybutyrate and serum insulin concentration were similar in the two groups. Mean blood acetoacetate and plasma free fatty acids (FFA) concentration were lower in the ESRD group.

Rodriguez et al. (1984) found that a 48-h fast blocked the glucose-specific effect on islet secretion and inhibited insulin and somatostatin secretion due to the insulin secretory defect; as previously reported by Boden et al. (1981). Starvation increases the secretion rate of glucagon, epinephrine and norepinephrine; which facilitates release of glucose from the liver, thereby contributing to the maintenance of the blood glucose concentration above the lower limit of the physiological range. The sympathetic nerves stimulate release of epinephrine and norepinephrine by adrenal medulla, resulting in release of glucose from the liver (Niijima, 1986).

Starvation for 72h affects the hormonal response of pancreatic islets in vitro by inhibiting insulin and somatostatin secretion without changing glucagon release (Efendic et al., 1976). Starvation has no effect on the time kinetics of the insulin response (Efendic et al., 1976). Bouman et al. (1979) reported that fasting for 24 and 72h caused inhibition of glucose-induced insulin secretion; both in vivo and in vitro. Also, increasing duration of fasting more than 72h delayed and inhibited the c-AMP response to glucose. Crespin et al. (1973) demonstrated that the insulin secretory response to glucose is already strongly impaired after 24h of food deprivation.

The pituitary, adrenal and thyroid glands, while not initiating FFA mobilization during fasting, nevertheless, modify the response; and optimal function of these glands is requisite for normal FFA mobilization due to food deprivation (Goodman and Knobil, 1961). Tegelman et al. (1986) reported that growth hormone secretion increases during

fasting. Mlekusch et al. (1975) reported an increased FFA level in rats during a 12-h fast due to a decrease in reesterification of FFA in the fat cell caused by lack of glycerol-3-phosphate.

Magnesium depletion in humans has been studied in association with various chronic illnesses (Flink, 1976; Flink, 1985). Magnesium depletion is induced experimentally in healthy subjects and in animals by using a magnesium-deficient diet (Drenick et al., 1969; Kruse et al., 1932). Subjects that fast for a long time excrete sizable quantities of magnesium and other minerals (Elia et al., 1984). An 18 days fast undertaken by 19 obese male subjects resulted in no change in plasma magnesium concentration, while urinary magnesium loss and muscular magnesium continued to be depleted (Drenick, 1964). Drenick et al. (1969) found that fasting 60d induces magnesium depletion, and the deficit may reach 20 percent of the total body magnesium content. Most of the magnesium loss occurs via the kidney; but in some subjects the intestine functions as an additional excretory organ for magnesium throughout fasting.

Fasting affects mineral metabolism by increasing magnesium and potassium excretion (Sapir et al., 1976; Stewart and Fleming, 1973). Elia et al. (1984) reported that plasma and urine calcium, magnesium, phosphate and sulphate were unchanged during four days of total starvation in five healthy non-obese young adults. Francesconi and Hubbard (1985) observed that food deprivation for 24, 48 or 72h and exercise (9.14 m/min) in the heat (35.5 ^{o}C) causes hypoglycemia in the rats. Those decrements in glucose concentrations were accompanied by decreases in circulating insulin values. Also, prolonged fasting, for 48 and 72h, caused significant hypertriglyceridaemia and hyperlactic acidemia. Sodium and potassium levels were unchanged.

Rapport et al. (1965) reported that plasma potassium concentration decreased during fasting. Also, Stewart and Fleming (1973) observed decreased erythrocyte potassium concentrations during 18 days of fasting in male patients who had a decreased plasma magnesium, but potassium did not change in patients given magnesium

supplements. Serum potassium concentration was higher in endstage renal disease patients on chronic hemodialysis during a 72-h fast (Dumbauld et al., 1983).

A strong relationship ($r= 0.69$, $p<0.001$) was found between degree of insulin resistance and fasting insulin levels in normal and diabetic subjects (Olefsky et al., 1973). When a known cause of insulin resistance is present, such as acromegaly, uremia, corticoid treatment, estrogen injection or lipodystrophy, it possibly will account for the correlation. Newman and Brodows (1983) showed that fasting markedly reduced glucose utilization at all insulin infusion rates, due to the affect of starvation which may alter the onset of insulin action or delys insulin delivery to its site of action.

A complex relationship exists between magnesium and glucose metabolism. Hypomagnesemia has been found in both insulin-dependent and noninsulin-dependent diabetic patients (Mather et al., 1979; Yajnik et al., 1984). Yajnik et al. (1984) found that fasting plasma magnesium was lowest in the insulin-treated diabetic group (mean, 2.0 mg/100ml), intermediate in the nondiabetic (2.2 mg/100ml) and highest in the noninsulin-treated diabetics (2.3 mg/100 ml). In all diabetic subjects, plasma magnesium concentrations were inversely related to plasma glucose values and in noninsulin-treated patients to plasma insulin concentration. Hypomagnesemia could cause the insulin resistance of diabetic ketoacidosis (Moles and McMullen, 1982). As mentioned earlier, magnesium has an important role in the action of insulin (Lostroh and Krahl, 1972). Magnesium is also involved in insulin secretion via regulation of the beta-cell function (Curry et al., 1977). Legrand et al. (1987) concluded that, in magnesium-deficient rats, magnesium exerts complex effects on glucose homeostasis, with magnesium deficiency decreasing pancreatic insulin store and secretion of insulin.

Starvation and diabetes lead to a catabolic state. Free fatty acids (FFA), acetoacetate and ß-hydroxybutyrate, compete as fuels for muscle metabolism. During prolonged starvation in man, free fatty acids displaced ketone bodies as the preferred fuels for oxidation in striated muscle (Owen and Reichard, 1971). Owen et al. (1973) concluded

that; during starvation or diabetic ketoacidosis; the ketone bodies concentration in blood exceeds those of FFA. Cahill et al. (1966) demonstrated the predominance of lipid as fuel during fasting and emphasized the lowering of glucose metabolism particularly by the brain in humans who survive prolonged starvation and spare protein as gluconeogenesis decreases. In addition, blood-brain concentration differentials for ketone bodies exceed those for free fatty acids; Hawkins et al. (1971) suggested that relative impermeability for these substrates into other tissues may be a factor limiting rate of their metabolism. Plasma ketone bodies, ß-Hydroxybutyrate and acetoacetate, were highest, and plasma glucose concentration was lowest after 48h of starvation (Owen et al., 1973). Fasting experiments have demonstrated the tendency to develop hypomagnesemia and the rapid production and utilization of ketones to spare glucose in young children (Lacers et al., 1985; Lacers et al., 1987; Nosadini et al., 1980; Saudubray et al., 1981).

A theory has evolved that during starvation, the production of ketone leads to stimulation of the secretion of insulin; which in turn acts to modulate the release of free fatty acids from adipose tissue (Madison et al., 1964). Jenkins (1967) discussed this theory and found that in rats, the ketone bodies stimulate insulin secretion and, after a period of time, cause diabetes on daily administration of sodium acetoacetate. Injestion of sodium acetoacetate lowered the fasting plasma free fatty acids and blood glucose in healthy humans.

Milk fat provides two metabolites, acetoacetate and ß-hydroxybutyrate. Edmond et al. (1985) support the concept that the developing brain prefers ketone bodies as a substrate for the supply of carbon for respiration and lipogenesis. The developing brain enhances enzymatic potential to use ketone bodies in the mitochondrial compartment to support respiration and in the cytosolic compartment to support lipogenesis. Aging is associated with increased susceptibility to fasting-induced hyperketonemia after 18h of fasting in humans (London et al., 1986). Intravenous infusion of sodium acetoacetate or by a 48-h fast shown that ketone bodies are major metabolic fuels for the brain of the suckling rat

under normal conditions (Hawkin et al., 1971). Thus, in the brain of suckling rats ketone bodies appeare to be as important as glucose as a source of metabolic fuel.

Ketogenic diet

Many earlier investigations showed which starvation and metabolic acidosis reduced convulsions in epileptic children. A ketogenic diet which produces metabolic acidosis reduces the incidence of seizures in epileptic children (Geyelin, 1971; Peterman, 1946; Wilder, 1921).

A ketogenic diet was introduced as a form of antileptic treatment by Wilder (1921). Ketogenic diets have been effective in the treatment of akinetic or myoclonic seizures and petit mal epilepsy (Dodson et al., 1976; Huttenlocher et al., 1971). This diet is most effective in 2-5 year-old children (Menkes, 1976). Ketogenic diets have anticonvulsant value, and in addition, mental function is not dulled as with the use of drugs (Talbot et al., 1926). McQuarrie and Keith (1957) reported that ketogenic diets forestalled mental deterioration and structural damage to brain by preventing seizures during the growth period. Berman (1975) confirmed this, and in addition, found that children became more alert, steady and sociable when their seizures were controlled by the diet. This was also one of Livingston's (1975) findings.

Ketogenic diets prevent electroshock seizures in animals (Appleton and DeVivo, 1974; Uhlemann and Neims, 1972). In children, the anticonvulsant effects of a ketogenic diet do not appear for a few days; even though blood ketone concentrations increase rapidly. Withrow (1980) concluded that the anticonvulsant effect of a ketogenic diet is associated with how blood ketone bodies are involved in brain energy metabolism and is not the result of water, electrolyte, acid-base or lipid fluctuations in the brain. Ingestion of excess glucose, which blocks ketone production; can rapidly negate the anticonvulsant effect of the diet (McQuarrie and Keith, 1957). Also, Talbot et al. (1926) and Hughes and Jabbour (1958) found that if an excess of any kind of carbohydrate is taken by the epileptic

patient, the ketosis disappears and seizure attacks recur. Edmond et al. (1985) demonstrated that ketone bodies, acetoacetate and ß-hydroxybutyrate are preferred substrates for the supply of carbon to respiration and lipogenesis in the developing brain of rats. Astrocytes oligodendrocytes and neurons from developing brain of rats have an excellent ability to use ketone bodies for respiration.

Convulsive attacks stop in children (ages 2-12 years) after a 24-h fast (Menkes, 1976; Mike, 1965). In epileptic children treated by fasting and ketogenic diet, there is an increase in the production of ketone bodies concentration and a decrease in the blood sugar concentration (Talbot et al., 1926), which are associated with a diminution or cessation of convulsion.

The use of medium-chain triglycerides (MCT) as an alternate fat for a ketogenic diet has been advocated by Huttenlocher et al. (1971). These triglycerides have primarily eight and ten carbon-atom fatty acids (three-fourth octanoic and one-fourth decanoic with trace amounts of caproic and lauric acid) (Senior, 1968) and are highly ketogenic (Bach et al., 1972). The fats have a low melting point, a small molecular size and a high solubility in water. Therefore, these fats are readily absorbed and hydrolyzed by intestinal mucosa (Greenberger and Skillman, 1969; Hashim et al., 1965). After MCT hydrolysis, almost 90 percent of free fatty acids are transferred directly to the liver through the portal vein (Bloom et al., 1951).

The commercial source of medium chain triglycerides is an oil consisting of mixed triglyceride of fatty acids with a chain length of six to ten carbons, approximately 71 percent octanoic acid, 23 percent decanoic acids with a minor amount of longer and shorter chain fatty acids. The greater ketogenic effect of MCT compared to other types of fat is attributed to their rapid absorption and metabolism (Greenberger and Skillman, 1969). MCT is absorbed and metabolized more quickly than other fats for several reasons. (1) There is some evidence that in humans, there exists a gastric lipase that hydrolyzes MCT more rapidly than other forms of fat (Cohen, 1971). (2) MCT does not require the action

of bile salts to be hydrolyzed to the free fatty acid form; interfacial tension between water and MCT is low, and MCT is thus more soluble and more easily dispersed in solution than other forms of fat (Senior, 1968). (3) Once hydrolyzed into the free fatty acid form, reesterification into the triglyceride form is not necessary for MCT to pass in and out of the epithelial cell as with other fats, and the medium-chain fatty acids can move about freely in the free fatty acid state in portal venous blood (Hashim et al., 1965). (4) Unlike other fats, MCT is transported directly into the liver via the portal vein, bypassing the normal route of delivery via the lymph system (Senior, 1968). (5) Once in the liver, MCT fatty acids are rapidly metabolized and degraded into two-carbon fragments. The perfusion of the liver with these two-carbon fragments may occur more rapidly than the tricarboxylic acid cycle can accommodate them (Senior, 1968) and ketosis occurs. Signore (1973) believed that the magnitude of ketosis in humans was the result of carbohydrate deficiency relative to the amount of fat entering the liver.

High-fat diets depress conversion of excess carbohydrate to fat in rat adipose tissue (Leveille, 1967). However, Theuer (1971) showed that rats fed a high MCT diet had enhanced lipogenesis. Infusion of MCT, octanoic (C_8), trinonanoic (C_9) and decanoic acid (C_{10}) into the blood of dogs increased serum ß-hydroxybutyrate significantly ($p<.002$), and induced significant hypomagnesemia (Guisard et al., 1973).

Bahnsen et al. (1984) reported that ketone bodies form an important fuel source in fasted humans. Skeletal and cardiac muscle show high utilization rates, and even nervous tissue, which normally uses glucose alone, will use ketone bodies in periods of chronic starvation. Ketone bodies have an added importance in that high circulating levels inhibit protein degradation and prevent unnecessary degradation of amino acids for conversion into glucose for direct oxidation.

Medium-chain triglycerides also enhanced calcium absorption in rats with bile duct ligation due to the "intraluminal solubilization of calcium by medium-chain length fatty

acid" (Kehayoglou et al., 1968). In contrast, Harrison et al. (1973) found no effect of MCT on the absorption of the calcium.

Medium-chain triglyceride also enhanced magnesium absorption in rats (Tadayyon and Lutwak, 1969). A positive correlation between fat absorption and calcium ($r= 0.84$, $p<0.001$) and magnesium absorption ($r= 0.54$ $p<0.005$); respectively; has been found in human infants (Tantibhedhyangkul and Hashim, 1978).

CHAPTER III

MATERIALS AND METHODS

Animal care

Male weanling Sprague-Dawley rats; 395 in number, (Simonsen Laboratories, Inc., Gilroy, California) were fed a control diet for at least a day and allowed to become acclimated before starting the experiments. They were housed individually in stainless-steel cages with wire fronts and bottoms, and deionized water was provided ad libitum in polyethylene bottles having rubber stoppers and stainless-steel lick spouts. Animal-room temperature was maintained between 20 and 23°C. Lights were on automatically from 0800 to 1900h daily. With and without diets consisted of 20 percent casein, 58-63 percent dextrose, 5 percent fat (as corn oil), 2 percent vitamin mixture (Table 1), 5-10 percent mineral mixture (Table 2) and 5 percent wood fiber (alpha-cellulose). The magnesium-deficient diet contained, by analysis, 44 mg of magnesium per kg diet. The control diet was prepared by adding 400 mg magnesium per kg as $MgCO_3$ to the deficient diet.

Animals were weighed before initiating feeding of the control and experimental diets and before testing for seizures. On day 17, audiogenic seizures were induced in susceptible animals by the method of Patton (1941) as modified by Buck et al. (1978), except in experiment 1, part 1, on days 12, 14, 16 and 17 on which audiogenic seizures were induced in susceptible animals. Audiogenic seizure testing was performed by placing the rats in a metal chamber that contained two school emergency buzzers producing 115 db for 90 seconds. Seizure scores were rated on a scale of 0 to 5. Zero means no seizure; 1 means rapid running; 2 means clonic seizures, characterized by full-body convulsion; 3 means tonic flexion, characterized by the animal on its side, rigid and kicking; 4 means tonic extension, which is characterized by rigidity and extended legs; and 5 means lethal seizure. Because of the loud noise, testing had to be done in another room across the hallway with all doors closed. Animals were placed in the testing chamber cage for a few

Table 1-- Vitamin mixture composition.

Vitamin	g/kg mixture	mg/kg diet
Vitamin A Acetate, 500,000 I.U./g	1.8	104,651 I.U/kg
Vitamin D, 850,000 I.U. Cholecalciferol/g	0.125	1,000,000 I.U/kg
DL-alpha-Tocopherol Acetate	22.0	440
Ascorbic Acid	45.0	900
Inositol	5.0	100
Choline Chloride	75.0	1,500
Menadione	2.25	45
P-Aminobenzoic Acid	5.0	100
Niacin	4.25	85
Riboflavin	1.0	20
Pyridoxine Hydrochloride	1.0	20
Thiamin Hydrochloride	1.0	20
Calcium Pantothenate	3.0	60
Biotin	0.020	0.4
Folic Acid	0.090	1.8
Vitamin. B-12	0.00135	0.027

I.U. = International units
ICN Biochemical Division of ICN Biomedicals, Inc. Cleveland, OH 44128 Catalog No. 904654 Lot No. 9261.

Table 2--Mineral mixture. *

Salt	Amount of salt, mg	Mineral	Mineral mg/kg diet
$MnSO_4$	137.0	Mn	49.90
KI	2.0	I	0.50
$FeSO_4.7H_2O$	174.0	Fe	35.10
$ZnSO_4.7H_2O$	53.0	Zn	12.00
$CuSO_4.5H_2O$	19.0	Cu	5.00
$Na_2MoO_4.2H_2O$	12.0	MoO_4	4.76
$CoCL_3.6H_2O$	4.0	Co	0.99
Na_2SeO_4	0.2	Se	0.09
$CrCL_3.6H_2O$	0.7	Cr	0.30
Na_2CO_3	1200.0	Na	510.0
Total	1601.9 mg	---	---
Dextrose	to make 100 g	---	---
$CaCO_3$#	12.5 g	Ca	5.0 g
NaH_2PO_4#	15.5 g	P	4.0 g
KCL#	6.2 g	K	3.6 g

* For control diet, Mg was supplied by adding 4.0 g $MgCO_3$/kg diet.

\# Added directly to the diet.

seconds before starting the buzzers. Death from seizures was usually prevented by chest massage until normal respiration had returned. On day 17, the animals were weighed, tested for seizures and blood samples collected by inserting capillary tubes via the retro-ocular capillary bed. The rats were killed by decapitation immediately after blood collection.

Chemical analysis

Calcium and magnesium were analyzed in all blood samples using an Instrumentation Laboratories Model 457 Atomic Absorption Spectrophotometer at 422.7 and 285.2 nm for calcium and magnesium, respectively. One percent of 100,000 ppm lanthanum solution was added to minimize phosphate interferences. The solution was made by dissolving 234.4 gm La_2O_3 in 440 ml HCL; then diluting to 2L with deionized water.

Phosphorus analyses were performed in all samples by method 22.042 of the A.O.A.C (1980) using molybdovanadate reagent; absorbance was measured at 400 nm with a Beckman DB-GT grating spectrophotometer.

Potassium and sodium were measured by emission spectroscopy using the Instrumentation Laboratories Model 457 Atomic Absorption Spectrophotometer at 766.5 and 589.0 for potassium and sodium, respectively.

Glucose was analyzed by Quantitative Calorimetric Procedure No. 635 (Sigma Chemical Company) in serum on the basis that, in the presence of heat and acid, O-toluidine reacts readily with glucose to form a colored complex. The intensity of the color formed was measured with a spectrophotometer at 635 nm and was proportional to the glucose concentration.

Beta-hydroxybutyric acid (BHB) was measured by Quantitative Enzymatic Procedure No. 310-UV (Sigma Chemical Company), based on the oxidation of BHB to acetoacetate with the assistance of ß-hydroxybutyrate dehydrogenase (BHBD). During this

oxidation, an equimolar amount of nicotinamide adenine dinucleotide (NAD) was reduced to NADH + H. The consequent increase in absorbance at 340 nm due to NADH was proportional to the BHB concentration.

Dietary and serum mineral analysis

Determination of minerals in the diet in the serum were made by ashing 1-2 g of diet in a porcelain crucible for 48h at 550°C. After cooling, 5-ml of 6N HCL was added, and the samples were slowly heated for 10-20 min to completely solubilize the ash. Then samples were diluted to 100-ml with demineralized water and analyzed for magnesium, calcium, phosphorus, potassium and sodium (Table 3).

Blood samples were allowed to clot in a refrigerator for approximately 2h to prevent glycolysis of glucose at room temperature. Then samples were centrifuged at 2000 rpm for 30 min (relative centrifugal force, gravities = 715). Serum was transferred to a new test tube using Pasteur pipettes and kept refrigerated for approximately 20h before dilution for mineral analyses. Glucose and BHB were analyzed directly after the serum was collected. The serum was diluted 1:100 by placing 0.1-ml serum into a 10-ml volumetric flask and diluting to volume with demineralized water. For magnesium analysis, 1-ml diluted serum was added to 0.1-ml of 100,000 ppm lanthanum solution in a 10-ml volumetric flask and brought to volume with demineralized water. This gave a final dilution of 1:1000. The same procedure was used for calcium.

Phosphorus analysis was conducted by adding 1 ml of 30 percent trichloroacetic acid in 5-ml of the diluted serum (1:1000), stirring with a Vortex mixer, and allowing to stand at room temperature for 20 min (A.O.A.C, 1980; method 20.138e). The samples were then centrifuged at 2000 rpm for 10 min (relative centrifugal force, gravities = 715). One ml of molybdovanadate reagent was added to 4-ml of the supernatant, and the color was allowed to develop for 15 min. Optical densities of the samples were determined at 400 nm.

Table 3--Concentration of minerals in diet of experiment 1 (mg/100g).

Diet	Mg*	Ca*	P*	K*	Na*
Mg-supplement (control) diet	0.35	5.52	6.21	3.64	3.09
Mg-deficient diet	0.01	5.43	5.93	3.72	3.06
Requirement for normal growth	0.04	5.60	4.40	2.00	6.00

*Analyzed values (mean of 3 samples).

Sodium and potassium were determined by diluting the serum samples 1:100 with demineralized water and were measured by emission spectroscopy using the Instrumentation Laboratories Model 457 Atomic Absorption Spectrophotometer.

Statistical analysis

Experimental data were analyzed statistically using a completely randomized design (ANOVA) (Dowdy and Wearden, 1983). A Chi-square test was used to analyze differences in seizure incidence among treatments; the 5 percent alpha level was used as the criterion for statistical significance. Analysis of variance (ANOVA) was performed to determine the differences in seizure score and serum metabolite and mineral concentrations within and among treatments. A factorial arrangement ANOVA of treatment (3x3) was used in experiments 2, 3 and 4 to determine the effects of various levels of dietary MCT and fasting times, and the effects of gavaging various levels of BHB and glucose were tested at different times after dosing on serum metabolites (BHB and glucose) and mineral concentrations (magnesium, calcium, phosphorus, potassium, and sodium) of magnesium-deficient rats. When "F" was significant ($p<0.05$), means were compared by least significant difference values (LSD).

Experimental Design

Experiment one. The objective of the first part of this experiment was to determine when magnesium-deficient rats become susceptible to audiogenic-induced seizuring. Thirty rats were assigned to four groups as follows: (1) Six rats were tested for seizure susceptibility on days 2, 4 and 6 of a magnesium-deficient diet; (2) Seven rats were tested on days 10 and 16; (3) Seven rats were tested on day 12; and (4) Seven rats were tested on day 14. All rats that had not convulsed on the assigned day were retested on day 17. Blood samples were collected for chemical analysis just after testing for seizures and just before killing the rats.

The objective of the second part of this experiment was to study the effect of a magnesium-deficient diet and fasting on seizure susceptibility. Fasting rats were assigned to groups of 10 and 30 rats. The 10 rats in group one were fed the magnesium-supplemented (control) diet, then divided into two subgroups of 5 rats each; one subgroup was fasted for 24h then tested for seizures; and the other subgroup was tested without fasting. The 30 rats in group two were divided into four groups and given the magnesium-deficient diet and fasted for 0, 6, 12 and 24-h, respectively, before seizure testing. Blood samples were collected just after testing for seizures and just before killing the rats.

Experiment two. It was found in the first experiment found that rats with high levels of BHB had low seizure incidence. Therefore, this experiment was designed to study the effects of different dietary concentrations of medium-chain triglyceride (MCT, ketogenic diet) on audiogenic-seizure susceptibility. MCT oil is the primary triglyceride fraction of coconut oil, consisting mostly of C_8 and C_{10} saturated fatty acids, approximately 68 percent octanoic acid, 24 percent decanoic acid, 5 percent longer than C_{10} and 3 percent shorter than C_8 fatty acids (Mead Johnson, Nutritional Division, Mead Johnson and company, Evansville, Indiana 47721 U.S.A.). Three diets were designed to

control for caloric density and percentage of energy as fat (Table 4). The first diet, 5 percent fat, was made up of 3 percent MCT oil and 2 percent corn oil (11.8 percent of the kcal from fat and 3.63 kcal/g caloric density). The second diet consisted of 28 percent MCT and 2 percent corn oil, resulting in 54.5 percent energy from fat and 3.56 kcal/g caloric density. The third diet consisted of 2 percent corn oil and 21.1 percent MCT with 53.2 percent of the energy from fat and 4.73 kcal/g caloric density. Ninety rats were randomly divided into 3 groups of 30 animals each. Then these 3 groups were fed a magnesium-deficient diet (~ 10g/day) with different dietary concentrations of medium-chain triglyceride. After receiving the respective diets for 17 days, each group was divided into 3 levels of fasting, 0, 12 and 24h, after which they were tested for seizure susceptibility.

