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Influence of magnesium deficiency on selected aspects of protein metabolism

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INFLUENCE OF MAGNESIUM DEFICIENCY ON SELECTED ASPECTS OF PROTEIN METABOLISM

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INTRODUCTION

Although the need of animals for magnesium was conclusively established by Kruse et al. in 1932, the mechanism through which magnesium functions in vivo has not been clearly defined.

Since 1927 when Erdtmann discovered that magnesium acted as an activator to the hydrolytic action of dialyzed mammalian kidney phosphatase, many studies have been published describing activation of other enzymes in vitro by magnesium. In many cases magnesium was not specific, other divalent cations substituting equally as well. However, the preponderance of magnesium over other divalent intracellular electrolytes, and the ubiquitous presence of magnesium in tissue appeared to designate this ion as a major physiological catalyst.

Many investigators have noted that animals deprived of magnesium failed to grow normally after a period of time. If they did not succumb to characteristic convulsive seizures, they eventually lost weight and exhibited general nutritional failure (Brookfield, 1934; Schrader et al., 1937 and Orent et al., 1932). Animals fed comparable amounts of food gained weight more slowly when magnesium was omitted from the diet than when it was supplied (Tufts and Greenberg, 1938a and Menaker and Kleiner, 1952).

Very little attempt has been made to correlate the effect of an inadequate supply of magnesium to aspects of protein metabolism other than growth and repletion of body weight following periods of protein-free diets.

Menaker (1954) found that magnesium deficient rats, depleted 25 per

cent below their initial body weights with protein-free diets, gained twice as much weight in 10 days when fed 7 per cent casein as when fed 14 per cent casein in the diet. Actually, the weight gain of magnesium deficient animals receiving the 7 per cent casein diet was not greatly different from that of control animals. In 14 day experiments, growth of magnesium deficient rats was depressed and mortality increased when their magnesium deficient diets were raised from 24 per cent to 50 per cent casein (Colby and Frye, 1951b). These experiments suggested that with low protein concentrations in diets for rats omission of magnesium made little difference; on the other hand, with high protein intakes effects of magnesium deficiency were prompt and deleterious.

The observations of Sauberlich and Baumann (1949) that mice excreted a greater proportion of ingested amino acids in the urine following a period of magnesium deprivation than when normal diets were fed, has suggested a relationship between utilization of amino acids and magnesium. Such a relationship has been supported by the observation that guinea pigs which have a high arginine requirement (Heinicke et al., 1955) also have a relatively great need for magnesium (Roine et al., 1949).

This study was designed to examine further the relationship of magnesium deficiency to protein utilization in albino rats. For this purpose, diets varying in casein content from 14 to 28 per cent, with and without magnesium added, were fed to weanling rats. Growth rates, food efficiency and nitrogen retention were studied in an attempt to assess effects of the experimental diets upon growth. Hemoglobin concentration, serum nitrogen and distribution of serum proteins, serum and hepatic transaminase activities and hepatic nitrogen were examined in an endeavor

to evaluate influence of magnesium deficiency upon other aspects of protein metabolism.

REVIEW OF LITERATURE

As an intracellular electrolyte, magnesium ranks quantitatively second only to potassium. Since the early days of nutrition research, magnesium has been generally accepted as one of the inorganic elements necessary for life because of its ubiquitous presence in body tissue and fluids. Yet Osborn and Mendel (1918) were unable to find evidence in support of this fact from their experimental studies. The "magnesium-free" diet they employed contained 0.012 per cent magnesium. On this ration a rat weighing 60 grams continued to grow for 306 days, after which it lost weight. When fed a control diet containing 0.08 per cent magnesium, it regained almost all weight lost. The experimenters expressed the belief that because a rat could complete its growth on a diet low in magnesium, this element was probably not essential to life.

Leroy (1926) reported that mice 26 days old ceased to grow and lived only 24 to 36 days when fed a diet providing only 0.00103 per cent magnesium. Somewhat later, Kruse et al. (1932a, 1932b) demonstrated that magnesium was also an essential element for life in the rat. These authors succeeded in preparing a diet with only 1.8 parts per million magnesium. Rats fed this diet developed spectacular deficiency symptoms which were described as: (1) vasodilation of the peripheral vascular bed within 3 to 5 days, during which time the exposed areas of the skin became vividly red; (2) hyperexcitability that increased progressively until the 18th day when any unusual noise threw the animals into tonic-clonic convulsions that resulted in death for many of them; (3) trophic changes in the skin and edema in advanced deficiency states.

In the period that followed the announcement by Kruse et al. of the essentiality of magnesium for rats their findings were confirmed and expanded by others (Barron, et al., 1948; Brookfield, 1934; Duckworth, et al., 1940; Tufts and Greenberg, 1938a) and observations were extended to other species (Bird, 1949; Kunkel and Pearson, 1948; Van Reen and Pearson, 1953). As with most nutritional deficiencies there were many similarities in response of various species to omission of magnesium from the diet. On the other hand marked differences also have been encountered.

Gross Symptoms of Magnesium Deficiency

Hyperemia, which was described by Kruse et al. (1932b) as one of the major symptoms of the deficiency syndrome in rats, has been observed in hamsters (Yamane and Singer, 1953) and dogs (Orent et al., 1932). Although hyperemia was visible in dogs over a period of 2 to 4 weeks the vasodilation was not as intense as that occurring in rats in which the hyperemic period lasted for only 5 to 7 days.

Hyperexcitability in some form was common to all animals studied except guinea pigs (O'Dell et al., 1960). Tonic-clonic convulsions followed a period of hyperirritability in hamsters (Yamane and Singer, 1953), dogs (Orent et al., 1932), chickens (Bird, 1949) and ducks (Van Reen and Pearson, 1953). Running fits that preceded the onset of convulsions in rats and rabbits did not develop in pigs, although these animals became tetonic. Calves reared on milk or milk plus various vitamin and mineral supplements, excluding magnesium, died in convulsive states (Duncan, 1935).

Attention has been called to the fact that "grass staggers" or "grass tetany", an endemic disease of cattle associated with hypomagnesemia,

resembled experimental magnesium deficiency in rats in that convulsive attacks were of the tonic-clonic type and that prior to attack animals showed signs of nervousness, restlessness and muscle twitching (Sjollema, 1932).

Various epidermal changes have been described. Rats have developed ringed-eye dermatitis (Tufts and Greenberg, 1938a) and erythema followed by eschar formation over the involved area (Schrader et al., 1937). Excessive keratinization of the tail was reported by Watchorn and McCance (1937) and Barron et al. (1948) described a sticky exudate on the feet, nose and ears of deficient rats. Poor feathering has been observed in chickens (Bird, 1949) and roughness and discoloration of the coat in rats by Duckworth et al. (1940) and Watchorn and McCance (1937).

Rats, rabbits and hamsters did not appear to be susceptible to gross muscle changes, but decreasing muscle tone in ducks and chickens led to progressive incoordination of movements. The birds squatted continually, arising only when excited (Bird, 1949; Van Reen and Pearson, 1953). Guinea pigs lost muscular coordination in the rear legs which O'Dell et al. (1960) felt was due to muscle stiffness. Muscle stiffness was differentiated from wrist stiffness that resulted from prolonged ingestion of certain diets, since administration of magnesium salts allowed the animals to regain the use of posterior limbs but had no effect on wrist stiffness.

Edema has been observed in the pedal portion of posterior extremities (Brookfield, 1934) and in the nasal region of rats (Barron et al., 1948).

Magnesium deficiency induced atrophy in bones of hamsters (Yamane and Singer, 1953), rats (Orent, et al., 1934) and guinea pigs (O'Dell et al., 1960) and, in addition, in the latter two species, incisor teeth became

soft and frequently broke off or loosened in their sockets and fell out. In rats incisors turned chalky white (Watchorn and McCance, 1937) while in the guinea pig they darkened with decay (O'Dell et al., 1960).

On gross inspection, the kidneys of rats deprived of magnesium frequently appeared enlarged and their surface was puckered or pitted with gray mottling or light patches, (Greenberg, et al., 1938). On the other hand, kidneys of guinea pigs were white in appearance and greatly enlarged, becoming twice as heavy as kidneys of control animals when the magnesium level of the diet was reduced to one third the control level (Maynard et al., 1958).

Magnesium Deficiency and Growth

Tufts and Greenberg (1938a) did not see any change in growth of rats during the first 2 to 3 weeks of reduced magnesium intake. Later, the growth rate of deprived rats decelerated, in part due to a decrease in food consumption to two thirds that of control animals. In paired-feeding experiments, animals weighing 100 grams and receiving magnesium in their diet gained 24.5 per cent more weight than those not receiving magnesium in a 33 day period; animals weighing 50 grams gained 32.3 per cent more weight than their magnesium-deficient pairmates in a 24 day period (Tufts and Greenberg, 1938a). Dick and Prior (1951) reported a significant decrease in average weight of experimental rats compared with their pair-fed controls within 15 days. Animals fed quantities of the control diet that would maintain body weights equal to those of animals deprived of magnesium required only 83 per cent as much food in a 60 day period as deficient animals (Kleiber et al., 1941).

When graded amounts of magnesium were added to a 10 per cent casein diet, weight gain in rats was maximal when magnesium concentration was 24 mg. per cent (Vitale et al., 1957c). Rats that had received 4 mg. per cent magnesium in the diet grew well for 10 weeks (Watchorn and McCance, 1937), but those that received 0.18 mg. per cent magnesium increased in weight for only two weeks (Kruse et al., 1932b).

Barron et al. (1948) found that control rats whose weights averaged 156 grams gained 13.9 grams per week in a five week period while magnesium deficient rats gained only 7.7 grams per week.

Garner et al. (1952) found that a low magnesium diet did not inhibit growth of sarcoma No. 10 or Walker carcinoma No. 256 transplants in rats. Magnesium deficient animals implanted with a second generation tumor from animals maintained for 50 days on diets low in magnesium also failed to show tumor inhibition. Tumors from depleted rats grew progressively, and equally as well in deficient animals as in their controls.

Growth inhibition produced by addition of thyroxine to diets of rats could be partially overcome by extra supplements of magnesium, and amounts of magnesium required were related to the concentration of thyroxin employed (Vitale et al., 1957b).

Magnesium and Protein Metabolism

A direct relationship of magnesium to protein metabolism has not been established but that such a relationship exists has been suggested by chemical and biological studies.

Kuby, Noda and Lardy (1954) have established that magnesium complexes of ATP and ADP in vitro were the active substrates for phosphorylases. In

addition, reaction velocity of ATP-creatine transphosphorylase was strikingly dependent upon magnesium ion concentration. By virtue of this association and requirement for ATP in the synthesis of peptide bonds, magnesium appeared to have a role in protein anabolism.

In 1955, Beechly and Happold found that magnesium ions greatly enhanced conversion of pyridoxamine phosphate to pyridoxal phosphate, the active cofactor for transaminases, but that other divalent metal ions had no effect. Later, Happold and Turner (1957) found that activity of partially purified preparations of glutamic-oxalacetic transaminase from heart muscle of sheep was increased 100 per cent by addition of magnesium ions.

Several studies have implied that magnesium may have a role in utilization of certain amino acids. For instance, either magnesium deficiency or arginine deficiency in chickens has produced cytopathological changes in hepatic cord cells (Jungherr et al., 1958). After a double deficiency of arginine and magnesium had been established, addition of arginine to the diet corrected the hypertrophied nucleolus and reversed hydrodystrophy of the cytoplasm, while addition of magnesium hydrated hepatic cytoplasm and minimized, but did not erase, morphological change in the nucleolus.

The difficulty encountered in finding a casein ration that would promote growth in guinea pigs equivalent to commercial rations was related to the relatively high need for arginine compared with the limited availability of this amino acid in casein (Heinicke et al., 1955). When casein diets were supplemented with arginine and methionine, the two most limiting amino acids in this protein for guinea pigs, satisfactory responses in weight gain could only be obtained when large amounts of magnesium and

potassium were provided (Heinicke et al., 1956).

Young mice, magnesium depleted for 55 days and losing weight at a rate of 0.08 grams per day, excreted a greater percentage of ingested amino acids in the urine than control animals (Sauberlich and Baumann, 1949). The loss of amino acids was not a function of weight loss alone as caloric deficient and riboflavin deficient animals, losing weight at more rapid rates, excreted smaller quantities of the amino acids studied. Loss of amino acids in the urine of magnesium depleted animals, not commensurate with weight loss, implied a failure to utilize amino acids in the absence of an adequate supply of magnesium.

After rats had been protein depleted until one-fourth of their body weight had been lost, magnesium deficiency did not appear to influence their ability to regain weight when they were offered a complete source of amino acids in the form of fibrin hydrolysate (Frost and Sandy, 1953). On the other hand, Menaker and Kleiner (1952) found that pair-fed rats receiving a 9 per cent casein ration with magnesium gained 40 per cent more weight in 10 days than those on magnesium deficient diets.

In 14-day experimental periods, raising casein in a magnesium deficient diet from 24 per cent to 50 per cent depressed growth in young rats (Colby and Frye, 1951b). When calcium was increased in the 24 per cent casein diet, low in magnesium, the growth of the animals was depressed to a greater extent than when calcium was fed at a normal level. Increased calcium in the 50 per cent casein diet reduced growth only slightly, however, there was a marked increase in mortality.

Histological Changes

Microscopic examination of the tissues of magnesium deficient rats has revealed histological lesions of the skin, kidney, liver, cardiovascular system, bones and teeth. Profuse hemorrhage and engorgement of the renal medulla, atrophy of tubular epithelium, glomerular degeneration accompanied by swollen cells and desquamation have been described in rats. At the cortico-medullary boundary zone, definite striations occurred in the region of greatest calcification. Although calcium deposits extended through the medulla they became progressively smaller as the tips of the pyramids were approached. Involvement was so extensive that practically all tubules were distended with calcareous material. Increased interstitial fibrotic material was observed, but there was no evidence of nephritis (Cramer, 1932; Greenberg et al., 1938; Barron et al., 1949). On the other hand, the majority of guinea pigs fed magnesium deficient rations did develop nephritis (Wickham, 1958), while rats were more susceptible to nephrosis-like degeneration (Greenberg et al., 1938).

In rats deprived of magnesium for 3 days Hess et al. (1959) observed pronounced mitochondrial swelling in epithelial cells of renal proximal convolutions. Six days later there was necrosis in areas corresponding to sites where mitochondrial swelling was most pronounced at the 3rd day. Fine deposits of calcium were observed in cells undergoing necrosis. As deficiency progressed, calcium deposits enlarged and became detached forming calcareous casts. The amount of calcium in kidneys of magnesium deficient rats has been reported as high as fifteen times that for control animals (Tufts and Greenberg, 1938a). Calcinosis has been observed in

myocardium (Vitale, et al., 1959), aorta (Wickham, 1958) and muscle (Tufts and Greenberg, 1938a). Guinea pigs appear to be especially susceptible to calcareous deposition in the kidney but a-many-fold increase in hepatic calcium has also been reported (Maynard, et al., 1958). Calcareous deposition in the calf occurred predominantly in the cardio-vascular system and spleen (Moore et al., 1938).

Hepatic tissue responded less to a decreased supply of dietary magnesium than did renal tissue. Cytoplasm of many cells disintegrated (Brookfield, 1934) and marked congestion was seen in hepatic vascular tissue of rats (Schrader et al., 1937) while guinea pigs developed focal hepatic necrosis in the absence of adequate magnesium in the diet (Maynard et al., 1958).

Myocardium, the aorta and coronary arteries of magnesium deficient dogs were susceptible to calcinosis, and moderate sudanophilic deposition appeared in the cardiac valves. Aortal placques were usually not lipid, but rather, fibrous in nature (Vitale et al., 1959), similar to those seen by Moore et al., (1938) in the yellow elastic fibers of the vascular system of calves raised on low magnesium rations.

Changes seen in rat incisor teeth after the 4th day of low magnesium intake were stratification of the predentin, progressive atrophy of odontoblasts, marked distortion of the enamel organ due to the presence of calcareous granules, and changes in the character of the cells of the paradentium. Molar teeth are less affected than incisors. (Irving, 1940 and Klein et al., 1935). Histological examination of the long bones of rats revealed replacement of cartilage by fibrous tissue (Dick and Prior, 1951).

Cerebellar changes described in chickens (Bird, 1949) and rabbits

(Barron et al., 1949) deprived of magnesium involved degeneration of Purkinje cells. Varying degrees of chromotolysis were observed, as well as decreased staining of the Nissel substance, swelling and nuclear changes.

Biochemical Changes in Magnesium Deficiency

Blood

Dietary deficiency of magnesium in rats produced a marked decrease in serum magnesium concentration, reaching 0.8 mg. per cent by the 4th or 5th day (Snyder and Tweedy, 1942). Magnesium in the red blood cells also decreased, but it dropped at a slower rate than the magnesium in the plasma, eventually reaching a concentration one half that of control animals (Tufts and Greenberg, 1938a). The low concentration of plasma magnesium was maintained until shortly before the onset of hyperexcitability, at which time it rose to near normal concentration then fell, within a few days, to sub-normal values. Hoobler et al. (1937) did not observe such a rise in serum magnesium in dogs. Hess et al., (1959) noted that plasma magnesium of rats fell progressively during the first 20 days, after which the concentration remained at a low value until 45 to 50 days. Toward the end of the experiment there was a tendency for values to increase slightly, but they remained in sub-normal range. In dogs, serum magnesium decreased over a period of 5 weeks, then remained at the low level (Kruse et al., 1933).

While serum magnesium dropped, calcium concentrations were reported within the normal range for rats (Colby and Frye, 1951a) and dogs (Kruse et al., 1933). However, a recent study with rats has confirmed the ob-

servations of Hoobler et al. (1937) that in dogs a marked hypercalcemia resulted in response to magnesium deprivation (MacIntyre and Davisson, 1958). Phosphorus concentrations remained unchanged in dogs after 17 weeks of magnesium deficiency (Kruse et al., 1933), and they were also unchanged in rats after 33 days on a low magnesium diet (Orent et al., 1934). Other serum electrolytes, chloride, sodium and potassium, as well as carbon dioxide and pH did not change in rats after 35 days of magnesium deprivation (Cotlove et al., 1951).

After minimum values of magnesium in the plasma of dogs had been reached, "fat clots" appeared. Analysis revealed abnormal amounts of certain plasma lipid fractions, particularly total and esterified cholesterol. However, the fatty acid fraction decreased concomitantly with the net result that total fat remained unchanged (Kruse et al., 1933).

Deprivation of magnesium caused a decrease in serum alkaline phosphatase in rats. This decrease coincided with the time that serum magnesium had reached its lowest concentration. Reduction in enzyme activity did not appear to be transitory since it was observed in several rats of different ages at various intervals throughout an experimental period of 29 days (Snyder and Tweedy, 1942). In contrast, no change in serum phosphatase activity was observed in magnesium deficient calves (Blaxter and Rook, 1955).

Increased blood values for lactic and pyruvic acids were found in magnesium deficient calves experiencing mild tetany (Blaxter and Rook, 1955). Since these blood values could be produced in normal calves with exercise, the elevated values probably were due to muscular activity brought about by tetany rather than to abnormality in carbohydrate

metabolism as the result of magnesium deprivation. Further support for the view that carbohydrate metabolism was normal was found in the fact that calves injected with glucose showed no difference in the rate of removal of the extra glucose, and no abnormal rise in blood pyruvate or lactate.

Soft tissues

Several investigators have reported that soft tissues of rats, particularly organ tissues were reluctant to give up their magnesium. Viscera and brain had not changed in magnesium content at the end of three weeks of deprivation, but muscle tissue had been progressively depleted of magnesium and potassium, accompanied by an increase in sodium and chloride (MacIntyre and Davisson, 1958). Earlier, Tufts and Greenberg (1938a) and Cotlove et al. (1951) had reported a slight decrease in muscle magnesium, but little change in the magnesium concentration of viscera as the result of a decreased supply of the mineral.

Some, but not all, enzymes in the soft tissues have been shown to be sensitive to magnesium deprivation. Alkaline phosphatase activity was lowered to 70 per cent of the control value in brain tissue of ducks by the 6th day of magnesium deficiency. Diphosphopyridinenucleotidase activity of the brain was significantly lowered after 10 days of deficiency, but activities of inorganic pyrophosphatase and cytochrome oxidase were not affected at either 6 or 10 days (Van Reen and Pearson, 1953). In subacute magnesium deficiency in rats phosphatase activity of kidney tissue and bone was normal (Watchorn and McCance, 1937). Histochemical studies of renal tissue of rats revealed an increase in various dehydrogenase and diaphorase activities on the 3rd day after a deficient diet was fed.

Three days later there was some loss in diphosphopyridinenucleotidase activity, and by the 9th day, there was marked reduction or complete loss of isocitric, malic, beta-hydroxy butyric and lactic dehydrogenase activities. Decreased enzyme activity occurred just prior to appearance of fine calcium deposits in the cell (Hess et al., 1959).

Skeletal tissues and teeth

A diet low in magnesium fed to young animals caused considerable change in the osseous system. Orent et al. (1934) demonstrated that dry bone weight increased at a faster rate in magnesium deficient rats than in control animals. The heavier bone weight, which was evident as early as the 5th or 6th day, was due to a greater quantity of ash found in bones of deficient animals than in control animals. Calcium deposition in excess of that found in control animals was responsible for the increased quantity of ash. After 30 days, magnesium concentration of the skeleton of rats was only one half that in the skeleton of control animals. Magnesium content of femora, tibiae, and fibulae of magnesium deficient animals 40 days of age sacrificed after induced convulsions, averaged 0.086 per cent while 0.16 per cent magnesium was found in bones of animals of the same age that were not convulsive.

In short term studies of 6 days Duckworth et al. (1940) did not observe increased weight of the femur of rats deprived of magnesium, but concentration of magnesium in the bone was only one half of that found in control animals. Percentage of calcium in skeletal ash was significantly higher on the dry, fat-free basis than in animals receiving magnesium. The authors suggested that mobilization of magnesium from skeletal tissues occurred very early in the deficiency and excess calcium was deposited as

a compensating mechanism. In the subacute magnesium deficiency produced in rats by Watchorn and McCance (1937) the bones examined had not changed in calcium or phosphorus at the end of 30 days, although, the magnesium concentration had been reduced to less than two thirds the value of the control animals.

Skeletal stores of magnesium were more labile than those of teeth. Magnesium content of the dry, fat-free rat tooth was not changed in the first 6 days of magnesium deficiency, although rate of weight increase was slightly subnormal (Duckworth and Godden, 1940). After 30 days of subacute magnesium deficiency teeth increased in water content by an average of 3.3 per cent, while the magnesium concentration fell to one-half the normal value and phosphorus declined only slightly (Watchorn and McCance, 1937).

Heat Production and Oxidative Phosphorylation

Magnesium deficiency in calves resulted in increased total heat production. The magnitude of the effect was related to serum magnesium concentration. As serum magnesium fell oxygen consumption increased. Increasing the magnesium of the ration caused a prompt increase in the serum magnesium concentration and a correlative decrease in the total heat production. At least part of the increased heat production might have been due to increased muscle activity as these calves exhibited mild tetany (Blaxter and Rook, 1955).

Increased basal heat production in magnesium deficient rats was not apparent until the 15th day after the low magnesium diet was started, became significantly greater by the 34th day, and reached a value of 25

per cent greater than control animals by the 58th day. These animals were not convulsive, nor did their activity differ appreciatively from littermate controls. The authors attributed the rise in energy expenditure to increased breakdown of tissue because of inability to secure sufficient magnesium out of intact tissues after the skeletal stores had been depleted (Kleiber et al., 1941).