Experiment three. Because the first two experiments and earlier investigations on human and animal studies indicated that increased serum levels of BHB, caused by ketogenic diet or fasting, were associated with decreased seizure incidence, this experiment was designed to study the effects of acute alteration of serum BHB. The effect of gavaging BHB on seizure susceptibility and serum metabolites (BHB and glucose) and minerals was determined. Fifty-two rats were fed a magnesium-deficient diet for 17 days and were randomly assigned to 3 groups of 17 rats each at the start of the experiment. These groups were assigned to receive 0.5, 1 or 2 millimoles of sodium-beta-hydroxybutyrate, respectively; by gavaging, the dose was inserted directly into the stomach using a long, curved, blunt, stainless-steel needle through the mouth. The rats were tested for audiogenic seizures after gavaging as follows: 5 rats in each group were tested after 30 min, 6 rats after 60 min and 6 rats after 120 min. Blood samples were collected just after testing for seizures and just before killing the rats.

Experiment four. All three earlier experiments indicated that rats that had high serum levels of glucose had high seizure incidence; therefore, this experiment was designed

Table 4--The composition and energy contribution of the ketogenic diets (MCT).

Diet ingredients	Diet I (3%) MCT			Diet II (21%) MCT			Diet III (28%) MCT		
	Percent in diet	Energy kcal/g	Calories %	Percent in diet	Energy kcal/g	Calories %	Percent in diet	Energy kcal/g	Calories %
MCT	3	0.25	6.9	21.1	1.76	49.4	28	2.32	49.4
C O	2	0.18	4.9	2.0	0.18	5.1	2	0.18	3.8
Total fat	5	0.43	11.8	23.1	1.93	54.5	30	2.50	53.2
Casein	20	0.80	22.04	20.0	0.80	22.0	20	0.80	17.0
Dextrose	60	2.4	66.1	20.4	0.82	23.0	35	1.4	29.8
Vit. mixture	2	0.0	0.0	2.0	0.00	0.0	2	0.00	0.00
Min.mixture	8	0.0	0.0	8.0	0.00	0.0	8	0.00	0.00
Fiber	5	0.0	0.0	26.5	0.00	0.0	5	0.00	0.00
Total	100	3.63	99.94	100	3.56	99	100	4.73	100

MCT, medium chain triglyceride, gives 8.3 kcal/g.
C O, corn oil, gives 9 kcal/g.
Protein, gives 4 kcal/g.

to study the effects of acute alteration of serum glucose levels on seizure susceptibility of 24-h fasted, magnesium-deficient rats. Ninety-six rats were divided into 2 groups: the first group; comprised of 63 rats that were randomly assigned into 3 subgroups, were gavaged with 1.4, 2.8 or 5.6 millimoles of a 50 percent alpha-D-glucose solution, 21 rats for each dose level. Then 7 rats from each dose level were tested for seizures at 30, 60 or 120 min after gavaging the glucose.

The remaining 33 rats were divided into 4 groups: 1) 8 unfasted rats were sham-gavaged with 0.9 percent saline as a positive control, 2) 8 rats were fasted for 24h and sham-gavaged with 0.9 percent saline as a negative control, 3) 8 unfasted rats were gavaged with 1.4 millimoles of glucose solution and then tested for seizures after 30 min and 4) 9 rats were fasted for 24h and gavaged with 2 millimoles of BHB. The gavaged rats were tested for seizures 30 min after receiving their respective doses. Blood samples were collected for chemical analysis just after testing for seizures and just before killing the rats.

Experiment five. The purpose of this experiment was to determine the effects of gavaging different doses of BHB and/or glucose on seizure susceptibility of magnesium-deficient rats. Therefore, 82 rats were divided into 8 groups as follows: group 1 was untreated as a control; group 2 was sham-gavaged (saline, 0.9 percent); group 3 was gavaged with 2.0 mmole BHB after 24-h of fasting; group 4 was gavaged with 5.6 mmole glucose; group 5 was gavaged with 0.5 mmole BHB and 5.6 mmole glucose; group 6 was gavaged with 2.0 mmole BHB and 5.6 mmole glucose; group 7 was gavaged with 0.5 mmole BHB and 1.4 mmole of glucose; and group 8 was gavaged with 2.0 mmole BHB and 1.4 mmole glucose of 24-h fasted rats.

CHAPTER IV

RESULTS

Experiment one

Part 1: The purpose of this experiment was to show when seizure susceptibility begins in weanling rats fed a magnesium-deficient diet. By days 5 to 7, the classic magnesium deficiency symptoms of erythema and edema of the ears, nose and claws, and skin lesions appeared. Edema and redness of the ears were very severe during days 7 to 12; then, after the 12th day, these symptoms decreased in severity. The effect of duration of magnesium deficiency on audiogenic seizure susceptibility is shown in Figure 1. Severity of seizures increased erratically over time during days 12 to 17 but was greatest on day 17 (Fig. 1). By day 17, 100 percent of the animals were susceptible to audiogenically-induced seizures (Fig. 1). Control animals fed magnesium-supplemented diet did not have audiogenic seizures.

Part 2: The purpose of this experiment was to study the effects of a magnesium-deficient diet and fasting on seizure susceptibility. The effect of feeding a magnesium-deficient diet for 17 days on seizure susceptibility of the animals fasted for 0, 6, 12, 18 or 24 hours is shown in Table 5. Compared with the animals fed magnesium-supplemented diet (control), all of the unfasted (0-h) magnesium-deficient rats had seizures; and none of the control animals had seizures (Table 5). Although the rats fasted 12 and 18h had reduced incidence of seizures, these reductions were not statistically significant compared to rats that were not fasted or were fasted for 6h. Rats fasted for 24h had reduced ($p<0.05$) seizure incidence compared with control and 6, 12 or 18-h fasting (Table 5). However, the correlation between hours of fasting and percentage of seizures was high, $r= -0.90$ (Fig. 2). Although the rats fasted for 24h had reduced seizure scores ($p<0.05$) compared with the unfasted rats, seizure scores of rats fasted 6, 12 or 18h were not reduced significantly

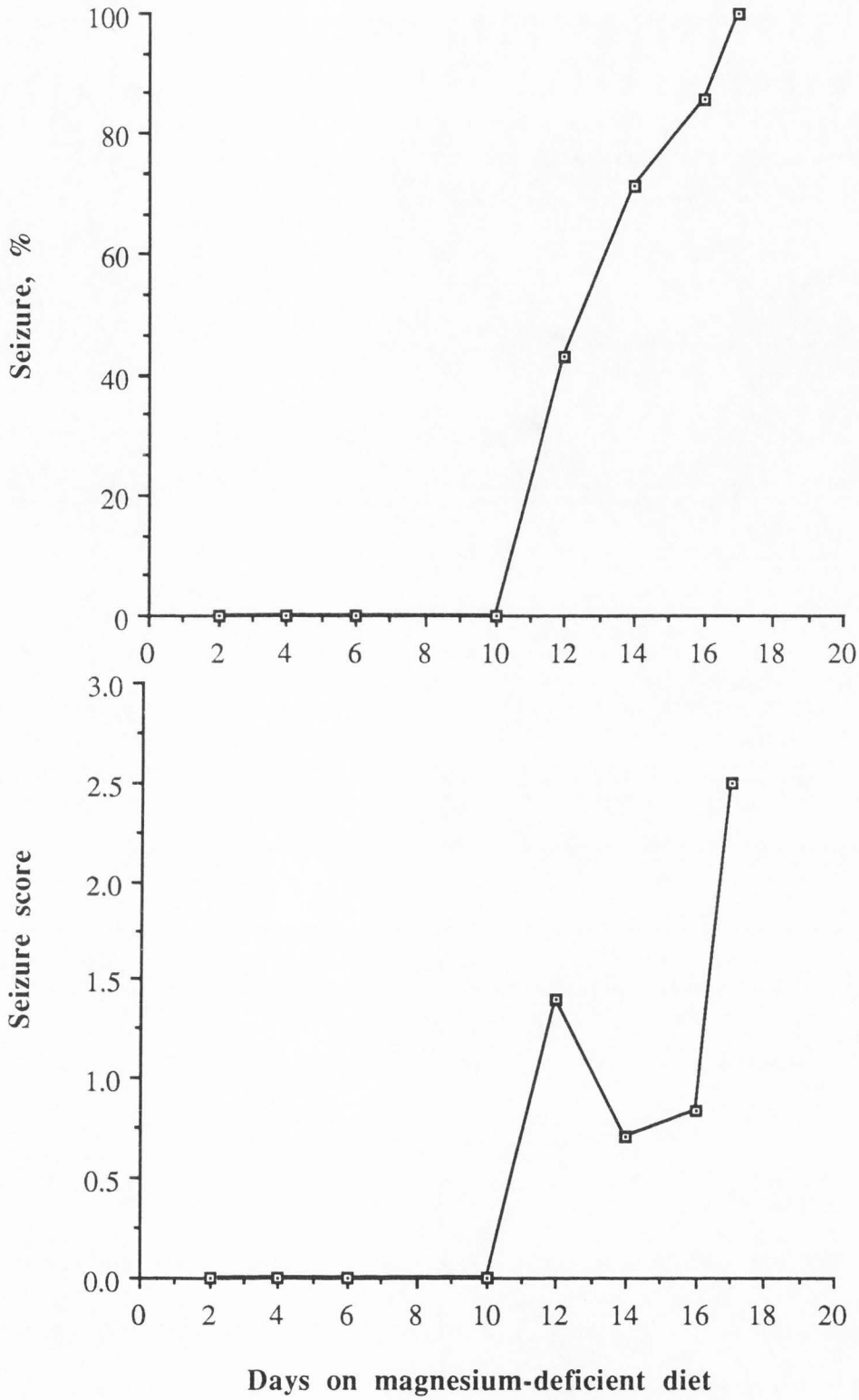

Fig. 1--Seizure percentage and scores of weanling rats fed a magnesium-deficient diet that seized when exposed to a 115 db buzzer (Experiment 1, part 1).

Table 5--Audiogenic seizures and serum BHB, glucose and minerals (mg/dl) of rats fed a magnesium-deficient or magnesium-supplemented (control) diet for 17 days (Experiment 1, part 2).

Diet Group	Fasting h	Number of rats	Seizure % #	Seizure Score*	BHB	Glucose	Mg	Ca	P	K	Na
CONTROL	0	5	0.0^{a}	0.0^{a}	3.30a	98^{a}	1.72^{a}	6.78	6.06	33.2	146
CONTROL	24	5	0.0^{a}	0.0^{a}	50.9^{b}	65^{b}	1.64^{b}	7.06	6.48	35.7^{a}	157
MG-DEF.	0	6	100^{b}	3.2^{b}	3.30^{a}	91^{a}	0.59^{c}	6.57	5.87	27.1^{bc}	151
MG-DEF.	6	6	100^{b}	2.8^{bc}	9.90^{a}	75^{ab}	0.67^{d}	6.90	6.48	25.5^{b}	150
MG-DEF.	12	6	83^{b}	1.6^{ac}	16.8^{ac}	61^{b}	0.57^{c}	6.60	5.22	28.7^{bc}	155
MG-DEF.	18	6	83^{b}	0.8^{a}	35.0^{bc}	46^{bc}	0.57^{c}	6.20	6.67	25.9^{bc}	158
MG-DEF.	24	6	50^{c}	1.2^{ac}	53.6^{b}	35^{c}	0.56^{c}	6.25 NS	6.98 NS	26.2^{bc}	154 NS

a,b,c Values having different superscripts are significantly different ($p<0.05$). See Appendix A, Table 32.
NS means there were no statistically significant ($p<0.05$) differences among the groups. See Appendix B, Table 38.
* The higher the score; the more severe the seizures were.
\# Chi-square values see Appendix C, Table 43.

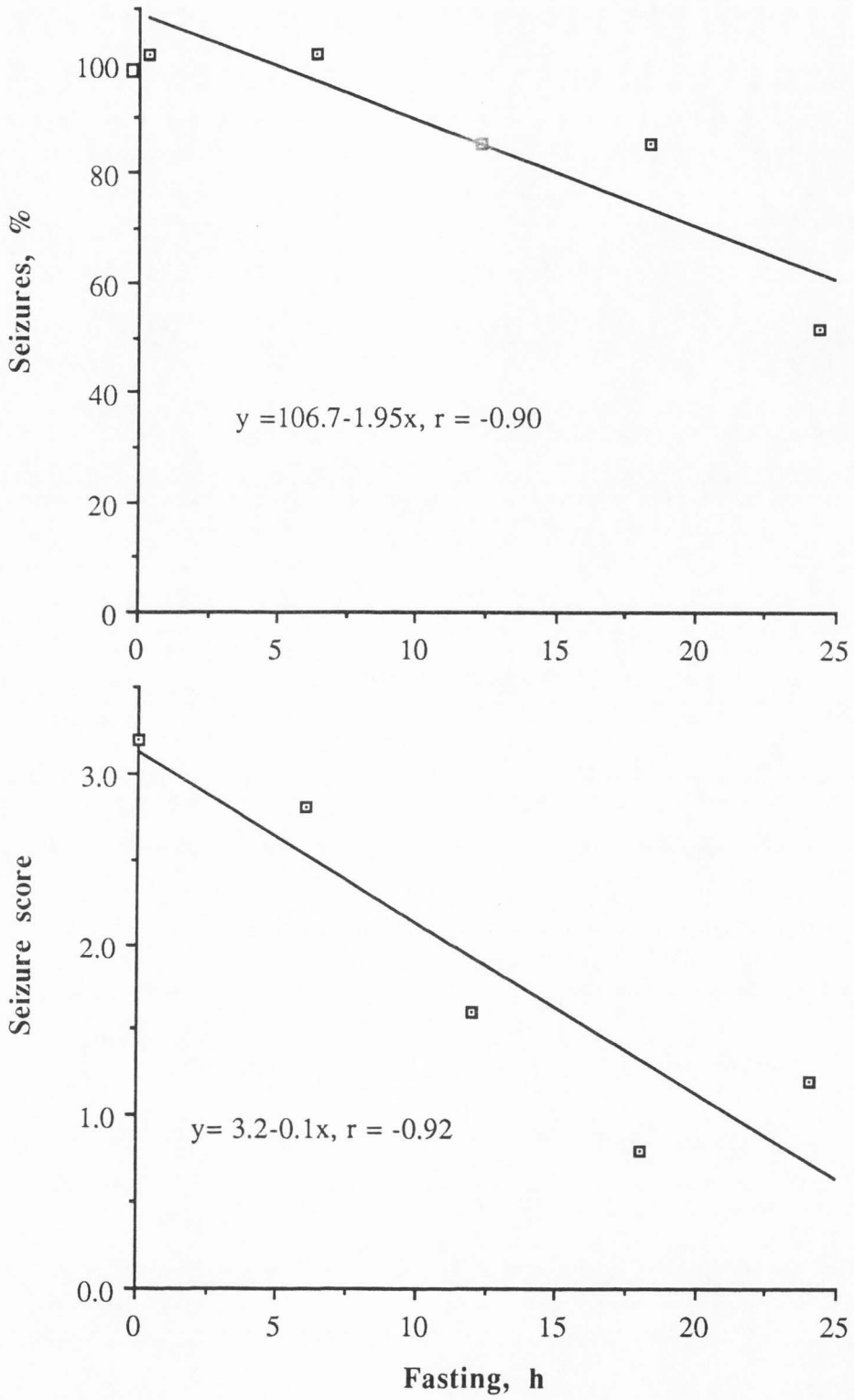

Fig. 2--Effect of fasting on percentage of seizures and seizure score of rats fed a magnesium-deficient diet for 17 days (Experiment 1, part 2).

as compared with the unfasted magnesium-deficient rats. However, seizure score decreased as duration of fasting increased, r= -0.92 (Fig. 2).

The effect of duration of fasting on serum BHB and glucose concentrations is shown in Table 5, Appendix D, Table 48 and Figure 3. As hours of fasting increased, serum BHB increased, r= 0.97, and serum glucose decreased, r= -1.00, significantly. While serum glucose was 91.2 mg/dl in the unfasted magnesium-deficient rats (0-h fasting) and 97.8 mg/dl in the unfasted control rats, by 24h fasting it had decreased to 35 mg/dl in magnesium-deficient rats and to 64.6 mg/dl in the control rats. In contrast, serum BHB increased from 3.3 mg/dl in the 0-h fasting and control rats to 53.6 mg/dl in the magnesium-deficient rats and to 50.9 mg/dl in the control rats after 24-h of fasting (Table 5). Serum magnesium concentrations were markedly decreased due to feeding magnesium-deficient diet. There were no significant differences in Ca, P and Na concentrations due to magnesium deficiency, but K concentration was significantly decreased in the magnesium-deficient rats due to magnesium deficiency.

Experiment two

The purpose of this experiment was to study the effects of ketogenic diet (MCT) and fasting on seizure susceptibility of weanling rats fed magnesium-deficient diet for 17 days. The effects of dietary MCT level (3, 21 or 28 percent in which the percentages of dietary energy from fat were 11.8, 54.5 or 53.2 percent, respectively, and the caloric densities of the diets were 3.63, 3.56 or 4.73 kcal/g) were studied (Table 4). The effects of different dietary concentrations of MCT and duration of fasting on seizure susceptibility of magnesium-deficient rats are shown in Tables 6-15. As dietary MCT level increased; seizure score and seizure incidence increased significantly. This effect was consistent, although not statistically significant between 21 and 28 percent MCT dietary levels, whether the rats were fasted 0, 12 or 24h (Tables 6 and 7). All of the nonfasted rats fed a 3 percent MCT diet (3.63 kcal/g caloric density) had seizures; but only 40 percent of those

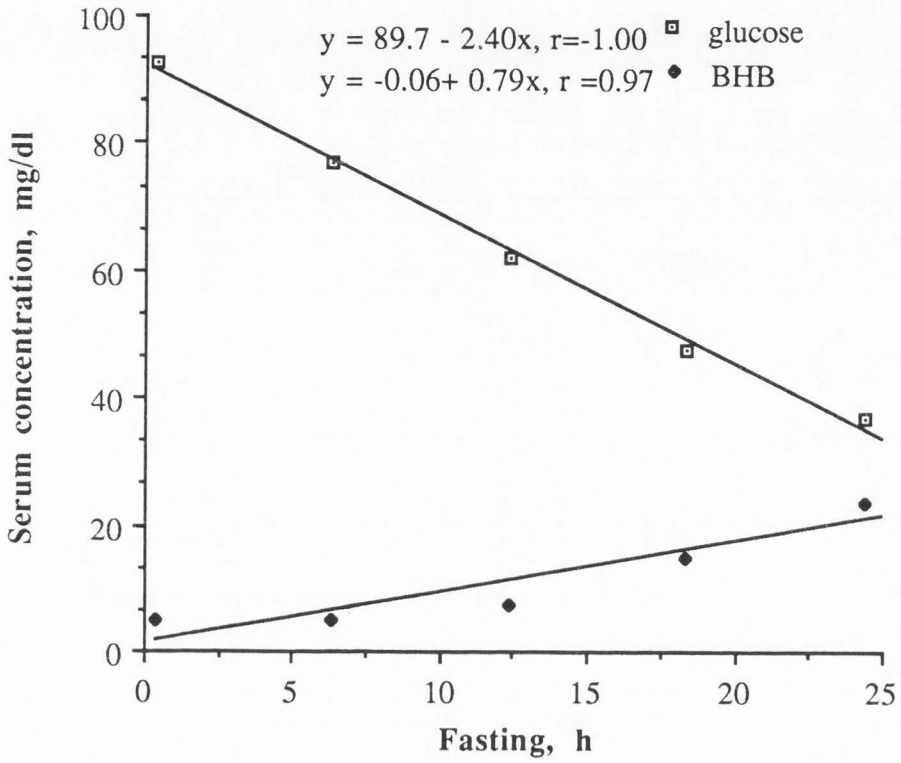

Fig. 3--The relationship between fasting times vs. serum BHB and glucose levels of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 1).

Table 6--The effect of dietary MCT levels and duration of fasting on audiogenic seizure susceptibility (seizure, %) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	100^{A}	50^{B}	40^{B}	63[1]
	12	90^{A}	25^{C}	22^{C}	46[2]
	24	50^{D}	29^{C}	13^{C}	31[2]
Main effect of MCT		80^{a}	35^{b}	25^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendix C, Table 44, for chi-square test.

Table 7--The effect of dietary MCT levels and duration of fasting on seizure score of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	3.7^{A}	2.0^{B}	1.8^{BC}	2.5[1]
	12	2.4^{B}	0.6^{CD}	0.6^{CD}	1.2[12]
	24	0.5^{D}	0.3^{D}	0.1^{D}	0.3[2]
Main effect of MCT		2.2^{a}	2.8^{a}	0.8^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39 for ANOVA and LSD analysis.

fed the 28 percent MCT diet were seized (4.73 kcal/g caloric density). Neither caloric density or percentage calories from fat, per se, affected seizure susceptibility. There were no significant differences in percentage seizures between the fasted and nonfasted rats fed the 21 percent or 28 percent dietary MCT. Increased duration of fasting decreased seizure percentages and seizure scores regardless of dietary MCT level (Tables 6 and 7).

Information on the effect of increasing dietary MCT and fasting on serum metabolites of rats fed a magnesium-deficient diet for 17 days is presented in Tables 8 and 9. The serum BHB concentration was elevated ($p<0.05$), from 7.1 mg/dl in the 0h-fasted rats fed a 3 percent MCT diet to 38.1 mg/dl in the 24h-fasted rats fed the 28 percent MCT diet. BHB was significantly increased within groups due to fasting and increased ($p<0.05$) among groups due to dietary MCT (Table 8). Glucose concentration was decreased ($p<0.05$) from 113 mg/dl in the 3 percent of dietary MCT to 90 mg/dl in the 28 percent of dietary MCT at 0-h fasting due to increase dietary MCT. Increasing duration of fasting to 24h decreased serum glucose ($p<0.05$) at all levels of dietary MCT (Table 9).

As dietary MCT level was increased, the percentage seizures, $r= -0.84$, and seizure score, $r= -0.94$; decreased (Fig. 4). As duration of fasting increased, seizure incidence and seizure score decreased at all levels of dietary MCT (Fig. 5). Serum BHB increased with fasting at all levels of dietary MCT, $r=0.98$ for 3 percent MCT, $r=0.99$ for 21 percent MCT and $r=0.98$ for 28 percent MCT, while serum glucose decreased with fasting at all levels of dietary MCT, $r= -0.97$ for 3 percent MCT, $r= -0.99$ for 21 percent MCT and $r= -0.98$ for 28 percent MCT (Fig. 6). As serum BHB increased, percentage of seizures decreased, $r= -0.88$, and seizure score decreased, $r= -0.93$, regardless of dietary MCT level or duration of fasting (Fig. 7, Appendix D, Table 49). Serum glucose increased as seizure percentage increased, $r= 0.69$, and as seizure score increased, $r= 0.91$ (Fig. 7).

The serum mineral concentrations of the animals were not affected ($p>0.05$) by MCT level (Tables 10-15). However, prolonged fasting decreased the serum magnesium,

Table 8--The effect of dietary MCT levels and duration of fasting on serum BHB (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	7.10^{A}	13.2^{B}	23.9^{C}	14.1[1]
	12	16.1^{B}	23.9^{C}	32.2^{D}	21.0[2]
	24	19.1^{B}	26.0^{C}	38.1^{D}	31.4[3]
Main effect of MCT		14.7^{a}	24.1^{b}	27.7^{c}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39 for ANOVA and LSD analysis.

Table 9--The effect of dietary MCT levels and duration of fasting on serum glucose (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	113[A]	77[B]	90[C]	93[1]
	12	89[B]	61[C]	73[D]	74[2]
	24	44[D]	50[D]	63[E]	52[3]
Main effect of MCT		82[a]	63[b]	75[b]	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39 for ANOVA and LSD analysis.