Recently Vitale et al. (1957a) have shown that mitochondrial preparations from hearts of young rats exhibited uncoupling of oxidative phosphorylation 4 days after a low magnesium diet was started, with maximal uncoupling occurring on the 8th day. Earlier, these experimenters had demonstrated that oxidative phosphorylation in mitochondrial preparations from hearts of thyroxine treated animals was impaired, but could be restored to normal by addition of magnesium ions to the reaction mixture (Vitale et al., 1957a). The effect of thyroxine upon basal metabolic rate in animals has been well established. In vitro experiments have demonstrated that this is due to "uncoupling" of phosphorylation from electron transfer along the respiratory chain (Martinus and Hess, 1951). This action could be reversed by addition of magnesium (Tapley and Cooper, 1956; Bain, 1954). Also, preliminary injection of magnesium into rats completely prevented the uncoupling action of thyroxine in heart mitochondrial preparations (Vitale et al., 1957a).

Kalant and Clamen (1959) were not able to demonstrate any effect on oxygen consumption of rats 2 or 4 weeks after addition of magnesium to basal diets containing thyroxine.

Magnesium did not reverse the uncoupling action of dinitrophenol in oxidative phosphorylation in rat liver mitochondia and supplied only

partial protection against uncoupling effects of calcium (Tapley and Cooper, 1956). It did reverse uncoupling effects of diiodothyronine and acetic acid analogues (Mudd et al., 1955).

METHOD OF PROCEDURE

Experimental Design

The objective of this investigation was to determine whether or not magnesium deficiency influenced protein metabolism in young albino rats.

The study was divided into three parts, reported here as Experiments I, II and III. In the first experiment four groups of animals were used in a preliminary study to observe the effect of magnesium deficiency upon their growth and general health when the diet contained either 19 or 28 per cent casein and 2.1 per cent salt mix (Hegsted et al., 1956). The second experiment was designed to study the influence of magnesium deficiency upon growth, general health and nitrogen balance of animals when the diet contained either 14 or 28 per cent casein and 3.8 per cent salt mix. The objective of the third experiment was to study in rats depleted of magnesium for 10 days, activities of glutamic-oxalacetic and glutamic-pyruvic transaminases of the serum and liver, concentrations of hemoglobin, packed red cell volumes, concentrations of nitrogen and magnesium in serum and distributions of serum proteins.

Experiment I

Two groups of 4 animals each were given 19 per cent casein diets, one of these receiving 18 mg. per cent magnesium added as the sulfate to the diet, the other receiving no added magnesium. Two other groups of 4 animals each were given 28 per cent casein diets, one of these receiving 18 mg. per cent magnesium, the other receiving no added magnesium.

Food intake and body weight were recorded daily so that growth curves could be plotted and food efficiencies calculated. At the end of 84 days,

animals were sacrificed and organs weighed and observed for gross changes. Hepatic nitrogen, and concentration of serum nitrogen and serum magnesium were determined. Serum proteins were separated by paper electrophoresis and the various fractions calculated as relative percentages by means of densitometry and planimetry.

Experiment II

In order to produce a larger difference between total nitrogen intake of animals on two basic diets than in the first experiment, casein was incorporated into diets at 14 per cent and 28 per cent by weight. The salt mix was increased over the concentration used in Experiment I to bring it nearer to the quantity customarily used in purified diets for rats in the Iowa State nutrition laboratory, to control the diarrhea in deficient animals which had developed when diets contained 2.1 per cent salt mix, and also to increase the calcium intake as an added stress in magnesium deficient animals. Four groups of 15 or 16 rats each were used. Two groups were given 14 per cent and 28 per cent casein diets, respectively, to which was added 36 mg. per cent magnesium. The quantity of magnesium in these diets was increased over that used in control diets of the earlier experiment in order to keep constant the ratio of magnesium to other minerals. The remaining two groups of animals were given diets containing either 14 per cent or 28 per cent casein to which no magnesium was added.

Nitrogen balances were obtained for two consecutive 4 day periods, the first coinciding with exhibition of hyperemia by deficient rats. Hyperemia appeared usually on the 6th day and began to fade on the 9th day. When deficient animals succumbed in the early part of the experimental period, their pair-mates were autopsied, but 9 rats receiving each diet containing

14 per cent casein and 9 or 10 rats receiving the diets containing 28 per cent casein were maintained on the experimental regimen for 28 days and 4 and 5 rats, respectively, for 42 days. Body weight and food intake were recorded daily so that growth curves could be plotted and food efficiencies calculated. Blood samples were obtained at autopsy for determination of serum nitrogen and magnesium. Conditions of the organs and their weights were recorded as in Experiment I, and livers were frozen until hepatic nitrogen was determined.

Experiment III

In order to obtain enough serum for analysis, duplicate sets of animals in four groups were raised on the same diets used in Experiment II. One set of animals was identified as subgroup A and the other as subgroup B. On the 11th experimental day, blood samples were drawn, the animal sacrificed, and the liver quickly excised, weighed and chilled. A suitable aliquot of homogenate prepared from the liver was centrifuged and the supernatant stored for enzymatic analysis on the following day. Hemoglobin concentrations and packed red blood cell volumes were measured within 3 hours of autopsy. Concentrations of serum magnesium and nitrogen, distribution of serum proteins and hepatic nitrogen were measured at a later time on samples obtained at autopsy.

Experimental Animals

Albino rats of Wistar stock, strain A from the Iowa State nutrition laboratory were used for this study. They had been inbred by brother and sister matings. Animals were weaned at 28 days, placed immediately in individual wire mesh cages and assigned randomly to the various diets.

Mean initial weight of animals are given in Table 1. Animals of subgroup A weighed, on the average, 2 to 4 grams less than subgroup B, otherwise averages of groups within an experiment were comparable.

Experimental Diets

Diets used in this study were composed of vitamin-free casein, cottonseed oil and dextrin, which furnished, respectively, protein, fat and carbohydrate. Minerals were added as a salt mix (Table 2), similar in composition to that recommended by Jones and Foster (1942) with the exception that magnesium sulfate was omitted because the diets were to be used to produce magnesium deficiency. Magnesium sulfate was added to control diets. Analytical grade salts were used. Vegetable cellulose provided bulk, and vitamins were incorporated into the diets as a dextrin-vitamin mix, the composition of which is given in Table 3. Composition of the experimental diets is given in Table 4.

Hereafter, diets A-Mg and A+Mg will be referred to as containing 19 per cent casein, diets B-Mg, B+Mg, C-Mg, and C+Mg as 28 per cent and diets D-Mg and D+Mg as 14 per cent casein.

The A and B diets contained 2.1 per cent salt mix; control diets, designated as A+Mg and B+Mg, contained in addition 0.24 per cent $MgSO_4 \cdot 7H_2O$, which furnished 18 mg. per cent magnesium. The C and D diets contained 3.8 per cent salt mix and in addition the control diets, C+Mg and D+Mg contained 0.42 per cent $MgSO_4 \cdot 7H_2O$, which furnished 36 mg. per cent magnesium. The calcium:magnesium ratio was constant in all control diets.

To mark the feces in the nitrogen balance experiments, 0.1 per cent

Table 1. Mean and range of initial body weights of control and magnesium deficient rats fed diets containing either 14, 19 or 28 per cent casein

Diet No.	Casein	No. of rats	Sex	Mean weight	Range
	(%)			(gm.)	(gm.)
Experiment I - 2.1% salt mix					
A-Mg	19	4	M & F	63.5	54-70
A+Mg	19	4	M & F	63.2	54-73
B-Mg	28	4	M & F	59.8	52-64
B+Mg	28	4	M & F	63.2	61-66
Experiment II - 3.8% salt mix					
C-Mg	28	16	M	61.9	57-67
C+Mg	28	15	M	61.5	57-70
D-Mg	14	15	M	60.6	57-69
D+Mg	14	15	M	61.5	56-68
Experiment III - 3.8% salt mix					
Subgroup A					
C-Mg	28	10	M	58.2	53-63
C+Mg	28	10	M	58.1	54-61
D-Mg	14	10	M	58.6	54-63
D+Mg	14	9	M	56.6	53-60
Subgroup B					
C-Mg	28	10	M	60.7	55-68
C+Mg	28	10	M	60.5	54-67
D-Mg	14	9	M	62.4	54-68
D+Mg	14	10	M	60.9	54-65

Table 2. Composition of salt mix[a]

Mineral salt[b]	Weight
	(gm.)
NaCl	146.25
KH_2PO_4	408.30
$CaCO_3$	400.40
$FeSO_4 \cdot 7H_2O$	28.30
KI	0.83
$MnSO_4 \cdot H_2O$	4.2245
$ZnCl_2$	0.2726
$CuSO_4$	0.3196
$CoCl_2 \cdot 6H_2O$	0.0238
Total	988.9205

[a]Jones, J. H. and Foster, C., J. Nutr. 24:245-256. 1942

[b]Magnesium sulfate omitted

Table 3. Composition of the vitamin mix

Vitamin	Weight
	(gm.)
Thiamin hydrochloride	0.040
Riboflavin	0.060
Pyridoxine hydrochloride	0.040
Calcium pantothenate	0.400
Niacin	0.500
Folic acid	0.008
Biotin in dextrin[a]	0.100
Vitamin B_{12} in mannitol[b]	0.750
Ascorbic acid	1.000
Choline chloride	10.000
Inositol	10.000
Para-aminobenzoic acid	10.000
Dextrin added to make 500 grams	

[a]Biotin in dextrin--1:100 parts biotin to dextrin

[b]Vitamin B_{12} in mannitol--1:1000 parts of vitamin to mannitol

Table 4. Composition of experimental diets

Dietary component	Diet							
	A-Mg	A+Mg	B-Mg	B+Mg	C-Mg	C+Mg	D-Mg	D+Mg
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Casein, vitamin free[a]	19.0	19.0	28.6	28.5	28.6	28.4	14.3	14.2
Dextrin[b]	66.2	66.0	56.7	56.5	55.0	54.8	69.3	69.0
Cottonseed oil[c]	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
Salt mix	2.1	2.1	2.1	2.1	3.8	3.8	3.8	3.8
Cystine[a]	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Cellulose[a]	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Vitamin mix	4.8	4.8	4.8	4.8	4.8	4.7	4.8	4.7
$MgSO_4 \cdot 7H_2O$	--	0.24	--	0.24	--	0.42	--	0.42
Total	99.9	99.9	100.0	99.9	100.0	99.9	100.0	99.9

[a]General Biochemicals, Inc., Chagrin Falls, Ohio

[b]Fisher Scientific Co., St. Louis, Mo.

[c]Wesson Oil Co., New Orleans, La.

ferric oxide was added to the experimental diets.

All diets were fed ad libitum and distilled water was available to the animal at all times. Vitamins A and D were provided as cod liver oil[1] and vitamin E as alpha-tocopherol[2] diluted in cottonseed oil[3] 1:49. One hundred milligrams of each of the fat soluble vitamin preparations, furnishing 180 U.S.P. units of vitamin A, 18 U.S.P. units of vitamin D and 2 mg. alpha-tocopherol, measured by calibrated droppers into small glass containers was presented to animals on alternate days. Animals always consumed these supplements, usually as soon as they were made available.

Experimental diets were analyzed for nitrogen by the macro-Kjeldahl procedure and results are presented in Table 5.

Plastic, enamel, or glass utensils were used for preparation of the diets. After the dry ingredients were mixed by hand or with a plastic spoon in large enamel pans, the cottonseed oil was added a few drops at a time. To mix the ingredients thoroughly diets were pressed through plastic window screening attached to a wooden frame. After two additional siftings diets were stored in dark glass bottles in a freezer (-20°C) until used.

Necropsy Procedure and Preparation of Tissues

Animals were anaethetized with a sodium pentabarbital solution (4.5 grains of Nembutal[4] diluted to 10 ml. with distilled water) injected

[1]Squibbs, medicinal

[2]General Biochemicals, Inc., Chagrin Falls, Ohio

[3]Wesson Oil

[4]Abbott Laboratory, Chicago, Ill.

Table 5. Nitrogen content of experimental diets

Diet	% Casein	Nitrogen
		(mg. N/gm.)
A-Mg	19.0	28.2
A+Mg	19.0	27.7
B-Mg	28.6	40.6
B+Mg	28.5	42.2
C-Mg	28.6	42.6
C+Mg	28.4	41.5
D-Mg	14.3	20.9
D+Mg	14.2	20.7

intraperitoneally.

In Experiment III samples from free-flowing tail blood were collected in clean capillary tubes for electrophoretic studies and in heparinized capillary tubes[5] for determination of packed red cell volume. Aliquots for hemoglobin determination were measured at this time.

Immediately after anesthesia was accomplished the abdominal and thoracic cavities were exposed. The abdomen was incised immediately to the right of the linea alba, and oblique incisions were made toward each auxilla from the midline starting immediately superior to the diaphragm.

[5]Aloe Scientific Company, St. Louis, Missouri

Blood, obtained by cardiac puncture using a 5 or 10 ml. syringe, fitted with a number 20 needle (3/4 inch with short bevel), was transferred to a centrifuge tube and allowed to stand at room temperature until a good clot had formed. To insure complete exsanguination the superior vena cava was severed.

The liver was removed, blotted free of blood and weighed immediately. Kidneys, and testes were removed, trimmed of all adhering fat and connective tissue, and weighed. Adrenals were removed, carefully trimmed of all extraneous tissue and weighed on a Roller-Smith torsion balance to the nearest 0.2 mg. Each organ was inspected for any visible abnormality. Kidneys were split in half lengthwise and examined in a well illuminated magnified field. Lungs were examined for the presence of infection and the remaining viscera removed and checked for hemorrhagic sites. Weight of the eviscerated carcass was recorded. Except in Experiment III in which enzyme determinations were made, the liver was wrapped in heavy aluminum foil and stored at -20°C until analysis for nitrogen was performed.

Preparation of tissues

Blood After a good clot was formed, the cardiac blood was centrifuged for 15 minutes at approximately 2000 r.p.m. The serum was removed to 1 dram vials. Samples for transaminase analysis were refrigerated overnight, other samples, frozen.

Capillary tubes containing the blood for electrophoretic analysis were centrifuged at the same time as the cardiac blood, after which cells and serum were separated by severing the tubes at their junction. Both ends of the portion of the tube containing serum were sealed with Seal-

Base, labeled and stored at -20°C.

Only one end of the heparinized tubes was sealed because the analysis for packed red blood cell volume was performed immediately.

Liver For the enzyme determinations the liver was trimmed quickly of adhering connective tissue, weighed and then chilled in an evaporating dish imbedded in an ice bath. After coarse mincing with scissors, samples of approximately 2.5 grams were weighed quickly in tared weighing bottles and transferred to a refrigerator. Later, the minced liver was homogenized in deionized water in all glass homogenizer. The sample was made to 100 ml. volume. A 20 ml. aliquot was centrifuged for 20 minutes at 2000 r.p.m. and the resultant supernatant was refrigerated overnight pending analysis. The remaining homogenate was stored in 4 oz. pharmacy bottles for nitrogen analysis. For enzymic analyses, 0.1 ml. of supernatant from homogenized liver was diluted to 10 ml. with distilled water.

Nitrogen Balance Procedure

On the first and last days of each balance period food was removed from the cage at 8 a.m. Rats were placed on balance at 1 p.m., collections being terminated 4 days later at the same time. At the initiation of the balance the rat was transferred to a metabolism cage and provided with a weighed amount of diet to which ferric oxide had been added to mark the feces. A freshly weighed portion of unmarked diet exceeding the rat's usual daily food intake was offered each day for the rest of the period. Weight of the rat was recorded at the time the fresh food was offered. Diet containing ferric oxide was given again on the day following the balance period.

Collection of urine

At the start of each period six 9 inch low-ash, low-nitrogen filter papers[6] were placed on pyrex plates beneath the cage. These papers had previously been soaked overnight in a solution of 900 ml. of 95 per cent ethyl alcohol solution and 100 ml. of glacial acetic acid, then air-dried. Each day the top filter paper was removed. Feces were separated and handled as described in the following section. Scattered food was scraped and/or brushed back into the food cup for weighing. The filter paper was brushed free of hair and preserved in a 1000 ml. wide-mouth Erlenmeyer flask in 200 ml. of 20 per cent hydrochloric acid. To control evaporation the flask was covered with a beaker. On termination of the balance period, the three remaining papers were added to the collection flask, and the animal removed either to a fresh metabolism cage or to a regular cage. The bottom and the sides of the cage up to a height of 2-1/2 inches were washed thoroughly with a stream of hot distilled water. Rinsings were collected in the pyrex plate and quantitatively transferred to the collection flask. Contents of flasks were autoclaved under 15 pounds of pressure per square inch for not less than one hour. After the digest had cooled, contents of the flasks were pressed through a sieve into a 1000 ml. volumetric flask and diluted to volume with water. After thorough mixing an aliquot was stored in a 12 ounce pharmacy bottle for subsequent nitrogen analysis.

To demonstrate ability to recover nitrogen quantitatively by this technique, a solution of $(NH_4)_2SO_4$ was dispersed over the bottom and lower area of the sides of metabolism cages, with a pipette twice daily for four

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[6] Schleicher and Schuell, no. 597, New York, New York

consecutive days to simulate the experimental conditions. Each day the top filter paper was removed to the collection flask as described above. Nitrogen recovery from these cages averaged 96.2 per cent and is recorded in Table 27 in the Appendix.

Collection of feces

Feces brushed free of food and hair were collected in covered 125 ml. Erlenmeyer flasks containing 50 ml. of 20 per cent hydrochloric acid. On the 1st day of the 1st balance period only red feces were saved as these indicated intestinal wastes that accumulated after the initiation of the balance regimen. At the end of the first period, all the brown feces were accumulated in the flask for the first period and all the red feces in the flask for the second period. On the day following termination of the balance experiment brown feces were saved and the red discarded. In this way, only fecal waste resulting from food eaten during test period was retained for analysis.

Fecal material was autoclaved by the procedure described for urine. After sieving, digests were made up to 250 ml. with distilled water and suitable aliquots preserved for nitrogen analysis. To insure prevention of evaporation tops of the bottles were coated with paraffin. All excreta digests were stored at room temperature.

Chemical Analyses

Nitrogen determination

Macro-Kjeldahl procedure Nitrogen content of diets used and excreta collected in the nitrogen balance experiments was determined by the Arnold-Gunning modification of the Kjeldahl procedure. Samples of diets

were weighed on aluminum foil and transferred quantitatively to digestion flasks. Aliquots of urinary and fecal digests, 25 and 50 ml., respectively, were pipetted directly into the flasks. Digestion was accomplished by means of concentrated sulfuric acid using a mixture of K_2SO_4 and HgO as a catalyst[7]. All samples were digested for one hour and fifteen minutes after clearing. Zinc dust was employed to reduce HgO in the catalyst according to the suggestion of Hiller et al. (1948) and an excess of saturated NaOH solution was used to neutralize the H_2SO_4. Distillate was collected in 50 ml. of 4 per cent boric acid solution (Meeker and Wagner, 1933) to which a mixed indicator (83 mg. per cent methylene blue and 123 mg. per cent methyl red in 95 per cent ethanol) had been added. Ammonia was titrated directly with a standard 0.1 N HCl solution. All samples and blanks were titrated to a grey endpoint.

Micro-Kjeldahl procedure Serum and hepatic nitrogen values were obtained by slight modification of the micro procedure recommended by the Association of Official Agricultural Chemists (1950). For serum analysis a sample of 0.1 ml. was used. Hepatic nitrogen was determined on 1 ml. aliquots of frozen homogenate (about 2.5:100). To each sample in a 30 ml. digestion flask a mixture of 1.8 grams K_2SO_4 and 0.2 gram of HgO was delivered by a calibrated spoon. Approximately 2 ml. of concentrated H_2SO_4 was added from a burette and flasks heated on an electrically controlled rack for one hour. After cooling, 5 ml. of deionized water were

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[7]Urine catalyst - 10 parts K_2SO_4:0.7 parts HgO. Food and fecal catalyst - 15 parts K_2SO_4:0.7 parts HgO

poured down the neck of each flask as it was rotated.

Distillation was performed in a Parnas-Wagner type apparatus heated with steam from a water reservoir equipped with an electrical control unit. The digested mixture was transferred with a minimum amount of wash water. After 8 to 10 ml. of a $NaOH$-$Na_2S_2O_3$ solution[8] was added to the distillation flask the steam was turned on and 20 ml. of distillate collected in 8 ml. of 4 per cent boric acid solution containing mixed indicator. A standard solution of 0.01 N HCl was employed for titration. All water used in solutions and for rinsing was deionized with Amberlite MB-3[9]. Agreement between replicates was within 3 per cent for hepatic nitrogen and 2 per cent for serum nitrogen. The author's ability to recover nitrogen from a creatinine solution was demonstrated (Table 29).

Measurement of transaminase activity

Glutamic-oxalacetic transaminase activity was measured by a modification of the Karmen (1955) procedure. Glutamic-pyruvic transaminase determination was modified from Wroblewski and La Due (1956). These determinations are based on the catalysis of the following reactions by the respective enzymes:

(1) Aspartic acid + alpha-ketoglutaric acid $\xrightleftharpoons{\text{Glutamic-oxalacetic transaminase}}$ Oxalacetic acid + glutamic acid

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[8] 210 grams NaOH and 25 grams $Na_2S_2O_3$ made to a volume of 500 ml.

[9] Rohm and Haas Company, Washington Square, Philadelphia, Pennsylvania

(2) Alanine + alpha-ketoglutaric acid $\xrightleftharpoons{\text{Glutamic-pyruvic transaminase}}$ Pyruvic acid + glutamic acid

No measurable changes in optical density occur in these reactions. However, reduced diphosphopyridine nucleotide (DPNH) has a high optical density at 340 mμ, and diphosphopyridine nucleotide (DPN) has no optical density at this wave length. By coupling the transamination reaction with the following reactions the rate of the transamination reaction can be followed.

(3) Oxalacetic acid + DPNH $\xrightleftharpoons{\text{Malic dehydrogenase}}$ Malic acid + DPN

(4) Pyruvic acid + DPNH $\xrightleftharpoons{\text{Lactic dehydrogenase}}$ Lactic acid + DPN

One unit of enzyme was defined as that amount of enzyme which would cause a decrease in optical density of 0.001 per minute per ml. of serum at 25°C per cm. of light path when measured at a wave length of 340 mμ.

All reagents used in this analysis, with the exception of the buffer salts, were obtained from the Sigma Chemical Company, St. Louis, Missouri. They were refrigerated until made into solution, after which they were frozen until used. Solutions of alpha-ketoglutarate, amino acids and buffer were stable, therefore, were made up only twice during the course of the experiment and were stored in 1 dram bottles so that only amounts needed were thawed at a time. Stock enzyme preparations were kept frozen, made into working solutions at frequent intervals (7 to 10 days) and were kept in the dark except during the brief periods they were in active use.

Aspartate solution, 0.2 M Exactly 2.662 grams l-aspartic acid were dissolved in approximately 70 ml. potassium phosphate buffer. The pH was

adjusted to 7.5 with approximately 20 ml. 1N NaOH. The solution was diluted to 100 ml. volume with additional buffer.

Alanine solution, 0.2 M In approximately 70 ml. phosphate buffer 1.782 grams l-alanine were dissolved, pH adjusted to 7.5 with 1N NaOH or 1 N HCl, diluted to 100 ml. volume with additional buffer.

Alpha-ketoglutarate solution, 0.1 M Approximately 70 ml. phosphate buffer was used to dissolve 1.47 grams alpha-ketoglutaric acid. The pH was adjusted to 7.5 with about 20 ml. 1 N NaOH and the volume made to 100 ml.

Potassium phosphate buffer, 0.1 M For 2 liter quantities of buffer, 27.94 grams $K_2HPO_4 \cdot 3H_2O$ and 5.38 grams anhydrous KH_2PO_4 were diluted nearly to volume, and the pH adjusted to 7.5 before the volume adjustment was completed.

Reduced diphosphopyridine nucleotide For each ml. of solution prepared 1 mg. DPNH was used. Usually 10 ml. of the solution was prepared at one time, using 0.01 N NaOH as the solvent.

Malic dehydrogenase The dry preparation of MDH containing 100,000 enzyme units was made into working solutions in two steps. First, a stock solution was made by adding sufficient ammonium sulfate solution (30 per cent by weight) to yield 10,000 units per ml. At this concentration, the preparation was stable for a month or more at 0°C or below. Working solutions were made to contain 2000 units per ml. by further dilution with the ammonium sulfate solution. At this dilution the reagent was stable only for one or two days.

Lactic dehydrogenase This enzyme preparation was supplied as a stabilized standarized solution ready for use with an activity of approxi-

mately 8000 units per ml.

Determination of glutamic-oxalacetic transaminase The following reagents were measured into suitable labeled test tubes, mixed well and incubated at 25°C for 12 to 20 minutes:

0.5 ml. 0.2 M aspartic acid solution

0.2 ml. DPNH solution

0.1 ml. malic dehydrogenase solution

1.8-1.9 ml. phosphate buffer (adjusted according to the quantity of tissue used in the analysis)

0.1 ml. serum or 0.2 ml. diluted liver homogenate supernatant

During the incubation period optical density of the reagent mixture changed due to reaction between pyruvate in the tissue and DPNH. The optical density was read at a wave length of 340 mμ in a Beckman Spectrophotometer, Model DU[10] attached to a constant temperature water bath. The instrument was adjusted to zero optical density with deionized H_2O. When no further change in optical density had been ascertained by successive readings of the mixture listed above, 0.2 ml. alpha-ketoglutaric acid was added with thorough mixing. Simultaneously with addition of the alpha-ketoglutarate to the first cell a stop watch was started. At the end of three minutes and exactly every two minutes thereafter optical density readings were recorded for a period of 18 to 20 minutes.

Determination of glutamic-pyruvic transaminase The following reagents were measured into test tubes as in the previous analysis:

0.5 ml. 0.2 M l-alanine solution

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[10]Acknowledgment is made to the Department of Biochemistry and Biophysics, Iowa State University of Science and Technology, for use of this instrument.

0.2 ml. DPNH solution

0.1 ml. lactic dehydrogenase solution

0.8 ml. distilled water

1.0-0.9 ml. phosphate buffer

0.2 ml. serum or 0.4 ml. diluted liver homogenate supernatant

The procedure used was similar to that for determination of glutamic-oxalacetic transaminase with the exception that sample aliquots were larger.

Calculation of transaminase activity Plots were made of change in optical density with time. For the most part these plots were linear. In a few instances temperature of the waterbath attached to the instrument differed from 25°C. A temperature correction factor, derived from data presented by Steinberg et al. (1956), was inserted into the denominator of the calculation formula.

Serum enzyme activity was calculated according to the following formula:

$$\text{Units per ml.} = \frac{\text{Decrease in O.D. x 1000}}{\text{Time x ml. serum x light path x temperature correction}}$$

Transaminase activity of the liver was calculated per ml. of diluted liver homogenate supernatant by substitution of the appropriate sample aliquot in the above formula. Enzyme activity per gram of liver was calculated according to the following:

$$\text{Units per gm. liver} = \frac{\text{dilution factor x units per ml. diluted supernatant}}{\text{gm. liver in homogenate}}$$

Total transaminase activity of the liver was calculated for each of the enzymes.

Magnesium

A modification was made in the colorimetric procedure suggested by Orange and Rhein (1951) using titan yellow as the dye and polyvinyl alcohol as a stabilizer of the colloidal color lake formed by the dye-magnesium hydroxide precipitate. This method gave good reproducibility and the standard curves indicated that the reaction followed Beer's Law in the narrow ranged employed in this study. Recovery of magnesium from rat sera obtained from stock animals was 99.1 per cent.

Preparation of reagents All solutions with the exception of the dye were made at the start in quantities needed to perform all analyses. Dye solution was made fresh every two weeks, and the polyvinyl alcohol-titan yellow working solution was made fresh daily in the quantity required for analyses performed that day.

Magnesium stock standard For each liter of solution, 10.131 grams of desiccator-dried $MgSO_4 \cdot 7H_2O$ and 0.5 ml. chloroform were used. One ml. is equivalent to 1 mg. magnesium.

Magnesium working standards Stock standard solution containing 1, 2, 3, 4, and 5 mg. of magnesium were diluted to 100 ml.

Trichloroacetic acid, 5 per cent Fifty grams of trichloroacetic acid were diluted to 1 liter.

Polyvinyl alcohol, 0.1 per cent For 500 ml. of solution 0.5 gram of polyvinyl alcohol[11] was stirred into approximately 300 ml. water over gentle heat (60°C) until the solution cleared; before dilution, a few drops of chloroform were added as a preservative.

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[11]DuPont Elvanol, Grade 70-05. E.I. duPont de Nemours and Company, (Inc.), Wilmington, Del.

Sodium hydroxide, 8 per cent Eight grams NaOH were diluted to 1 liter and stored in a bottle provided with a calcium chloride tube.

Titan yellow, 7.5 mg. per cent Seventy five mg. of the dye[12] were diluted to one liter and stored in a dark bottle in a refrigerator.

Polyvinyl alcohol-titan yellow working solution Equal portions of the polyvinyl alcohol and titan yellow solutions were mixed and kept in the dark until used for color development.

Serum samples of 0.2 ml. were deproteinated with 1.8 ml. of 5 per cent trichloroacetic acid. After centrifugation 1 ml. aliquots were transferred to 5 ml. volumetric flasks and the following reagents added with thorough mixing after each addition:

2 ml. polyvinyl alcohol-titan yellow working solution

1 ml. NaOH

Water to volume

Color developed immediately and was stable for more than 4 hours. The optical densities were read in a Beckman, Model B, spectrophotometer at a wave length of 560 mμ after the instrument was adjusted to zero optical density with a reagent blank.

A standard curve was run daily, using solutions containing 0.01, 0.03 and 0.05 mg. of magnesium per ml. Slopes of the curves were constant, but the intercepts varied from day to day.

Paper electrophoresis

Separation of serum proteins was accomplished by paper strip electrophoresis in a three compartment apparatus manufactured by the E-C

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[12]Eastman Kodak #P4454. Distillation Products Industries, Rochester, N. Y.

Apparatus Company, 23 Haven Avenue, New York 32, New York.[13]

All separations were made using barbital buffer with a pH of 8.6 and ionic strength of 0.1. This was made up in 5 liter batches using 14 grams of diethylbarbituric acid and 103 grams of sodium barbital[14]. Each of the three compartments of the apparatus was filled with buffer to within 1 to 2 mm. of the top. Levels were equilibrated by opening the leveling tubes between compartments.

Whatman No. 3 MM filter paper[15] cut into strips 2.5 inches by 18.5 inches was used in all runs. Even moistening of paper with buffer was accomplished by placing three dry strips of paper on a teflon sheet on the lower cooling plate of the apparatus with ends extending over the outer walls of the buffer reservoir. Horizontal portions were moistened by dribbling 10 ml. of buffer as evenly as possible over the surface. Three dry strips were laid on top of the wet ones, and pressure was applied evenly by placing a foam rubber pad in position and clamping the lid of the apparatus in place. After five minutes strips were separated and approximately 0.01 ml. of sample applied with a capillary tube, in a narrow band between two points located 7 cm. from the center of the strip. Points had been previously marked on papers with a pencil. Two such bands were applied to each strip. A solution of 0.9% sodium chloride was used to rinse the capillary tube between samples. After placing strips in the machine with the pressure pad between, ends were tucked into the buffer compartments and the lid clamped into place. Separations were carried out

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[13]Acknowledgment is made to the Department of Biochemistry and Biophysics, Iowa State University of Science and Technology, for use of this instrument.

[14]Fisher Scientific Co., Chicago, Illinois

[15]George T. Walker Co., Minneapolis, Minnesota

with a potential of 180 volts for 14 hours. The apparatus was cooled by water of 30°C running through the plates continuously.

Immediately following the separation, portions of the strips dipping into the buffer were torn off. Strips were then removed together with the teflon sheeting to an oven of 100°C where they were dried for 30 minutes. Dry strips were placed in the dye bath for 16 to 24 hours. The procedure outlined by Block (1955) was followed. Dye solution was made by diluting 0.1 gram bromphenol blue, 50 grams zinc sulfate and 50 ml. glacial acetic acid to 1 liter with distilled water. Strips were washed three times in 2 per cent acetic acid solution (v/v) for 5, 10, and 10 minutes, respectively, and dye was fixed with a 2 minute wash of 10 per cent acetic acid-2 per cent sodium acetate. Strips were blotted with filter paper to remove excess moisture and hung vertically to dry at 100°C for 10 to 15 minutes.

Optical densities of strips were read at 2 mm. intervals using a photometer, Model no. 512A, and a scanner, Model no. 52-0, manufactured by the Photovolt Corporation, New York, New York[16]. Plots were made directly on graph paper placed on the moveable table attached to the scanner.

Relative concentration of each serum protein fraction was obtained by dropping perpendiculars from the lowest portions of the curve, using the strip as a guide where the fractions did not form a clearcut trough. Area measurements of these sections were obtained by planimetry using an instrument made by the Los Angeles Scientific Instrument Co., Model no. 123-a, Serial no. 14394, Los Angeles, California. Areas were then con-

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[16]Acknowledgment is made to the Department of Dairy and Food Industry, Iowa State University of Science and Technology, for use of these instruments.

verted to relative percentage.

Preliminary studies were carried out to establish the conditions of temperature, time, voltage and ionic strength for best separation of the proteins. Hog blood known to be high in α-globulin was used as a positive control to establish the position of this band under conditions of this experiment. Reproducibility of this procedure was uneven, but for the most part, the difference between replicate values for the samples was less than 5 per cent. Serums used in this study had been held in frozen storage approximately a year before analysis was performed. In the course of the preliminary investigations serums from stock animals were examined in the fresh state and after frozen storage for varying lengths of time. No change in the serum due to freezing or to storage could be detected.

Hematological Determinations

Hemoglobin determination

Hemoglobin concentrations were measured in duplicate by a modification of the procedure of Wintrobe et al. (1936, p. 383). Twenty microliters of tail blood were collected for each replicate in a calibrated blood pipette, which was emptied into a small flask containing 10 ml. of 0.5 per cent ammonium hydroxide solution, and the pipette flushed with the solution several times. Optical density of solutions were read in a Beckman DU spectrophotometer at a wave length of 540 mμ within four hours of the collection of the sample. The oxyhemoglobin concentration was calculated according to the following formula:

$$\text{Grams per cent hemoglobin} = \frac{\text{Total volume x reading}}{9.18 \text{ x volume of blood sample}}$$

The factor had been obtained by standardization of a Beckman DU spectrophotometer using the oxygen capacity method of Peters and Van Slyke (1932, p. 263). Replicates varied by no more than 3 per cent.

Packed red cell volume

Determinations of packed red cell volume were made, in duplicate on tail blood collected in heparinized capillary tubes as described in an earlier section. The tubes were spun at 20,000 revolutions per minute for five minutes in an International microcapillary centrifuge[17] and read in an Adams microhematocrit reader no. A-2970[18]. All samples were free of hemolysis. Replicates agreed within 3 per cent of each other and in most instances deviation between them was less than 1 per cent.

Statistical Analyses

Statistical differences between means were determined by the t-test. Probabilities less than 0.05 were considered significant; and those less than 0.01 were considered highly significant.

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[17]International Equipment Co., Boston, Massachusetts

[18]Clay Adams, Inc., New York 10, New York. Acknowledgment is made to the Department of Veterinary Physiology and Pharmacology, Iowa State University of Science and Technology, for use of these instruments

RESULTS AND DISCUSSION

Mortality

Control animals survived in all experiments. In Experiment I, all animals which had received diets containing either 19 per cent or 28 per cent casein and 2.1 per cent salt mix without magnesium survived an experimental period of 84 days.

In Experiment II when the amount of casein in the diet was either 14 per cent or 28 per cent and salt mix was 3.8 per cent, one fourth of the animals receiving 28 per cent and one half of the animals receiving 14 per cent casein in the diet died before the 16th experimental day (Table 6). In Experiment III, in which diets similar to those of Experiment II were used, one third of the deficient animals died within 10 days regardless of amount of protein in the diet.

Since all groups of animals in this study were of similar mean body weight and age at the beginning of the experiments it was not likely that initial body stores of magnesium differed enough to account for differences in mortality rates found in this study.

Higher salt concentration in diets used in Experiments II and III than in Experiment I appeared to be responsible for the higher mortality in these 2 later experiments compared to the earlier one. This finding supports the observation of Tufts and Greenberg (1938a) that animals receiving magnesium deficient diets containing 1.16 per cent calcium had a shorter life span than animals receiving 0.87 per cent calcium.

Higher mortality rates were observed in Experiment II for animals receiving 14 per cent than for those receiving 28 per cent casein, however

Table 6. Mortality rates of control and magnesium deficient rats fed diets containing 14, 19 or 28 per cent casein

Season of year	Diet	Casein (%)	Animals started	Deaths	Mortality (%)
Experiment I - 2.1% salt mix					
Spring	A-Mg	19	4	0	0
	A+Mg	19	4	0	0
	B-Mg	28	4	0	0
	B+Mg	28	4	0	0
Experiment II - 3.8% salt mix					
Fall	C-Mg	28	16	4	25
	C+Mg	28	15	0	0
	D-Mg	14	15	7	47
	D+Mg	14	15	0	0
Experiment III - 3.8% salt mix					
Spring	C-Mg	28	28	9	32
	C+Mg	28	20	0	0
	D-Mg	14	29	9	31
	D+Mg	14	19	0	0

in Experiment III, in which the same experimental diets were used, mortality rates were the same for animals fed the different quantities of casein. In Experiment III, rats died at an earlier time than did the rats in Experiment II. There were only two spontaneous deaths, one on the lower and one on the higher casein diet without magnesium, in the first 10 experimental days in Experiment II. When similar periods of time are considered, mortality rates were higher in Experiment III than they were in Experiment II.

Animals for Experiment II were raised starting in the fall of the year and extending through the winter, while, Experiment III was started in April. The season and/or room temperature and humidity may have exerted some influence. Locations of cages were changed during the course of all experiments in order to compensate for variation in environment within the laboratory. Even during the experiment which lasted 10 days, positions were changed every 2 to 3 days.

No effect of season per se on symptoms of magnesium deficiency could be found in the literature; however, a relationship between body temperature and serum magnesium concentration has been observed. Increased serum magnesium during hibernation has been well documented, and serum magnesium concentration of non-hibernating species may be increased by chilling the body. Such an increase has been produced in dogs (Axelrod and Bass, 1956), rats and cats (Platner and Hosko, 1953). Animals awakened from hibernation by raising their body temperature experienced a prompt reduction of serum magnesium to that characteristic of active animals (Riedesel and Folk, 1955).

Excretion of magnesium in the urine of rats raised in an environment

of 5°C was found to be less than that of control animals housed at room temperature (Mefferd et al., 1958). Although the animals in this study were not subject to large changes in room temperature, the possibility that even small changes might affect magnesium metabolism must be considered.

Autopsy Observations

A thorough examination of the viscera of all animals was made at autopsy and weights of carcass, liver, kidneys, adrenals and testes were recorded. These are reported in Tables 7, 8 and 9 as absolute weight and in Tables 10, 11 and 12 as organ weight per 100 grams of body weight.

None of the pathology observed could be related to the amount of casein in the diet, therefore, pathological changes will be discussed in terms of the presence or absence of magnesium from the diet and the length of the experimental period.

Viscera of all control animals were normal in appearance. Four control animals did have infection in portions of their lungs. Only one experimental animal had some infection in its lungs, hence the small amount of lung involvement seen in these experiments was not related to the experimental diets.

In Experiment I all magnesium deficient animals had pathological changes in the kidneys. Surfaces were pitted and dull instead of smooth and glistening, and in three fourths of the kidneys a row of white granules was visible at the cortico-medullary boundary.

Pitted kidney surfaces and abnormal deposits in the cortico-medullary zone were also seen in almost all deficient animals sacrificed at 28 and

Table 7. Mean and range of organ weights of control and magnesium deficient rats fed diets containing either 19 or 28 per cent casein. Experiment I

Diet no.	Casein	Length of exp.	No. of rats	Body weight	Carcass weight	Organ weights			
						Liver	Kidneys	Adrenals	Testes[a]
	(%)	(days)		(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)
Experiment I - 2.1% salt mix									
A-Mg	19	84	4	143 (131-158)[b]	107 (98-115.5)	5.24 (4.58-6.04)	1.44 (1.25-1.72)	50.0 (46.3-55.6)	3.30 (3.02-3.58)
A+Mg	19	84	4	225 (166-290)	179 (131-236)	7.08 (5.94-8.54)	1.72 (1.39-2.08)	48.5 (38.8-57.8)	3.12 (2.99-3.24)
B-Mg	28	84	4	136 (114-157)	101 (86-116)	5.80 (4.77-6.72)	1.64 (1.26-2.21)	48.8 (41.4-58.0)	4.05 (3.96-4.14)
B+Mg	28	84	4	224 (183-302)	185 (146-243)	7.26 (6.07-9.00)	1.87 (1.68-2.32)	53.0 (50.4-55.8)	2.98 (2.78-3.18)

[a]Mean value for 2 animals
[b](No.) = range

Table 8. Mean and range of organ weights of control and magnesium deficient rats fed diets containing either 14 or 28 per cent casein. Experiment II

Diet no.	Casein	Length of exp.	No. of rats	Body weight	Carcass weight	Organ weights			
						Liver	Kidneys	Adrenals	Testes
	(%)	(days)		(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)
Experiment II - 3.8% salt mix									
C-Mg	28	42	4	118 (109-124)[a]	85 (76-92)	5.30 (5.05-5.77)	1.50 (1.42-1.62)	29.4 (23.8-33.8)	3.03 (2.92-3.15)
C+Mg	28	42	4	224 (202-243)	177 (162-191)	7.47 (6.05-8.70)	1.62 (1.01-1.99)	37.1 (24.6-45.8)	2.82 (2.55-2.96)
D-Mg	14	42	3	109 (103-117)	77 (74-83)	4.85 (4.22-5.98)	1.36 (1.20-1.62)	28.7 (26.4-29.8)	2.42 (2.16-2.63)
D+Mg	14	42	3	216 (196-234)	173 (157-188)	6.93 (6.29-7.28)	1.67 (1.45-1.86)	33.5 (30.4-38.0)	2.68 (2.62-2.76)
C-Mg	28	28	5	112 (106-117)	82 (78-86)	4.63 (5.22-5.90)	1.40 (1.26-1.50)	28.8 (24.4-36.0)	2.64 (2.41-2.83)
C+Mg	28	28	4	155 (170-199)	139 (128-154)	6.86 (6.00-7.90)	1.66 (1.55-1.84)	33.4 (28.2-41.4)	2.40 (2.30-2.52)
D-Mg	14	28	4	98 (87-109)	71 (65-81)	4.60 (3.78-5.15)	1.25 (1.00-1.40)	27.4 (22.2-31.2)	2.44 (2.26-2.61)
D+Mg	14	28	4	169 (153-180)	130 (122-139)	6.48 (5.01-7.75)	1.38 (1.23-1.50)	25.0 (23.8-26.6)	1.99 (0.84-2.50)

[a](No.) = range

Table 9. Mean and range of organ weights of control and magnesium deficient rats fed diets containing either 14 or 28 per cent casein. Experiment III

Diet no.	Casein	Length of exp.	No. of rats	Body weight	Carcass weight	Organ weights			
						Liver	Kidneys	Adrenals	Testes
	(%)	(days)		(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)
Experiment III - A & B - 3.8% salt mix									
C-Mg	28	10	19	85 ± 2[a] (74 -96)[b]	62 ± 1 (54-74)	3.69 ± 0.09 (3.04-4.17)	0.90 ± 0.01 (0.85-1.00)	23.0 ± 0.4 (18.4-26.0)	1.17 ± 0.02 (0.98-1.31)
C+Mg	28	10	20	101 ± 2 (81-114)	75 ± 4 (57-86)	4.55 ± 0.17 (3.29-5.76)	1.00 ± 0.02 (0.84-1.14)	23.7 ± 0.5 (19.2-27.8)	1.11 ± 0.01 (1.00-1.20)
D-Mg	14	10	19	82 ± 2 (65-97)	60 ± 1 (47-72)	3.48 ± 0.10 (2.23-4.09)	0.81 ± 0.02 (0.69-0.96)	22.0 ± 0.9 (17.6-36.4)	1.21 ± 0.03 (1.04-1.56)
D+Mg	14	10	19	94 ± 2 (81-105)	70 ± 1 (60-78)	4.04 ± 0.14 (3.25-5.43)	0.82 ± 0.02 (0.68-0.98)	21.6 ± 1.0 (11.6-29.4)	1.10 ± 0.03 (0.92-1.30)

[a]Standard error of the mean

[b](No.) = range

Table 10. Mean and range of organ weights expressed as per cent of body weight of control and magnesium deficient rats fed diets containing either 19 or 28 per cent casein. Experiment I

Diet no.	Casein	Length of exp.	No. of rats	Per cent of body weight				
				Carcass	Liver	Kidneys	Adrenals	Testes
	(%)	(days)		(%)	(%)	(%)	(%)	(%)
Experiment I - 2.1% salt mix								
A-Mg	19	84	4	75.0 (73.1-78.9)[a]	3.66 (3.22-4.08)	1.01 (0.88-1.09)	0.036 (0.029-0.040)	2.22 (1.91-2.52)
A+Mg	19	84	4	79.5 (78.4-81.4)	3.23 (2.94-3.58)	0.78 (0.71-0.84)	0.022 (0.015-0.032)	1.12 (1.11-1.12)
B-Mg	28	84	4	74.2 (73.5-75.4)	4.28 (3.95-4.69)	1.18 (1.08-1.41)	0.036 (0.027-0.042)	2.63 (2.52-2.74)
B+Mg	28	84	4	82.2 (78.4-89.7)	3.29 (2.71-3.93)	0.85 (0.80-0.92)	0.025 (0.017-0.030)	1.14 (1.05-1.24)

[a](No.) = range

Table 11. Mean and range of organ weights expressed as per cent of body weight of control and magnesium deficient rats fed diets containing either 14 or 28 per cent casein. Experiment II

Diet no.	Casein	Length of exp.	No. of rats	Per cent of body weight				
				Carcass	Liver	Kidneys	Adrenals	Testes
	(%)	(days)		(%)	(%)	(%)	(%)	(%)
Experiment II - 3.8% salt mix								
C-Mg	28	42	4	71.2 (69.7-74.2)[a]	4.49 (4.14-4.85)	1.26 (1.17-1.36)	0.025 (0.019-0.028)	2.56 (2.44-2.68)
C+Mg	28	42	4	79.2 (78.6-80.2)	3.32 (3.00-3.58)	0.73 (0.44-0.83)	0.016 (0.012-0.020)	1.26 (1.22-1.29)
D-Mg	14	42	3	70.8 (68.5-72.8)	4.41 (4.03-5.11)	1.23 (1.11-1.38)	0.026 (0.023-0.029)	2.23 (1.85-2.44)
D+Mg	14	42	3	80.3 (79.4-80.3)	3.21 (3.11-3.31)	0.77 (0.74-0.80)	0.016 (0.014-0.017)	1.25 (1.27-1.34)
C-Mg	28	28	5	73.7 (70.7-78.2)	5.05 (4.79-5.38)	1.25 (1.16-1.34)	0.026 (0.023-0.031)	2.36 (2.08-2.57)
C+Mg	28	28	4	77.2 (75.3-79.1)	3.84 (3.28-4.65)	0.92 (0.89-0.96)	0.018 (0.016-0.021)	1.33 (1.27-1.38)
D-Mg	14	28	4	72.8 (70.0-74.7)	4.70 (4.34-5.12)	1.28 (1.15-1.49)	0.028 (0.020-0.032)	2.51 (2.39-2.60)
D+Mg	14	28	4	77.0 (74.4-79.7)	3.82 (3.27-4.40)	0.81 (0.78-0.85)	0.015 (0.014-0.016)	1.15 (0.55-1.39)

[a](No.) = range

Table 12. Mean and range of organ weights expressed as per cent of body weight of control and magnesium deficient rats fed diets containing either 14 or 28 per cent casein. Experiment III

Diet no.	Casein	Length of exp.	No. of rats	Per cent of body weight				
				Carcass	Liver	Kidneys	Adrenals	Testes
	(%)	(days)		(%)	(%)	(%)	(%)	(%)
Experiment III - 3.8% salt mix								
C-Mg	28	10	19	72.5 (70.1-77.1)[a]	4.32 (3.54-5.02)	1.05 (0.91-1.20)	0.028 (0.022-0.040)	1.38 (1.20-1.58)
C+Mg	28	10	20	74.4 (68.6-88.5)	4.50 (3.73-5.19)	1.00 (0.89-1.08)	0.024 (0.019-0.029)	1.10 (0.95-1.41)
D-Mg	14	10	19	73.1 (71.6-76.2)	4.22 (3.43-5.11)	0.99 (0.86-1.20)	0.027 (0.021-0.047)	1.48 (1.30-1.66)
D+Mg	14	10	19	73.7 (72.0-76.4)	4.29 (3.76-5.22)	0.87 (0.79-0.94)	0.023 (0.011-0.035)	1.17 (0.99-1.32)

[a](No.) = range

42 days in Experiment II. However, in Experiment III when the experiment was terminated after 10 days, renal deposits were seen in only half of the animals, and the quantity of granules visible was considerably less than in animals in Experiments I and II.