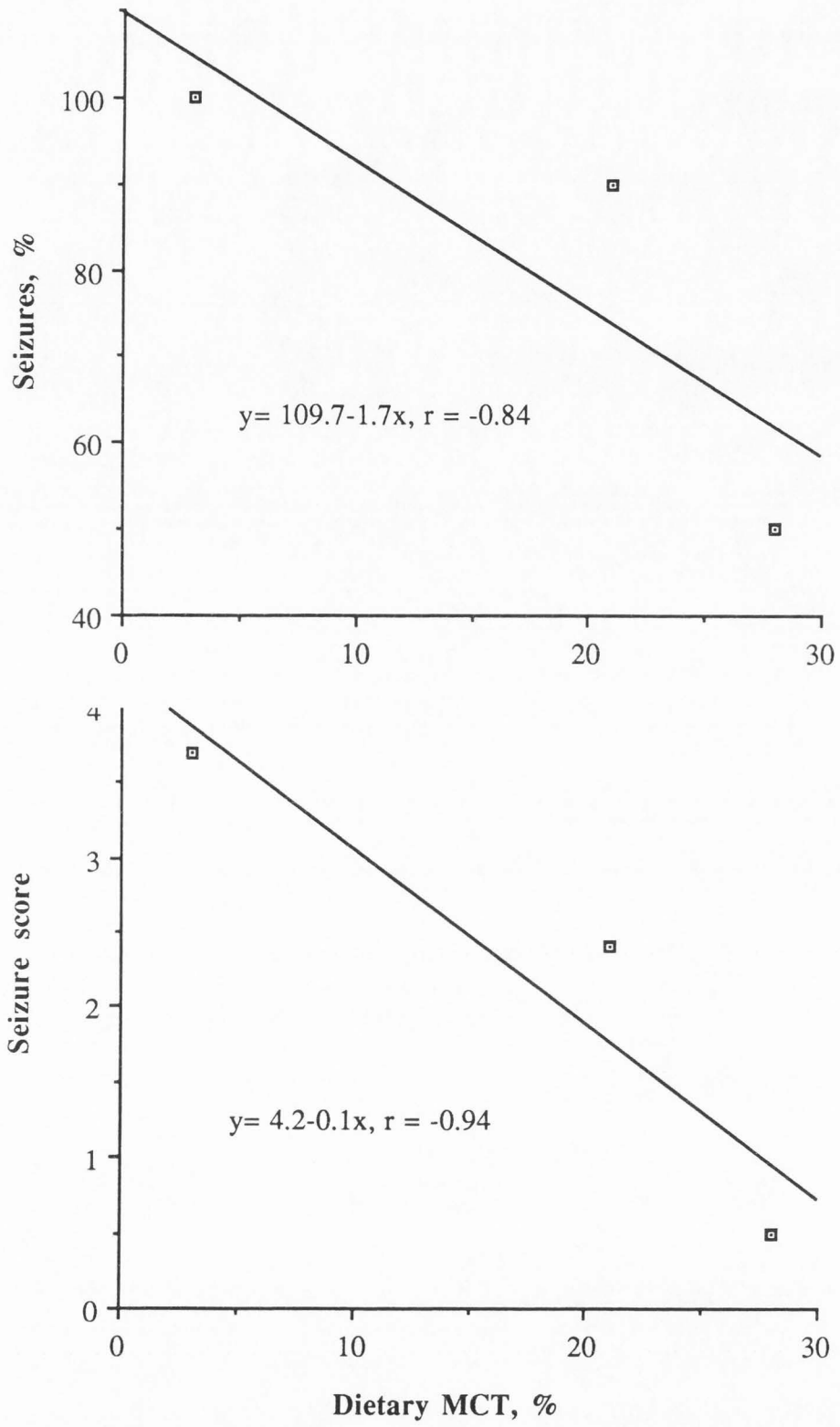

Fig. 4--Effect of MCT levels in diet on the percentage of seizures and seizure score of rats that had audiogenic seizures after consuming a magnesium-deficient diet for 17 days (Experiment 2).

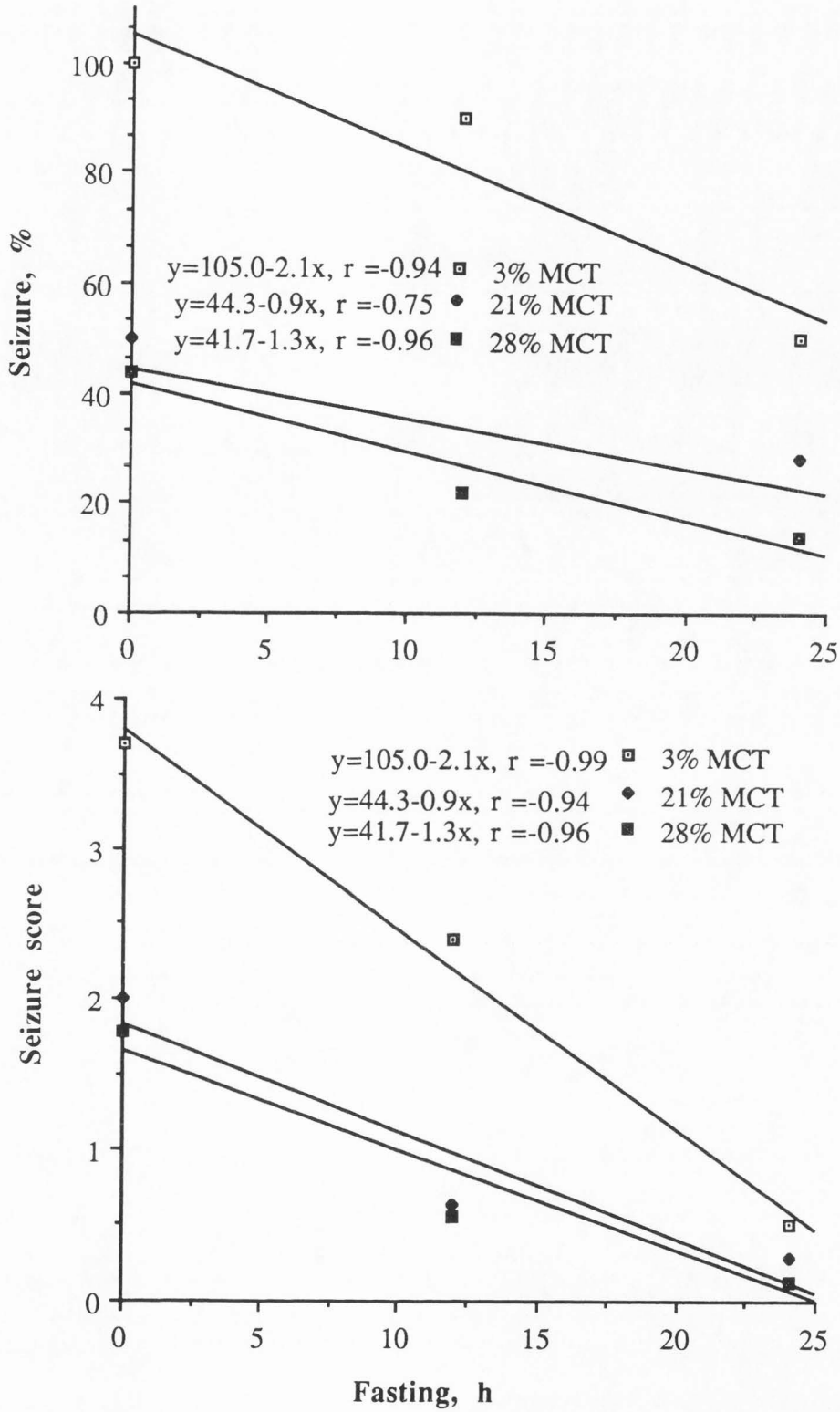

Fig. 5--The effect of fasting time and dietary level of MCT on the percentage of seizures and seizure score of rats that consumed a magnesium-deficient diet for 17 days (Experiment 2).

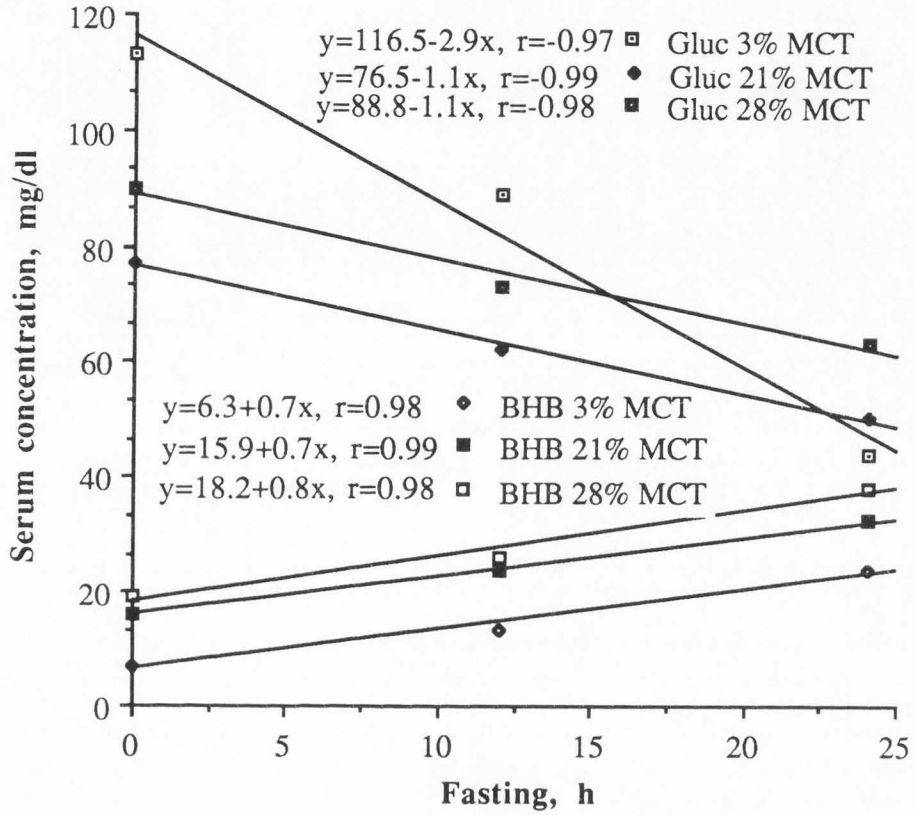

Fig. 6--The effect of fasting time and dietary level of MCT on serum glucose and BHB of weanling rats after consuming a magnesium-deficient diet for 17 days (Experiment 2).

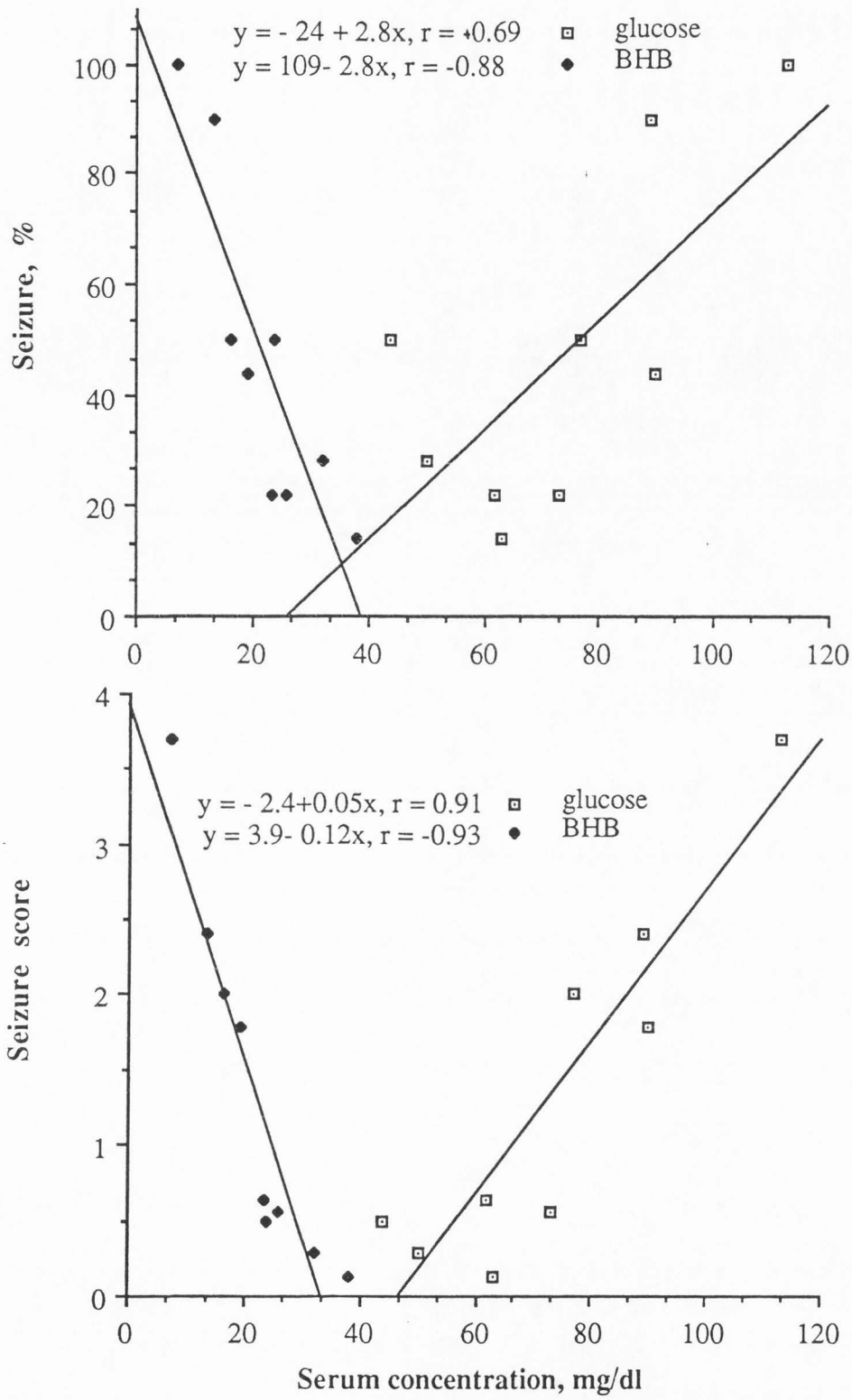

Fig. 7--The relationships between serum BHB and glucose vs. percentage of seizures and seizure score of weanling rats fed a magnesium-deficient diets with 3, 21 or 28 percent MCT and fasted 6, 12 or 24h (Experiment 2).

calcium, phosphorus, potassium and sodium levels ($p<0.05$) in all dietary MCT groups (Tables 10-15).

Experiment three

The purpose of this experiment was to determine the effects of gavaging BHB on serum BHB and glucose and seizure susceptibility of magnesium-deficient rats. The effects of gavaging 0.5, 1.0 or 2.0 mmoles of BHB and testing for seizure at 30, 60 and 120 minutes after dosing on seizure incidence, serum BHB, glucose and mineral levels of magnesium-deficient rats are presented in Tables (16-21). The 1 and 2 mmole BHB doses resulted in decreased seizure incidence and seizure scores ($p<0.05$) 30 min after gavaging; compared with the 0.5 mmole dose (Tables 16 and 17). Waiting 120 minutes before seizure testing resulted in increased seizure incidence; especially in rats gavaged with 2 mmole BHB. There was a high association between BHB dose and the percentage of seizures ($p<0.05$) regardless of testing time (Fig. 8). However, this association was weakest in those groups that were tested 120 min after dosing, $r= -0.64$ or -0.79, respectively.

Tables 18 and 19 also show the effect of different BHB doses on serum metabolites. Serum BHB was 3.5 mg/dl in the rats gavaged with 0.5 mmole BHB and was 43.3 mg/dl in the rats gavaged with 2.0 mmole BHB; but this effect was diminished with increasing time of dosing (Table 18). Conversely, serum glucose concentration in the animals given the 0.5 mmole BHB dose, 118 mg/dl, was higher ($p<0.05$) than in rats gavaged with the 2.0 mmole BHB and tested 30 min later, 80 mg/dl, and this effect was also diminished with increasing time after dosing (Table 19). There were high positive correlations between serum glucose and seizure percentage, $r= 0.96$, and seizure score, $r= 0.88$, and high negative correlations between serum BHB and seizure percentage, $r= -0.88$, and seizure score, $r= -0.81$, (Fig. 9, Appendix D, Table 50). There was also a high negative association between serum BHB and serum glucose, $r= -0.93$ (Fig. 10).

Table 10--The effect of dietary MCT levels and duration of fasting on serum magnesium (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	0.76^{A}	0.79^{A}	0.78^{A}	0.78[1]
	12	0.67^{B}	0.72^{C}	0.58^{D}	0.66[2]
	24	0.59^{C}	0.67^{E}	0.65^{E}	0.64[2]
Main effect of MCT		0.67^{a}	0.73^{b}	0.67^{a}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39, for ANOVA and LSD analysis.

Table 11--The effect of dietary MCT levels and duration of fasting on serum calcium (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	10.5^{A}	10.9^{A}	9.29^{B}	10.2[1]
	12	7.13^{C}	9.40^{B}	9.10^{B}	8.54[2]
	24	6.02^{B}	6.83^{C}	8.39^{D}	7.08[3]
Main effect of MCT		7.88^{a}	9.04^{b}	8.93^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39, for ANOVA and LSD analysis.

Table 12--The effect of dietary MCT levels and duration of fasting on serum phosphorus (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	11.0^{A}	11.2^{A}	11.4^{A}	11.2[1]
	12	9.31^{B}	9.81^{C}	9.49^{B}	9.54[2]
	24	7.94^{C}	7.91^{D}	7.51^{E}	7.78[3]
Main effect of MCT		9.42^{a}	9.64^{a}	9.46^{a}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39, for ANOVA and LSD analysis.

Table 13--The effect of dietary MCT levels and duration of fasting on serum potassium (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	28.4^{A}	28.9^{A}	29.0^{A}	28.8[1]
	12	26.2^{B}	26.3^{B}	27.9^{C}	26.8[2]
	24	27.8^{A}	25.7^{B}	26.5^{B}	26.7[2]
Main effect of MCT		27.5^{a}	26.9^{a}	27.8^{a}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39, for ANOVA and LSD analysis.

Table 14--The effect of dietary MCT levels and duration of fasting on serum sodium (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2)[1].

		MCT, %			Main effect of fasting
		3	21	28	
Fasting, h	0	191^{A}	192^{A}	191^{A}	191[1]
	12	181^{B}	174^{C}	185^{E}	180[2]
	24	159^{C}	170^{E}	178^{D}	169[3]
Main effect of MCT		177^{a}	179^{b}	184^{c}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39, for ANOVA and LSD analysis.

Table 15--Summary of the effect of dietary MCT level and duration of fasting on audiogenic seizure susceptibility and serum BHB, glucose and minerals of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 2).

MCT, %	3			21			28		
Fasting, h	0	12	24	0	12	24	0	12	24
Number of rats	10	10	10	8	8	7	9	9	8
Seizure score*	3.7^{a}	2.4^{b}	0.5^{c}	2.0^{b}	0.6^{c}	0.3^{c}	1.8^{b}	0.6^{c}	0.1^{c}
Seizure, %#	100^{a}	90^{a}	50^{b}	50^{b}	25^{b}	29^{b}	40^{b}	22^{b}	13^{c}
BHB, mg/dl	7.10^{a}	13.2^{b}	23.9^{c}	16.1^{b}	23.9^{c}	32.2^{d}	19.1^{b}	26.0^{c}	38.1^{d}
Glucose, mg/dl	113^{a}	89^{b}	44^{c}	77^{d}	61^{e}	50^{f}	90^{b}	73^{d}	63^{e}
Mg, mg/dl	0.76^{a}	0.67^{b}	0.59^{c}	0.79^{a}	0.72^{b}	0.67^{c}	0.78^{a}	0.58^{b}	0.65^{c}
Ca, mg/dl	10.5^{a}	7.13^{d}	6.02^{c}	10.9^{a}	9.40^{b}	6.83^{c}	9.29^{b}	9.10^{b}	8.39^{c}
P, mg/dl	11.0^{a}	9.31^{b}	7.94^{c}	11.2^{a}	9.81^{b}	7.91^{c}	11.4^{a}	9.49^{b}	7.51^{c}
K, mg/dl	28.4^{a}	26.2^{b}	27.8^{a}	28.9^{a}	26.3^{b}	25.7^{b}	29.0^{a}	27.9^{a}	26.5^{b}
Na, mg/dl	191^{a}	181^{c}	159^{c}	192^{a}	174^{e}	170^{f}	191^{a}	185^{b}	178^{d}

a,b,c,d,e,f, Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 33 and 39 for ANOVA and LSD analysis.

* The higher the score; the more severe the seizures were.

\# The data were analyzed by Chi-square test (see Appendix C, Table 44).

Table 16--The effect of gavaging different BHB doses and different time after dosing on audiogenic seizure susceptibility (seizure, %) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 3)[1].

		BHB, mmoles			Main effect of min after dosing
		0.5	1.0	2.0	
Min after dosing	30	100^{A}	40^{B}	17^{C}	52[1]
	60	80^{A}	50^{B}	40^{B}	57[1]
	120	100^{A}	60^{B}	67^{D}	76[1]
Main effect of BHB		93^{a}	50^{b}	41^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendix C, Table 45, for Chi-square test.

Table 17--The effect of gavaging different BHB doses and different time after dosing on seizure score of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 3)[1].

Min after dosing	BHB, mmoles 0.5	1.0	2.0	Main effect of min after dosing
30	3.8^{A}	1.0^{B}	0.7^{B}	1.8[1]
60	2.8^{A}	0.8^{B}	1.0^{B}	1.5[1]
120	3.4^{A}	1.8^{A}	1.7^{A}	2.3[1]
Main effect of BHB	3.3^{a}	3.6^{a}	1.1^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 34 and 40 for ANOVA and LSD analysis.

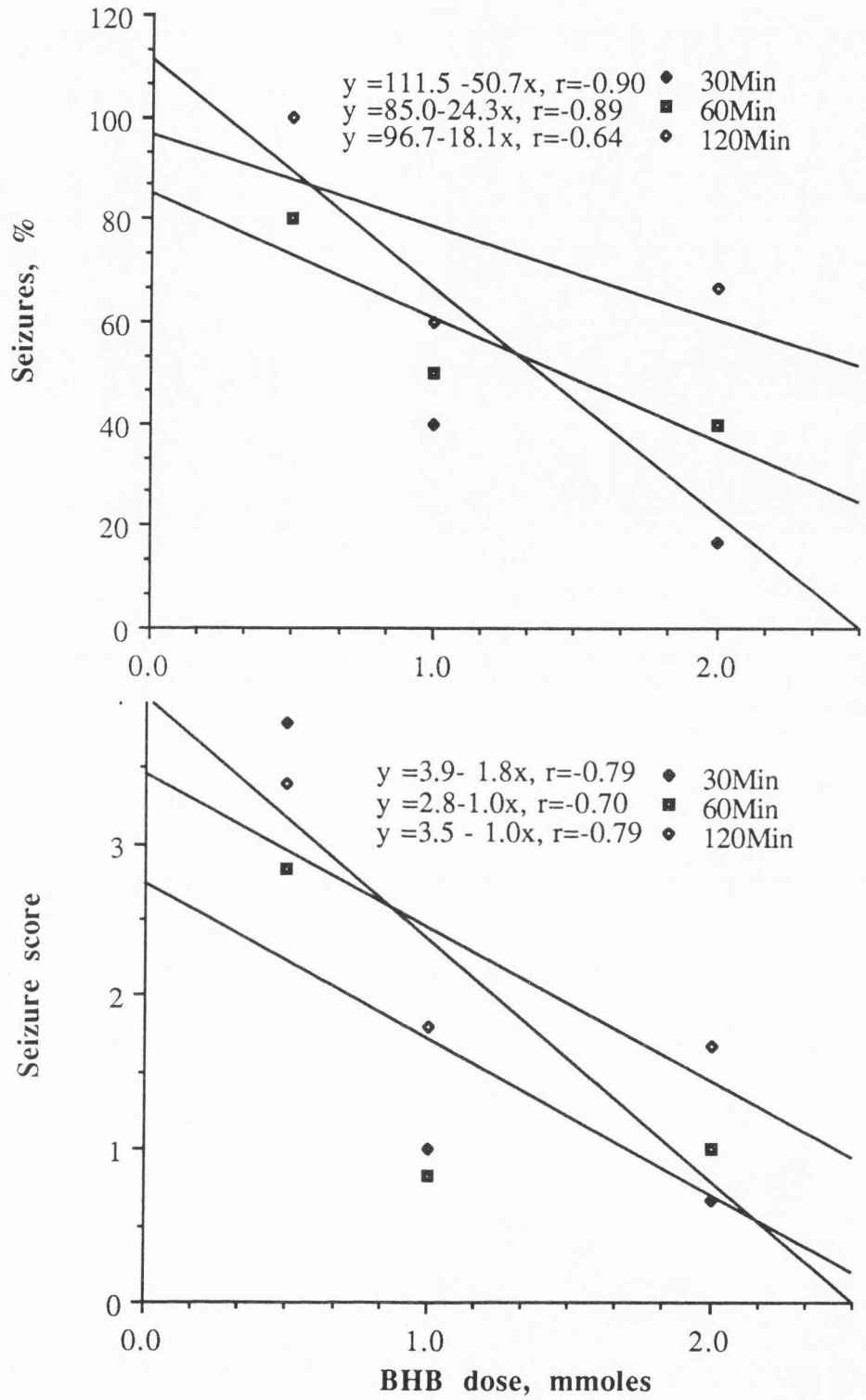

Fig. 8--Effect of gavaging 0.5, 1.0 or 2.0 mmoles of BHB on seizure % and seizure score of magnesium-deficient rats tested at 30, 60 or 120 minutes later (Experiment 3).

Table 18--The effect of gavaging different BHB doses and different time after dosing on serum BHB (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 3)[1].

		BHB, mmoles			Main effect of min after dosing
		0.5	1.0	2.0	
Min after dosing	30	3.50^{A}	8.30^{A}	43.3^{B}	18.4[1]
	60	2.40^{A}	5.50^{A}	14.7^{C}	7.5[2]
	120	1.20^{A}	4.30^{A}	9.40^{A}	5.0[2]
Main effect of BHB		2.40^{a}	6.03^{a}	22.5^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 34 and 40 for ANOVA and LSD analysis.