Except for animals on diets for 10 days, mean absolute weights of kidneys were greater in magnesium deficient animals than in control animals, and greater, for both control and deficient animals, when 28 per cent casein diets were fed than when animals received 14 per cent or 19 per cent casein for equal lengths of time. In relation to body weight kidneys were heavier in deficient animals than control animals, and sometimes, but not always larger when the higher than the lower concentrations of casein were fed.

One of the outstanding characteristics of magnesium deficiency has been pathological changes in kidneys. Both Cramer (1932) and Dick and Prior (1951) observed enlarged kidneys with puckered surfaces and Cramer reported grey surface mottling after 6 weeks of magnesium deprivation.

To the author's knowledge no other investigator has reported visible renal deposits in magnesium deficient rats, although several histological studies have revealed progressive degeneration of the tubule epithelium with calcification (Barron et al., 1949; Greenberg et al., 1938 and Cramer, 1932). That calcium was deposited in kidneys of magnesium deprived animals has been established by chemical analyses (MacIntyre and Davisson, 1958) and histochemical studies (Hess et al., 1959).

Since food consumption of deficient animals was less than that of control animals, and body size smaller, enlarged kidneys were contrary to expectation. Curtailment of food intake and malnutrition have often been

associated with decreased renal size, paralleling decreased body weight (Rivero-Fontan et al., 1952 and Mulinos and Pomerantz, 1940).

The liver in many animals appeared normal. Mean hepatic weight of deficient animals was less than that of control animals but greater per 100 grams of body weight than that of animals receiving diets with added magnesium. There appeared to be an association between hepatic size and quantity of casein fed. In deficient animals receiving the diets for 42 or 84 days, livers were larger, both in absolute weight and in relation to body weight when animals received 28 per cent casein than those of animals fed 14 or 19 per cent casein for equal lengths of time.

Changes in size and composition of liver of magnesium deficient rats have not been reported by other investigators, and deposition of calcium has been reported only in guinea pig hepatic tissue (Maynard et al.,1958). Rats given a low salt ration developed livers that were proportionately larger with respect to body weight than when normal quantities of salt were fed (Eppright and Smith, 1937), on the other hand, Mulinos and Pomerantz (1940) found that restriction of food intake to one fourth the normal amount caused a relatively greater loss in hepatic weight than in body weight. Hence, the relative increase in hepatic size in deficient animals in the present study appeared to be related to factors other than quantity of food eaten.

Absolute mean weights of adrenal glands of magnesium deficient animals were not unequivocally larger than those of control animals, however, per 100 grams of body weight adrenal tissue was greater in all groups of animals deprived of magnesium than those fed the mineral in the diet. Adrenal size could not be related to quantity of casein fed.

Increased adrenal size, indicative of stress, may in this case have been caused by decreased food consumption of deficient animals compared with that of control animals, as restriction of caloric intake of rats has been found to induce a "relative hypertrophy" of adrenal glands (Rivero-Fontan et al., 1952). But undoubtedly additional stress was imposed on deficient animals by the muscular responses and internal blood loss observed in some animals.

In the present study mean absolute weights of testes of deficient animals were equal to or heavier than those of control animals, and when expressed relative to body size, were in most instances approximately twice the weight of control animals if they had been on the experimental regimen for longer than 10 days. At 84 and 42 days, testicular weights of deficient animals expressed both in absolute values and in relation to body size, were greater when the higher than the lower casein diets were fed. Increase in testicular weight in the magnesium deficient rats was not expected as Rivero-Fontan et al. (1952) observed decreased testicular weight following caloric restriction, although Siperstein (1921) reported weight of the testes was lost more slowly than total body weight.

The teeth of animals receiving magnesium in the diet appeared to be normal; on the other hand, changes in dental structures occurred after the animal had been fed diets deficient in magnesium. In Experiment I incisors became chalky white in appearance and loose in their sockets and by the end of the experiment one fourth of the animals had lost a tooth and exhibited dental decay, also hyperemia and hyperplasia of gum tissue similar to that described by Kruse et al. (1932b), appeared. On the other hand, in Experiment II, incisor teeth of 3 animals had loosened but no

decay developed.

It is not certain whether the shorter experimental period in Experiment II than that used in Experiment I or the different proportion of mineral mix in the ration was responsible for the better oral health of the animals.

General Health of the Animals

Within 1 to 3 days all magnesium deficient animals fed diets containing 2.1 per cent salt mix developed diarrhea that persisted 20 days or longer. No control animals developed diarrhea.

In Experiments II and III when the diets contained 3.8 per cent salt mix diarrhea occurred in less than half the animals deprived of magnesium. In these animals diarrhea lasted only one or two days, so its mildness was striking in contrast to that seen in Experiment I. Amount of protein in the diet did not appear to affect either the incidence or duration of diarrhea, and again, control animals did not exhibit diarrhea. The decreased incidence of diarrhea in Experiments II and III compared with that in Experiment I undoubtedly was influenced by the larger amount of mineral mix used in the diets over that used in the first experiment.

Diarrhea has been reported in magnesium deficient rats, fed a diet containing 5 per cent salts (Watchorn and McCance, 1937) and in magnesium deficient rabbits consuming diets with 3.6 per cent salts (Kunkel and Pearson, 1948).

Hyperemia never appeared among control rats, but after 5 to 9 days all animals deprived of magnesium exhibited generalized peripheral vasodilation, which persisted 4 to 6 days. Although hyperemia was most ob-

vious in the feet and ears, it could also be seen beneath the fur of the animals.

The amount of mineral mix did not influence the time of onset and duration of hyperemia, but in all experiments hyperemia appeared later, and continued a shorter period of time when the amount of casein was reduced below 28 per cent. All animals fed diets containing 2.1 per cent salt mix exhibited less severe vasodilation than animals receiving the diet with 3.8 per cent salt mix.

Tufts and Greenberg (1938b) and Colby and Frye (1951a) found that increasing the concentration of calcium in magnesium deficient diets increased severity of vasodilation and shortened the time before its onset. In this study, added calcium did not effect time of onset of hyperemia, but its severity was less on lower than on higher calcium intakes.

No suitable explanation of hyperemia in the magnesium deficiency syndrome has been proposed. Hyperemia appeared at the time serum magnesium concentration was undergoing a sharp decrease (Tufts and Greenberg, 1938a, Orent et al., 1934), and Kashiwa and Hungerford (1958) established that vasodilation coincided with the peak of leucocytosis, while Belanger et al. (1957) associated it with reduced counts and degranulation of dermal mast cells.

Coincidental to fading of hyperemia, animals fed magnesium deficient diets appeared considerably more irritable than earlier. Signs of hyperirritability, mild at first, became more marked with time. Increased irritability of rats as magnesium deficiency progressed has been observed by Kruse et al. (1932b) and Barron et al. (1948). The occurrence of hyperexcitability in relation to hyperemia has varied with different experi-

mental conditions. Deficient animals, 25 to 39 days of age at the start of the deficiency regime became hyperirritable while they were still in the hyperemic period (Snyder and Tweedy, 1942, Schrader et al., 1937) while rats, 65 to 107 grams initially, became convulsive starting on the 28th day although they had exhibited no vasodilation (Brookfield, 1934).

An increased cardiac rate in experimental animals was perceptible to this investigator, however no attempt was made to measure this rate. Tufts and Greenberg (1938c) reported increased cardiac rate of 20 beats per minute over the control animals after 5 days of magnesium deficiency and an increase of 40 beats per minute after 10 to 40 days of deprivation.

In the first unusual behavior pattern noticed by the investigator in the present study, the animals rose upon their hind limbs and shook the anterior portion of the body. Later the animals were subject to more severe seizures characterized by a preliminary phase of running wildly about the cage and culminating in tonic-clonic convulsions similar to those described in the literature (Kruse et al., 1932b and Brookfield, 1934). These attacks sometimes resulted in death. Many animals found dead had their teeth clamped around one of the wires of the cage, a position often assumed during a seizure.

No difference in behavior could be attributed to amount of casein in the diet but when diets contained 2.1 per cent salts, hyperirritability occurred at a later time and was much less severe than when the diet contained 3.8 per cent salts. Autopsy performed on 3 animals immediately after fatal seizure, revealed massive hemorrhage in the chest of each. Lungs of 2 of these animals were also hemorrhagic, and lung hemorrhage was present in more than 40 per cent of the animals fed magnesium deficient

diets.

In the present study edema most frequently involved the extremities, although some animals had swelling about the face that could not be associated with any abnormal condition of the oral cavity. For the majority of animals receiving deficient diets containing 3.8 per cent mineral mix, edema did not become obvious until the 13th to 15th days. When the diet contained 2.1 per cent salt mix, edema appeared later, about the 35th day, and for most of the animals not until after the 42nd day.

Other observers have reported appearance of edema between the 3rd and 6th week after feeding a magnesium deficient diet. When diets containing 20 per cent casein and 5.9 per cent salts were fed to rats weighing 65-107 grams initially, edema appeared on the 36th day (Brookfield, 1934), and when fed to 34-45 gram rats edema appeared between the 4th and 6th week (Kruse et al., 1932b). With diets furnishing 22 per cent casein and 3.6 per cent salts 150 to 300 gram rats became edematous in the 3rd week (Barron et al., 1948).

Porphyrin staining, scaliness of tails and feet, coarse, matted and discolored hair with general thinning of the coat, but no denuded areas as described by Kruse et al. (1932b), appeared as the experiment progressed. Ringed-eye dermatitis reported by Tufts and Greenberg (1938a) was observed and in the 5th and 6th weeks of the experiment the animals walked only on the digital portion of the rear feet which elevated the posterior end of the body. Hematuria appeared in only one deficient animal, but deeply colored urine in the last few days of Experiment I was observed in all animals deprived of magnesium. Darkening of the urine had been observed by Kruse et al. (1932b). In Experiment I, male rats appeared to fare

worse than female rats. Priapism seen in all males by the 5th experimental week, confirmed observations of Watchorn and McCance (1937).

In Experiment I, progression of the experimental animals toward depletion of their magnesium stores was slow, orderly and unspectacular. On the other hand, in Experiment II the animals went through two stages similar to those described by Tufts and Greenberg (1938a). Vasodilation resulting in hyperemia and development of hyperexcitability were the predominant symptoms of the 1st stage of the deficiency syndrome, while the 2nd phase was characterized by reduction in rats of growth, cachexia, and development of malnutrition.

Weight Gain, Food Consumption and Food Efficiency

Growth curves for animals in Experiment I are shown in Figure 1 and for animals in Experiments II and III in Figure 2.

Growth of control animals receiving 14, 19, or 28 per cent casein in the diet compared favorably with animals fed stock diet; and as expected, on all experimental diets females gained less weight than did males.

In Experiment I, all animals gained weight during the first 10 weeks of the experiment. However, in the last 2 weeks only one-fourth of the deficient animals, and all control animals continued to gain, while remaining deficient animals lost weight.

Differences in mean body weight between deficient animals and control animals in Experiments II and III was significant after 10 days of feeding, and was highly significant after 4 weeks. There was no significant difference in weight gained by control or deficient animals fed

Figure 1. Growth curves for control and magnesium deficient rats fed diets containing either 19 per cent or 28 per cent casein and 2.1 per cent salt mix.

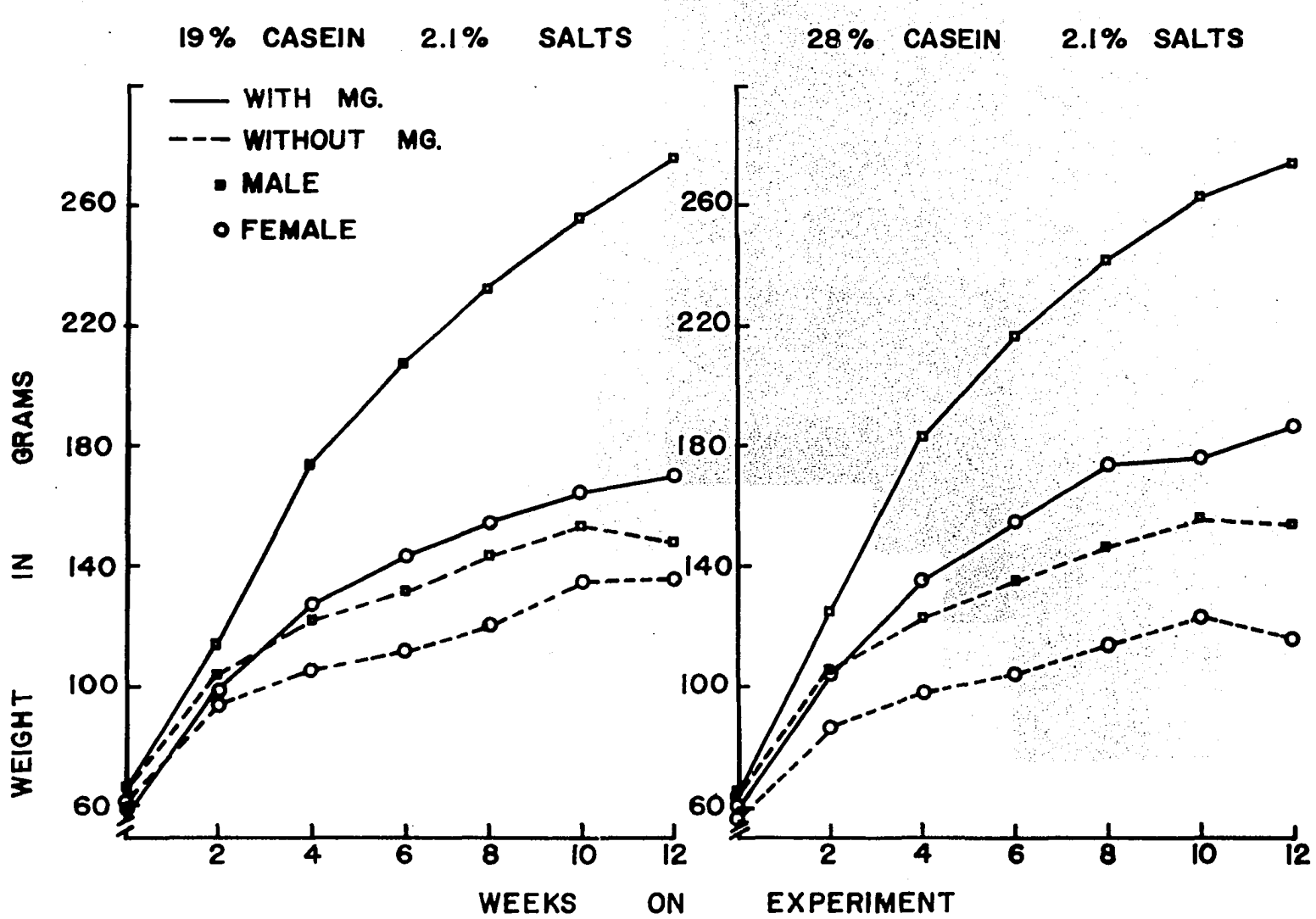
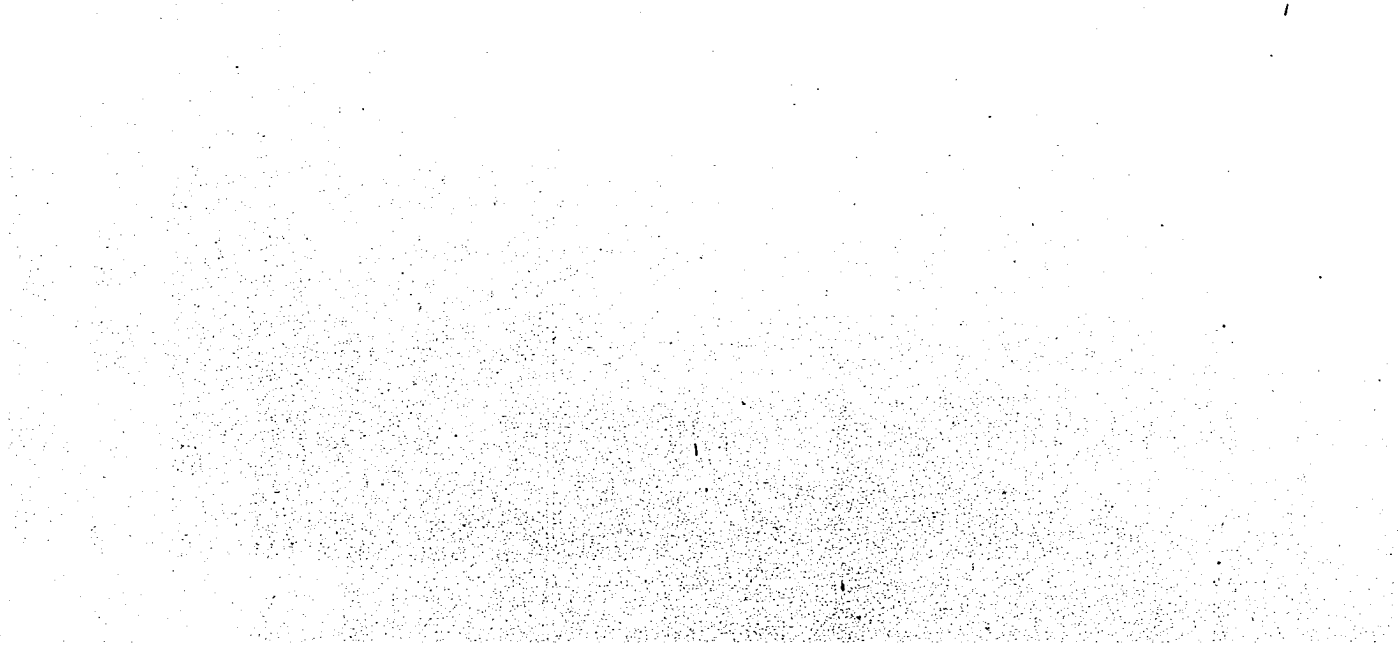
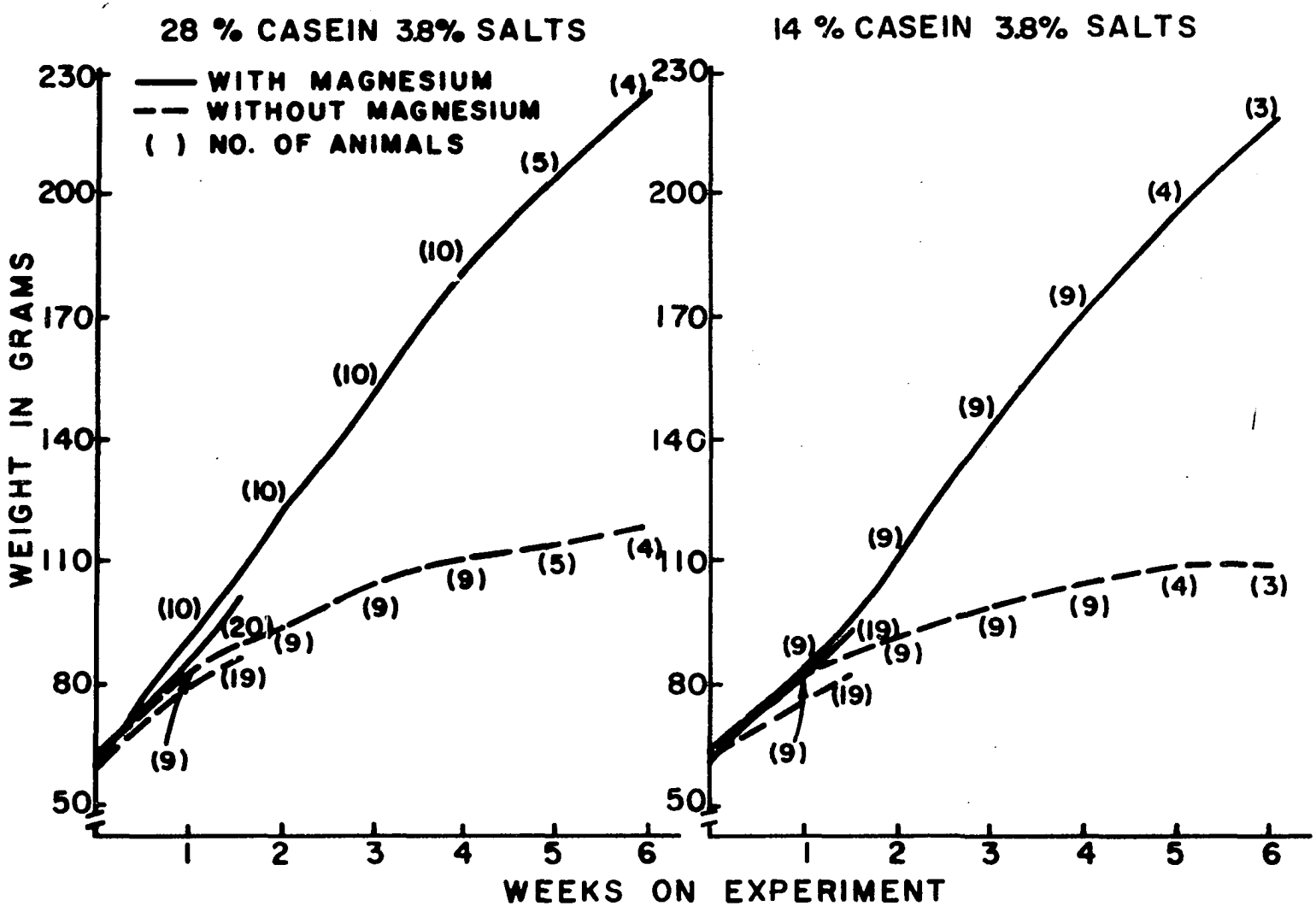

19% CASEIN 2.1% SALTS
28% CASEIN 2.1% SALTS
WITH MG.
WITHOUT MG.
MALE
FEMALE
WEIGHT IN GRAMS
60
100
140
180
220
260
2
4
6
8
10
12
WEEKS ON EXPERIMENT

Figure 2. Growth curves for control and magnesium deficient rats fed diets containing either 14 per cent or 28 per cent casein and 3.8 per cent salt mix




28 % CASEIN 3.8% SALTS
14 % CASEIN 3.8% SALTS
WITH MAGNESIUM
WITHOUT MAGNESIUM
( ) NO. OF ANIMALS
WEIGHT IN GRAMS
230
200
170
140
110
80
50
1
2
3
4
5
6
WEEKS ON EXPERIMENT
(10)
(10)
(10)
(5)
(4)
(20)
(19)
(9)
(9)
(9)
(5)
(4)
(9)
(9)
(9)
(4)
(3)
(19)
(9)
(9)
(9)
(9)
(4)
(3)

different amounts of dietary casein.

Decreased rate of weight gain in deficient animals and development of hyperirritability were concurrent in the present investigation. The muscular spasms that accompanied hyperirritability undoubtedly increased expenditure of energy, which in part, accounted for slower weight gains in deficient than in control animals.

Procedures concerned with assessment of growth usually have been patterned after the growth response test first used by Osborn, Mendel and Ferry (1919), in which both weight gain and food consumption were considered to account for diversity in body size due to variable food intakes of animals being compared. In this study food efficiency ratio (FER) was defined as grams of body weight gained by an animal per 100 grams of food consumed.

Cumulative mean weight gains, food consumptions and FER for animals in Experiment I and Experiment II at the end of the 1st, 2nd and 4th experimental week are reported in Table 13 and Figure 3.

In both experiments, higher casein diets promoted greater FER, both when magnesium was present and when it was absent from the diet, than the lower quantities of casein, and control animals had higher FER than did deficient animals.

During the period of four weeks, FER decreased with time in magnesium deficient animals. FER also decreased somewhat with time when magnesium was fed in the diet; the amount of decrease was greater when the diet contained 28 per cent casein than when either 14 per cent or 19 per cent casein was fed.

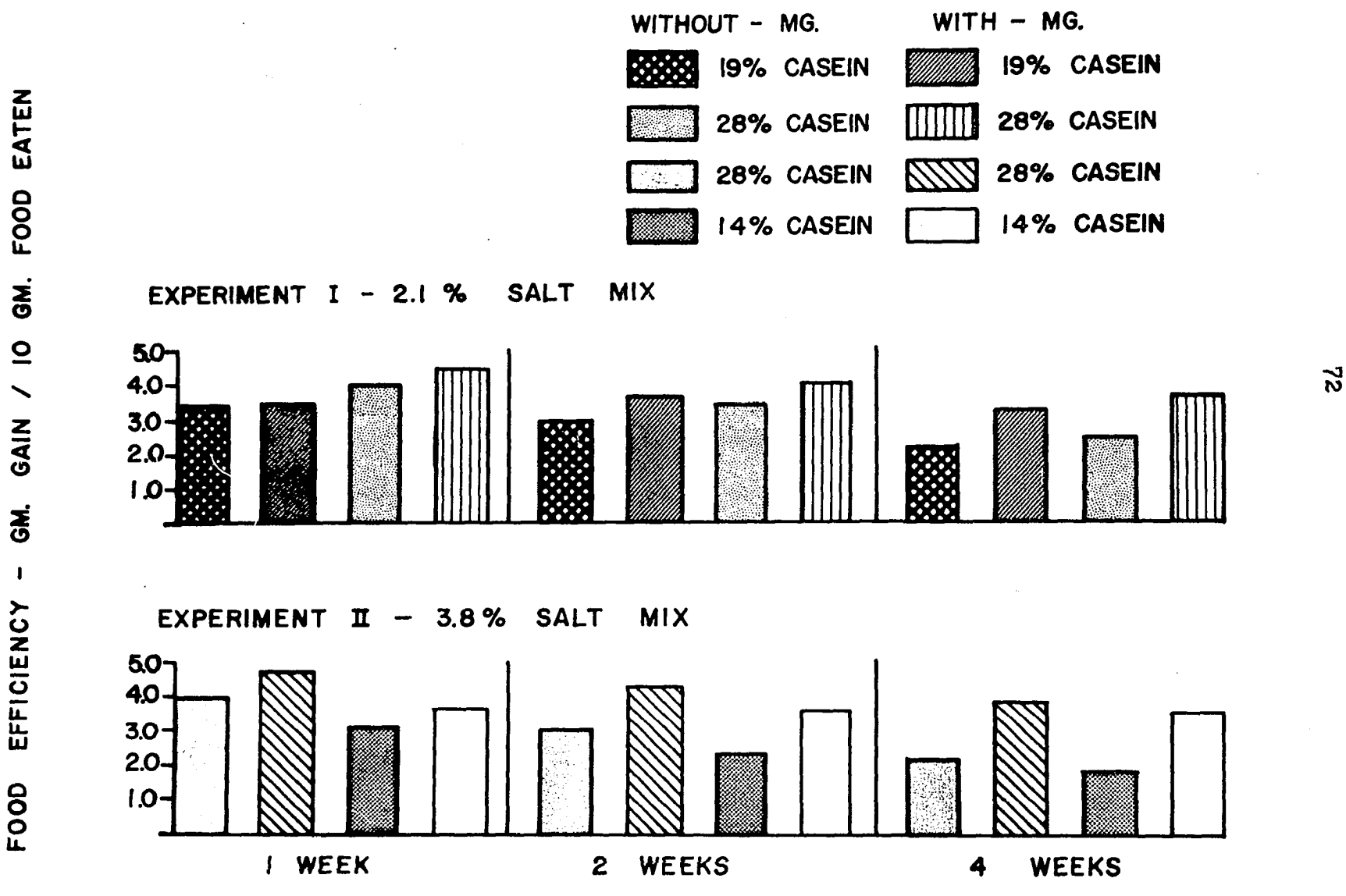
Better growth responses found at higher than at lower concentrations

Table 13. Mean weight gained, food consumed and food efficiency ratio at 1, 2 and 4 weeks of control and magnesium deficient rats fed diets containing 14, 19 or 28 per cent casein

Diet no.	Casein	Sex	1 week				2 weeks				4 weeks			
			No. of rats	Wt. gained	Food consumed	F.E.R.[a]	No. of rats	Wt. gained	Food consumed	F.E.R.	No. of rats	Wt. gained	Food consumed	F.E.R.
	(%)			(gm.)	(gm.)			(gm.)	(gm.)			(gm.)	(gm.)	
Experiment I - 2.1% salt mix														
A-Mg	19	M&F	4	19	54	3.5	4	35	117	3.0	4	50	238	2.1
A+Mg	19	M&F	4	21	58	3.6	4	45	125	3.6	4	86	268	3.2
B-Mg	28	M&F	4	20	48	4.0	4	34	102	3.3	4	50	209	2.4
B+Mg	28	M&F	4	24	54	4.4	4	48	120	4.0	4	94	262	3.6
Experiment II - 3.8% salt mix														
C-Mg	14	M	16	21	51	4.0	15	32	108	3.0	10	48	224	2.2
C+Mg	14	M	15	28	58	4.7	14	59	137	4.3	9	119	309	3.8
D-Mg	28	M	15	17	54	3.2	13	26	110	2.3	9	40	227	1.8
D+Mg	28	M	15	22	60	3.7	13	50	138	3.6	9	109	310	3.5

[a]Food efficiency ratio - expressed as grams of weight gained per 10 grams of food consumed

Figure 3. Mean food efficiency ratios at 1, 2, and 4 weeks for control and magnesium deficient rats fed diets containing 14, 19 or 28 per cent casein


WITHOUT - MG.
WITH - MG.
19% CASEIN
19% CASEIN
28% CASEIN
28% CASEIN
28% CASEIN
28% CASEIN
14% CASEIN
14% CASEIN
EXPERIMENT I - 2.1 % SALT MIX
EXPERIMENT II - 3.8 % SALT MIX
FOOD EFFICIENCY - GM. GAIN / 10 GM. FOOD EATEN
5.0
4.0
3.0
2.0
1.0
1 WEEK
2 WEEKS
4 WEEKS

of casein in the diets of magnesium deficient rats disagreed with observations reported by Menaker (1954), who found that rats fed 7 per cent casein without added magnesium gained more in a 10 day experimental period than those fed 14 per cent casein. However, the present study supported an earlier study of Menaker and Kleiner (1952) in which rats fed 9 per cent casein diets without magnesium gained almost twice as much in a 10 day period as those fed 7 per cent casein in the study mentioned above; and gained more weight when magnesium was present in the diet than when the incomplete mineral ration was fed.

The decreased FER found in the present study when magnesium was not supplied in the diet over that found with animals that had an adequate supply of this mineral confirmed the observations of Kleiber et al. (1941) who found that utilization of food was more efficient when magnesium was present in the diet. By their calculations magnesium deficient rats required 103 grams more food in 57 days to maintain the same body weight as control animals. By failure to gain as much body weight as control animals, the magnesium deficient rats wasted 9.8 kilocalories per day. This wasted energy amounted to 18.5 per cent of the mean energy content of the food consumed by these animals.

Nitrogen Balance

Nitrogen balance was obtained in two consecutive 4 day balance period, the first period coinciding with the appearance of hyperemia in the deficient animals. Mean nitrogen intake, excretion and retention for period I is given in Table 14 and for period II in Table 15. Results for the two balance periods are expressed graphically in Figure 4.

Table 14. Mean nitrogen intake, urinary and fecal nitrogen expressed in milligrams per rat per 4 day period and per cent of consumed nitrogen retained for control and magnesium deficient rats fed diets containing 14 per cent or 28 per cent casein. Experiment II Period I

Diet no.	Casein	No. of rats	Nitrogen				Per cent of consumed N retained
			Intake	In urine	In feces	Retention	
	(%)		(mg.)	(mg.)	(mg.)	(mg.)	(%)
Period I							
C-Mg	28	9	1323 ± 12[a] (1252-1372)[b]	737 ± 26 (563-861)	78 ± 5 (58-103)	508 ± 33 (289-684)	38 (23-50)
C+Mg	28	8	1771 ± 34 (1664-1876)	841 ± 38 (722-1046)	111 ± 7 (82-132)	820 ± 56 (590-1062)	46 (34-57)
D-Mg	14	9	664 ± 17 (568 - 723)	235 ± 12 (162-305)	76 ± 4 (58-96)	353 ± 17 (289-413)	52 (35-62)
D+Mg	14	9	876 ± 29 (735-1025)	169 ± 12 (144-259)	111 ± 5 (77-127)	596 ± 29 (489-739)	68 (57-73)

[a]Standard error of the mean

[b](No.) = range

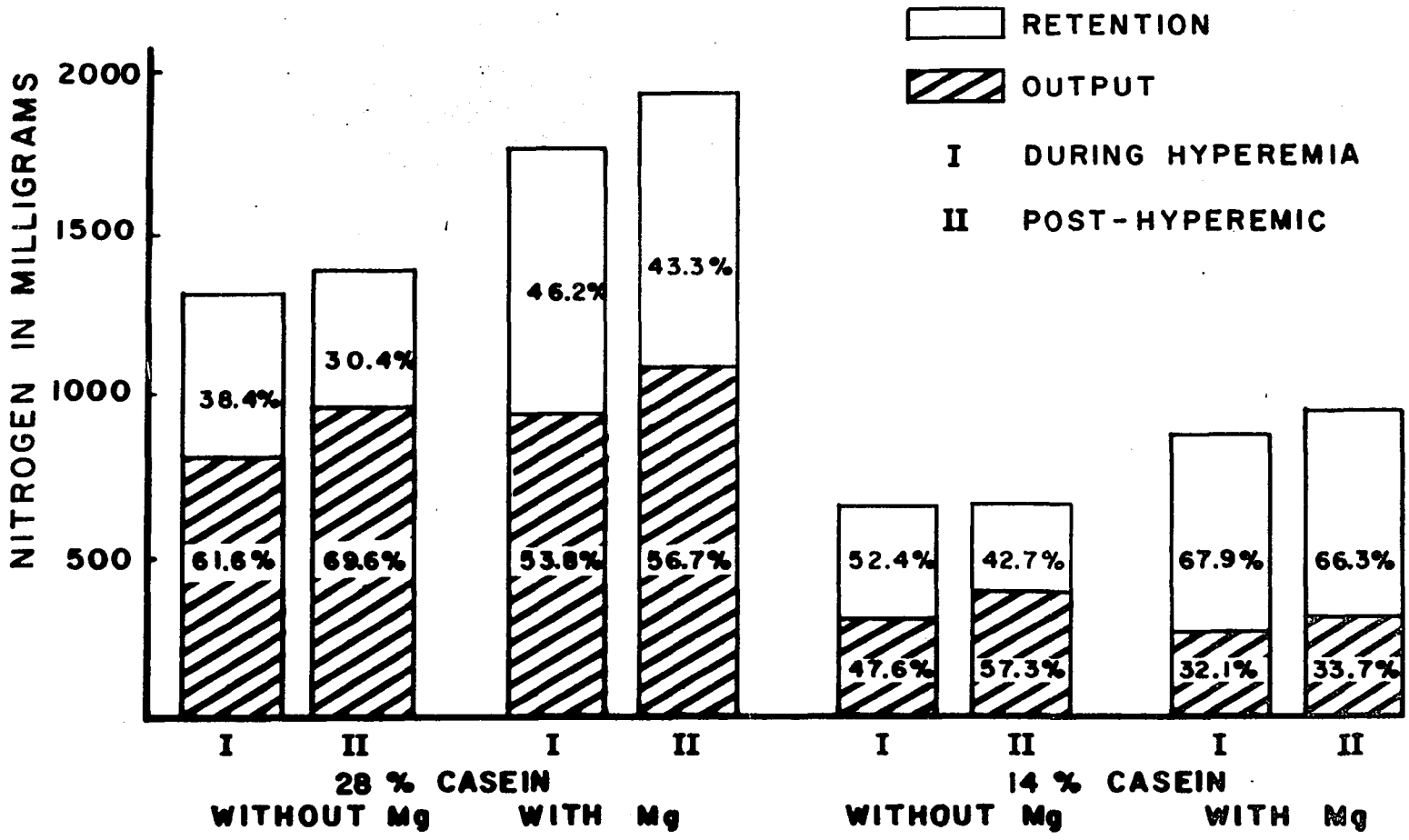
Table 15. Mean nitrogen intake, urinary and fecal nitrogen expressed in milligrams per rat per 4 day period and per cent of consumed nitrogen retained for control and magnesium deficient rats fed diets containing 14 per cent or 28 per cent casein. Experiment II Period II

Diet no.	Casein	No. of rats	Nitrogen				Per cent of consumed N retained
			Intake	In urine	In feces	Retention	
	(%)		(mg.)	(mg.)	(mg.)	(mg.)	(%)
Period II							
C-Mg	28	9	1397 ± 20[a] (1342-1546)[b]	870 ± 12 (834-958)	97 ± 3 (78-111)	429 ± 17 (368-496)	30 (26-35)
C+Mg	28	8	1920 ± 4 (1780-2200)	978 ± 28 (816-1092)	111 ± 8 (68-142)	830 ± 25 (712-978)	43 (40-49)
D-Mg	14	7	666 ± 3 (512-790)	289 ± 15 (210-345)	90 ± 6 (72-108)	287 ± 22 (145-339)	43 (28-52)
D+Mg	14	9	948 ± 29 (791-1083)	201 ± 8 (168-248)	118 ± 9 (86-150)	629 ± 20 (537-704)	66 (63-70)

[a]Standard error of the mean

[b](No.) = range

Figure 4. Nitrogen intake, excretion and retention of control and magnesium deficient rats fed diets containing either 14 per cent or 28 per cent casein. Period I and Period II


RETENTION
OUTPUT
I DURING HYPEREMIA
II POST-HYPEREMIC
NITROGEN IN MILLIGRAMS
2000
1500
1000
500
38.4%
61.6%
30.4%
69.6%
46.2%
53.8%
43.3%
56.7%
52.4%
47.6%
42.7%
57.3%
67.9%
32.1%
66.3%
33.7%
I
II
I
II
I
II
I
II
28 % CASEIN
WITHOUT Mg
WITH Mg
14 % CASEIN
WITHOUT Mg
WITH Mg

All animals were in positive nitrogen balance during the periods measured. In both balance periods, control and magnesium deficient animals on the 28 per cent casein diets consumed twice as much nitrogen as those on 14 per cent casein diets. Similar amounts of nitrogen were consumed by deficient animals in both periods, but control animals consumed more nitrogen in period II than period I.

In each balance period, magnesium deficient animals lost 3 times as much, while control animals lost 5 times as much nitrogen in the urine when the higher casein diet was fed than when the lower casein diet was furnished.

Urinary nitrogen was greater in period II than period I for all groups, but increment in loss in period II over period I was greater for animals fed the higher than the lower concentration of dietary casein.

Fecal nitrogen was unaffected by amount of casein in the diet, although it was somewhat higher in the presence of magnesium than in its absence. For deficient animals, fecal loss in period II was somewhat higher than in period I. The absolute amounts of fecal nitrogen were similar for animals receiving different quantities of casein, but nitrogen intake was smaller for animals fed 14 per cent than those fed 28 per cent casein. Therefore, the percentage of ingested nitrogen lost in the feces was greater with 14 per cent casein than with 28 per cent casein.

Nitrogen retention was greater in animals receiving the higher casein diets than the lower casein diets. In periods I and II, respectively, on the 28 per cent casein diet deficient animals retained 1.4 and 1.5 times as much, and control animals 1.4 and 1.3 times as much nitrogen as animals on the lower casein diet. Deficient animals retained less,

while control animals retained more nitrogen in the 2nd balance period than in the 1st. Magnitude of the decrease in retention of deficient animals was about the same for both concentrations of casein studied, and increased retention for control animals was very small regardless of amount of casein fed.

All groups of animals excreted in the urine a greater percentage of ingested nitrogen in the 2nd balance period than in the 1st. Animals receiving 28 per cent casein diets excreted a greater proportion of consumed nitrogen than those receiving the lower quantity of casein, and deficient animals lost a greater proportion of ingested nitrogen in the urine than control animals. However, with 14 per cent casein in the diet, actual excretion of nitrogen in both balance periods was less when magnesium was present than when it was absent from the diet.

With larger protein intakes, both in magnesium deficient and control animals, lower proportions of ingested nitrogen were retained than when diets furnished less protein, but the presence of magnesium in the diet enabled animals to retain a greater quantity of ingested nitrogen than when it was absent.

Mean weight gains in the combined balance periods was 38.3 grams for control animals fed 28 per cent casein and 32.4 grams for animals fed 14 per cent casein, while deficient animals gained 15.6 and 11.0 grams respectively (Table 16). With deficient diets there was little difference in mean weight gain per gram of nitrogen retained between animals fed either 14 or 28 per cent casein, but when animals were supplied with magnesium, mean weight gain per gram of nitrogen retained by animals fed the lower casein diet was 14 per cent greater than that of animals receiving

Table 16. Mean weight gain, nitrogen retention and weight gain per gram of nitrogen retained by control and magnesium deficient rats fed either 14 or 28 per cent casein for 8 days

	Diet			
	28% casein without Mg.	28% casein with Mg.	14% casein without Mg.	14% casein with Mg.
Weight gain, gm.	15.6	38.3	11.0	32.4
Nitrogen retention, mg.	937	1650	640	1225
Weight gain, gm./gm. nitrogen retained	16.6	23.2	17.2	26.4

28 per cent casein. Thus, nitrogen efficiency on the lower casein diet was greater than on the higher in the presence of magnesium, but not in its absence.

Positive nitrogen balances found in the present investigation indicated that all animals were gaining body protein. The extent of growth, indicated by magnitude of weight gain and nitrogen retention, was greater on higher than lower casein diets and greater when magnesium intake was not restricted that when it was restricted. Of course, animals not restricted in magnesium intake had better appetites, hence consumed more nitrogen than those whose magnesium intake was very low. However, when animals had an adequate supply of magnesium they appeared to be able to use more efficiently the nitrogen consumed than animals fed magnesium deficient diets, as a greater proportion of ingested nitrogen was retained by the former than the latter. This relationship appeared to be true for animals fed either 14 per cent or 28 per cent casein.

Hyperirritability developed in deficient animals during period II

and might have accounted, at least in part, for the lack of food consumption increase in these animals in the 2nd balance period. Depression of appetite was also associated with hyperirritability by Tufts and Greenberg (1938a).

The observations presented in this study confirmed reports of others (Allison et al., 1946 and Barnes and Bosshardt, 1946) that amount of nitrogen retained is greater with higher than with lower nitrogen intake. In the present study this relationship appeared to hold when magnesium was omitted from the diet as well as when animals were fed a complete mineral ration, as deficient animals consumed and retained a greater quantity of nitrogen when 28 per cent casein than when 14 per cent casein diets were fed.

Hemoglobin Concentration and Packed Red Blood Cell Volumes

Hemoglobin concentration and packed red blood cell volumes were measured in Experiment III. Because serum samples from subgroups within this experiment were used for analysis either of distribution of proteins and transaminase activity or of magnesium and nitrogen, means were obtained for each of the subgroups as well as for the combined subgroups (Table 17). Mean packed red blood cell volumes found in this experiment for control animals were 40.2 per cent and 38.9 per cent, respectively, for animals receiving 28 per cent and 14 per cent casein in the diet, a difference which was not significant.

Quantity of casein fed did not influence packed red blood cell volume of either subgroup A or subgroup B. But in the latter subgroup, magnesium

Table 17. Mean and range of hemoglobin concentration and packed red blood cell volume of control and magnesium deficient rats fed diets containing either 14 per cent or 28 per cent casein Experiment III

Diet no.	Casein	Hemoglobin		Packed red blood cell volume	
		Mean	Range	Mean	Range
	(%)	(gm./100 ml.)	(gm./100 ml.)	(%)	(%)
Subgroup A					
C-Mg	28	11.44 ± 0.15[a]	10.78-12.32	43.5 ± 1.26	40.5-49.2
C+Mg	28	11.19 ± 0.21	10.18-12.46	42.1 ± 0.96	39.9-46.2
D-Mg	14	11.20 ± 0.20	10.46-12.44	40.4 ± 1.36	35.5-45.2
D+Mg	14	11.23 ± 0.22	10.08-12.30	39.8 ± 0.96	35.1-45.7
Subgroup B					
C-Mg	28	10.57 ± 0.15	9.90-11.24	41.4 ± 0.58	37.9-42.9
C+Mg	28	10.45 ± 0.33	8.68-11.54	38.9 ± 0.87	34.5-43.5
D-Mg	14	10.75 ± 0.32	9.64-12.30	40.8 ± 1.35	38.0-46.5
D+Mg	14	10.19 ± 0.25	8.90-11.46	38.1 ± 0.28	36.9-40.1
Combined group					
C-Mg	28	11.05 ± 0.15	9.90-12.32	42.3 ± 0.67	37.9-49.2
C+Mg	28	10.84 ± 0.21	8.68-12.46	40.2 ± 0.76	34.5-46.2
D-Mg	14	11.00 ± 0.18	9.64-12.44	40.6 ± 0.91	35.5-46.5
D+Mg	14	10.68 ± 0.20	8.90-12.30	38.9 ± 0.50	35.1-45.7

[a]Standard error of the mean

deficient animals had significantly higher packed red blood cell volumes than control animals. There was no difference in packed red blood cell volumes between deficient and control animals in subgroup A.

Magnesium deficient rats in subgroup B were more irritable than those in subgroup A; 47 per cent of the former experienced seizures, while 33 per cent of the latter displayed overt symptoms of hyperirritability. Although convulsive attacks undoubtedly occurred in the absence of an observer, they were most likely to occur at the time animals were handled. In addition, lung hemorrhage, present in two thirds of the animals in this study that died spontaneously, was found in a greater number of deficient animals in subgroup B than in subgroup A. Thus it appeared that hyperexcitability was more severe in subgroup B than in subgroup A, and might have been the cause of the higher packed red blood cell volumes in the deficient animals in the former subgroup.

Values of packed red blood cell volumes found in this experiment are within the range of those reported in the literature. For male rats, values of 38.0 per cent (Wintrobe et al., 1936) and 43.5 per cent (Cameron and Watson, 1949) have been observed, while Farris and Griffith (1949) reported 50 per cent as the average value for adult rats.

There was no significant difference in hemoglobin concentration of animals in either subgroup A or subgroup B due to quantity of casein in the diet, nor did magnesium deficiency have any effect upon this blood constituent.

Mean hemoglobin concentrations of all animals in subgroup B appeared to be somewhat lower than those of subgroup A, but differences between groups fed similar diets were not significant. Yet, one would expect

higher mean hemoglobin concentrations in deficient animals of subgroup B than of subgroup A because of the higher mean packed red blood cell volume observed in the former group.