Table 19--The effect of gavaging different BHB doses and different time after dosing on serum glucose (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 3)[1].

		BHB, mmoles			Main effect of min after dosing
		0.5	1.0	2.0	
Min after dosing	30	118^{A}	95^{B}	80^{C}	98
	60	122^{A}	102^{B}	93^{C}	106[1]
	120	124^{A}	106^{B}	110^{D}	113[1]
Main effect of BHB		121^{a}	106^{b}	94^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 34 and 40 for ANOVA and LSD analysis.

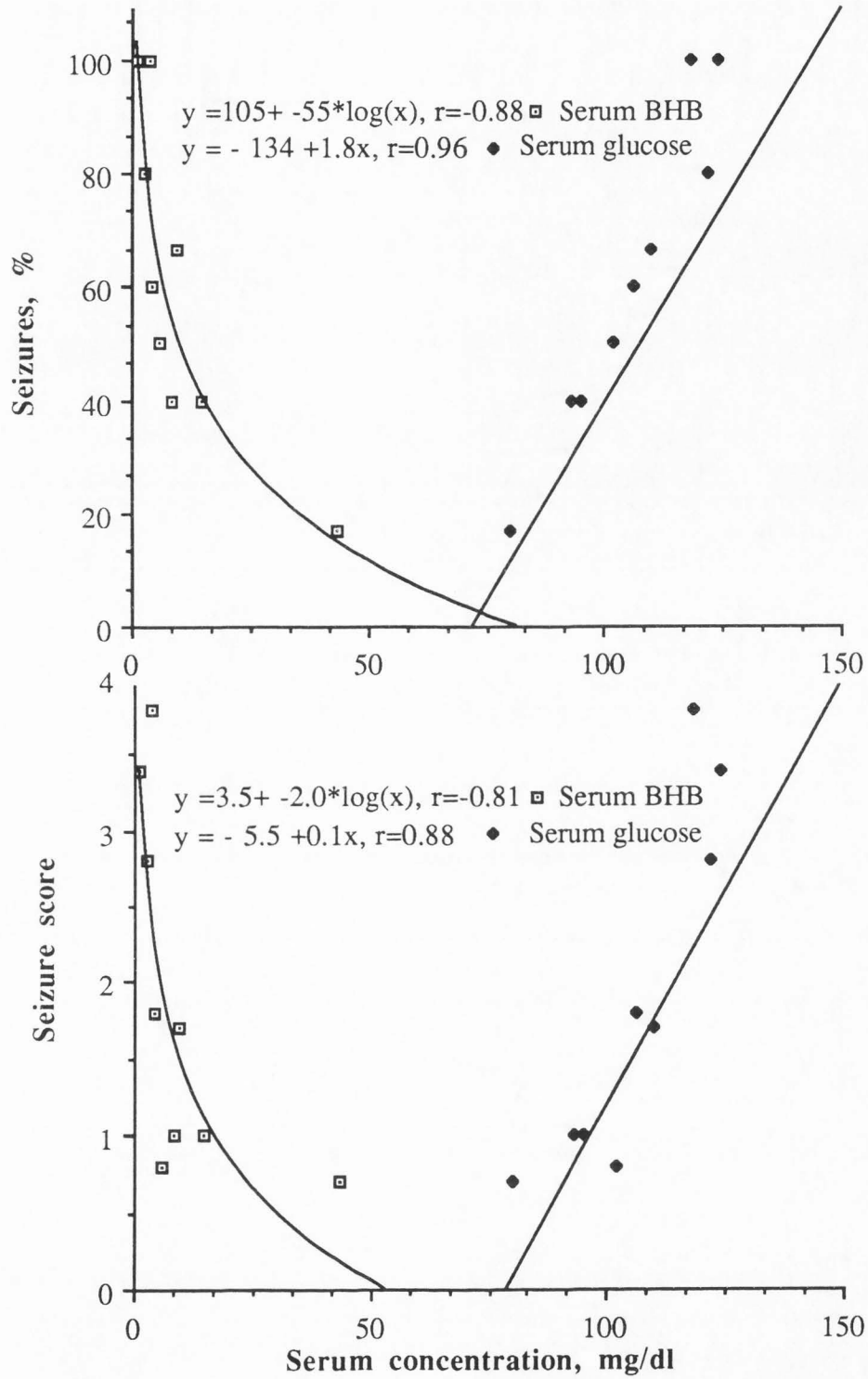

Fig. 9--The relationships between serum BHB and glucose vs. percentag of seizures and seizure scores of rats fed a magnesium-deficient diet for 17 days, gavaged with 0.5, 1.0 or 2.0 mmoles of BHB and tested 30, 60 or 120 minutes later (Experiment 3).

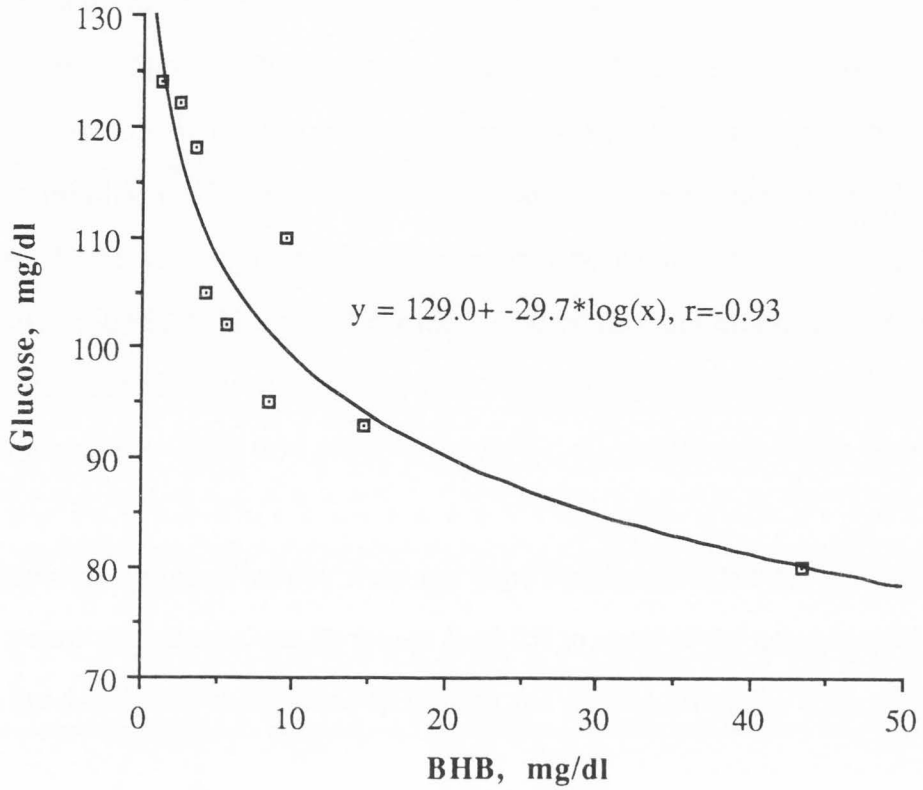

Fig. 10--The relationship between serum BHB and glucose of rats fed a magnesium-deficient diet for 17 days and then gavaged with BHB before seizure testing (Experiment 3).

Figure 11 illustrates the effects of gavaging various levels of BHB and time-after-dosing on seizure percentage. There were high correlations between BHB dose and seizure percentages, 30 min waiting after dosing, r= 0.98, or 0.99 for 1.0, or 2.0 mmoles BHB, respectively, and low correlation with low dose 0.5 mmole of BHB, r= 0.19 (Fig. 11). In the groups tested 60 or 120 minutes after gavaging BHB, serum glucose level was significantly ($p<0.05$) higher and serum BHB was significantly lower ($p<0.05$) compared with the 30-min levels (Table 19). This was especially true for the 1.0 and 2.0 mmole BHB doses. There was a high negative association between time after gavaging BHB and serum BHB, r= -0.99, -0.92 or -0.84 for the 30, 60 or 120-min times, respectively (Fig. 12). In contrast, there was a high positive association between time after gavaging BHB and serum glucose, r= 0.93, 0.94, or 0.99 for the 30, 60, or 120-min times, respectively, (Fig. 12).

The serum mineral values were not significantly affected by gavaging BHB. However, serum phosphorus was decreased ($p<0.05$) in some of the groups tested at 60 or 120 min after dosing due to increased BHB dose and waiting time after dosing (Tables 20 and 21).

Experiment four

In previous experiments; fasting, ketogenic diet and gavaging BHB reduced audiogenic seizure susceptibility in magnesium-deficient rats. The purpose of this experiment was to determine if audiogenic seizure susceptibility would be increased in 24h-fasted animals by gavaging glucose 1.4, 2.8 or 5.6 mmoles. Subgroups were tested for seizure susceptibility at 30, 60 or 120 min after dosing (Groups 5-13, Table 22). The control groups were unfasted sham-gavaged (Group 1) and fasted sham-gavaged (Group 2).

All of the unfasted sham-gavaged rats (Group 1) had audiogenic seizures; and 50 percent of the fasted sham-gavaged rats (Group 2) had seizures (Table 23).

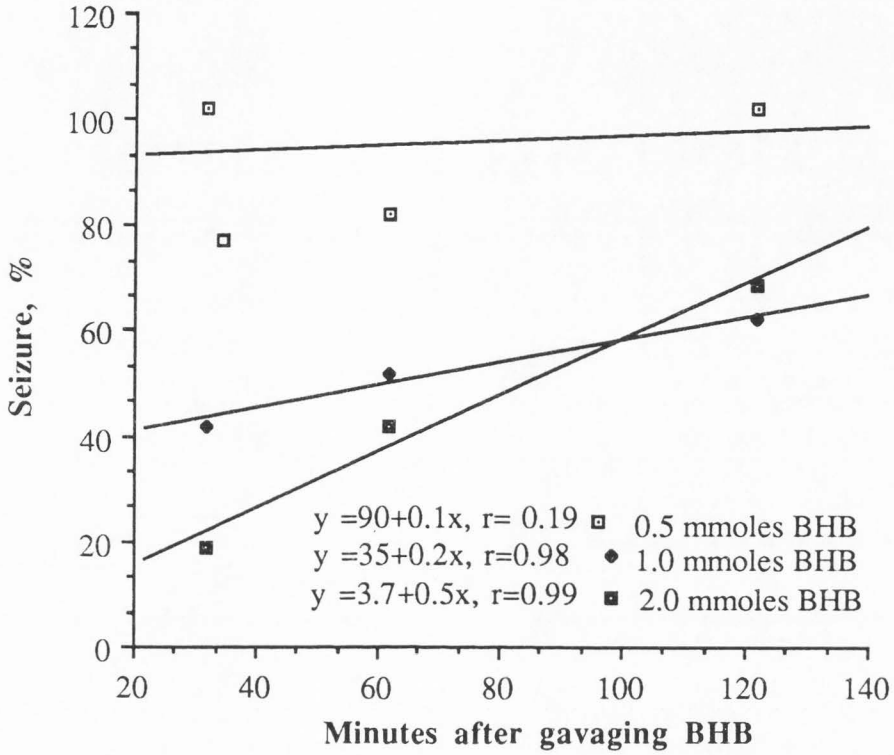

Fig. 11--Effect of gavaging various levels of BHB doses and time after dosing on seizure % of rats fed a magnesium-deficient diet for 17 days (Experiment 3).

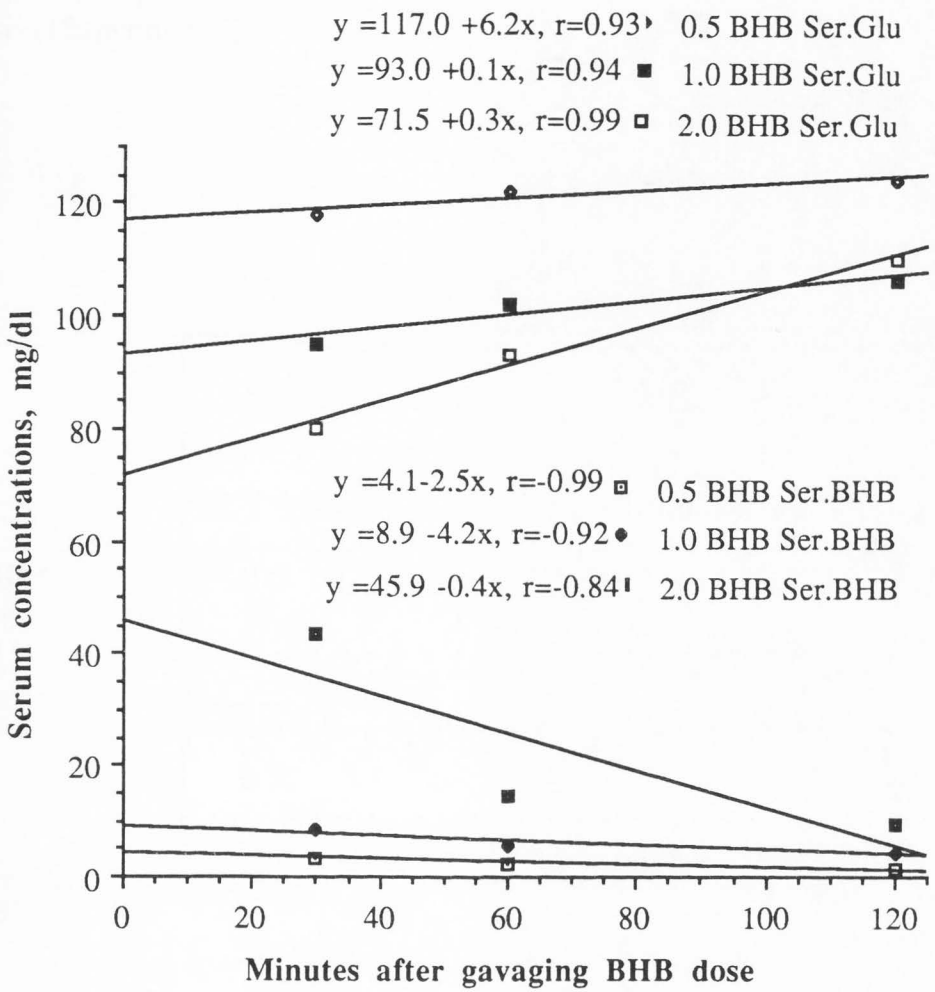

Fig. 12--Effect of waiting time after gavaging 0.5, 1 or 2 mmoles BHB on serum BHB and glucose of rats fed a magnesium-deficient diet for 17 days (Experiment 3).

Table 20--The effect of gavaging different BHB doses and different time after dosing on serum phosphorus (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 3)[1].

		BHB, mmoles			Main effect of min after dosing
		0.5	1.0	2.0	
Min after dosing	30	10.7^{A}	11.4^{B}	10.7^{A}	10.9[1]
	60	10.9^{A}	10.7^{AC}	10.2^{BC}	10.6[1]
	120	9.65^{B}	10.7^{C}	9.81^{B}	10.1[2]
Main effect of BHB		10.4^{a}	10.9^{b}	10.2^{a}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 34 and 40 for ANOVA and LSD analysis.

Table 21--Summary of the effect of gavaging different BHB doses and different time on seizure %, seizure score and serum BHB, glucose and mineral concentrations of weanling rats fed a magnesium-deficient diet (Experiment 3).

Group number	1	2	3	4	5	6	7	8	9	
BHB, mmoles	0.5	0.5	0.5	1	1	1	2	2	2	
Minutes after dosing *	30	60	120	30	60	120	30	60	120	
Number of rats	5	6	5	5	6	5	6	5	6	
Seizure score	3.8[a]	2.8[a]	3.4[a]	1.0[b]	0.8[b]	1.8[b]	0.7[c]	1.0[b]	1.7[b]	
Seizure, % #	100[a]	80[a]	100[a]	40[b]	50[a]	60[a]	17[c]	40[b]	67[a]	
BHB, mg/dl	3.50[a]	2.40[a]	1.20[a]	8.30[ab]	5.50[ac]	4.30[ad]	43.3[e]	14.7[b]	9.40[bcd]	
Glucose, mg/dl	118[a]	122[a]	124[a]	95[b]	102[b]	106[b]	80[b]	93[b]	110[c]	
Mg, mg/dl	0.52	0.52	0.52	0.57	0.59	0.58	0.56	0.58	0.57	NS
Ca, mg/dl	6.93	7.05	6.79	6.95	7.08	7.09	7.26	6.93	7.01	NS
P, mg/dl	10.7[a]	10.9[a]	9.65[b]	11.4[b]	10.7[a]	10.7[a]	10.7[a]	10.2[b]	9.81[b]	
K, mg/dl	27.8	27.0	27.2	27.2	27.7	27.5	27.7	27.2	26.8	NS
Na, mg/dl	151	152	151	152	152	151	152	152	152	NS

[a,b,c,d] Values having different superscripts are significantly different ($p<0.05$). See Appendix B, table17.

NS Means that treatment "F" was not statistically significant ($p< 0.05$).

\# The data were analyzed by Chi-square (see Appendix C, Table 22).

* Minutes after gavaging the BHB.

Table 22--The effect of gavaging n-saline (sham-gavaged), 2.0 mmole ß-hydroxybutyrate (BHB) or 1.4, 2.8 or 5.6 mmoles of glucose on seizure %, seizure score and serum levels of BHB, glucose and minerals of weanling rats fed a magnesium-deficient diet for 17 days and fasted 0 or 24h before dosing (Experiment 4).

Group number	1	2	3	4	5	6	7	8	9	10	11	12	13	
BHB, mmoles	--	--	2.0	--	--	--	--	--	--	--	--	--	--	
Glucose, mmoles	--	--	--	1.4	1.4	1.4	1.4	2.8	2.8	2.8	5.6	5.6	5.6	
Saline, %	0.9	0.9	--	--	--	--	--	--	--	--	--	--	--	
Fasting, h	0	24	24	0	24	24	24	24	24	24	24	24	24	
Minutes after dose	30	30	30	30	30	60	120	30	60	120	30	60	120	
Number of rats	8	8	8	8	7	7	6	7	7	6	6	7	6	
Seizure score	1.8^{a}	0.9^{ac}	0.1^{bc}	2.0^{a}	0.4^{a}	0.6^{ab}	1.8^{bc}	1.7^{abc}	1.3^{ab}	2.5^{bcd}	3.0^{cde}	4.0^{e}	3.5^{de}	
Seizure % #	100^{a}	50^{b}	14^{c}	86^{a}	43^{a}	57^{a}	100^{b}	86^{c}	86^{c}	100^{c}	100^{c}	100^{c}	86^{c}	
BHB, mg/dl	0.70^{a}	24.5^{b}	45.1^{c}	0.60^{a}	20.0^{a}	15.1^{a}	1.40^{b}	5.50^{b}	5.20^{b}	4.90^{b}	0.90^{b}	0.63^{b}	0.60^{b}	
Glucose, mg/dl	91^{a}	44^{b}	79^{c}	117^{d}	45^{a}	53^{a}	83^{b}	108^{c}	109^{c}	116^{c}	132^{d}	144^{d}	161^{d}	
Mg, mg/dl	0.55	0.56	0.56	0.56	0.55	0.58	0.65	0.55	0.54	0.53	0.54	0.52	0.53	NS
Ca, mg/dl	6.87	6.70	7.27	7.06	6.89	6.79	7.22	6.69	6.37	7.24	6.85	7.35	7.04	NS
P, mg/dl	7.34^{a}	7.05^{a}	10.9^{b}	7.24^{a}	7.69^{a}	6.95^{b}	7.13^{a}	6.40^{b}	6.59^{b}	6.87^{b}	6.87^{b}	6.66^{b}	6.35^{b}	
K, mg/dl	26.3^{a}	26.6^{a}	27.8^{b}	26.4^{a}	26.8^{a}	27.0^{a}	28.0^{a}	25.9^{a}	28.4^{b}	28.5^{b}	29.2^{b}	28.7^{b}	28.6^{b}	
Na, mg/dl	155^{a}	155^{a}	152^{a}	155^{a}	160^{a}	158^{ab}	153^{bc}	152^{cd}	154^{bc}	152^{cd}	150^{cd}	147^{d}	149^{cd}	

^{a,b,c,d} Values in the same row of the same block having different superscripts are significantly different ($p<0.05$). See Appendix B, table 41.

NS Means that treatment "F" was not statistically significant ($p<0.05$). See Appendix A, Tables 35 and 36.

\# The data were analyzed by Chi-square test (see Appendix C, Table 46).

Table 23--The effect of gavaging n-saline (sham-gavaged), 2.0 mmoles ß-hydroxybutyra (BHB) or 1.4 mmoles of glucose on seizure %, seizure score and serum levels of BHB, glucose and minerals of weanling rats fed a magnesium-deficient diet for 17 days and fasted 0 or 24h before dosing (Experiment 4).

Group number	1	2	3	4	
BHB, mmoles	--	--	2.0	--	
Glucose, mmoles	--	--	--	1.4	
Saline, %	0.9	0.9	--	--	
Fasting, h	0	24	24	0	
Minutes after dose	30	30	30	30	LSD
Number of rats	8	8	8	8	8,8
Seizure score	1.8[a]	0.9[ac]	0.1[bc]	2.0[a]	1.30
Seizures, % *	100[a]	50[b]	14[c]	86[a]	--
BHB, mg/dl	0.70[a]	24.5[b]	45.1[c]	0.60[a]	12.9
Glucose, mg/dl	91[a]	44[b]	79[c]	117[d]	11.5
Mg, mg/dl	0.55[a]	0.56[a]	0.56[a]	0.56[a]	0.05
Ca, mg/dl	6.87[a]	6.70[a]	7.27[b]	7.06[b]	0.40
P, mg/dl	7.34[a]	7.05[a]	10.9[b]	7.24[a]	0.50
K, mg/dl	26.3[a]	26.6[a]	27.8[b]	26.4[a]	1.10
Na, mg/dl	155[a]	155[a]	152[a]	155[a]	3.40

[a,b,c,d] Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 35 and 41 for ANOVA and LSD analysis.

* The data were analyzed by Chi-square (see Appendix C, Table 46).

Serum BHB was 0.7 mg/dl in the unfasted sham-gavaged rats as compared with 24.5 mg/dl in the sham-gavaged, 24-h fasted rats (Table 23, Groups 1 vs Group 2). In contrast, serum glucose was 91 mg/dl in the sham-gavaged, unfasted rats as compared with 44 mg/dl in the sham-gavaged, 24-h fasted ones (Table 23, Group 1 vs. Group 2). There were no significant effects on serum minerals due to fasting.

Fasting and gavaging with BHB reduced seizure susceptibility (Table 23, Group 3). Rats that received 2.0 mmoles BHB and were fasted for 24h (Group 3) had decreased ($p<0.05$) seizure score, 0.1, and seizure incidence, 14 percent, compared with sham-gavaged rats (Group 2); although the reduction in seizure score was not statistically significant. The 24-h fasted rats gavaged with 2.0 mmoles BHB (Group 3) had higher ($p<0.05$) serum BHB 45.1 mg/dl, and lower ($p<0.05$) serum glucose, 79 mg/dl, as compared with the fasted, sham-gavaged (Group 2) that had 24.5 and 44.0 mg/dl of BHB and glucose, respectively, and as compared with the unfasted sham-gavaged rats (Group 1) that had very low BHB, 0.7 mg/dl; and high glucose, 91 mg/dl (Table 23). Rats that received 2.0 mmole BHB increased ($p<0.05$) serum phosphorus compared with rats that received saline (Table 23, Group 2 vs. 3).

Gavaging glucose to unfasted rats did not affect ($p>0.05$) seizure incidence or seizure score (Table 23, Group 1 vs. 4). The unfasted rats that received 1.4 mmoles glucose (Group 4) had higher ($p<0.05$) seizure incidence and seizure score as seizure incidence and seizure score in unfasted sham-gavaged rats (Group 1). Serum glucose was higher ($p<0.05$); while serum BHB was not different ($p<0.05$) from the sham-gavaged rats (Group 1).

The effect of increased serum glucose levels on seizure susceptibility of rats fasted for 24-h after being fed a magnesium-deficient diet for 17 days is shown in (Table 22, Groups 5-13). The rats were tested for audiogenic seizures 30, 60 or 120 minutes after gavaging 1.4, 2.8 or 5.6 mmoles of glucose. Seizure incidence increased ($p<0.05$) from 43 percent to 86 percent, to 100 percent in the rats dosed with 1.4, 2.8 or 5.6 mmoles glucose;

respectively, and tested 30 min later (Table 24). Consistently, seizure score also increased ($p<0.05$) from 0.4 to 1.7 to 3.0 with glucose dose (Table 25, Groups 5, 8 and 11 in Table 22). In addition, increasing the testing time after glucose dosing to 120 min increased ($p<.05$) seizure incidence and seizure score at all levels of glucose dosing (Tables 24 and 25, Groups 7, 10 and 13 in Table 24). Fig. 13 depicts the acute effects of gavaging glucose on seizure incidence and seizure score. There were high correlations between glucose doses 30 or 60 min after gavaging, and seizure percentages, $r= 0.99$, or 0.94 for 1.4 or 2.8 mmoles glucose doses, respectively. There were also high correlations between glucose doses 30 or 60 minutes after gavaging, and seizure score, $r= 0.97$, 0.78 for 1.4 or 2.8 mmoles glucose doses, respectively (Fig. 13). In contrast, poor correlations were found between glucose dose among the groups of rats tested 120 minutes after gavaging glucose vs. percent seizure and seizure score, $r= 0.0$ and 0.33, respectively (Fig. 13).