Hemoglobin concentrations determined in this study were lower than those reported in the literature. Cameron and Watson (1949) found hemoglobin concentrations for 4 month old male rats averaged 14.6 grams per 100 ml. of blood, while Wintrobe et al., (1936) reported 12.0 grams per 100 ml. blood. No reference to hemoglobin values for young rats was found, but 4 weanlings from the stock colony at Iowa State had a mean of 9.9 grams of hemoglobin per 100 ml. of blood, while mean hemoglobin concentration of 4 animals fed the usual stock diet for 10 days after weaning was 11.2 grams, a value which compares favorably with those found in this study.

Slightly higher hemoglobin values found for deficient animals than those fed the complete diet, although not large enough to be significant, probably reflected a tendency toward hemoconcentration in these animals. Unfortunately, hemoglobin concentrations were not obtained for animals in Experiment I, in which all deficient animals were edematous.

The experimental period used in Experiment III might not have been long enough to show changes in concentration of this blood constituent that would appear after extended periods of magnesium deficiency. Hypochromic anemia has been produced by feeding a low protein diet, but hemoglobin values lower than normal did not appear until the 76th day. Orten and Orten (1943) and Albanese et al. (1947) have demonstrated that certain amino acid deficiencies did not influence blood proteins or hemoglobin although producing sub-optimal growth of immature rats. These observations indicated that hemoglobin would not be expected to change in short

term studies.

Serum Magnesium

Serum magnesium concentrations of animals fed the various experimental diets for differing lengths of time are reported in Table 18.

Hypomagnesemia was observed in all animals fed magnesium deficient diets, and appeared to be most severe in animals that had been on the deficient diets for only a short time. Animals deprived of magnesium for 10 days had mean serum magnesium concentrations of less than 1 mg. per 100 ml. of serum regardless of quantity of casein fed. There appeared to be a higher magnesium concentration in animals fed the lower casein diet than those fed the larger quantity of casein. This difference was not significant, however.

Animals on the experimental regimen for 28 days or longer appeared to have higher serum magnesium concentrations than animals deficient for only 10 days. Significance in these differences could not be demonstrated, however, due to the large variance of individual values, particularly among the 10 day animals. Range of serum magnesium values was large not only after 10 days of magnesium deficiency, but also was large for control animals of this age. Wide variations in serum values have been seen in the young of other species. After measuring the plasma magnesium concentration every 1 to 2 weeks over a period of 18 months, Duncan et al. (1938) concluded that the magnesium of calves could not be regarded as constant.

No significant differences were noted between serum magnesium of animals fed deficient diets for 28 or 42 days, however these groups were small, and, had larger groups of animals been examined statistically

Table 18. Mean and range of serum magnesium of control and magnesium deficient rats fed diets containing 14, 19 or 28 per cent casein for 10, 28, 42 or 84 days

Diet no.	Protein	Season	Length of exp.	No. of animals	Magnesium	
					Mean	Range
	(%)		(days)		(Mg./100 ml.)	(Mg./100 ml.)
Experiment I - 2.1% salt mix						
A-Mg	19	Spring	84	4	1.55	1.25-1.92
A+Mg	19	Spring	84	3	2.73	2.33-3.02
B-Mg	28	Spring	84	4	1.60	1.50-1.72
B+Mg	28	Spring	84	4	2.68	2.38-3.08
Experiment II - 3.8% salt mix						
C-Mg	28	Fall	42	4	1.66	1.55-1.75
C+Mg	28	Fall	42	4	2.89	2.62-3.25
D-Mg	14	Fall	42	3	1.33	1.15-1.45
D+Mg	14	Fall	42	4	2.61	1.95-3.20
C-Mg	28	Fall	28	5	1.28	0.92-1.75
C+Mg	28	Fall	28	4	3.04	2.75-3.25
D-Mg	14	Fall	28	4	1.39	1.00-1.90
D+Mg	14	Fall	28	5	2.89	2.62-3.15
Experiment III-B-3.8% salt mix						
C-Mg	28	Spring	10	9	0.87 ± 0.20[a]	0.30-1.75
C+Mg	28	Spring	10	9	2.81 ± 0.18	1.94-3.50
D-Mg	14	Spring	10	9	0.93 ± 0.24	0.30-1.90
D+Mg	14	Spring	10	10	2.54 ± 0.19	1.40-3.28

[a]Standard error of the mean

significant differences might have appeared.

Quantity of casein in the diet did not influence significantly serum magnesium concentrations of either deficient or control animals, nor did concentration of mineral mix used, season of the year and age of animals, but in each experiment differences between control and magnesium deficient animals were highly significant.

The large variety of methods used for magnesium analysis made it difficult to compare serum magnesium concentrations found in the literature. Normal serum and plasma magnesium values reported for rats, along with method of analysis follows:

	Method	Age	Sex	Magnesium (mg./100 ml.)
Serum				
Orent et al. (1934)	Phosphate precipitation	25-55 days		2.9-3.3
Vitale et al. (1957c)	Titan yellow	24-26 days		1.33-1.75
Watchorn (1933)	Phosphate precipitation	3-4 months	M	5.63
		3-4 months	F	4.39
		5 1/2-16 months	M	4.43
		5 1/2-16 months	F	4.43
Watchorn and McCance (1937)	Phosphate precipitation	3 months	M&F	4.26
Snyder and Tweedy (1942)	Hydroxy-quinoline	30-55 days	M&F	1.5-3.6
Plasma				
Kleiber et al. (1941)	Not given	77 days		3.9
Tufts and Greenberg (1938b)	Hydroxy-quinoline	77-156 days	Pooled	3.10-3.65
Hess et al. (1959)	Flame Photometer		F	1.74

The titan yellow method used in this study gave an overall average of 2.74 mg. magnesium per 100 ml. serum for control animals. This was higher than that found by Vitale et al. (1957c) using a similar method, but lower than values obtained by other methods.

Decreased plasma or serum magnesium concentrations were reported in magnesium deficient animals by Snyder and Tweedy (1942) and Tufts and Greenberg (1938a), but differences in the conditions of these experiments from those of the present investigation and in methods of analysis used make if difficult to compare actual magnesium values obtained.

Data presented in the present study support the observation of Colby and Frye (1951a) that magnesium concentration of the blood was greatly reduced by magnesium deficiency but no further decrease was noted when calcium was added to the magnesium deficient ration.

Orent et al. (1934) found that changes in age from 25 to 58 days did not influence concentrations of serum magnesium in rats. On the other hand, Watchorn (1933) observed lower serum magnesium values in male rats 3 to 4 months of age, than in animals of the same sex 8.5 to 16 months old. Calves attained adult concentrations of serum magnesium within 3 months after birth (Alcroft and Godden, 1934).

In Experiment I, in which animals were equally divided between the sexes, no sex difference was observed; however, the number of animals used in this experiment was small.

Watchorn (1933) found that female rats 3 to 4 months of age had significantly lower magnesium concentrations than males of the same age, but that animals 8.5 to 16 months of age did not differ in serum magnesium concentration between males and females. Simonsen et al. (1947) have

reported higher values of serum magnesium for men than women.

Serum Nitrogen and Protein Distribution

Determination of serum nitrogen was made on rats in all experiments and distribution of serum proteins was measured in rats of Experiments I and in animals of subgroup B in Experiment III.

Mean serum nitrogen concentrations for animals in the various experiments are given in Table 19. For experiment II and III in which animals received the same four diets, but for differing lengths of time, values are expressed graphically in Figure 5.

Quantity of casein fed did not influence mean serum nitrogen concentration either in animals deprived of magnesium or in control animals, regardless of the length of time the experimental diets had been fed.

In all experiments, mean serum nitrogen concentrations were lower for deficient than for control animals. Difference in serum nitrogen concentration between deficient and control animals became significant after 10 days of feeding the 28 per cent, but not the 14 per cent casein diet. Since packed red blood cell volume was significantly higher for deficient than control animals receiving the latter diet, it was possible that differences in total circulating nitrogen were masked by a decreased blood volume in these animals.

All deficient animals in Experiments I and II were edematous at the time samples were obtained for nitrogen analysis. In the 10 day experiment, however, only 2 deficient animals, 1 on the 28 per cent and 1 on the 14 per cent casein diet, were edematous at the time of autopsy.

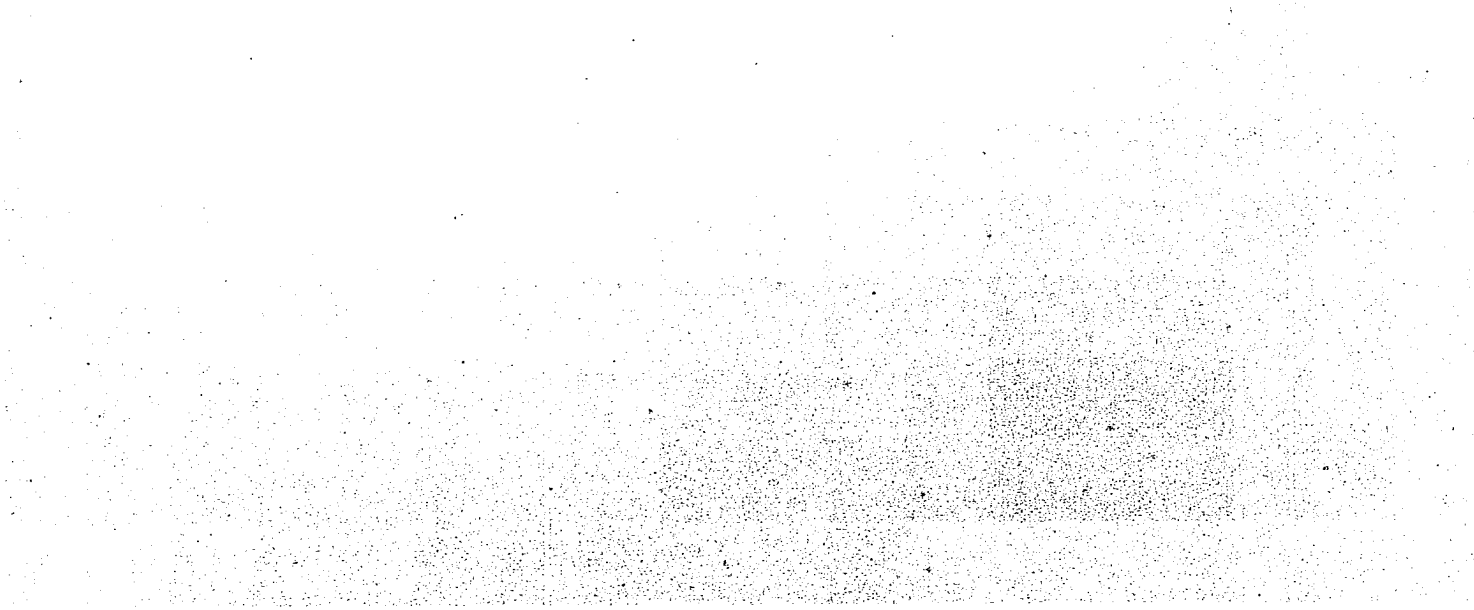
The ability of blood to maintain balance of water between intra-

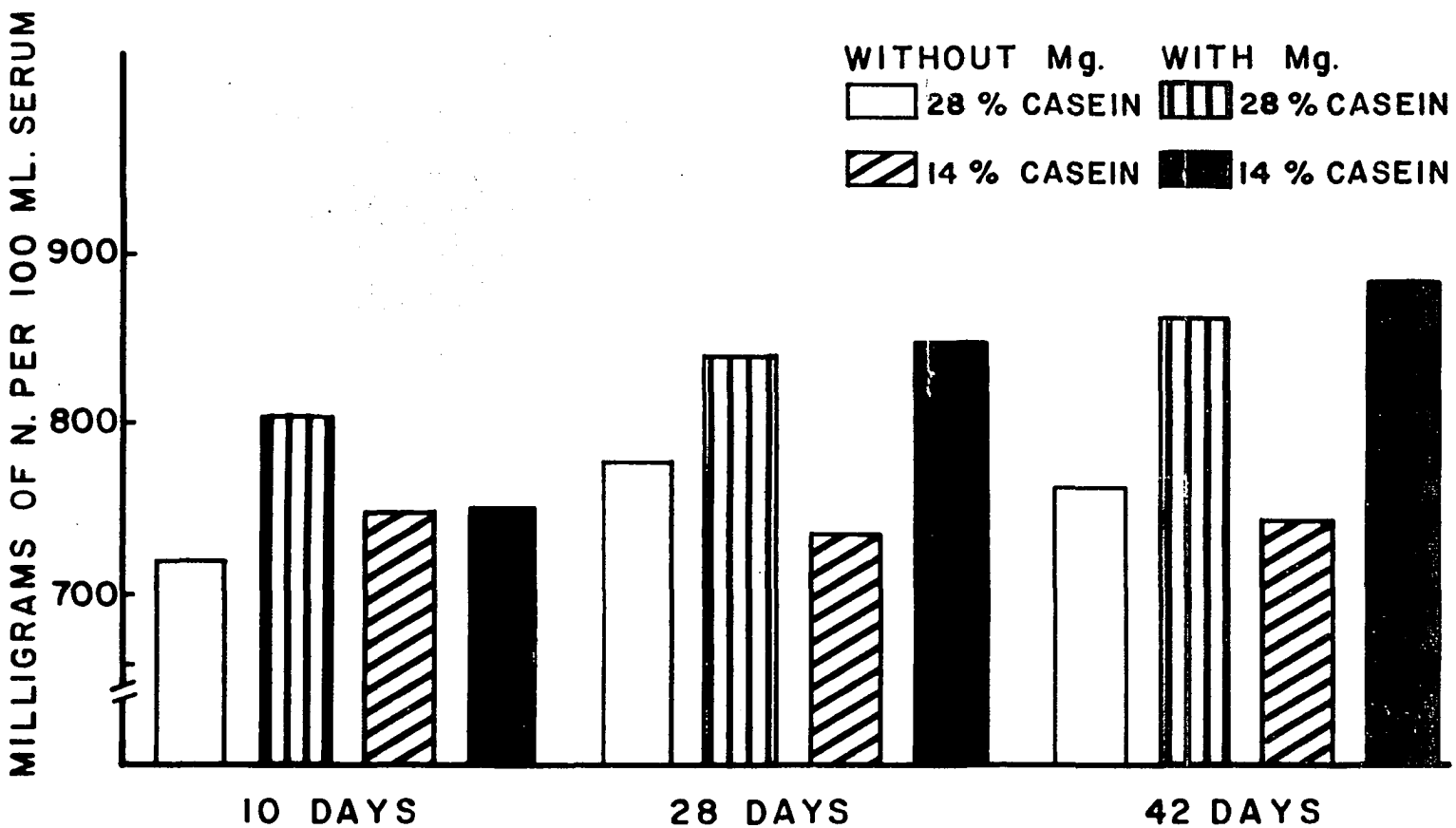
Table 19. Mean and range of total serum nitrogen of control and magnesium deficient rats fed diets containing 14, 19 or 28 per cent casein for 10, 28, 42 or 84 days

Diet no.	Protein	Length of exp.	No. of animals	Nitrogen	
				Average	Range
	(%)	(days)		(Mg./100 ml.)	(Mg./100 ml.)
Experiment I - 2.1% salt mix					
A-Mg	19	84	4	831	754-878
A+Mg	19	84	4	1037	942-1133
B-Mg	28	84	4	794	735-844
B+Mg	28	84	4	1061	962-1235
Experiment II - 3.8% salt mix					
C-Mg	28	42	4	767	697-804
C+Mg	28	42	5	868	851-890
D-Mg	14	42	3	745	731-753
D+Mg	14	42	3	897	869-927
C-Mg	28	28	5	779	626-888
C+Mg	28	28	4	840	777-930
D-Mg	14	28	4	736	629-827
D+Mg	14	28	5	851	794-941
Experiment III-B-3.8% salt mix					
C-Mg	28	10	9	718 ± 25[a]	594-851
C+Mg	28	10	9	806 ± 29	721-998
D-Mg	14	10	9	752 ± 32	657-991
D+Mg	14	10	10	750 ± 8	714-802

[a]Standard error of the mean

Figure 5. Serum nitrogen in control and magnesium deficient rats fed 14, 19 or 28 per cent casein for 10, 28, 42 or 84 days




WITHOUT Mg.
28 % CASEIN
14 % CASEIN
WITH Mg.
28 % CASEIN
14 % CASEIN
MILLIGRAMS OF N. PER 100 ML. SERUM
900
800
700
10 DAYS
28 DAYS
42 DAYS

cellular and extracellular compartment is due in part to its protein concentration. Although many factors may lead to the development of edema, the influence of protein concentration is regarded as a major one because of its influence upon osmotic pressure of the blood. Lowering of the plasma protein concentration lowers the osmotic differential between the blood and interstitial fluid and thereby decreases the rate of transfer of fluid from the tissues.

In this study serum nitrogen, not serum protein was measured. In normal animals, however, the greatest portion of the serum nitrogen has been found to be in the form of protein (Dukes, 1955). A small fraction of non-protein nitrogen was always found present, the amount being more or less dependent upon ability of the kidney to excrete end-products of protein metabolism (Guyton, 1956).

The present study has shown that many animals, limited in growth due to inability to utilize efficiently the dietary nitrogen, had decreased serum nitrogen concentrations. This conditions might have resulted from failure to replace blood proteins at a rate commensurate with their removal, and might have reflected inability of the body to synthesize needed tissue protein when the supply of magnesium was limited. On the other hand, with degenerative changes of the kidney tubule occurring within 9 days from the beginning of magnesium deprivation (Hess et al., 1959) and with appearance of albumin in the urine after two weeks of feeding magnesium deficient diets (Greenberg et al., 1938), consideration must be given to the fact that the lowered serum nitrogen concentration found in this study might have resulted from loss of protein following renal damage.

When the serum proteins were separated by paper electrophoresis the 5 bands that appeared on all papers were designated as albumin$_1$, albumin$_2$, alpha$_1$-, beta- and gamma-globulin in descending order of their mobility. Mean distribution of protein fractions for animals in Experiment I are given in Table 20. In Experiment III-A, the small band of protein appearing between alpha-globulin and beta-globulin was designated as alpha$_2$-globulin. It appeared on 25 per cent of the papers of magnesium deficient animals but was never separated in the sera of control animals (Table 21). Data in this study did not indicate whether this protein was of different composition than any found in the blood of control animals or whether serum proteins in the two groups of animals were similar, but composition of the sera of the deficient animals was such that mobility of certain protein fractions was changed.

In Experiment III-A magnesium deficient animals had a lower percentage of total serum albumin than control animals, but the mean difference was significant only for animals fed 14 per cent casein. There was no difference in per cent of total albumin within either deficient or control animals that could be related to the amount of casein furnished in the diets.

Neither quantity of casein nor of magnesium in the diet influenced the proportions of other proteins found in serum. Mean values for the alpha$_2$-globulin fraction separated in sera of deficient animals were 5.2 per cent and 2.2 per cent, respectively, for animals receiving 28 per cent and 14 per cent casein diets. Since the number of sera in which the alpha$_2$-globulin separated was small, the difference between these two values is probably not significant.

The A:G ratio was not changed by feeding different amounts of dietary

Table 20. Mean and range of relative distribution of serum protein fractions in per cent of total serum protein of rats fed diets containing either 19 per cent or 28 per cent casein and 2.1 per cent salt mix for 84 days. Experiment I

Diet no.	Casein	No. of rats	$Albumin_1$	$Albumin_2$	Globulins			A:G
					$Alpha_1$	Beta	Gamma	
	(%)		(%)	(%)	(%)	(%)	(%)	
A-Mg	19	4	23.8 (20.8-27.0)[a]	19.9 (17.6-22.0)	14.3 (9.0-17.2)	25.2 (22.6-28.4)	16.9 (13.4-20.6)	0.78 (0.62-0.90)
A+Mg	19	4	23.6 (19.6-27.8)	20.4 (18.9-21.4)	11.1 (10.7-11.8)	22.9 (21.1-27.4)	22.2 (19.6-26.2)	0.79 (0.68-0.97)
B-Mg	28	4	24.4 (21.8-27.2)	18.5 (18.0-19.4)	17.8 (17.2-18.8)	21.4 (17.8-24.8)	17.8 (16.8-19.4)	0.76 (0.66-0.87)
B+Mg	28	4	23.7 (19.6-26.6)	20.4 (18.2-21.4)	11.0 (9.4-14.7)	22.2 (18.7-27.2)	22.6 (21.8-23.4)	0.79 (0.73-0.89)

[a](No.) = range

Table 21. Mean and range of relative distribution of serum protein fractions in per cent of total serum protein of rats fed diets containing either 14 per cent or 28 per cent casein and 3.8 per cent salt mix for 10 days. Experiment III

Diet no.	Casein	No. of rats	$Albumin_1$	$Albumin_2$	Globulins				A:G
					$Alpha_1$	$Alpha_2$	Beta	Gamma	
	(%)		(%)	(%)	(%)	(%)	(%)	(%)	
C-Mg	28	10	25.1 ± 0.9[a] (21.2-29.2)[b]	17.6 ± 0.7 (12.8-20.0)	15.1 ± 1.0 (11.4-22.0)	5.2 (0-12.5)	20.7 ± 0.5 (18.1-22.9)	16.2 ± 1.0 (10.8-22.2)	0.75 (0.64-0.97)
C+Mg	28	10	25.1 ± 0.6 (20.4-27.4)	19.9 ± 0.9 (14.0-22.6)	16.9 ± 0.8 (13.2-20.5)	-	21.2 ± 0.7 (17.4-23.9)	16.8 ± 1.0 (12.4-21.2)	0.82 (0.69-0.93)
D-Mg	14	10	23.3 ± 0.5 (22.0-25.2)	18.5 ± 0.9 (15.2-23.1)	15.2 ± 1.3 (10.8-20.0)	2.2 (0-12.7)	23.5 ± 0.7 (21.0-27.0)	17.3 ± 1.2 (13.0-23.3)	0.72 (0.60-0.85)
D+Mg	14	9	25.7 ± 0.7 (22.8-27.9)	20.1 ± 0.5 (18.4-23.3)	15.6 ± 0.9 (12.3-19.4)	-	21.6 ± 0.6 (19.7-25.6)	16.9 ± 0.9 (14.3-23.0)	0.85 (0.71-1.06)

[a]Standard error of the mean

[b](No.) = range

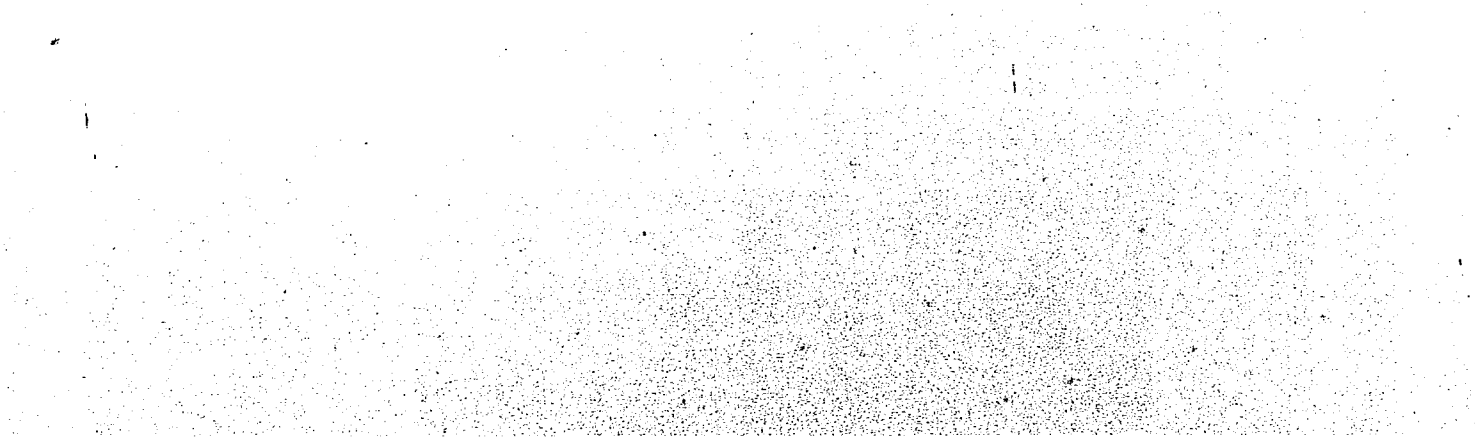
casein, but magnesium deficient animals receiving 14 per cent casein had lower ratios of albumin to globulin than was found in control animals. The change in albumin caused an increase in the proportion of total serum globulins. The magnitude of these changes were small, and the increased globulin appeared to be distributed between the beta-globulin and gamma-globulin.