Gavaging glucose drastically affected ($p<0.05$) serum metabolites of the 24-h fasted, magnesium-deficient rats (Tables 26 and 27). Serum glucose ranged from 45 to 161 mg/dl; varying with glucose dose and time of seizure testing (Table 22). In contrast, as glucose dose increased, serum BHB decreased ($p<0.05$); ranging from 20.0 to 0.6 mg/dl depending on glucose dose and time of testing (Table 26). As glucose dose was increased, serum glucose increased ($r= 0.98$) and serum BHB decreased ($r= -0.99$, Fig. 14) 30 minutes after gavaging. Increasing time after gavaging resulted in higher serum glucose concentrations and lower serum BHB concentrations at all levels of glucose gavaged (Tables 26 and 27). For all of the data combined, there was a high negative correlation between serum glucose and serum BHB, $r= -0.86$ (Fig. 15). For all of the data combined, animals that seized had low serum BHB and high serum glucose (Appendix D, Table 51).

Serum magnesium and calcium concentrations were not affected in the 24-h fasted rats by any of the glucose doses ($p>0.05$) or time after gavaging (Table 22). There were

Table 24--The effect of gavaging different glucose doses and different time after dosing on audiogenic seizure susceptibility (seizure, %) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 4)[1].

Min after dosing	glucose, mmoles 1.4	2.8	5.6	Main effect of min after dosing
30	43^{A}	86^{BC}	100^{BC}	76
60	57^{C}	86^{BC}	100^{BC}	81[1]
120	100^{BC}	100^{BC}	86^{BC}	95[1]
Main effect of glucose	67^{a}	91^{b}	95^{c}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendix C, Table 46 for Chi-square test.

Table 25--The effect of gavaging different glucose doses and different time after dosing on seizure score of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 4)[1].

Min after dosing	glucose, mmoles 1.4	2.8	5.6	Main effect of min after dosing
30	0.4^{A}	1.7^{B}	3.0^{C}	1.7[1]
60	0.6^{A}	1.3^{B}	4.0^{C}	1.9[1]
120	1.8^{B}	2.5^{B}	3.5^{C}	2.6[1]
Main effect of glucose	0.9^{a}	1.8^{ab}	3.5^{ab}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 36 and 41 for ANOVA and LSD analysis.

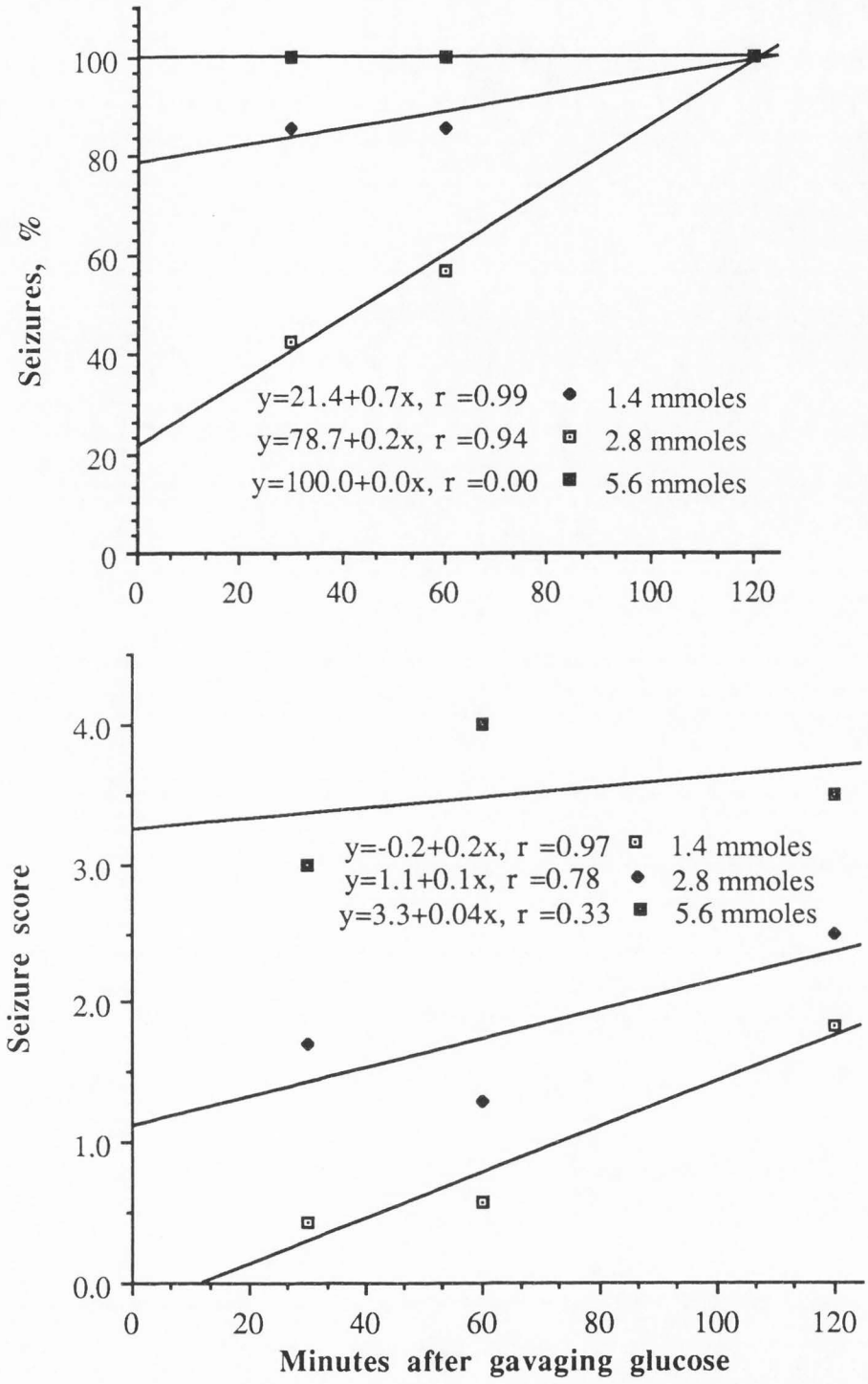

Fig. 13--Effect of gavaging 1.4, 2.8 or 5.6 mmoles of glucose on the percentage of seizures and seizure scores of rats fed a magnesium-deficient diet for 17 days and fasted for 24h before dosing (Experiment 4).

Table 26--The effect of gavaging different glucose doses and different time after dosing on serum glucose (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 4)[1].

Min after dosing	glucose, mmoles 1.4	2.8	5.6	Main effect of min after dosing
30	45^{A}	108^{B}	132^{C}	95[1]
60	53^{A}	109^{B}	144^{C}	102[1]
120	83^{D}	116^{B}	161^{C}	120[2]
Main effect of glucose	60^{a}	111^{b}	146^{c}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 36 and 41 for ANOVA and LSD analysis.

Table 27--The effect of gavaging different glucose doses and different time after dosing on serum BHB (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 4)[1].

Min after dosing	glucose, mmoles 1.4	2.8	5.6	Main effect of min after dosing
30	20.0^{A}	5.50^{AB}	0.90^{AB}	8.80[1]
60	15.1^{AB}	5.20^{AB}	0.63^{AB}	7.00[1]
120	1.4^{AB}	4.9^{AB}	0.60^{AB}	2.3[2]
Main effect of glucose	12.2^{a}	5.2^{b}	0.7^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 36 and 41 for ANOVA and LSD analysis.

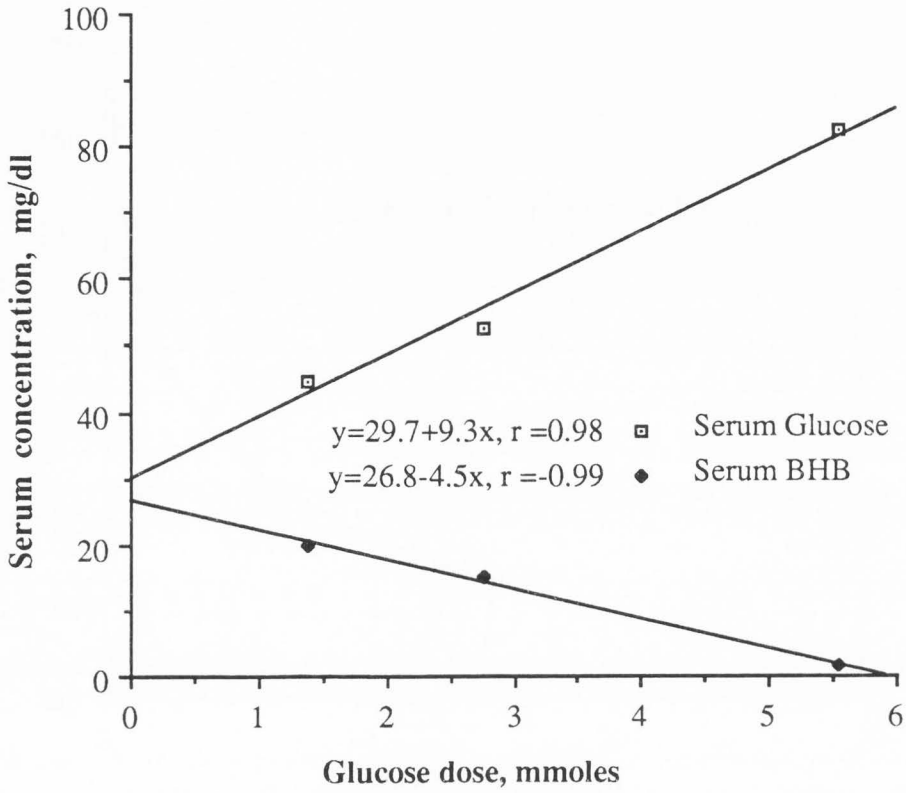

Fig. 14--The effect of gavaging 1.4, 2.8 or 5.6 mmoles of glucose on serum BHB and glucose concentrations of rats fed a magnesium-deficient diet for 17 days and fasted for 24h (Experiment 4).

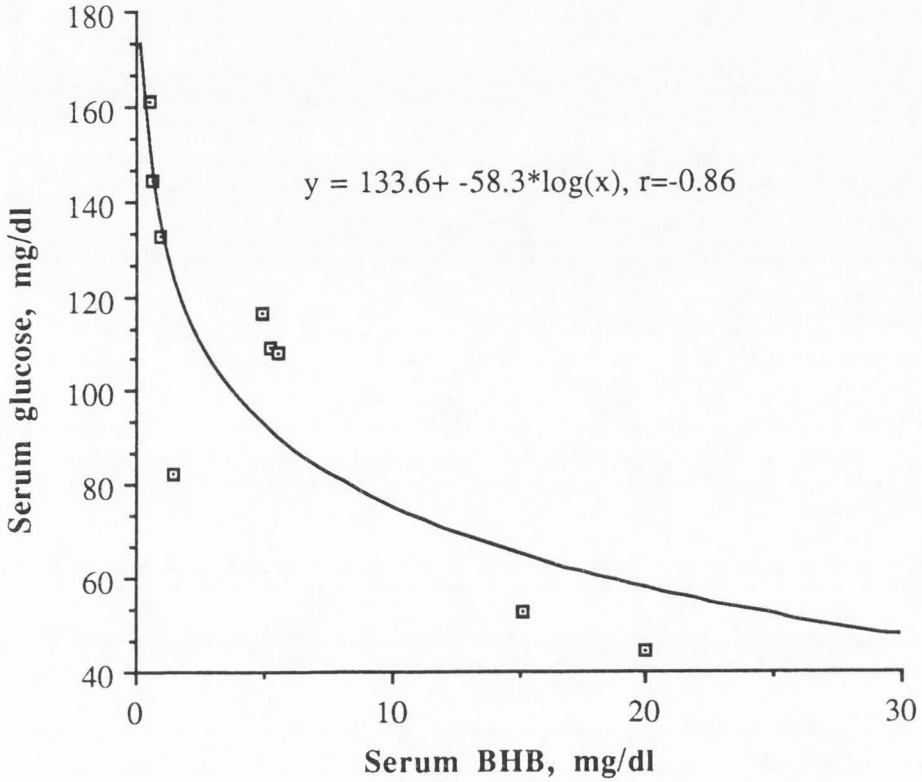

Fig. 15--The relationship between serum BHB and glucose after gavaging glucose to rats fed a magnesium-deficient diet for 17 days and fasted for 24h (Experiment 4).

significant increases in the potassium (Table 28) and significant decreases in the phosphorus and sodium by increasing glucose dose (Tables 29 and 30).

Rats gavaged with 2.8 or 5.6 mmoles glucose (Groups 8 or 11 in Table 22) had higher seizure incidence, 86 or 100 percent, as compared with 43 percent seizure in the 24-h fasted rats given 1.4 mmoles glucose (Group 5) when tested 30 min after gavaging (Table 22). Seizure score was also higher ($p<0.05$); 1.7 or 3.0 (Groups 8 or 11); as compared with a score of 0.4 for the rats given 1.4 mmole glucose (Group 5) paralleling seizure incidence.

Fasting clearly decreased percentage of seizures and seizure scores, 43 percent and 0.4, in the rats gavaged with 1.4 mmole glucose (Group 5); as compared with 86 percent seizures and 2.0 seizure score without fasting (Group 4 in Table 22). Similarly, 24-h fasted rats given 1.4 mmole glucose (Group 5) had a lower percentage of seizures and seizure score than the unfasted sham-gavaged rats (Group 1). Conversely, there was no significant difference in seizure incidence and seizure score between rats given 1.4 mmole glucose and then sham-gavaged after a 24-h fast (Groups 5 vs. 2 in Table 22).

Fasting decreased ($p<0.05$) the serum glucose and increased the serum BHB (Group 1 vs. 2). Serum glucose was 117 mg/dl in the unfasted rats given 1.4 mmole glucose (Group 4); as compared with 45 mg/dl in the 24-h fasted rats (Group 5), and BHB, 0.6 mg/dl, was lower ($p<0.05$) in the unfasted rats given 1.4 mmoles glucose (Group 4); as compared with 20.0 mg/dl in the 24-h fasted rats (Group 5) as shown in Table 22.

Experiment five

As noted in prior experiments, rats that had high seizure incidence had low serum BHB and high glucose. Thus, in this experiment both glucose and BHB were gavaged to weanling rats fed a magnesium-deficient diet and fasted for 24h to see if rats with high serum levels of both glucose and BHB were susceptible to audiogenic seizures (Table 31).

Table 28--The effect of gavaging different glucose doses and different time after dosing on serum potassium (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 4)[1].

Min after dosing	glucose, mmoles 1.4	2.8	5.6	Main effect of min after dosing
30	26.8^{A}	25.9^{A}	29.2^{B}	27.3[1]
60	27.0^{A}	28.4^{B}	28.7^{B}	28.0[1]
120	28.0^{B}	28.5^{B}	28.6^{B}	28.6[2]
Main effect of glucose	27.3^{a}	27.6^{a}	28.8^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 36 and 41 for ANOVA and LSD analysis.

Table 29--The effect of gavaging different glucose doses and different time after dosing on serum phosphorus (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 4)[1].

		glucose, mmoles			Main effect of min after dosing
		1.4	2.8	5.6	
Min after dosing	30	7.69^{A}	6.40^{B}	6.87^{B}	6.99[1]
	60	6.95^{B}	6.59^{B}	6.66^{B}	6.73[1]
	120	7.13^{AB}	6.87^{B}	6.35^{B}	6.78[1]
Main effect of glucose		7.25^{a}	6.62^{b}	6.63^{b}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 36 and 41 for ANOVA and LSD analysis.

Table 30--The effect of gavaging different glucose doses and different time after dosing on serum sodium (mg/dl) of weanling rats fed a magnesium-deficient diet for 17 days (Experiment 4)[1].

Min after dosing	glucose, mmoles 1.4	2.8	5.6	Main effect of min after dosing
30	160^{B}	152^{AB}	150^{AB}	154[1]
60	158^{AB}	154^{AB}	147^{AB}	153[1]
120	153^{AB}	152^{AB}	149^{AB}	151[1]
Main effect of glucose	157^{a}	153^{b}	149^{c}	

[1]Values having different superscripts are significantly different ($p<0.05$). See Appendices A and B, Tables 36 and 41 for ANOVA and LSD analysis.

Table 31--The effect of gavaging 0, 0.5 or 2.0 mmoles ß-hydroxybutyrate (BHB) and/or 0, 1.4 or 5.6 mmoles of glucose in 24h fasted rats fed magnesium-deficient diet for 17 days on seizure %, seizure score and serum BHB, glucose and mineral levels (Experiment 5) @.

Group number	1^	2#	3	4	5	6	7	8	
Fasting, h	0	0	24	24	24	24	24	24	
Number of rats	10	9	8	9	10	9	10	9	
BHB, mmoles	--	--	2.0	0	0.5	2.0	0.5	2.0	
Glucose, mmoles	--	--	--	5.6	5.6	5.6	1.4	1.4	
Saline, %	--	.9	--	--	--	--	--	--	LSD
Seizure score	3.4^{a}	2.4^{a}	0.3^{b}	2.8^{a}	2.9^{a}	2.4^{a}	2.1^{a}	1.8^{a}	
Seizure, % *	100^{a}	89^{a}	25^{b}	89^{a}	80^{a}	78^{a}	56^{c}	50^{c}	
BHB, mg/dl	0.80^{a}	0.60^{a}	40.6^{b}	0.80^{a}	1.40^{a}	7.70^{c}	20.6^{d}	20.9^{d}	
Glucose, mg/dl	93^{a}	92^{a}	63^{b}	136^{c}	142^{c}	120^{d}	93^{a}	95^{a}	
Mg, mg/dl	0.59	0.57	0.61	0.59	0.58	0.52	0.59	0.59	NS
Ca, mg/dl	5.75	6.42	6.15	6.62	6.45	6.09	6.32	6.04	NS
P, mg/dl	6.44^{a}	6.01^{a}	10.1^{b}	6.69^{ac}	7.21^{ad}	7.86^{cde}	8.88^{e}	8.78^{e}	
K, mg/dl	27.6^{a}	26.8^{a}	24.7^{b}	27.1^{a}	26.5^{a}	26.8^{a}	27.3^{a}	26.4^{a}	
Na, mg/dl	152	152	151	154	157	154	154	153	NS

a,b,c,d Values having different superscripts are significantly different ($p<0.05$) see Appendix B, Table 42.

NS means that treatment "F" was not statistically significant ($p<0.05$) see Appendix A, Table 37.

\# Sham-gavaged controls given 0.9 % saline

^ Untreated controls.

@ The animals were tested for seizures 30 min after gavaging and then blood was drawn

* The data were analyzed by Chi-square (see Appendix C, Table 47).

The effect of gavaging glucose and BHB on seizure incidence, serum BHB, glucose and mineral levels of 24-h fasted, magnesium-deficient rats is presented in Table 31.

Rats gavaged with normal saline did not differ ($p>0.05$) in seizure susceptibility, serum metabolites or serum minerals; as compared with magnesium-deficient rats (Table 31, Groups 1 vs. 2). Therefore, gavaging has no effect on audiogenic seizure susceptibility of unfasted rats.

Fasted rats gavaged with 2.0 mmole BHB (Group 3) had a lower ($p<0.05$) seizure score, 0.3, and seizure incidence, 25 percent, than unfasted magnesium-deficient rats (Groups 1 and 2) that had high seizure scores and seizure incidence, 3.4 and 2.4 and 100 percent or 89 percent, respectively (Table 31).

Rats gavaged with 5.6 mmol glucose (Group 4) showed a higher ($p<0.05$) seizure score, 2.8 vs. 0.3, and seizures, 89 percent, vs. 25 percent, compared with rats gavaged with 2 mmol BHB (Group 3). Fasted rats gavaged with 5.6 mmoles glucose (Group 4) had higher serum glucose ($p<0.05$), 136 mg/dl, and low serum BHB, 0.8 mg/dl, (Group 4 vs. 3, Table 31). There was a high positive association between serum glucose and seizure percentage or seizure score, $r= 0.92$ and 0.95; respectively (Fig. 16). There were no significant effects on serum Mg, Ca or Na by gavaging 5.6 mmole glucose to fasted, magnesium-deficient rats, but the group gavaged with 2.0 mmole BHB had increased serum phosphorus and lower serum potassium (Table 31). Fig. 17 shows a high association between serum glucose and serum phosphorus, $r= -0.98$, and a modest association between serum glucose vs. serum calcium and potassium, $r= 0.61$ and 0.65, respectively. Fig. 18 shows the negative correlation between serum phosphorus and potassium, $r= -0.67$. In addition, there was a high negative association between serum phosphorus and seizure percentage, $r= -0.98$, and a slightly positive association with potassium, $r= 0.76$ (Fig. 19).

In Fig. 20, rats given 5.6 mmole glucose and 2 mmol of BHB in the same dose (Group 5) had a high seizure score, 2.4, compared with rats given just 2 mmole BHB

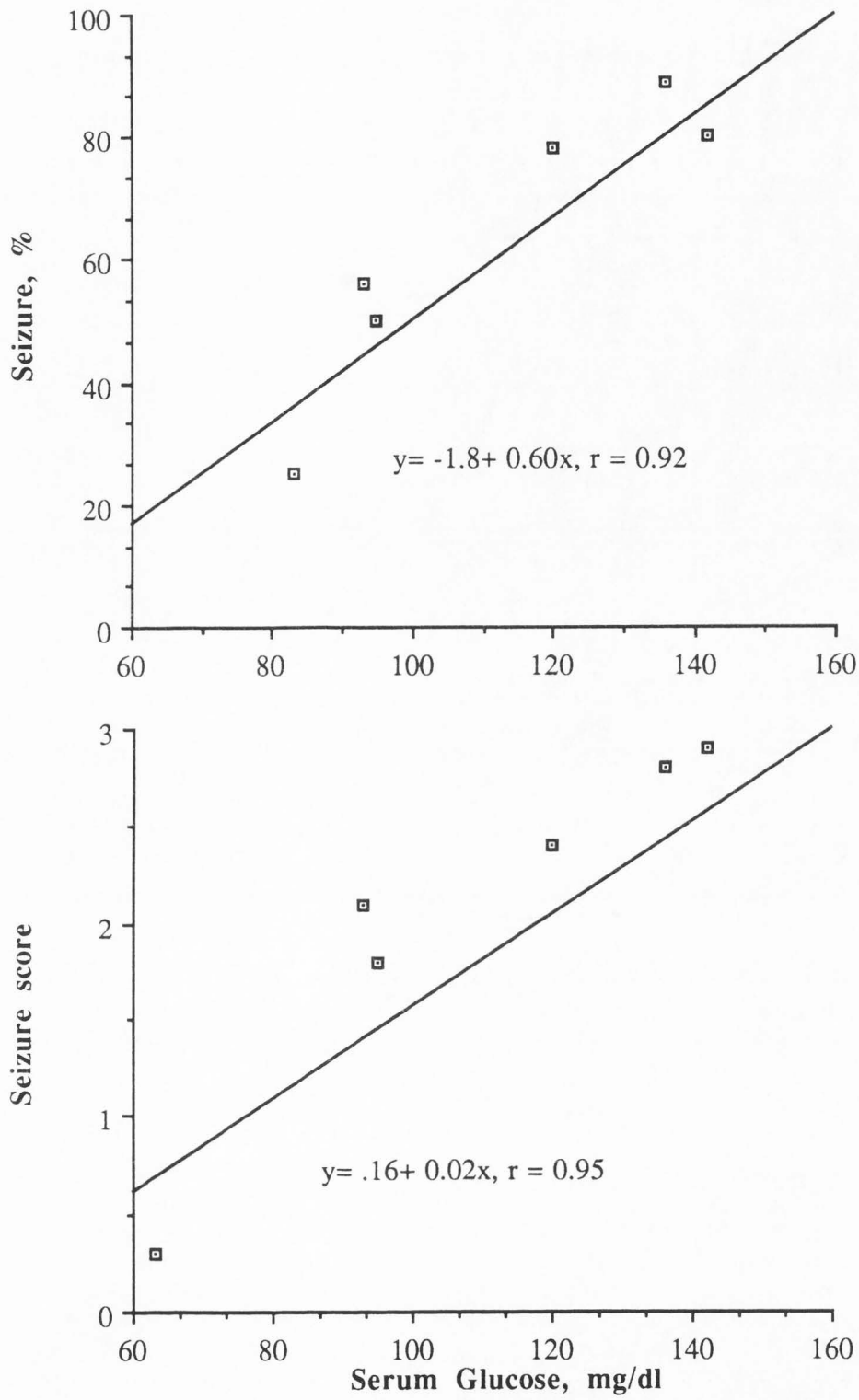

Fig. 16--The relationship between serum glucose and percentage of seizures or seizure score after gavaging glucose to rats fed a magnesium-deficient diet for 17 days and fasted for 24h (Experiment 5).