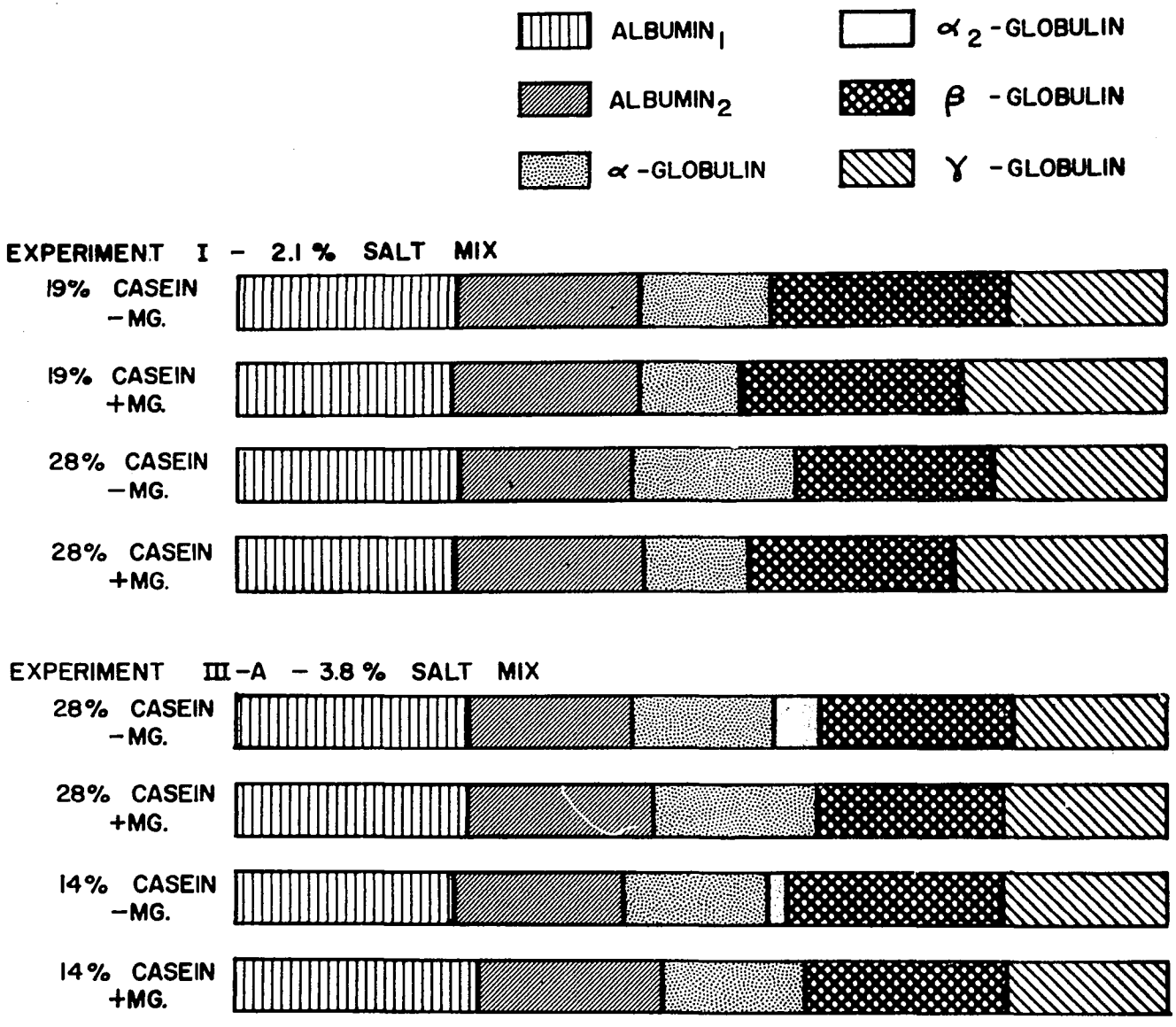
In Experiment I differences in total albumin between magnesium deficient animals and control animals were not significant, and as expected the A:G ratio was slightly lower than in Experiment III-A due to the fact that the former animals were older when blood samples were taken and total serum globulins in rats has been shown to increase with age (Zawadski and Smith, 1932).

In sera of animals deprived of magnesium, the percentage of gamma-globulin decreased, while that of alpha-globulin increased with both concentrations of dietary casein fed, when compared with the same fractions from control animals. When the diet contained 14 per cent casein, the sera of deficient animals increased slightly in beta-globulin, but when the diet contained 28 per cent casein very little change in this fraction occurred between animals receiving no magnesium in the diet and their controls. Quantitatively, distribution of globulins in the sera of older deficient animals receiving 28 per cent casein and 2.1 per cent salt mix resembled that of younger control animals receiving a similar amount of casein and 3.8 per cent salt mix (Figure 6).

In Experiment I, the smaller amount of gamma-globulin in deficient animals than in their controls was interesting in light of the rather strongly held view that reduction of total quantity of serum proteins involved a reduction of serum albumin. Such a reduction would cause a

Figure 6. Distribution of serum proteins in control and magnesium deficient rats fed diets containing 14, 19 or 28 per cent casein




ALBUMIN 1
ALBUMIN 2
α -GLOBULIN
α 2 -GLOBULIN
β -GLOBULIN
γ -GLOBULIN
EXPERIMENT I - 2.1 % SALT MIX
19% CASEIN −MG.
19% CASEIN +MG.
28% CASEIN −MG.
28% CASEIN +MG.
EXPERIMENT III-A - 3.8 % SALT MIX
28% CASEIN −MG.
28% CASEIN +MG.
14% CASEIN −MG.
14% CASEIN +MG.

proportionate increase in total serum globulin when expressed on a percentage basis. This view has been supported by protein depletion studies in which regardless of method used for depletion, the A:G ratio was decreased (Weimer and Nishihara, 1957; Barrows and Chow, 1959).

Although the proportion of serum albumin in deficient animals in Experiment I was the same as control animals, this does not mean that total circulating albumin was the same, since total serum nitrogen concentration was considerably lower for deficient animals than for animals receiving magnesium.

If one assumed that there were no changes in the relative amounts of non-protein nitrogen in the sera of magnesium deficient animals, the actual quantity of albumin present would be lower than that of control animals due to decreased quantity of total nitrogen. This assumption may not be valid since derangement of kidney morphology has been demonstrated before the 10th experimental day of magnesium deficiency (Hess et al., 1959). Moreover, mean non-protein nitrogen determinations on 2 magnesium deficient dogs, examined weekly for 6 weeks, were 35.7 and 31.7 mg. per 100 cc. of blood, respectively, compared to 26.7 mg. in a control dog. More likely, non-protein nitrogen in blood of deficient animals would be elevated, in which case the quantity of serum albumin would be even less in deficient animals than in their controls.

Whether changes in serum nitrogen and concentration of albumin was due to loss of serum proteins in the urine or whether animals failed to synthesize needed blood proteins when the supply of magnesium was limited cannot be answered by this study.

There is little agreement among previously reported values for rat

serum protein fractions that had been separated by paper electrophoresis. Erwin (1960) found the following values for rats 86 days of age: albumin 64.2 per cent, alpha-globulin 7.8 per cent, beta-globulin 16.3 per cent, and gamma-globulin 11.7 per cent. On the other hand, Weimer and his co-workers who studied serum protein changes during protein depletion, have reported that albumin concentrations of sera from rats weighing 400 grams was only 33.8 per cent (Weimer et al., 1959b). In a later communication the various fractions in sera of similar rats were reported as: albumin 34.5 per cent, $alpha_1$-globulin 25.9 per cent, $alpha_2$-globulin 12.1 per cent, beta-globulin 19.0 per cent and gamma-globulin 8.6 per cent (Weimer et al., 1959a). Reasons for discrepancies in values reported perhaps can be ascribed to technical difficulties in the analytical procedure. Factors which have influenced the separation of serum proteins include weight of paper used, ionic strength of buffer, time and strength of the current applied, and type of stain used for visualizing the separate fraction. Only comparisons made within a laboratory have been feasible since methods have not been standardized.

In this study, failure to supply adequate amounts of dietary magnesium appeared to limit the usual increase in gamma-globulin as animals grew older. Recently Erwin (1960) has demonstrated that a similar inhibition could be produced by feeding gluten, an incomplete protein, to weanling rats. Also, protein depleted dogs showed a slight decrease in gamma-globulin as well as reduction of the albumin fraction after the animals were subjected to plasmaphoresis (Chow et al., 1945). Hence, gamma-globulin of blood may be more sensitive to dietary change than was believed formerly.

Hepatic Nitrogen

Weight, concentration of nitrogen and total quantity of nitrogen in livers of rats in the various experiments are given in Tables 22, 23 and 24.

As stated previously, livers of animals deprived of magnesium tended to be larger when the diet contained 28 per cent than 14 per cent casein. With larger livers than found in animals fed the lower quantity of casein, one could expect more total hepatic nitrogen in rats fed 28 per cent casein than in those fed 14 per cent casein, and proved to be so in the present investigation. After 10, and again after 28 days, this difference was highly significant. The higher casein diets not only permitted more total hepatic nitrogen in the latter than the former animals, but also, generally permitted greater nitrogen concentration in their livers than in those fed the lower quantity of dietary casein. Where no difference in nitrogen concentration was found, groups were small and variability within the group large.

The same relationship of hepatic size and nitrogen concentration was found in control animals as was found in the deficient animals, animals with higher casein intake having greater hepatic nitrogen stores, both in terms of concentration and total hepatic nitrogen, than animals fed less casein. Animals receiving 14 per cent casein with added magnesium generally had the lowest nitrogen concentrations of any of the groups in an experiment.

Magnesium deficient animals generally had the same or higher hepatic nitrogen concentration than control animals. However, total hepatic nitrogen was less in the former animals than the latter due to the small amount of hepatic tissue found in deficient animals.

Table 22. Mean and range of hepatic weight, nitrogen concentration and total nitrogen of control and magnesium deficient rats fed diets containing 19 per cent or 28 per cent casein. Experiment I

Diet no.	Casein	Length of exp.	No. of rats	Hepatic weight	Nitrogen	
					Concentration	Total
	(%)	(days)		(gm.)	(gm./100 gm.)	(mg.)
Experiment I - 2.1% salt mix						
A-Mg	19	84	4	5.24 (4.97-6.04)[a]	3.65 (3.44-3.83)	191 (175-208)
A+Mg	19	84	4	7.08 (5.94-8.54)	3.43 (3.19-3.67)	242 (212-272)
B-Mg	28	84	4	5.80 (4.77-6.72)	3.57 (3.46-3.73)	208 (178-247)
B+Mg	28	84	4	7.26 (6.07-9.00)	3.58 (3.32-3.86)	258 (238-313)

[a](No.) = range

Table 23. Mean and range of hepatic weight, nitrogen concentration and total nitrogen of control and magnesium deficient rats fed diets containing 14 per cent or 28 per cent casein. Experiment II

Diet no.	Casein	Length of exp.	No. of rats	Hepatic weight	Nitrogen	
					Concentration	Total
	(%)	(days)		(gm.)	(gm./100 gm.)	(mg.)
Experiment II - 3.8% salt mix						
C-Mg	28	42	4	5.30 (5.05-5.77)[a]	3.58 (3.44-3.75)	190 (182-198)
C+Mg	28	42	4	7.27 (6.05-7.90)	3.59 (3.52-3.74)	260 (226-281)
D-Mg	14	42	4	4.78 (4.22-5.98)	3.60 (3.22-3.87)	170 (154-193)
D+Mg	14	42	4	7.00 (6.29-7.28)	3.32 (3.29-3.36)	232 (211-240)
C-Mg	28	28	5	5.63 (5.22-5.90)	3.55 (3.03-3.86)	200 (179-215)
C+Mg	28	28	4	6.86 (6.00-7.90)	3.45 (3.18-3.69)	236 (221-251)
D-Mg	14	28	4	4.60 (3.78-5.15)	3.38 (2.96-3.63)	154 (152-177)
D+Mg	14	28	4	7.20 (5.93-7.88)	3.10 (2.96-3.50)	222 (208-233)

[a](No.) = range

Table 24. Mean and range of hepatic weight, nitrogen concentration and total nitrogen of control and magnesium deficient rats fed diets containing 14 per cent or 28 per cent casein. Experiment III

Diet no.	Casein	Length of exp.	No. of rats	Hepatic weight	Nitrogen	
					Concentration	Total
	(%)	(days)		(gm.)	(gm./100 gm.)	(mg.)
Experiment III - 3.8% salt mix						
C-Mg	28	10	19	3.69 ± 0.08[a] (3.04-4.17)[b]	3.58 ± 0.04 (3.19-4.01)	132 ± 3 (107-155)
C+Mg	28	10	19	4.55 ± 0.16 (3.29-5.76)	3.36 ± 0.05 (2.98-3.85)	153 ± 5 (119-185)
D-Mg	14	10	19	3.48 ± 0.09 (2.23-4.09)	3.39 ± 0.04 (3.10-3.67)	116 ± 4 (77-142)
D+Mg	14	10	19	4.04 ± 0.14 (3.25-5.43)	3.26 ± 0.06 (2.76-38.2)	130 ± 2 (107-145)

[a]Standard error of the mean

[b](No.) = range

Data presented in this study agreed with reports in the literature that reduction in liver weight and concentration of liver nitrogen reflect quantity of protein fed (Widdowson and McCance, 1957; Benditt et al., 1949).

Hepatic and Serum Transaminase Activities

Activity of glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase was measured in liver and serum of animals fed 14 per cent or 28 per cent casein diets for 10 days. Mean activities of the enzymes were obtained per total liver, per gram of liver and per mg. of hepatic nitrogen and are given in Table 25.

Mean hepatic glutamic-oxalacetic transaminase activity did not differ significantly among the various groups of animals studied, either in regard to activity per gram of liver or per gram of hepatic nitrogen.

Absence of magnesium from the diet appeared to have little influence upon hepatic glutamic-oxalacetic transaminase activity of animals receiving 28 per cent casein, but there was a tendency to greater activity when magnesium was included in the 14 per cent casein diets than when it was omitted. Livers of control animals, being larger than those of deficient animals, had more total activity than deficient animals, but differences were not significant and variation among individual values was wide.

Awapara (1953) did not find a change in activity of glutamic-oxalacetic transaminase in hepatic tissue of adult rats after 20 days of feeding 8 per cent casein compared with rats fed 25 per cent casein. However, Duncan (1959) in this laboratory had observed a significant decrease in hepatic glutamic-oxalacetic activity, both on the basis of activity per

Table 25. Mean and range of hepatic glutamic-oxalacetic and glutamic-pyruvic transaminase activities of control and magnesium deficient rats fed diets containing either 14 per cent or 28 per cent casein. Experiment III

Diet no.	Casein	Hepatic glutamic-oxalacetic transaminase			Hepatic glutamic-pyruvic transaminase		
		Total	Concentration	Per mg. N	Total	Concentration	Total
	(%)	(units x 10^{-4})[a]	(units x 10^{-4} per gm.)	(units x 10^{-3} per mg. N)	(units x 10^{-4})	(units x 10^{-4} per gm.)	(units x 10^{-3} per mg. N)
C-Mg	28	59.0 ± 5.72[b] (27.3-75.9)	16.5 ± 1.7 (8.1-19.8)	4.57 ± 0.42 (2.35-6.52)	14.7 ± 2.2 (5.5-28.6)	4.2 ± 0.6 (1.3-8.0)	1.49 ± 0.28 (0.53-3.70)
C+Mg	28	71.6 ± 5.6 (28.7-89.6)	17.4 ± 1.8 (6.7-27.2)	4.96 ± 0.53 (1.90-7.49)	19.1 ± 3.0 (10.6-40.0)	4.5 ± 0.7 (2.6-9.2)	1.40 ± 0.24 (0.64-3.02)
D-Mg	14	53.4 ± 3.3 (32.7-69.2)	16.0 ± 1.1 (8.7-19.1)	4.73 ± 0.27 (2.73-5.72)	10.5 ± 1.7 (6.0-12.6)	3.2 ± 0.6 (1.7-8.0)	0.94 ± 0.17 (0.53-2.37)
D+Mg	14	70.4 ± 4.2 (57.6-91.0)	18.3 ± 1.3 (14.4-26.6)	5.54 ± 0.24 (4.50-6.96)	12.7 ± 2.2 (4.7-22.9)	3.4 ± 0.6 (1.3-6.7)	1.00 ± 0.17 (0.39-1.75)

[a]Units as stated have been divided by 10^4

[b]Standard error of the mean

gram of liver and per gram of hepatic nitrogen, when 3 per cent protein or protein-free diets were fed to adult rats than when 25 per cent protein diets were fed.

Activity of glutamic-pyruvic transaminase appeared to increase both on the basis of activity per unit of hepatic weight and in relation to hepatic nitrogen with increase in dietary protein. Again, there was large variability in the groups and statistical significance was not obtained.

The larger livers of animals fed the higher casein diets showed more total glutamic-pyruvic transaminase activity than the smaller livers of those furnished diets containing lower quantities of casein.

Control animals, having larger amounts of hepatic tissue than deficient animals, exhibited more total glutamic-pyruvic transaminase activity than the latter. No difference was observed, however, in concentration of the enzyme in hepatic tissues or amount of enzyme per mg. of hepatic nitrogen between the two groups of animals.

Awapara (1953) reported no significant change in alanine-glutamic transaminase after 20 days of feeding 8 per cent casein to rats, but activity of this enzyme was decreased 50 per cent in four days when the diet was protein-free. On the other hand, Kaplanskii et al. (1945) found alanine-glutamic transaminase was decreased in livers of rats fed 8 per cent protein for 25 to 30 days, in comparison with rats with an adequate supply of protein. Young rats fed a 2 per cent casein diet for 30 days had less transaminating capacity than control animals fed 20 per cent casein in the diet (Srinivasan and Patwardhan, 1955). It has also been reported that rats fed protein deficient diets long enough to produce hypoproteinemia have markedly reduced ability to transfer amino groups from glutamic acid

to pyruvic acid (Kaplansky, 1943).

The present study did not support the observation of Cohen and Hekhuis (1941) that hepatic glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase activity was related inversely to protein synthesis.

This study has shown that diets containing 28 per cent casein promoted greater weight gains and nitrogen retention than 14 per cent casein, and that activity of hepatic glutamic-pyruvic transaminase was also increased when 28 per cent casein was fed, but was not related to whether magnesium was present or absent from the diet.

The data presented here suggest that glutamic pyruvic transaminase is more sensitive to change in response to variation in protein content of the diet than is glutamic-oxalacetic transaminase. This observation is supported by the study of Beaton et al. (1957) in which glutamic-pyruvic transaminase activity changed following administration of protein-free diets but glutamic-oxalacetic activity remained the same.

Mean serum glutamic-oxalacetic and glutamic-pyruvic transaminase activities are given in Table 26.

Neither the quantity of casein in the diet nor the absence of magnesium from the ration influenced serum transaminase activities. With both enzymes studied the variability among individual determinations was great, particularly for deficient animals fed 14 per cent casein diets.

A search of the literature revealed little information on transaminating activity in rat serum. Although Spector (1956) gave transaminase values for several tissues in this animal, he did not include this information for serum.

Physical condition of the animal prior to the collection of the blood

Table 26. Mean and range of serum glutamic-oxalacetic and glutamic-pyruvic transaminase of control and magnesium deficient rats fed diets containing either 14 per cent or 28 per cent casein

Diet no.	Casein	Glutamic-oxalacetic transaminase	Glutamic-pyruvic transaminase
	(%)	(units/ml.)	(units/ml.)
C-Mg	28	86.9 ± 8.1[a] (44-120)	10.8 ± 0.7 (8.0-14.5)
C+Mg	28	103.0 ± 10.2 (39-156)	12.0 ± 1.1 (7.5-19.3)
D-Mg	14	109.4 ± 13.2 (38-196)	13.0 ± 1.9 (7.5-26.2)
D+Mg	14	85.9 ± 5.3 (57-109)	12.6 ± 0.9 (8.5-15.5)

[a]Standard error of the mean

sample did not appear to be related to concentration of either glutamic-oxalacetic transaminase or glutamic-pyruvic transaminase in the serum. Cope and Polis (1959) have associated various kinds of stress with lowered glutamic-oxalacetic transaminase in plasma of monkeys. In the present investigation animals that appeared to be under the greatest amount of stress, i.e., observed in a siezure just prior to autopsy, were not the animals that had the greatest serum enzyme concentrations.

SUMMARY AND CONCLUSIONS

Very little information was available on the relationship of magnesium to protein in the intact animal, with the exception of demonstrations by Colby and Frye (1951) and Menaker (1954) that magnesium deficient rats gained less weight when the quantity of protein in the diet was increased than when a smaller amount of protein was fed. However, empirical bases for such a relationship could be found in _in vitro_ studies in which magnesium ions were required by biological systems concerned with protein metabolism. For instance, a magnesium-ATP complex appeared to be the reactive species for all phosphorylations involving transfer of high energy bonds from ATP, a reaction which activates many amino acids. Moreover, activity of glutamic-oxalacetic transaminase was increased by the addition of magnesium ions.

The present study examined selected aspects of protein metabolism in magnesium deficient rats fed different quantities of casein in the diet.

A preliminary experiment, Experiment I, used 4 rats in each group, equally divided as to sex, fed diets containing 19 per cent or 28 per cent casein, with and without the addition of magnesium, and 2.1 per cent salt mix. Growth and food efficiency were studied until the 84th day when viscera were inspected for pathological changes and serum samples were obtained for nitrogen, protein distribution and magnesium measurements.

In the second and again in the third experiments, in which only male animals were used, differences between quantities of protein in the diets were increased by using casein concentrations of 14 per cent and 28 per cent. The amount of mineral mix was increased to 3.8 per cent in order

to control diarrhea which had developed on the 2.1 per cent salt intake.

In Experiment II, growth and food efficiency were studied, and nitrogen balances were obtained while the rats were hyperemic and in the period immediately following hyperemia. Total serum nitrogen, serum magnesium and hepatic nitrogen were measured in animals sacrificed on the 28th and 42nd experimental day.

Glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase activities were measured in serum and livers of animals in subgroup A of Experiment III after 10 days of feeding the experimental diets. Data on hepatic nitrogen, packed red blood cell volume, hemoglobin concentration and relative proportions of the various blood proteins after separation on paper by electrophoresis were also obtained for these animals. Data obtained for subgroup B animals consisted of hemoglobin concentration, packed red blood cell volume, hepatic nitrogen and concentration of serum nitrogen and magnesium.

The following statements summarize the results of this study:

1. Animals deprived of magnesium developed classical symptoms of vasodilation and hyperemia followed by a period of hyperexcitability. Pathological changes were observed in kidneys, and hemorrhage was sometimes present in the lungs and intestinal tract of magnesium deficient animals.

2. After 10 days absolute weights of kidneys and testes, and after 28 days absolute weights of livers, were heavier when the deficient animals received 28 per cent casein than when the lower casein diets were fed.

3. Weights of liver, kidneys, adrenals and testes were heavier in proportion to body weight, at each concentration of casein studied, when animals were deprived of magnesium than when the complete diet was fed.

4. In the absence of magnesium, concentration of dietary casein made little difference in weight gain of the animals, however weight gained at each casein concentration was less than for control animals.

5. Food efficiency was greater with the 28 per cent than with the 14 or 19 per cent casein diets, and greater at each concentration of casein studied when magnesium was present in the diet than when it was absent.