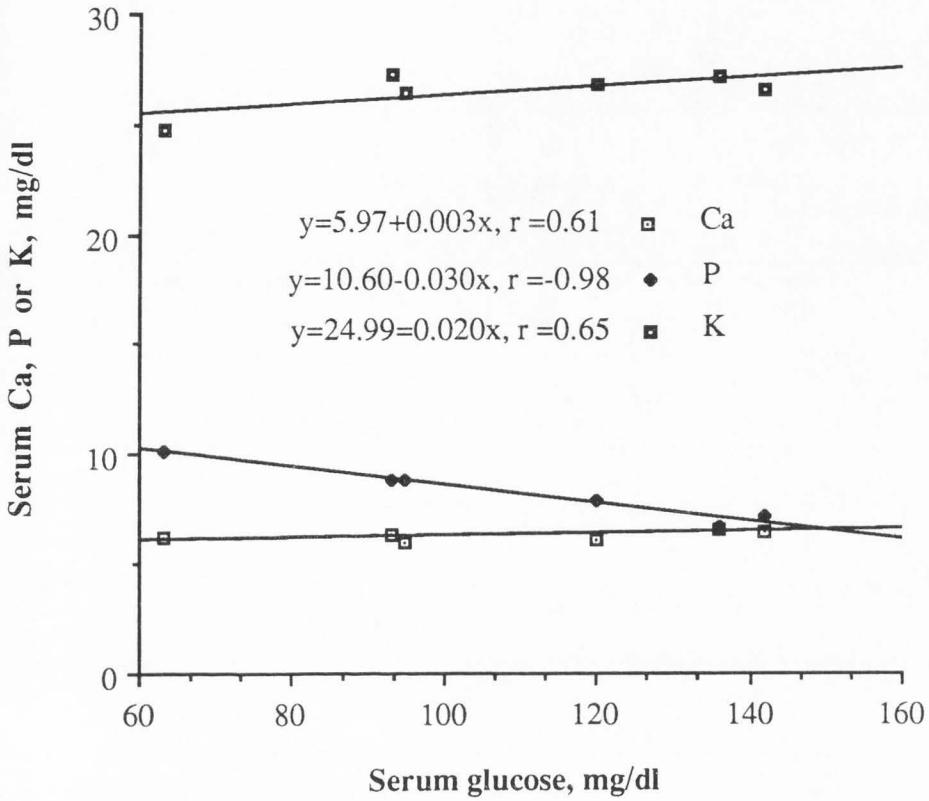

Fig. 17--The relationship between serum glucose and minerals after gavaging glucose to rats fed a magnesium-deficient diet for 17 days and fasted for 24h (Experiment 5).

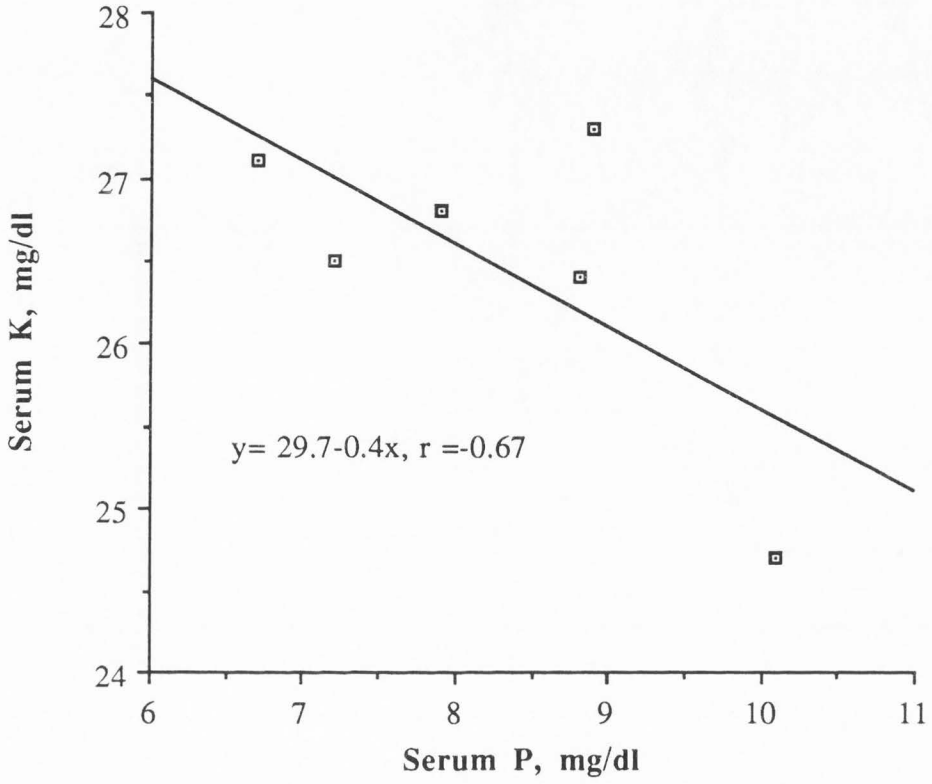

Fig. 18--The relationship between serum phosphorus (P) and potassium (K) after gavaging glucose to rats fed a magnesium-deficient diet for 17 days and fasted for 24h (Experiment 5).

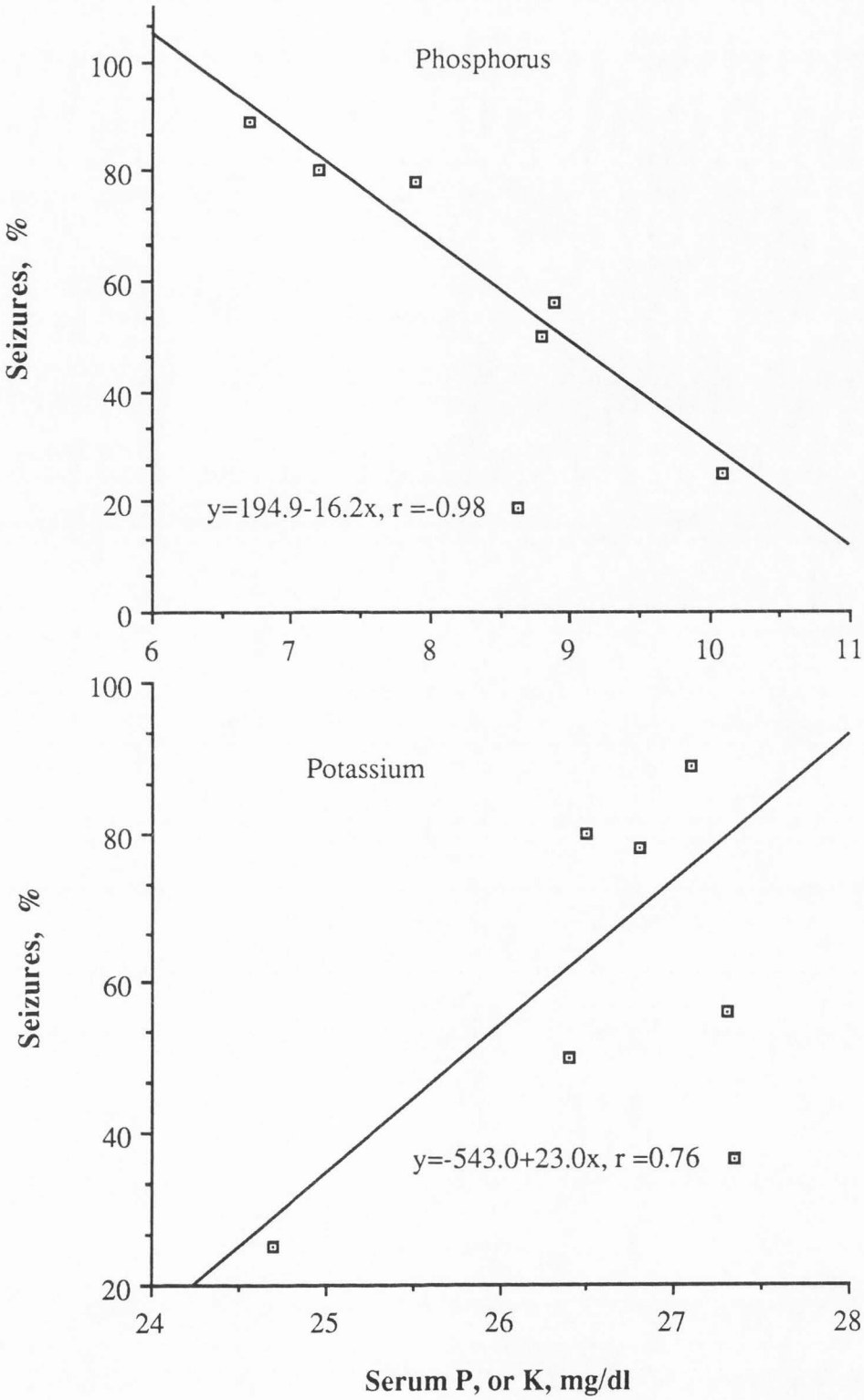

Fig. 19--The relationship between serum phosphorus or potassium and seizure % after gavaging glucose to rats fed a magnesium-deficient diet for 17 days and fasted for 24h (Experiment 5).

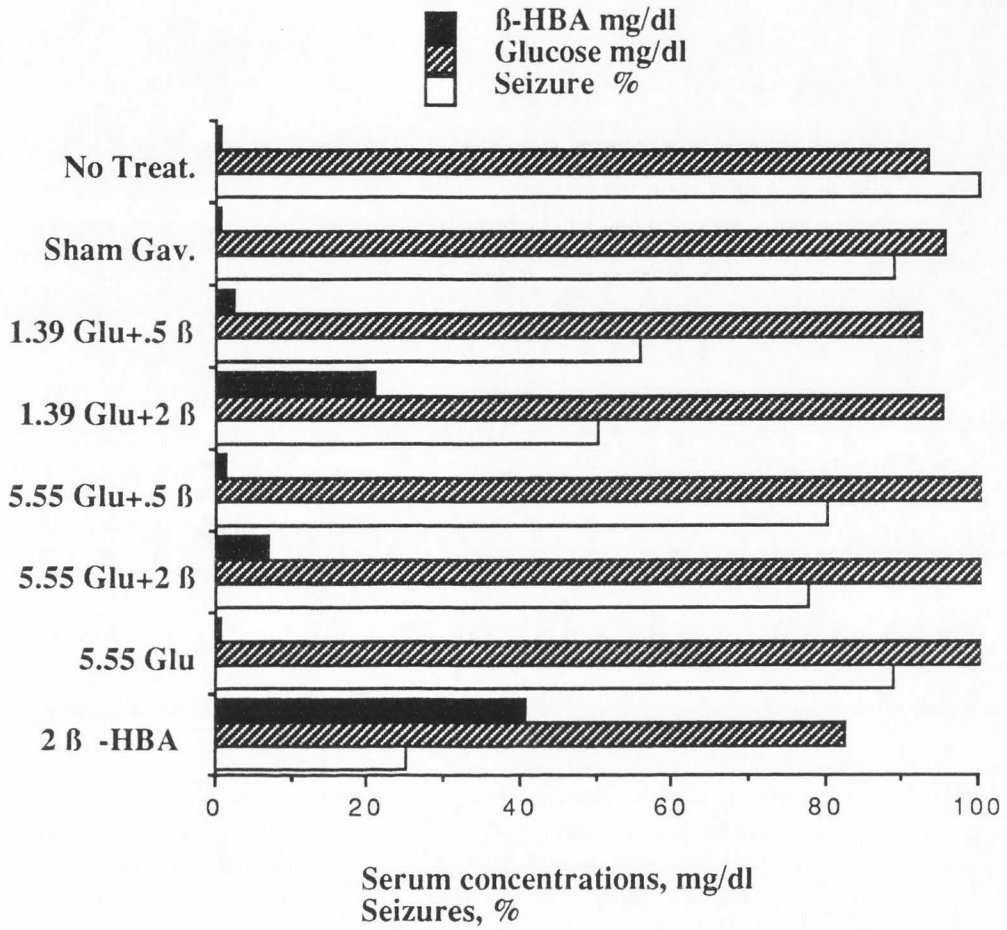

Fig. 20--Effect of gavaging 0, 0.5 or 2.0 mmoles BHB and/or 0, 1.4 or 5.6 mmoles glucose on seizure %, serum glucose and serum BHB levels of rats fed a magnesium-deficient diet for 17 days and fasted for 24h. The untreated and sham-gavaged (0.9% saline) animals were not fasted. The animals were tested for seizures 30 min after gavaging and then blood was drawn. (Experiment 5).

(Group 3) that had a seizure score of 0.3. In contrast, there was no significant difference in seizure score by comparing group 5 with rats given 5.6 mmoles glucose only (Group 4). Consistently, rats given 5.6 mmole glucose and 2 mmole BHB in the same dose (Group 5) had high seizure incidence, 78 percent, compared with rats given just 2 mmoles BHB (Group 3) that had a seizure incidence of 25 percent (Table 31, Fig. 20). In contrast, there was no significant difference in seizure incidence by comparing group 5 with rats given 5.6 mmole glucose (Group 4) only (Table 31, Fig. 20). Rats gavaged with 5.6 mmole glucose with 0.5 mmole BHB (Group 6) had a higher ($p<.05$) seizure score, 2.9, and seizure incidence, 80 percent as compared with rats given just 2 mmole BHB (Group 3) that had a 0.3 seizure score and 25 percent seizure incidence. In contrast, rats given 5.6 mmole glucose and 0.5 mmoles BHB (Group 6) showed no significant difference in seizure score and seizure incidence; as compared with rats that received just 5.6 mmole glucose (Group 5) due to the lowering BHB in the dose. Rats given 1.4 mmole glucose and 2 mmole BHB (Group 7) had a 1.8 seizure score and 50 percent seizure incidence; as compared with rats given 5.6 mmole glucose (Group 4) and 2 mmole BHB (Group 3) or as compared with rats that received 5.6 mmole glucose only (Group 4). Rats given 0.5 mmole BHB and 1.4 mmole glucose in the same dose (Group 8) did not have a significantly altered seizure score and seizure incidence; as compared with Groups 4, 5, 6, and 7 (Table 31). In contrast, there was a significant difference in seizure incidence and seizure score between rats that received 2.0 mmole BHB and 1.4 mmole glucose (Group 6) and rats which received 2.0 mmole BHB alone (Group 3). Serum metabolites were affected by increasing or decreasing the BHB or glucose dosed (Table 31, Fig. 20). Clearly, gavaging high or low glucose with high or low BHB in the same dose decreased serum BHB and increased serum glucose significantly (Table 31 and Fig. 18). Rats gavaged with 5.6 mmole glucose and 2 mmole BHB (Group 5) had decreased ($p<0.05$) serum BHB and increased ($p<0.05$) serum glucose; as compared with the rats given 1.4 mmole glucose and 2 mmole BHB (Group 7). Rats gavaged with 0.5 mmole BHB and 5.6 mmole glucose (Group 6) did not

different ($p>0.05$) serum BHB, while serum glucose was different ($p<0.05$) as compared with the rats given 0.5 mmole BHB and 1.4 mmole glucose (Group 8).

From the previous comparisons, two conditions appear necessary to prevent seizure incidence: 1) high serum BHB and 2) low serum glucose. This conclusion is based on the following: 1) Group 8 (Table 31), which was gavaged with high BHB and low glucose, had low seizure incidence. 2) Group 5 (Table 31), gavaged with low BHB and high glucose, had high seizure incidence. 3) Group 7 (Table 31), gavaged with low BHB and low glucose, had medium seizure incidence. 4) Group 5 (Table 31), gavaged with high BHB and high glucose, had medium seizure incidence. From these observations; high serum BHB and high serum glucose were tried as an anticonvulsant but failed due to the inhibition of ketone bodies by glucose (Appendix D, Table 52).

There was a high association between serum concentrations of BHB or glucose and seizure percentage in all groups (Fig. 16). Increasing serum BHB to 40 mg/dl by gavaging the rats with 2 mmole BHB (Group 3) decreased seizure percentage to 25. In contrast, seizure percentage increased drastically to 100 in magnesium-deficient rats (Group 1), sham-gavaged rats (Group 2) and in rats given 5.6 mmole glucose (Group 4); which had low serum BHB 0.8, 0.6 or 0.8 and high serum glucose, 93, 92 or 136, respectively; (Fig. 20) and Fig. (16) also show a high positive correlation between serum glucose and percent seizures, $r = 0.92$. A similar association was found in the rats gavaged with a combination of glucose and BHB. Higher serum BHB, 20.9 mg/dl, was found in rats given 1.4 mmoles glucose (Group 7) and 2 mmole BHB; which had low seizure incidence, 50 percent, compared with rats given 5.6 mmole glucose and 0.5 mmole BHB (Group 6) that had a seizure incidence of 80 percent (Table 31).

CHAPTER V
DISCUSSION

Methodology

Seizures reportedly happen in rats after 2-7 days on magnesium-deficient diets (Chutkow, 1972; Chutkow and Grabow, 1972; Chutkow and Meyers, 1968). However, others have reported that seizures did not occur until after 12 days of a magnesium-deficient diet (Aikawa, 1973; Buck, et al., 1976; Caddell, 1978). Therefore, the objective of part one of the first experiment was to see when magnesium-deficient rats start to seize. Seizure susceptibility began after 12 days of feeding the magnesium-deficient diet (Fig. 1). In addition, the severity of seizures was greatest on day 17 (Table 5). This finding is consistent with the findings of Buck et al. (1978) and Caddell (1978). A number of investigators reported lowered magnesium concentrations in serum, cerebrospinal fluid or body tissues due to low dietary magnesium. The lowering of magnesium appears first in serum, followed by a lowering in cerebrospinal fluid concentrations (Allsop and Pauli, 1985; Buck et al., 1976; Fisher et al., 1985; Leaver et al., 1987). A sharp decrease in serum magnesium, with change occurring in cerebrospinal fluid, is accompanied by a marked increase in nervous system hyperexcitability and seizure activity (Bartrup and Stone, 1988; Buck et al., 1978; Chutkow, 1974; Leaver et al., 1987). The increase in seizure susceptibility after 12d was probably associated with the reduction in CSF magnesium occurring at about this time (Buck et al., 1978).

Handling and gavaging animals might affect audiogenic seizure susceptibility. Sham gavaging (saline, 0.9 percent Nacl) of non-fasted, magnesium-deficient rats (Table 23, Group 1) did not affect seizure incidence because all of the animals had seizures; as would be expected of untreated magnesium-deficient rats (Table 23). This gives us confidence that handling and gavaging per se did not affect seizure incidence in these studies. Differences in caloric density or percentage of the calories from fat might affect

seizure susceptibility. However, no evidence of this was found (Table 15). The effects on seizure susceptibility was associated only with the effects of these diets on serum BHB and glucose.

Magnesium effect

Magnesium-deficient diet clearly induced seizure susceptibility (Tables 5, 15, 22, 23 and 31); a fact consistent with the findings of other researchers (Anderson et al., 1986; Buck et al., 1976, 1978; Chutkow, 1974; Coan and Collingridge, 1985; Kruse et al., 1932; Leaver et al., 1987; Mahoney et al., 1983). Magnesium deficiency would strongly potentiate any subthreshold depolarization; due to either endogenous transmitter release or pathological conditions in the central nervous system (CNS). The generation of excitability and maintenance of prolonged seizure events (Kruse et al., 1932) are due to the following neurological mechanisms: first, enhancement of neuronal excitability to levels that induce and maintain abnormal forms of neuronal discharges due to increased ease of transmission of field potential across synapses of the central nervous system (Coan and Collingridge, 1985; Herron et al., 1985). Second, axonal hyperexcitability results from lowered depolarization thresholds or resting potential. Both mechanisms cause nerve cells to be hypersensitive to stimulation from any source and to be more susceptible to premature firing. Physiological concentrations of magnesium ion appear to exert a voltage-dependent blockage of the ionophore associated with excitatory amino acid receptors of the N-methyl-D-aspartate (NMDA) type (Mayer et al., 1984; Nowak et al., 1984). The occurrence of spontaneous depolarization in the hippocampus; as well as the stimulated synaptic transmission occurring in magnesium-depleted medium; has been explained by removal of the blockage (Ascher and Nowak, 1988; Coan and Collingridge, 1985; Herron et al., 1985). A similar mechanism may be involved in the genesis of paroxysmal events in the entorhinal cortex, since both spontaneously occurring and evoked events were blocked by NMDA receptor antagonists (Hegstad et al., 1989; Jones and Heinemann, 1988). Thus,

the NMDA receptor-mediated excitation is large and prolonged and may also be involved in recurrent or feed-forward excitation (Jones and Heinemann, 1988). In addition, reducing extracellular magnesium concentration of the medium by bathing a rat hippocampal slice preparation reduced ability of adenosine to inhibit the synaptically-evoked propagation potential (Bartrup and Stone, 1988). However, other factors; such as a lowered threshold for spike generation (McLaughlin et al., 1971) and stimulation of presynaptic transmitter release (Herron et al., 1985); contribute to the epileptogenic effect of magnesium ion depletion.

Fasting effect

Fasting for 24h reduced the susceptibility of magnesium-deficient rats to audiogenic seizures (Tables 5, 15, 22, 23 and 31). Hughes and Jabbour (1958), Mahoney et al. (1983) and Peterman (1924) also observed that fasting decreased seizure susceptibility in rats. High serum ketone body (BHB) concentrations were observed after 18h or 24h but not after 6h or 12h of fasting in magnesium-deficient rats (Table 5). Similarly, reduction in seizure incidence did not occur until after 18h of fasting. A significant time-dependency in serum was noted because high serum BHB levels did not occur until 18h of fasting (Fig. 3). In addition, low serum glucose was observed after 18 or 24h but not during the first 12-h period of fasting (Table 5). During starvation, carbohydrates supply the energy required for body function for about 12h; which leads to reduced glucose stores in the body. A lack of availability of carbohydrates automatically increases rate of removal of free fatty acids from adipose tissues, and in addition, several hormonal responses; such as increased secretion of corticotropin, glucocorticoid, glucagon and decreased secretion of insulin (Cahill et al., 1966; Efendic et al. 1976; Rodriguez et al., 1984). The lessened availability of glucose then initiates a series of events that leads to ketosis. Ketone bodies, like BHB, can cross the blood-brain barrier and can be utilized by the brain cells for energy (Persson et al., 1972). Fasting experiments in young children (Lacers et al., 1987; London

et al., 1986; Sokoloff, 1973) and in rats (Hawkins et al., 1971) demonstrated the tendency to develop hypoglycemia and the rapid production and utilization of ketone to spare glucose. Fasting increased serum BHB and decreased serum glucose; BHB contributes to the energy metabolism of the brain (Bossi et al., 1989; Edmond et al., 1985; Hawkins et al., 1971; Krilowicz, 1985; Lacers et al., 1987; London et al., 1986; Peterman, 1946; Talbot et al., 1926). Prolonged fasting; both in humans (London et al., 1986; Owen et al., 1973) and animals (Hawkins et al., 1971; Krilowicz, 1985); results in a striking change in metabolism of the brain tissues that acquire the ability to utilize BHB directly as a substrate for oxidative metabolism (Edmond et al., 1985; Hawkins et al., 1971; Huttenlocher et al., 1971; Sokoloff, 1973). This change in brain energy metabolism may well be associated with alterations in neuronal excitability in this study.

A decrease in the blood glucose concentration is associated with increases in ketone bodies (BHB) during fasting; which was associated with a diminution or cessation of seizure incidence (Tables 5, 15, 22, 23 and 31, Figs. 2 and 3). This finding confirmed earlier findings by many investigators (Hughes and Jobbour, 1958; Peterman, 1946; Talbot et al., 1926; Wilder, 1921).

Ketogenic diet (MCT) effect

Ketogenic diet decreased seizure susceptibility of the magnesium-deficient rats. This seems to be due to the ketone production associated with a high MCT diet; which is consistent with previous results of the effects of high-fat diet on seizure susceptibility in animals (Appleton and DeVivo, 1974; Huttenlocher et al., 1971; Uhlemann and Neims, 1972). The effect of dietary MCT on seizure incidence may be due to a direct effect of the fatty acids contained in MCT; including octanoic and decanoic acid, which has been previously demonstrated (Huttenlocher et al., 1971). MCT is rapidly hydrolyzed in the gut; and the component fatty acids are readily absorbed. These fatty acids are transported to the liver via the hepatic portal circulation as free fatty acids (Greenberger and Skillman,

1969; Senior, 1968). These fatty acids are rapidly metabolized by the liver (Pi-Sunyer, 1971; Senior, 1968). Thus, dietary MCT rapidly induces ketosis. However, Mahoney et al. (1983) reported that gavaging MCT did not reduce seizure incidence when the rats were tested 1h later. This may be due to their methods of treatment, since they gavaged magnesium-deficient rats with MCT, as well as the timing of their test 1h after gavaging; which may have produced ketosis for such a short duration that brain energy metabolism remained unaltered and did not effect seizure incidence. Moreover, insufficient data about serum ketone bodies may not explain the effect of MCT on seizure incidence. In this study, the serum BHB and glucose were measured, and it was found that increasing dietary MCT and duration of fasting decreased seizure incidence due to increased serum ketone bodies measured as BHB and decreased glucose concentrations (Figs. 5, and 6). This is consistent with the effects of ketogenic diet and fasting on epileptic children found by Talbot et al. (1927). The 28 percent MCT diet increased serum BHB dramatically due to the inhibition of glucose metabolism in the cell by increasing acetyl Co-A and citrate (Jenkins, 1967; Madison et al., 1964; Randle et al., 1964) and decreased serum glucose slightly (Table 15, Figs. 5 and 6). This is consistent with the findings of others (Bach et al., 1972; Flatt et al., 1985; Guisard et al., 1973; Theuer, 1971).

Serum metabolites effect

This study shows that increased serum ketone bodies measured as BHB; decreased serum glucose due to a fasting, ketogenic diet (MCT) or gavaging BHB are related to the reduction of audiogenic seizure susceptibility in all the experiments. Waiting for 60 or 120 min after gavaging BHB increased seizure incidence, which is consistent with the findings of Mahoney et al. (1983); who found that gavaging MCT did not reduce seizure incidence 1h later. In addition, this study also shows rats that had low seizure score always had high serum BHB and low serum glucose (Appendix D, Tables 48-52). Rats that had not seized always had high serum BHB and low serum glucose whether due to increasing in fasting

hrs (Table 48, Fig. 2), increasing dietary MCT (Table 49, Fig. 2), gavaging high dose of BHB to fed animals (Table 50, Fig. 8) or gavaging low dose of glucose to fasted animals (Tables 51 and 52, Figs. 13 and 16).

In humans, infusion of ketone bodies depresses blood glucose (Jenkins, 1967). In animals, ketone-body infusions also depress blood glucose (Bach, et al., 1972; Bossi et al., 1989). Madison et al. (1964) demonstrated in dogs that ketone bodies stimulate pancreatic insulin release and thus lower plasma FFA and blood glucose. This is consistent with this study in which there was always an inverse relationship between serum BHB and serum glucose (Figs. 10 and 15).

Gavaging BHB produces ketonemia and may alter brain energy metabolism even of short duration (Huttenlocher et al., 1971). Ketone bodies are the preferred substrate in supplying carbon for respiration and lipogenesis in developing brain (Edmond et al., 1985; Lacers et al., 1987). Sokoloff (1973) confirmed that the brain can utilize BHB and acetoacetate as fuels. A study by Bossi et al. (1989) in dissociated whole-brain cell cultures from newborn mice reported the rapid production and utilization of ketone as BHB when glucose was lacking. Astrocytes, oligodendrocytes and neurons from developing brains all have high capacity to use ketone bodies for respiration (Edmond et al., 1985). Hawkins et al. (1971) reported large blood-brain concentration gradients for ketone bodies and suggested that relative impermeability of ketone bodies into brain tissue may be a factor limiting the rate of their metabolism in brain tissues. Also, obese subjects have induced tolerance to hypoglycemia after fasting; and this is associated with increased cerebral uptake of BHB and acetoacetate (Owen et al., 1973).

Elevated ketone concentration that accompanies fasting inhibits glucose oxidation in rats (Randle et al., 1964). Metabolism of ketone bodies or free fatty acids increases cellular acetyl-CoA concentration. This increase in acetyl-CoA results in an increase in intracellular citrate level that inhibits phosphofructokinase activity. Therefore, glucose-6-phosphate accumulates within the cell. High glucose-6-phosphate levels inhibits hexokinase that leads

to glucose build-up within the cell. This build-up causes decreased glucose uptake by the cell (Randle et al., 1964). In this study, increased serum ketone bodies measured as BHB were always associated with decreased serum glucose; whether due to fasting, increased dietary MCT or gavaging BHB (Tables 5, 8, 9, 15, 18 and 19). The increase in serum ketone bodies and the decrease of serum glucose are associated with the reduction of seizure susceptibility (Figs. 7 and 9). Ketone bodies like BHB might be used as a source of energy for the brain cells; thereby preventing seizures when a low level of glucose is available.

In the first three experiments, serum glucose decreased due to fasting or increasing dietary MCT (Tables 5, 15 or 19). This reduction in serum glucose is associated with the reduction in seizure incidence (Fig. 9). Therefore, in experiment four, glucose was gavaged to fasted magnesium-deficient rats that would normally have a low seizure incidence. Gavaging glucose resulted in increased seizure incidence in fasted, magnesium-deficient rats (Tables 23 and 31); gavaging glucose and testing 30 min after dosing resulted in a drastic increase in seizure incidence and seizure score (Table 22, Fig. 13). This result confirms the earlier findings by Hughes and Jabbour (1958) and Talbot et al. (1927). Also, Mahoney et al. (1983) reported that gavaging glucose did not reduce seizure incidence in non-fasted, magnesium-deficient rats that have a high seizure incidence when tested 1h later. Thus, any ketosis that may have been caused by fasting appears to have been depressed by gavaging glucose, thereby allowing seizure susceptibility to recur.

In all of these observations, rats that had low seizure incidence always had high serum BHB and low serum glucose. To try to separate the effects of BHB from glucose, the objective of experiment five was to gavage both BHB and glucose simultaneously to achieve high serum BHB and glucose; however, it was not possible to get high BHB and glucose in the same treatment (Table 31). In all cases, whenever serum BHB was increased; serum glucose was decreased. Therefore, it appears that serum BHB has to be

at least 20 mg/dl and serum glucose has to be less than 63 mg/dl to reduce audiogenic seizure susceptibility in magnesium-deficient rats (Table 5, 15, 22, 23 and 31).

Seizures and acidosis

In this study, tonic-clonic seizures caused death of some animals. This may be due to a combination of respiratory insufficiency that results from generalized; intense; sustained muscular contraction and also from excessive cellular energy needs of the brain that may affect functions of the brain tissues. Also, intense muscular activity takes place during the seizure, lactic acid accumulates in the plasma; which leads to metabolic respiratory acidosis (Howse, 1979). In experimental animals, hypercapnia is sufficient to block seizure discharges. The CO_2 accumulation reduces extension to flexion (E/F) ratio of mice caused to have maximal electroshock seizures (MES) and increases the latency time required for 50 percent of the animals to recover from a first maximal electro-shock seizure to have another MES. In contrast, hypoxia increased the E/F ratio and decreased the recovery time that lead to enhanced seizures (White et al., 1986). In addition, lactic acidosis following prolonged seizures enhances the activity of the Na^+/H^+ exchanges and exaggerates entry of Na^+ as well as the coupled influx of Cl^- and water. This series of events during seizure leads to astrocytic swelling (White et al., 1986).

Serum minerals and seizures

A combination of both magnesium deficiency and acute exposure to stressors leads to a stimulation of central nervous and neurohumoral mechanisms. The concentration of a number of hormones and transmitters is usually elevated, including acetylcholine, gluco- and mineral corticoids, thyroid and gonadal hormones, vasopressin and catecholamines (Classen, 1981; Leaver et al., 1987), which in turn may induce secondary metabolic alterations and affect electrolyte metabolism. This alteration may explain the abnormalities

in serum mineral concentrations, including calcium, potassium and phosphorus in this study.

Seizure induction is associated with an increase in potassium and sodium and a decrease in calcium and magnesium concentrations in the extracellular space. The drop in calcium levels may increase neuronal excitability by decreasing the inhibitory influence of potassium afflux (Davies and Peterson, 1989; Lux et al., 1986). The abnormalities in sodium, potassium, calcium and phosphorus may be related to the epileptogenic process. One possible mechanism for the abnormal electrolyte metabolism in epileptic brain is linked to decreased (Na^+,K^+)-ATPase activity (Pedemonte and Beauge, 1986; Rapport et al., 1975). Inhibition of (Na^+,K^+)-ATPase can lead to membrane depolarization; resulting in stimulation of transmitter release (Meyer and Cooper, 1981). Ouabain, a specific inhibitor of (Na^+,K^+)-ATPase, results in seizures in rats when injected intraventricullarly into the brain (Chutkow, 1974; Rangaraj and Kalant, 1979). The (Na^+,K^+)-ATPase enzyme acts as a trigger for neurotransmitter release at the synaptic cleft (Hexum, 1977) and for maintaining the cell's resting potential. Catecholamines may protect against seizures; norepinephrine may act as an endogenous anticonvulsant because decreased norepinephrine was associated with reduced (Na^+,K^+)-ATPase activities (Hexum, 1977). Thus, decreased norepinephrine conceivably induces convulsions in a normal brain. Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter; produces an increase in membrane permeability to chloride ions; which increases membrane conductance. When GABA contacts membranes that have a specific receptor, chloride ions distribute across the membrane due to the chloride equilibrium potential, which is similar to the resting potential of the cell. By opening ionic channels, GABA acts on postsynaptic sites like chemical voltage clamps, allowing suitable anionic pumping that causes hyperpolarization of the excitable membrane to occur. GABA action obviously is a counterbalance to the depolarizing influences exerted on cell membranes by passive cation leakage and by the excitatory influences (Roberts, 1975). Thus; the increase in serum phosphorus ions may

have contributed to decreased seizure incidence seen in this study (Table 31, Fig. 19). In addition, calcium in the presence of calmodulin stimulated the release of norepinephrine, acetylcholine and acetylcholinesterase from calmodulin-dependent vesicles. This effect of calcium on vesicle neurotransmitter release were mediated by calmodulin and required an intact biological system that was dependent on magnesium and ATP (DeLorenzo, 1986). Therefore, decreased magnesium and calcium in all experiments in this study may decrease norepinephrine and acetylcholine; thereby causing seizure susceptibility to be increased; as suggested by DeLorenzo (1986) and Heinemann et al. (1986). Except in experiments 2 and 5, serum calcium was significantly higher in rats fed a 28 percent MCT diet and fasted for 24h than rats fed a 3 percent MCT diet (Table 15). This increase in calcium concentration is due to a dietary MCT-containing diet that improves the absorption of calcium. The improvement in calcium absorption is positively associated with fat absorption (Kehayoglou et al., 1968; Tantibhedhyangkul and Hashim, 1978). This increase in calcium is associated with the reduction of seizure incidence (Table 15) and seizure score (Appendix D, Table 49); in addition, ketosis accompanies fasting and increases the dietary MCT effect. This is consistent with the findings of Chaistitwanich et al. (1987); Heinemann et al. (1986) and Lux et al. (1986) who found that increasing calcium and magnesium concentrations can reduce seizure incidence in animals and in vitro studies. In experiments 1, 3, 4 and 5, reductions in calcium occurred due to the magnesium-deficient diet (Flink, 1985; Fisher et al., 1985; Graber and Schulman, 1986). This reduction in calcium level is associated with reduction in seizure incidence (Tables 5, 21, 22, 23 and 31). In contrast, in all experiments increases in calcium level is associated with reduction in seizure score (Appendix D, Tables 48-52). However, increased serum ketone bodies accompany fasting and gavaging BHB; which may alleviate seizures even with low calcium.

Serum phosphorus and potassium were significantly higher in rats gavaged with 5.6 mmole glucose as compared with rats that were gavaged with 1.5 mmoles glucose

(Experiments 4 and 5, Tables 22, 23 and 31, Fig. 17). There was a negative correlation between serum phosphorus and potassium (Fig. 18). In addition, there was a high negative correlation between serum phosphorus and seizure incidence (Tables 22 and 23, Fig. 19). Consistently, rats that had not seized had high serum phosphorus compared with rats that had high seizure score (Appendix D, Tables 51 and 52). Serum potassium was increased in the rats gavaged with glucose; and there was a positive correlation between serum potassium and seizure incidence (Fig. 19). Consistently, rats that had not seized had low serum potassium compared with rats that had high seizure score (Appendix D, Tables 48-52). This is consistent with the elevated extracellular K^+ associated with increased neuronal excitability and spread of epileptic activity (Hablitz and Lundervold, 1981; Lux et al., 1986); dependent both on the depolarizing effects of K^+ accumulation on neuronal membranes and on the reduced driving force for K^+ outward currents as a result of a depolarizing shift of the K^+ equilibrium potential (Hablitz and Lundervold, 1981). This recruitment may be further facilitated through the excitability-increasing effects associated with reduction of $[Ca^{+2}]$ concentration (Nicholson, 1980). Elevated K^+ concentration has a possible significant release of synaptic transmitters from presynaptic terminals because afferent impulses arriving in a depolarized terminal would be of abnormally low amplitude. However, Cooke and Quastel (1973) found that, at the neuromuscular junction of the frog, elevated K^+ could cause an increase of acetylcholine release by each motor nerve impulse. A moderate elevation of K^+ leads to enhancement of excitatory synaptic transmission by increasing output of transmitter and by mild depolarization of postsynaptic target cells (Somjen et al., 1986). Increased phosphorus concentration increased the inhibitory transmitter, especially GABA liberated on dendrites close to a cell body or on an axon segment increase permeability to anions, thereby making excitable membranes more resistant to depolarization, accelerating the return-to-resting potential of membranes that the transmitter contacts (Roberts, 1975). These observations of increased GABA, anions and high permeability may explain the decrease of seizure percent to 50, 56 or 25 associated

with increases in levels of 8.87, 8.88 or 10.1 mg/dl of serum phosphorus, respectively (Table 31, Groups 8, 7, and 2, Fig. 19).

P-magnetic resonance spectroscopy studies of adult guinea-pig brain tissues showed a decrease in phosphocreatine content at 0.2 mM glucose in the superfusing medium under normoxia and at 0.5 mM glucose under hypoxia (Cox et al., 1983). Insulin-induced hypoglycemia or hypoxic hypoxia induced a decrease in phosphocreatine peak coincident with an increase of the inorganic phosphorus peak in the brain of adult rabbits. The behavior of the phosphodiester peak in hypoglycemia suggested the generation of energy from alternate sources (Prichard et al., 1983). Dissociated whole-brain cells cultured from newborn mice showed that during glucose deprivation under normoxia, D-ß-hydroxybutyrate is increasingly used for energy production (Bossi et al., 1989). These studies are consistent with the present findings that increased serum phosphorus associated with gavaging low glucose compared with high dose (Table 22) was associated with decreased seizure incidence. As serum BHB increased due to decreased glucose metabolism; then seizure incidence decreased.

Unique associations of serum phosphorus and potassium exist with respect to seizure susceptibility (Experiments 1, 2 and 5). At the same time, the changes in serum levels of calcium, phosphorus, potassium and sodium were not uniquely associated with respect to seizure susceptibility (Experiments 4 and 5). Therefore, fasting, ketogenic diet (MCT) and gavaging BHB or glucose did not affect serum minerals in a consistent manner. Therefore, the changes in serum minerals do not account for this effect on seizure susceptibility. Finally, increases in serum BHB and decreases in serum glucose due to a fasting, ketogenic diet (MCT) and gavaging BHB were consistently associated with seizure susceptibility of rats fed a magnesium-deficient diet for 17 days.

CHAPTER IV

SUMMARY AND CONCLUSIONS

From this research, magnesium-deficient diets caused decreased serum magnesium concentrations in the first experiment, compared with magnesium-supplemented diet (control). Weanling rats fed a magnesium-deficient diet are highly susceptible to audiogenic seizures.

Fasting magnesium-deficient rats for 24h decreased seizure incidence and seizure score as compared with unfasted magnesium-deficient rats.

Fasting increased serum BHB in magnesium-deficient or magnesium-supplemented rats; respectively; and decreased serum glucose in magnesium-deficient or magnesium-supplemented rats, respectively.

Increasing levels of dietary MCT decreased seizure incidence in weanling rats. There was an additive; anticonvulsant effect of fasting plus high dietary level of MCT that increased serum BHB and decreased serum glucose; which in turn is related to decreased seizure incidence.

Audiogenic seizure susceptible rats, in general, had lower serum BHB and higher serum glucose concentrations than those that did not convulse due to fasting or dietary MCT.

Changes in the serum levels of magnesium, calcium, phosphorus, potassium and sodium were not uniquely associated with seizure susceptibility. But there were significant differences in serum levels of these minerals that could modify the anticonvulsant effects of fasting, dietary MCT and gavaging BHB and/or glucose.

Gavaging BHB decreased seizure incidence, evidence that BHB is associated with reduced seizure incidence.

Serum BHB and glucose were affected significantly due to gavaging BHB. There were high negative correlations between serum BHB and glucose concentrations. Serum

BHB has to be 20 mg/dl or higher to have an anticonvulsant effect; in contrast, serum glucose has to be 63 mg/dl or higher to have an anticonvulsant effect.

Gavaging glucose to magnesium-deficient rats that were fasted for 24-h increased seizure incidence to what would be expected for unfasted rats, evidence that glucose has a negative effect on seizure susceptibility.

Gavaging BHB and glucose simultaneously affected seizure incidence. Increasing BHB to 2 mmoles and decreasing glucose to 1.4 mmoles decreased seizure incidence significantly (Table 31). In contrast, increasing glucose dose to 5.6 mmoles and decreasing BHB to 0.5 mmoles in the dose caused seizure incidence to increase significantly (Table 31).

In conclusion: A magnesium-deficient diet is reflected in decreased serum magnesium concentrations compared with magnesium-supplemented diet (control). Weanling rats fed magnesium-deficient diet are highly susceptible to audiogenic seizure compared with magnesium-supplemented rats.

Fasting magnesium-deficient rats for 24h increased serum BHB and decreased serum glucose which is associated with the reduction of seizure incidence.

Increasing level of dietary MCT to 28% decreased seizure incidence due to increased serum BHB and decrease serum glucose.

Gavaging BHB decreased seizure incidence, while gavaging glucose increased seizure incidence.

Audiogenic seizure susceptible rats in general, had lower serum BHB and higher serum glucose concentrations than those which did not seize due to fasting, dietary MCT, gavaging BHB and glucose.

Although, some treatments affected serum minerals, these effects were not consistent among experiments. Therefore, it was concluded that fasting, ketogenic diet (MCT), and gavaging BHB and glucose did not affect serum minerals consistently and that

modifications in serum minerals caused by these treatments did not account for this effect on seizure incidence and severity.

CHAPTER VII

RECOMMENDATIONS

In this study, the relationship between a fasting, ketogenic diet MCT) and audiogenic seizure susceptibility revealed that seizures were reduced with increasing duration of fasting to 24-h and by increasing the concentration of dietary MCT to 28 percent. This reduction in seizure incidence by both fasting and dietary MCT was due to increased production of ketone bodies (BHB, acetoacetate). Many investigators found that most of the organism tissues; even the brain; prefer BHB and acetoacetate as a substrate for energy supply. Therefore, determination of serum and cerebrospinal-fluid (CSF) BHB and acetoacetate concentrations in relation with seizure susceptibility may be beneficial in the interpretations of future experiments.

Determination of the free fatty acids in serum and cerebrospinal-fluid (CSF) per se may be beneficial also as a major factor in limiting ketone-body oxidation.

Determination of the pH and lactic acidosis after audiogenic seizure susceptibility may aid examination of concomitant electrolyte changes.

Determination of the serum minerals before and after seizure testing may help identify the disturbance of minerals during seizure. The affect of bleeding and handling animals before seizure testing may affect seizure incidence.

In all experiments; increased serum BHB and decreased serum glucose increased susceptibility to audiogenic seizures of magnesium-deficient rats. Gavaging BHB and glucose simultaneously in Experiment (5) confirmed that increasing the glucose to 5.6 mmoles; even with 2 or 0.5 mmoles of BHB to the rats; increased seizure incidence significantly when compared with gavaging BHB alone; which had low seizure incidence due to the inhibition of ketone bodies by glucose. Therefore, it will be beneficial to study the effect separately and by treating with anti-insulin antibodies to see the effect of those

antibodies on the inhibition of insulin and their effect on serum glucose and BHB in relation to seizure incidence of magnesium-deficient rats.

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APPENDICES

Appendix A

Table 32--Analysis of variance as tests of significance for effects of magnesium-deficient diets on seizure score and serum BHB, glucose and minerals (Experiment 1, Table 5).

S.V	DF	MS	F-ratio	S-L	MS	F-ratio	S-L
		Glucose			BHB		
Treatment	6	2958.7	15.36	0.000	2149.8	6.274	0.000
Error	32	192.6			342.6		
		Seizure score			Magnesium		
Treatment	6	8.843	4.675	0.002	1.504	875.00	0.000
Error	32	1.892			0.002		
		Calcium			Phosphorus		
Treatment	6	0.575	0.848	0.543	1.866	2.246	0.064
Error	32	0.678			0.831		
		Potassium			Sodium		
Treatment	6	83.269	11.382	0.000	101.871	0.984	0.452
Error	32	7.316			103.514		

S.V= Sourse of variation.
DF= Degree of fredom.
MS= Mean of square.
S-L= Significant level.

Table 33--Analysis of variance as tests of significance for the effect of different dietary MCT levels and duration of fasting on seizure score and serum BHB, glucose and minerals (Experiment 2, Tables 6-15).

S.V	df	MS	F-ratio	S-L	MS	F-ratio	S-L
		Glucose			BHB		
MCT (M)	2	2511.85	45.55	0.000	1263.46	33.93	0.000
Fasting(F)	2	10745.2	194.85	0.000	1933.61	51.93	0.000
MF	4	1582.8	28.0	0.000	8.537	0.23	0.921
Error	70	55.14			37.233		
		Seizure score			Magnesium		
MCT (M)	2	16.079	8.34	0.001	0.024	14.26	0.000
Fasting(F)	2	31.198	16.18	0.000	0.159	94.76	0.000
MF	4	2.345	1.22	0.312	0.017	10.02	0.007
Error	70	1.928			0.002	1.928	
		Calcium			Phosphorus		
MCT (M)	2	15.95	34.74	0.000	0.334	2.19	0.119
Fasting(F)	2	81.72	178.0	0.000	74.055	485.6	0.000
MF	4	6.39	13.9	0.000	0.586	3.842	0.007
Error	70	0.459			0.152	0.459	
		Potassium			Sodium		
MCT (M)	2	3.939	3.497	0.036	429.8	74.5	0.000
Fasting(F)	2	37.274	33.09	0.000	3254.7	564.3	0.000
MF	4	7.327	6.504	0.000	359.4	62.3	0.000
Error	70	1.127			5.767	1.1265	

S.V= source of variation.
df= Degree of freedom.
MS= Mean of square.
S-L= significant levels.

Table 34--Analysis of variance as tests of significance for the effect of gavaging different BHB doses at different times on seizure score and serum BHB, glucose and minerals (Experiment 3, Tables 16-21).

S.V	df	MS	F-ratio	S-L	MS	F-ratio	S-L
		Glucose				BHB	
BHB (B)	2	3091.68	23.6	0.000	1885.3	54.65	0.000
Waiting time(W)	2	952.84	7.27	0.002	810.57	23.5	0.000
BW	4	226.38	1.73	0.163	521.88	15.13	0.000
Error	40	130.98			34.5	130.98	
		Seizure score				Magnesium	
BHB (B)	2	25.57	9.92	0.000	0.024	5.114	0.011
Waiting time(W)	2	2.24	0.87	0.427	0.001	0.21	0.811
BW	4	0.919	0.356	0.838	0.001	0.122	0.974
Error	40	2.578			0.005	2.578	
		Calcium				Phosphorus	
BHB (B)	2	0.114	1.114	0.338	2.76	9.576	0.000
Waiting time(W)	2	0.024	0.232	0.794	3.327	11.54	0.000
BW	4	0.122	1.183	0.333	0.355	1.231	0.313
Error	40	0.103			0.288	0.1028	
		Potassium				Sodium	
BHB (B)	2	0.192	0.12	0.887	1.908	0.993	0.379
Waiting time(W)	2	0.727	0.456	0.637	1.549	0.806	0.454
BW	4	0.777	0.488	0.745	0.027	0.014	1.000
Error	40	1.592			1.921	1.592	

S.V= Sources of variation.
df= Degree of freedom.
MS= Mean square.
S-L= Significant levels.

Table 35--Analysis of variance as tests of significance for the effect of gavaging n- saline (sham-gavage), BHB or glucose on seizure score and serum BHB, glucose and minerals (Experiment 4, Table 23. Groups 1-4).

S.V	df	MS	F-ratio	S-L	MS	F-ratio	S-L
		Glucose			BHB		
Treatment	3	7457.6	59.34	0.000	3679.9	23.16	0.000
Error	28	125.67			158.91		
		Seizure score			Magnesium		
Treatment	3	5.875	3.481	0.029	0.003	1.037	0.391
Error	28	1.688			0.002		
		Calcium			Phosphorus		
Treatment	3	0.489	3.557	0.027	126.819	137.63	0.000
Error	28	0.137			0.195		
		Potassium			Sodium		
Treatment	3	4.37	3.635	0.025	14.708	1.304	0.293
Error	28	1.202			11.277		

S.V= Source of variation.
df= Degree of freedom.
MS= Mean of square.
S-L= Significant levels

Table 36--Analysis of variance as tests of significance for the effect of gavaging 1.4, 2.8 or 5.6 mmoles of glucose at different time on seizure score and serum BHB, glucose and minerals (Experiment 4, Tables 22 and 24-30).

S.V	df	MS	F-ratio	S-L	MS	F-ratio	S-L
			Glucose			BHB	
Glucose(G)	2	36403	124.3	0.000	652.8	17.95	0.000
Waiting time(W)	2	3121.6	10.65	0.000	212.09	5.83	0.005
GW	4	407.5	1.391	0.25	184.63	5.08	0.002
Error	50	292.95			36.375	292.95	
			Seizure score			Magnesium	
Glucose (G)	2	32.48	23.16	0.000	0.112	1.629	0.206
Waiting time(W)	2	4.038	2.88	0.065	0.062	0.904	0.412
GW	4	1.91	1.362	0.26	0.065	0.949	0.444
Error	50	1.402			0.069		
			Calcium			Phosphorus	
Glucose(G)	2	0.474	2.28	0.113	2.88	7.325	0.002
Waiting time(W)	2	0.76	3.651	0.033	0.443	1.126	0.332
GW	4	0.617	2.966	0.028	0.695	1.768	0.15
Error	50	0.208			0.393	0.2081	
			Potassium			Sodium	
Glucose(G)	2	26.96	15.24	0.000	344.34	15.06	0.000
Waiting time(W)	2	0.839	0.474	0.625	23.38	1.023	0.367
GW	4	6.609	3.735	0.01	33.07	1.446	0.233
Error	50	1.769			22.865		

S.V= Source of variation.
df = Degree of freedom.
MS= Mean of square.
S-L= Significant levels.

Table 37--Analysis of variance as test of significance for the effect of gavaging BHB and/or glucose on seizure score and serum BHB, glucose and minerals (Experiment 5, Table 31).

S.V	df	MS	F-ratio	Sig-level	MS	F-ratio	Sig-level
		Glucose				BHB	
Treatment	3	7457.6	59.34	0.000	3679.90	23.16	0.000
Error	28	125.67			158.91		
		Seizure score				Magnesium	
Treatment	3	5.875	3.481	0.029	0.003	1.037	0.391
Error	28	1.688			0.002		
		Calcium				Phosphorus	
Treatment	3	0.489	3.557	0.027	26.819	137.63	0.000
Error	28	0.137			0.195		
		Potassium				Sodium	
Treatment	3	4.37	3.635	0.025	14.708	1.304	0.293
Error	28	1.202			11.277		

S.V= Source of variation.
df= Degree of freedom.
MS= Mean of square.

Appendix B

Table 38--Least significant difference analysis of the effects of magnesium-deficient diet on seizure score and serum BHB, glucose and minerals (Experiment 1, Table 5).

	LSD (ni,nj)		
	6,6	5,5	6,5
Seizure score	1.62	1.78	1.70
BHB (mg/dl)	21.9	23.9	22.9
Glucose (mg/dl)	16.4	17.9	17.2
Mg (mg/dl)	0.05	0.05	0.05
Ca (mg/dl)	0.97	0.97	1.02
P (mg/dl)	1.75	1.18	1.13
K (mg/dl)	3.19	3.49	3.34
Na (mg/dl)	12.0	13.1	12.6

Table 39--Least significant difference analysis of the effects of dietary MCT level and duration of fasting on seizure score and serum BHB, glucose and minerals (Experiment 2, Tables 6-15).

	LSD (ni,nj)										LSD (ni,nj) for interaction					
	10,9	10,8	10,7	9,8	9,7	8,7	10,10	7,7	8,8	9,9	27,30	27,23	27,26	25,30	25,23	25,26
Seizure score	1.27	1.31	1.36	1.34	1.39	1.43	1.20	1.50	1.40	1.30	1.60	1.70	1.60	1.60	1.70	1.70
BHB, mg/dl	5.60	5.60	5.98	5.90	6.10	6.30	5.40	6.50	6.10	5.70	6.90	7.40	7.20	7.10	7.60	7.40
Glucose, mg/dl	6.79	7.01	7.30	7.20	7.50	7.60	6.60	7.90	7.40	6.97	8.50	9.10	8.80	8.60	9.20	8.90
Mg, mg/dl	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.05	0.05	0.05	0.05	0.05	0.05
Ca, mg/dl	0.62	0.64	0.66	0.66	0.68	0.69	0.60	0.70	0.67	0.64	0.80	0.80	0.80	0.80	0.80	0.80
P, mg/dl	0.36	0.37	0.38	0.38	0.39	0.40	0.40	0.42	0.38	0.37	0.40	0.50	0.50	0.50	0.50	0.50
K, mg/dl	0.97	1.00	1.04	1.03	1.06	1.09	0.94	1.10	1.10	0.99	1.20	1.30	1.30	1.20	1.30	1.30
Na, mg/dl	2.20	2.3	2.40	2.30	2.40	2.50	2.10	2.60	2.40	2.30	2.70	2.70	2.80	2.80	2.90	2.90

LSD= Least significant differences; where n_i is the number of rats in the first group and n_j the number of rats in the second group.

Table 40--Least significant difference analysis of the effects of gavaging different BHB doses on seizure score and serum BHB, glucose and minerals (Experiment 3, Tables 16-21).

	LSD (n_i,n_j)			LSD for interaction	
	5,5	6,6	5,6	16,16	16,17
Seizure score	2.10	1.87	1.96	1.2	1.1
BHB (mg/dl)	7.51	6.90	7.20	4.2	4.1
Glucose (mg/dl)	14.63	13.40	14.00	8.2	8.1
Mg (mg/dl)	0.09	0.08	0.08	0.001	0.001
Ca (mg/dl)	0.40	0.37	0.39	0.23	0.23
P (mg/dl)	0.69	0.63	0.66	0.38	0.38
K (mg/dl)	1.60	1.50	1.50	0.9	0.9
Na (mg/dl)	1.80	1.60	1.70	1.0	1.0

LSD= Least of significant; where n_i is the number of rats in the first group and nj the number of rats in the second group.

Table 41--Least significant difference analysis of the effects of gavaging 1.4, 2.8 or 5.6 mmole of glucose on seizure score and serum BHB, glucose and minerals (Experiment 4, Tables 22 and 24-30).

	LSD (n_i,n_j)						LSD (n_i,n_j) for interaction				
	6,6	7,7	8,8	6,8	7,6	7,8	18,19	18,20	18,21	19,20	19,21
Seizure score	1.30	1.3	1.30	1.30	1.3	1.30	0.80	0.80	0.80	0.80	0.80
BHB(mg/dl)	7.00	17.2	12.90	10.30	6.75	10.50	4.20	4.20	4.20	4.20	4.20
Glucose (mg/dl)	19.90	18.4	11.5	16.3	19.1	15.40	11.8	11.7	11.6	11.5	11.3
P (mg/dl)	0.30	0.70	0.50	0.61	0.7	0.60	0.43	0.43	0.43	0.43	0.43
K (mg/dl)	1.50	1.43	1.10	1.40	1.5	1.30	0.90	0.90	0.90	0.90	0.90
Na (mg/dl)	5.60	5.10	3.40	4.60	5.3	4.40	3.30	3.30	3.30	3.30	3.30

LSD= Least significant differences; where n_i is the number of rats in the first group and n_j the number of rats in the second group.

Table 42--Least significant difference analysis of the effect of gavaging BHB and/or glucose on seizure score and serum BHB, glucose and minerals (Experiment 5, Table 31).

	LSD (ni,nj)				
	10,10	9, 9	10,9	10,8	9,8
Seizure score	1.50	1.60	1.50	1.60	1.60
BHB(mg/dl)	4.80	5.03	5.10	5.1	5.20
Glucose (mg/dl)	10.50	11.10	10.8	11.2	11.40
Mg (mg/dl)	0.03	0.03	0.03	0.03	0.03
Ca (mg/dl)	0.50	0.60	0.6	0.60	0.60
P (mg/dl)	1.30	1.30	1.3	1.40	1.40
K (mg/dl)	1.30	1.40	1.4	1.40	1.40
Na (mg/dl)	3.10	3.98	3.90	4.00	4.10

LSD= Least significant differences; where n_i is the number of rats in the first group and n_j the number of rats in the second group.

Appendix C

Table 43--Chi-square test as test of significance for seizure % (Experiment 1, Table 5).

Comparisons	x^2	sig.-level
1 vs 2	0.00	NS
1 vs 3	10.4	S
1 vs 4	10.8	S
1 vs 5	7.82	S
1 vs 6	7.82	S
1 vs 7	5.00	S
2 vs 3	10.8	S
2 vs 4	11.6	S
2 vs 5	6.21	S
2 vs 6	7.82	S
2 vs 7	4.17	S
3 vs 4	0.00	NS
3 vs 5	1.34	NS
3 vs 6	2.00	NS
3 vs 7	4.00	S
4 vs 5	2.00	NS
4 vs 6	2.00	NS
4 vs 7	4.00	S
5 vs 6	0.02	NS
5 vs 7	4.00	S
6 vs 7	4.00	S

x^2 Chi-square test; where x^2, 0.05, 1=3.841.
1 rat on Mg-supplemented diet and 0-h fasting.
2 rat on Mg-supplemented diet and 24-h fasting.
3 rat on Mg-deficient diet and 0-h fasting.
4 rat on Mg-deficient diet and 6-h fasting.
5 rat on Mg-deficient diet and 12-h fasting.
6 rat on Mg-deficient diet and 18-h fasting.
7 rat on Mg-deficient diet and 24-h fasting.

Table 44--Chi-square test as test of significance for seizure % (Experiment 2, Tables 7 and15).

Comparisons	x^2	sig.-level
1 vs 2	1.05	NS
1 vs 3	6.67	S
1 vs 4	6.29	S
1 vs 7	7.35	S
2 vs 3	3.81	NS
2 vs 5	4.87	S
2 vs 8	8.87	S
3 vs 6	0.81	NS
3 vs 9	2.92	NS
4 vs 5	1.07	NS
4 vs 6	0.71	NS
4 vs 7	0.22	NS
5 vs 6	0.01	NS
5 vs 8	0.01	NS
6 vs 9	0.60	NS
7 vs 8	0.99	NS
7 vs 9	0.23	NS
8 vs 9	0.26	NS
1,2,3 vs 4,5,6	4.39	S
1,2,3 vs 7,8,9	4.08	S
4,5,6 vs 7,8,9	0.35	NS
1,4,7 vs 2,5,8	1.90	NS
1,4,7 vs 3,6,9	6.24	S
2,5,8 vs 3,6,9	1.40	NS

x^2 Chi-square test; where x^2, 0.05, 1=3.841.
1 rat on 3% MCT and 0-h fasting.
2 rat on 3% MCT and 12-h fasting.
3 rat on 3% MCT and 24-h fasting.
4 rat on 21% MCT and 0-h fasting.
5 rat on 21% MCT and 12-h fasting.
6 rat on 21% MCT and 24-h fasting.
7 rat on 28% MCT and 0-h fasting.
8 rat on 28% MCT and 12-h fasting.
9 rat on 28% MCT and 24-h fasting.

Table 45--Chi-square test as test of significance for seizure % (Experiment 3, Table 16).

Comparisons	x^2	sig.-level
1 vs 2	1.10	NS
1 vs 3	0.00	NS
1 vs 4	4.29	S
1 vs 5	3.29	NS
1 vs 6	2.50	NS
1 vs 7	7.82	S
1 vs 8	3.71	NS
1 vs 9	1.99	NS
2 vs 3	1.30	NS
2 vs 4	2.28	NS
2 vs 5	1.50	NS
2 vs 6	0.66	NS
2 vs 7	5.33	S
2 vs 8	2.28	NS
2 vs 9	0.44	NS
3 vs 4	4.29	S
3 vs 5	3.61	NS
3 vs 6	3.50	NS
3 vs 7	8.52	S
3 vs 8	3.14	NS
3 vs 9	2.61	NS
4 vs 5	0.13	NS
4 vs 6	0.03	NS
4 vs 7	4.66	S
4 vs 8	0.00	NS
4 vs 9	0.79	NS
5 vs 6	0.13	NS
5 vs 7	1.50	NS
5 vs 8	0.36	NS
5 vs 9	0.34	NS
6 vs 7	2.28	NS
6 vs 8	2.40	NS
6 vs 9	0.60	NS
7 vs 8	0.66	NS
7 vs 9	3.81	S
8 vs 9	0.43	NS

x^2 Chi-square test; where x^2, 0.05, 1=3.841.
1 rat on 3% MCT and 0-h fasting.
2 rat on 3% MCT and 12-h fasting.
3 rat on 3% MCT and 24-h fasting.
4 rat on 21% MCT and 0-h fasting.
5 rat on 21% MCT and 12-h fasting.
6 rat on 21% MCT and 24-h fasting.
7 rat on 28% MCT and 0-h fasting.
8 rat on 28% MCT and 12-h fasting.
9 rat on 28% MCT and 24-h fasting.

Table 46--Chi-square test as test of significance for seizure % (Experiment 4, Tables 23 and 24).

Comparisons	x^2	sig.-level
1 vs 2	5.30	S
1 vs 3	4.87	S
1 vs 4	12.4	S
2 vs 3	5.82	S
2 vs 4	5.82	S
3 vs 4	9.00	NS
4 vs 5	1.66	S
1 vs 5	6.03	NS
2 vs 5	0.09	S
3 vs 5	5.40	NS
5 vs 6	0.29	NS
5 vs 7	2.90	NS
6 vs 7	3.40	NS
8 vs 9	0.00	NS
8 vs 10	1.08	NS
9 vs 10	1.08	NS
11 vs 12	0.00	NS
11 vs 13	0.00	NS
12 vs 13	0.00	NS
5,6,7 vs 8,9,10 vs 11,12,13	9.73	S
5,8,11 vs 6,9,12 vs 7,10,13	4.79	S

x^2 Chi-square test; where x^2, 0.05, 1=3.841.
1 rat treated with sham-gavage.
2 rat treated with sham-gavage and 24-h fasting.
3 rat treated with 2 mM BHB and 24-h fasting.
4 rat treated with 5.6 mM BHB without fasting.
5 rat treated with 1.4 mM glucose; waiting 30 min after dosing.
6 rat treated with 1.4 mM glucose; waiting 60 min after dosing.
7 rat treated with 1.4 mM glucose; waiting 120 min after dosing.
8 rat treated with 2.8 mM glucose; waiting 30 min after dosing.
9 rat treated with 2.8 mM glucose; waiting 60 min after dosing.
10 rat treated with 2.8 mM glucose; waiting 120 min after dosing.
11 rat treated with 5.6 mM glucose; waiting 30 min after dosing.
12 rat treated with 5.6 mM glucose; waiting 60 min after dosing.
13 rat treated with 5.6 mM glucose; waiting 120 min after dosing.

Table 47--Chi-square test as test of significance for seizure % (Experiment 5, Table 31).

Comparisons	x^2	Sig-level
3 vs 4	7.150	S
3 vs 5	3.070	NS
3 vs 6	5.440	S
3 vs 7	1.150	NS
3 vs 8	1.650	NS
3 vs 2	7.110	S
3 vs 1	4.970	S
4 vs 5	1.290	NS
4 vs 6	0.250	NS
4 vs 7	3.170	NS
4 vs 8	2.490	NS
4 vs 2	0.000	NS
4 vs 1	1.060	NS
5 vs 6	0.390	NS
5 vs 7	0.550	NS
5 vs 8	0.230	NS
5 vs 2	1.290	NS
5 vs 1	2.960	S
6 vs 7	1.980	NS
6 vs 8	1.410	NS
4 vs 2	0.250	NS
4 vs 1	2.220	NS
7 vs 8	0.076	NS
5 vs 2	3.310	NS
8 vs 2	2.400	NS
6 vs 1	5.600	S
2 vs 1	1.056	NS
1 vs 8	4.090	S

x^2 Chi-square test; where x^2, 0.05, 1= 3.841.
1 animal treated with Mg-deficient diet.
2 animal treated with 0.9% saline (sham-gavage).
3 animal treated with 2 mmoles BHB.
4 animal treated with 5.6 mmoles glucose.
5 animal treated with 2 mmoles BHB & 5.6 mmoles glucose.
6 animal treated with 0.5 mmoles BHB & 5.6 mmoles glucose.
7 animal treated with 2 mmoles BHB & 1.4 mmoles glucose.
8 animal treated with 0.5 mmoles BHB & 1.4 mmoles glucose.

Appendix D

Table 48--The relationship between seizure score vs. serum BHB, glucose and minerals (mg/dl) of magnesium-deficient rats (Experiment 1).

Fasting, h	0			6			12			18		24			
Seiz. score	1	3	4	1	2	4	0	1	4	0	1	0	1	2	4
No. of rats	2	1	3	2	1	3	1	3	1	1	5	3	1	1	1
BHB	5.2	2.1	2.1	14.6	12.5	5.9	18.7	18.0	11.4	96.7	22.7	70.2	62.4	30.1	18.7
Glucose	72	67	112	73	78	76	56	59	69	42	47	31	33	33	29
Mg	0.68	0.56	0.53	0.61	0.61	0.53	0.56	0.58	0.58	0.56	0.57	0.58	0.58	0.53	0.53
Ca	7.3	6.9	6.8	7.3	6.5	6.8	7.7	6.6	6.5	6.9	6.1	6.9	6.4	5.2	5.2
P	7.0	5.3	5.8	6.8	7.1	5.9	6.4	5.3	3.9	7.6	6.5	7.4	7.6	6.6	5.6
K	25.7	26.2	28.3	25.1	26.2	25.5	27.5	28.7	29.8	23.5	26.4	25.2	26.4	27.5	27.5
Na	152	149	152	143	147	155	141	158	160	151	159	151	156	159	155

Table 49--The relationship between seizure score vs. serum BHB, glucose and minerals (mg/dl) of magnesium-deficient rats fed 3, 21 or 28 percent MCT and fasted 0, 12 or 24h before testing (Experiment 2).

MCT, %	3							21						
Fasting, h	0		12			24		0		12			24	
Seiz. score	1	4	0	1	4	0	1	0	4	0	1	4	0	1
No. of rats	1	9	1	4	5	5	5	4	4	6	1	1	5	2
BHB	9.40	6.80	15.6	14.3	11.9	32.2	15.6	18.7	13.5	24.8	22.9	13.5	35.9	22.9
Glucose	117	113	97	92	85	31	57	82	72	64	62	48	54	41
Mg	0.80	0.76	0.72	0.66	0.67	0.59	0.61	0.85	0.75	0.75	0.64	0.61	0.68	0.64
Ca	11.1	10.5	7.29	7.39	6.89	6.11	5.73	10.8	10.8	9.57	9.60	8.13	6.97	6.48
P	11.4	10.9	9.30	9.38	9.26	8.04	7.83	11.3	11.1	10.1	9.40	8.30	8.36	6.80
K	27.5	28.5	25.5	26.0	26.5	26.8	28.8	28.7	29.3	26.2	27.5	26.2	25.8	25.7
Na	190	192	183	186	177	159	158	192	192	174	173	173	170	170

Table 49--Continued.

MCT, %	28						
Fasting, h	0		12			24	
Seiz. score	1	4	0	1	4	0	1
No. of rats	1	9	1	4	5	5	5
BHB	22.7	14.6	28.1	22.9	14.6	40.3	22.9
Glucose	94	85	75	69	69	62	69
Mg	0.81	0.75	0.56	0.70	0.55	0.57	0.66
Ca	10.6	10.0	9.39	8.10	8.10	7.42	7.71
P	11.4	11.5	9.50	9.40	9.20	6.61	7.20
K	28.7	29.5	27.8	27.5	28.7	23.1	27.5
Na	191	190	186	183	183	156	175

Table 50--The relationship between seizure score vs. serum BHB, glucose and minerals (mg/dl) of magnesium- deficient rats gavaged with 0.5, 1.0 or 2.0 mmoles of BHB and tested at 30, 60 or 120 min after gavaging (Experiment 3).

BHB, mM	0.5							1.0								
Tested, min	30		60			120		30			60			120		
Seiz. score	3	4	0	1	4	1	4	0	1	4	0	1	3	0	1	4
No. of rats	1	4	1	1	4	1	4	3	1	1	3	2	1	2	1	2
BHB	3.1	3.6	2.1	2.6	2.5	1.0	1.3	8.7	9.4	6.2	5.7	5.5	5.2	4.2	4.6	4.2
Glucose	109	120	109	112	126	118	124	94	95	99	101	100	113	103	105	109
Mg	0.56	0.51	0.58	0.56	0.50	0.56	0.51	0.60	0.50	0.56	0.64	0.56	0.50	0.68	0.56	0.50
Ca	7.33	6.83	7.32	7.31	6.91	7.32	6.66	7.04	7.02	6.63	7.24	6.90	6.91	7.37	7.31	6.70
P	11.2	10.5	10.8	11.04	10.8	9.32	9.73	11.5	11.2	11.2	10.6	10.9	10.4	10.1	10.8	11.2
K	26.2	28.3	25.2	25.2	27.9	25.2	27.7	27.1	27.5	28.7	27.0	28.1	28.7	26.2	27.5	28.7
Na	150	151	150	152	152	150	151	152	150	153	151	151	153	149	151	153

Table 50--Continued.

BHB, mM	2.0							
Tested min	30		60			120		
Seiz. score	0	4	0	1	4	0	1	4
No. of rats	5	1	3	1	1	2	2	2
BHB	79.0	17.7	16.3	12.5	12.0	10.3	9.50	8.30
Glucose	48	85	78	115	116	85	120	125
Mg	0.58	0.50	0.62	0.53	0.50	0.66	0.55	0.50
Ca	7.33	6.91	7.20	6.51	6.52	7.16	6.92	6.97
P	10.9	9.52	10.7	9.52	9.33	10.6	9.32	9.50
K	27.5	28.7	26.3	28.5	28.7	25.5	26.2	28.7
Na	152	153	151	153	153	150	152	152

Table 51--The relationship between seizure score vs. serum BHB, glucose and minerals (mg/dl) of magnesium- deficient rats gavaged with 1.4, 2.8 or 5.6 mmoles glucose and tested 30, 60 or 120 min after gavaging (Experiment 4).

Glucose,mM	1.4							2.8							
Tested min	30		60		120			30			60			120	
Seiz. score	0	1	0	1	1	3	4	0	1	4	0	1	4	1	4
No. of rats	4	3	3	4	4	1	1	1	4	2	1	5	1	3	3
BHB	29.7	7.20	28.9	7.50	1.60	1.60	0.80	16.1	5.30	0.83	6.80	5.70	1.00	6.10	3.70
Glucose	42	49	45	59	77.4	96	90	75	109	123	99	109	120	110	122
Mg	0.61	0.54	0.60	0.54	0.55	0.56	0.53	0.58	0.53	0.52	0.58	0.54	0.50	0.54	0.51
Ca	7.23	6.44	7.20	6.49	7.26	7.30	7.01	7.70	6.59	6.41	7.30	6.35	5.54	7.40	7.04
P	7.75	7.60	7.40	6.60	7.90	5.60	5.60	7.60	6.50	5.60	7.60	6.60	5.50	6.90	6.83
K	26.2	27.5	26.3	27.6	25.9	26.2	26.2	24.6	25.4	27.5	23.5	26.6	28.7	27.1	29.8
Na	157	164	153	162	153	153	156	143	151	157	143	153	167	152	151

Table 51--Continued.

Glucose, mM	5.6				
Tested min	30		60	120	
Seiz. score	1	4	4	1	4
No. of rats	2	4	7	1	5
BHB	1.60	0.55	0.60	0.50	0.57
Glucose	126	133	144	131	167
Mg	0.56	0.52	0.52	0.56	0.52
Ca	7.16	6.49	7.35	7.70	6.91
P	7.10	6.70	6.66	6.90	6.24
K	28.1	29.4	28.7	27.5	28.8
Na	147	154	147	146	149

Table 52--The relationship between seizure score vs. serum BHB, glucose and minerals (mg/dl) of magnesium-deficient rats gavaged with 0.5 or 2.0 mmoles of BHB and/or 1.4 or 5.6 mmoles of glucose and tested 30 min after gavaging (Experiment 5).

Glucose,mM	5.6			5.6			1.4			1.4		
BHB, mM	2.0			0.5			2.0			0.5		
Seiz. score	0	3	4	0	3	4	0	1	4	0	3	4
No. of rats	1	1	4	2	3	5	5	1	4	4	2	3
BHB	17.6	10.5	3.90	3.00	1.10	0.80	27.6	14.6	14.1	21.7	21.0	16.5
Glucose	100	99	130	121	143	151	83	107	108	85	88	106
Mg	0.66	0.60	0.55	0.66	0.56	0.56	0.62	0.58	0.56	0.63	0.58	0.56
Ca	6.70	6.60	5.28	7.60	6.27	6.10	6.64	6.00	5.50	6.18	6.15	6.63
P	9.50	9.30	7.10	9.40	7.40	6.22	10.6	9.50	6.33	10.8	8.45	6.87
K	22.5	25.5	28.1	25.1	26.8	26.9	25.2	28.7	27.4	26.5	28.1	27.9
Na	150	150	159	149	159	158	153	158	154	152	152	158

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