6. Nitrogen retention was greater, both in magnesium deficient and control animals when 28 per cent than when 14 per cent casein diets were fed. With magnesium in the diet, animals not only consumed more food, but they also retained a greater proportion of the nitrogen consumed.

7. Magnesium deficient rats retained less nitrogen in the 2nd balance period than in the first period; on the other hand, control animals stored similar amounts of nitrogen in both balance periods.

8. When diets were deficient in magnesium there was little difference in mean weight gain per gram of nitrogen retained by animals receiving different concentrations of dietary casein. When animals were supplied with magnesium, however, mean weight gain per gram of nitrogen retained was greater for animals receiving the lower quantity of casein in the diet than for those that received the larger amounts.

9. Hemoglobin concentration was not affected by magnesium deficiency or quantity of casein in the diet.

10. Absence of magnesium from the diet caused a highly significant decrease in serum magnesium by the 10th experimental day.

11. Serum nitrogen was not affected by quantity of casein fed in any of the experimental periods studied, although it was lower for deficient animals than their controls.

12. There was a lower proportion of serum albumin in magnesium deficient animals fed the experimental diets for 10 days, than in their controls, although the difference was significant only for animals receiving the 14 per cent casein diet. A small band of protein, designated as alpha$_2$-globulin, was separated in one-fourth of the sera of deficient animals; however this band never appeared in sera of control animals.

13. After 84 days of very low magnesium intake, the per cent of gamma-globulin decreased and that of alpha-globulin increased while albumin did not change. Quantitatively, distribution of globulins in the sera of older deficient animals receiving 28 per cent casein and 2.1 per cent salt mix resembled that of younger control animals receiving a similar quantity of casein and 3.8 per cent salt mix.

14. Total hepatic nitrogen and hepatic nitrogen concentration were generally greater in magnesium deficient animals when 28 per cent casein than when 14 per cent casein diets were fed.

15. In deficient and control animals, activity of hepatic glutamic-pyruvic, but not glutamic-oxalacetic, transaminase appeared to be greater, both on the basis of activity per unit of hepatic weight and per mg. of hepatic nitrogen when 28 per cent casein than when 14 per cent casein was supplied; on the other hand magnesium deficiency did not affect activity of these enzymes.

16. Neither quantity of casein in the diet nor the absence of magnesium from the ration influenced serum glutamic-oxalacetic and glutamic-pyruvic transaminase activity.

It can be concluded from the data herein reported that magnesium deficiency influenced protein metabolism by (1) decreasing nitrogen retention

and weight gain per gram of nitrogen retained, thus preventing as good a rate of growth as seen with control animals, (2) causing a decrease in total serum nitrogen and in proportion of serum albumin, and (3) preventing an increase of serum gamma-globulin as animals grew older.

In this investigation animals fed 14 per cent casein without magnesium were less well off than deficient animals receiving 28 per cent casein because they had significantly less total hepatic nitrogen, lower serum albumin and a smaller A:G ratio than animals fed larger quantities of casein. In addition, in deficient animals fed the lower quantity of casein organ weights tended to be smaller, food efficiency was somewhat less and there was a tendency to less hepatic glutamic-pyruvic transaminase activity than when the diet contained 28 per cent casein. There was little or no change, however, in hemoglobin concentration, weight gain per gram of nitrogen retained, serum magnesium or serum nitrogen concentration in deficient animals fed differing quantities of casein.

Thus, data presented in this study did not support observations of Menaker (1954) and Colby and Frye (1957b) that increased quantities of casein in diets of magnesium deficient rats were more deleterious than when lower concentrations were used. The difference in findings between the present investigation and those reported earlier might have been due to the casein concentrations employed in the various experiments. The 14, 19, and 28 per cent casein diets fed in the present investigation, although as much as 50 per cent different from each other, were closer to the rat's requirement for protein than either the 7 per cent or 50 per cent casein diets used by Menaker and Colby and Frye, respectively.

The author would like to suggest that the present investigation be extended to include observation of protein metabolism in magnesium deficiency after experimental diets containing 14 per cent and 28 per cent casein have been administered for longer than 10 days. To decrease mortality of deficient animals, a salt concentration lower than 3.8 per cent in these diets is recommended. Further, protein metabolism should be investigated when very low concentrations of dietary casein are employed.

LITERATURE CITED

Albanese, A. A., Holt, L. E., Jr., Irby, V., Snyderman, S. E., and Lein M.
1947 Studies on protein metabolism of infants. II. Tryptophane requirement of the infant.
Bull. Johns Hopkins Hosp. 80: 158-165.

Alcroft, W. M. and Godden, W.
1934 Changes in the calcium and magnesium of the serum and in inorganic phosphorus of the blood of cows at calving and of the calf during early life.
Biochem. J. 28: 1004-1007.

Allison, J. B., Anderson, J. A., and Seeley, R. D.
1946 The determination of the nitrogen balance index in normal and hypoproteinemic dogs.
Ann. N. Y. Acad. Sci. 47: 245-271.

Association of Official Agricultural Chemists
1950 Official methods of analysis.
7th ed. Washington, D. C. Author.

Awapara, J.
1953 Effect of protein depletion on transaminating activities of some rat organs.
J. Biol. Chem. 200: 537-541.

Axelrod, D. R. and Bass, D. E.
1956 Electrolytes and acid-base balance in hypothermia.
Amer. J. Physiol. 186: 31-34.

Bain, J. A.
1954 The effect of magnesium upon thyroxine inhibition of phosphorylation.
J. Pharm. Exp. Therap. 110: 2-3.

Barnes, R. H. and Bosshardt, D. K.
1946 The evaluation of protein quality in the normal animal.
Ann. N. Y. Acad. Sci. 47: 273-296.

Barron, G. P., Brown, S. O., and Pearson, P. B.
1949 Histologic manifestations of a magnesium deficiency in the rat and rabbit.
Proc. Soc. Exp. Biol. Med. 70: 220-223.

Barron, G. P., Pearson, P. B., and Brown, S. O.
1948 Magnesium deficiency in the sexually mature rat.
Proc. Soc. Exp. Biol. Med. 69: 128-130.

Barrows, C. H., Jr. and Chow, B. F.
1959 Dietary proteins and synthesis of tissue protein. In Albanese, A. A., ed. Protein and amino acid nutrition. pp. 117-142. New York, N. Y., Academic Press.

Beaton, G. H., Curry, D. M., and Veen, M. J.
1957 Alanine glutamic transaminase activity and protein metabolism. Arch. Biochem. Biophys. 70: 288-290.

Beechey, R. B. and Happold, F. C.
1955 The enzymatic interconversion of pyridoxal phosphate and pyridoxamine phosphate by aqueous extracts of E. Coli. Biochem. J. 61: 20.

Belanger, L. F., Van Erkel, G. A., and Jakerow, A.
1957 Behavior of the dermal mast cells in magnesium-deficient rats. Science 126: 29-30.

Benditt, E. P., Wissler, R. W., Wollridge, R. L., Rowley, D. A., and
1949 Steffee, C. H.
Loss of body protein by rats on low protein diets. Proc. Soc. Exp. Biol. Med. 70: 240-243.

Bird, F. H.
1949 Magnesium deficiency in the chick. J. Nutrition 39: 13-29.

Blaxter, K. L. and Rook, J. A. F.
1955 Energy and carbohydrate metabolism in magnesium deficient calves. Brit. J. Nutrition 9: 121-132.

Block, R. J., Durrum, E. L., and Zweig, G.
1958 A manual of paper chromatography and paper electrophoresis. 2nd ed. New York, N. Y., Acad. Press.

Brookfield, R. W.
1934 Magnesium deficiency in the rat. Brit. Med. J. 1: 848-849.

Cameron, D. G. and Watson, G. M.
1949 The blood count of the adult albino rat. Blood 4: 816-818.

Chow, B. F., Allison, J. B., Cole, W. H., and Seeley, R. D.
1945 Effect of protein depletion on plasma proteins in the dog measured by electrophoretic analysis. Proc. Soc. Exp. Biol. Med. 60: 14-17.

Cohen, P. P. and Hekhuis, G. L.
1941 Transamination in tumors, fetal tissue and regenerating liver. Cancer Res. 1: 620-626.

Colby, R. W. and Frye, C. M.
1951a Effect of feeding various levels of calcium, potassium, and magnesium to rats.
Amer. J. Physiol. 166: 209-212.

Colby, R. W. and Frye, C. M.
1951b Effect of feeding high levels of protein and calcium in rat rations on magnesium deficiency syndrome.
Amer. J. Physiol. 166: 408-412.

Cope, F. W. and Polis, B. D.
1959 Increased plasma glutamic-oxalacetic transaminase activity in monkeys due to non-specific stress.
J. Aviation Med. 30: 90-96. (Original not available for examination; abstracted in Chem. Abstr. 53: 2235. 1959).

Cotlove, E., Halliday, M. A., Schwartz, R., and Wallace, W. M.
1951 Effects of electrolyte depletion and acid-base disturbance on muscle cations.
Amer. J. Physiol. 167: 665-675.

Cramer, W.
1932 Experimental production of kidney lesions by diet.
Lancet 223: 174-175.

Dick, H. J. and Prior, J. T.
1951 Experimental acute metastatic calcification in the kidney of rats.
Amer. J. Clin. Path. 21: 409-422.

Duckworth, J. and Godden, W.
1940 The influence of diets low in magnesium upon the chemical composition of the incisor tooth of the rat.
J. Physiol. 99: 1-7.

Duckworth, J., Godden, W., and Warnock, G. M.
1940 The effect of acute magnesium deficiency on bone formation in rats.
Biochem. J. 34: 97-108.

Dukes, H. H.
1955 The physiology of domestic animals. 7th ed.
Ithaca, N. Y. Comstock Publishing Assoc.

Duncan, B. J.
1959 Influence of dietary protein on the activity of serum and liver transaminase of the rat.
Unpublished M. S. Thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.

Duncan, C. W., Huffman, C. F., and Robinson, S. C.
1935 Magnesium studies in calves. I. Tetany produced by a ration of milk or milk with various supplements.
J. Biol. Chem. 108: 35-44.

Duncan, C. W., Lightfoot, C. C., and Huffman, C. F.
1938 Studies of the composition of bovine blood. I. The magnesium content of the blood plasma of the normal dairy calf.
J. Dairy Sci. 21: 689-696.

Eppright, E. S. and Smith, A. H.
1937 The influence of specific mineral deficiencies on the growth of body and organs of the rat.
J. Nutrition 14: 21-33.

Erdtmann, H.
1927 Glycerophosphatspaltung durch Nierenphosphatase und irhe Aktivierung.
Hoppe-Seyler's Ztschr. 172: 182-198.

Erwin, E. S.
1960 Effect of dietary protein quality on alteration of serum proteins and lipoproteins in the rat.
Proc. Soc. Exp. Biol. Med. 103: 396-398.

Farris, E. J. and Griffith, J. Q.
1949 The rat in laboratory investigation.
2d. ed. Philadelphia, Pa., J. B. Lippincott Co.

Frost, D. V. and Sandy, H. R.
1953 Effects of mineral deficiencies on amino acid utilization: critical role of potassium and phosphorus.
Proc. Soc. Exp. Biol. Med. 83: 102-105.

Garner, R. E., Orent, E. R., McCollum, E. V., and Hyde, R. R.
1932 No inhibition of a transplantable sarcoma and carcinoma in the rat after depletion of magnesium by diet.
Amer. J. Hyg. 16: 323-324.

Greenberg, D. M., Lucia, S. P., and Tufts, E. V.
1938 The effect of magnesium deprivation on renal function.
Amer. J. Physiol. 121: 424-430.

Guyton, A. C.
1956 Textbook of medical physiology.
Philadelphia, Pa., W. B. Saunders Co.

Happold, F. C. and Turner, J. M.
1957 Effect of magnesium ions on heart muscle transaminase.
Nature 179: 155-156.

Hegsted, D. M., Vitale, J. J., and McGrath, H.
1956 Effect of low temperature and dietary calcium upon magnesium requirements.
J. Nutrition 58: 175-188.

Heinicke, H. R., Harper, A. E., and Elvehjem, C. A.
1955 Protein and amino acid requirements of the guinea pig. I. Effect of carbohydrate, protein level and amino acid supplementation.
J. Nutrition 57: 483-496.

Heinicke, H. R., Harper, A. E., and Elvehjem, C. A.
1956 Protein and amino acid requirement of the guinea pig. II. Effect of age, potassium and magnesium and type of protein.
J. Nutrition 58: 269-280.

Hess, R., MacIntyre, I., Alcock, N., and Pearse, A. G. E.
1959 Histochemical changes in rat kidney in magnesium deprivation.
Brit. J. Exp. Path. 40: 80-86.

Hiller, A., Plazin, J., and Van Slyke, D. D.
1948 A study of conditions for Kjeldahl determinations of nitrogen in protein.
J. Biol. Chem. 176: 1401-1419.

Hoobler, S., Kruse, H., and McCollum, E.
1937 Studies on magnesium deficiency in animals. VIII. Effect of magnesium deprivation on the total and untrafilterable calcium and magnesium of the serum.
Amer. J. Hyg. 25: 86-106.

Irving, J. T.
1940 The influence of diets low in magnesium upon the histological appearance of the incisor tooth of the rat.
Jr. Physiol. 99: 8-17.

Jones, J. H. and Foster, C.
1942 A salt mixture for use with basal diets either low or high in phosphorus.
J. Nutrition 24: 245-256.

Jungherr, E. L., Snyder, J. M., and Scott, H. M.
1958 Cytopathologic changes in liver cord cells of arginine deficient chicks.
J. Nutrition 65: 281-292.

Kalant, U. and Clamen, M.
1959 Dietary magnesium and oxygen consumption.
Arch. Biochem. Biophys. 85: 563-564.

Kaplansky, S. J.
1943 J. Nervopathologii i Psykhiatrii. No. 5-6.
(Original not available for examination; cited in Braunstein, A. E. Transamination and the integrative functions of the dicarboxylic acids in nitrogen metabolism. Adv. in Prot. Chem. 3: 2-52b. 1947).

Kaplanskii, S., Berezovskaya, N., and Shmerling, Z. L.
1945 Influence of a diet low in protein on deamination, transamination on and synthesis of amino acids in the liver and kidney. (In Russian:English summary). Biokhimiya 10: 401-406. (Original not available for examination; abstracted in Chem. Abstr. 40: 3174. 1946).

Karmen, A.
1955 A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum.
J. Clin. Invest. 34: 131-133.

Kashiwa, H. K. and Hungerford, G. F.
1958 Blood leucocyte response in rats fed a magnesium deficient diet.
Proc. Soc. Exp. Biol. Med. 99: 441-443.

Kleiber, M., Boelter, M. D. D., and Greenberg, D. M.
1941 Fasting catabolism and food utilization of magnesium deficient rats.
J. Nutrition 21: 363-372.

Klein, H., Orent, E., and McCollum, E. V.
1935 The effects of magnesium deficiency in the teeth and their supporting structure in rats.
Amer. J. Physiol. 112: 256-262.

Kruse, H. D., Orent, E., and McCollum, E. V.
1932a Chemical changes in the blood following magnesium deprivation.
J. Biol. Chem. 97: 3-4.

Kruse, H. D., Orent, E. R., and McCollum, E. V.
1932b Studies on magnesium deficiency in animals. I. Symptomatology resulting from magnesium deprivation.
J. Biol. Chem. 96: 519-539.

Kruse, H. D., Orent, E., and McCollum, E. V.
1933 Studies in magnesium deficiency animals. III. Chemical changes in blood following magnesium deprivation.
J. Biol. Chem. 100: 603-643.

Kuby, S. A., Noda, L., and Lardy, H. A.
1954 Adenosinetriphosphate-creatine transphosphorylase. III. Kinetic studies.
J. Biol. Chem. 210: 65-82.

Kunkel, H. O. and Pearson, P. B.
1948 Magnesium in the nutrition of the rabbit.
J. Nutrition 36: 657-666.

LeRoy, J.
1926 Necessite du magnesium pour la croissance de la sauris.
C. R. Soc. Biol. 94: 431-433.
(Original not available for examination; cited in Kruse, H. D., Orent, N. R., and McCollum, E. V. Studies on Magnesium deficiency in animals. J. Biol. Chem. 96: 519-539. 1932).

MacIntyre, I. and Davisson, D.
1958 The production of secondary potassium depletion, sodium retention nephrocalcinosis and hypercalcaemia by magnesium deficiency.
Biochem. J. 70: 456-462.

Martinus, C. and Hess, B.
1951 The mode of action of thyroxine.
Arch. Biolchem. Biophys. 33: 486-487.

Maynard, L. A., Boggs, D. F., Fisk, G., and Seguin, D.
1958 Dietary mineral interrelations as a cause of soft tissue calcification in guinea pigs.
J. Nutrition 64: 85-97.

Meeker, E. W. and Wagner, E. C.
1933 Titration of ammonia in presence of boric acid.
Ind. Eng. Chem. Anal. Ed. 5: 396-398.

Mefferd, R. B., Jr., Hale, H. B., and Martens, H. H.
1958 Nitrogen and electrolyte excretion of rats chronically exposed to adverse environments.
Amer. J. Physiol. 192: 209-218.

Menaker, W.
1954 Influence of protein intake on magnesium requirement during protein synthesis.
Proc. Soc. Exp. Biol. Med. 85: 149-151.

Menaker, W. and Kleiner, I. S.
1952 Effect of deficiency of magnesium and other minerals on protein synthesis.
Proc. Soc. Exp. Biol. Med. 81: 377-379.

Moore, L. A., Hallman, E. T., and Sholl, L. B.
1938 Cardiovascular and other lesions in calves fed diets low in magnesium.
Arch. Path. 26: 820-838.

Mudd, S. H., Park, J. H., and Lippman, F.
1955 Magnesium antagonism of the uncoupling of oxidative phosphorlation by iodo-thyronines.
Natl. Acad. Sci. Proc. 41: 571-576.

Mulinos, M. G. and Pomerantz, L.
1940 Pseudo-hypophysectomy: a condition resembling hyphophysectomy produced by malnutrition.
J. Nutrition 19: 493-504.

O'Dell, B. L., Morris, E. R., and Regan, W. O.
1960 Magnesium requirement of guinea pigs and rats; effect of calcium and phosphorus and symptoms of magnesium deficiency.
J. Nutrition 70: 103-111.

Orange, M. and Rhein, H. C.
1951 Microestimation of magnesium in body fluids.
J. Biol. Chem. 189: 379-386.

Orent, E., Kruse, H. D., and McCollum, E. V.
1932 Studies on magnesium deficiency in animals. II. Species variation in symptomatology of magnesium deprivation.
Amer. J. Physiol. 101: 454-461.

Orent, E. R., Kruse, H. D., and McCollum, E. V.
1934 Studies on magnesium deficiency in animals. VI. Chemical changes in bone, with associated blood changes, resulting from magnesium deprivation.
J. Biol. Chem. 106: 573-593.

Orten, A. V. and Orten, J. M.
1943 The role of dietary protein in hemoglobin formation.
J. Nutrition 26: 21-31.

Osborn, T. B. and Mendel, L. B.
1918 The inorganic elements in nutrition.
J. Biol. Chem. 34: 131-139.

Osborn, T. B., Mendel, B., and Ferry, E. L.
1919 A method of expressing numerically the growth promoting value of proteins.
J. Biol. Chem. 37: 223-229.

Peters, J. P. and Van Slyke, D. D.
1932 Quantitative clinical chemistry. 2nd ed.
Baltimore, Md., Williams and Wilkins Co.

Platner, W. S. and Hosko, N. J., Jr.
1953 Mobility of serum magnesium in hypothermia.
Amer. J. Physiol. 174: 273-276.

Riedesel, M. L. and Folk, G. E., Jr.
1955 Serum magnesium and hibernation.
Federation Proc. 15: 151.

Rivero-Fontan, J., Paschkis, K. E., West, E., and Cantarow, A.
1952 Influence of different protein levels in starvation on the endocrine system of male and female rats.
Endocrinol. 51: 100-109.

Roine, P., Booth, A. N., Elvehjem, C. A., and Hart, E. B.
1949 Importance of potassium and magnesium in nutrition of guinea pigs.
Proc. Soc. Exp. Biol. Med. 71: 90-91.

Sauberlich, H. E. and Baumann, C. A.
1949 Excretion of amino acids by mice fed certain deficient diets.
Arch. Biochem. 20: 305-314.

Schrader, G. A., Prickett, C. O., and Salmon, W. D.
1937 Symptomatology and pathology of potassium and magnesium deficiencies in the rat.
J. Nutrition 14: 85-109.

Simonsen, D. S., Westover, L. M., and Wertman, M.
1947 The determination of serum magnesium by the molybdivanate method for phosphate.
J. Biol. Chem. 169: 39-47.

Siperstein, D. M.
1921 The effect of acute and chronic inanition upon the development and structure of the testis in the albino rat.
Anat. Rec. 20: 355-381.

Sjollema, B.
1932 Nutritional and metabolic disorders in cattle.
Nut. Abst. Rev. 1: 621-632.

Snyder, F. H. and Tweedy, W. R.
1942 The effects of a magnesium-deficient diet on the serum phosphotase activity in the albino rat.
J. Biol. Chem. 146: 639-647.

Spector, W. S., editor.
1956 Handbook of biological data.
Philadelphia, Pa., W. B. Saunders Co.

Srinivasan, R. R. and Patwardhan, N. V.
1955 The effect of protein deficiency on some liver constituents and enzymes in liver, pancreas and blood plasma in albino rats.
Indian J. Med. Res. 43: 1-13.

Steinberg, D., Baldwin, D., and Ostrow, B. H.
1956 A clinical method for the assay of serum glutamic-oxalacetic transaminase.
J. Lab. Clin. Med. 48: 144-151.

Tapley, D. F. and Cooper, C.
1956 The effect of thyroxine and related compounds on oxidative phosphorylation.
J. Biol. Chem. 222: 341-349.

Tufts, E. V. and Greenberg, D. M.
1938a The biochemistry of magnesium deficiency. I. Chemical changes resulting from magnesium deprivation.
J. Biol. Chem. 122: 693-714.

Tufts, E. V. and Greenberg, D. M.
1938b The biochemistry of magnesium deficiency. II. The minimum magnesium requirement for growth, gestation, and lactation, and effect of dietary calcium level thereon.
J. Biol. Chem. 122: 715-726.

Tufts, E. V. and Greenberg, D. M.
1938c Nature of magnesium tetany.
Amer. J. Physiol. 121: 416-423.

Van Reen, R. and Pearson, P. B.
1953 Magnesium deficiency in the duck.
J. Nutrition 51: 191-203.

Vitale, J. J., Hegsted, D. M., and Nakamara, M.
1957a The effect of magnesium deficiency on oxidative phosphorylation.
J. Biol. Chem. 228: 573-576.

Vitale, J. J., Hegsted, D. M., Nakamara, M., and Connors, P.
1957b The effect of thyroxine on magnesium requirement.
J. Biol. Chem. 226: 597-601.

Vitale, J. J., Hellerstein, E. E., Nakamara, M., Lown, B., Hegsted, D. M.,
1959 and Caner, J.
Arteriosclerosis in puppies fed a magnesium deficient diet.
Federation Proc. 18: 550.

Vitale, J. J., White, P. L., Nakamura, M., Hegsted, D. M., Zamcheck, M.,
1957c and Hellerstein, E. E.
Interrelationships between experimental hypercholesteremia, magnesium requirement, and experimental atherosclerosis.
J. Exp. Med. 106: 757-766.

Watchorn, E.
1933 The normal serum-calcium and magnesium of the rat: their relation to sex and age.
Biochem. J. 27: 1875-1878.

Watchorn, E. and McCance, R. A.
1937 Subacute magnesium deficiency in rats.
Biochem. J. 31: 1379-1389.

Weimer, H. E., Bell, R. T., and Nishihara, H.
1959a Dietary protein and serum electrophoretic patterns of the adult rat.
Proc. Soc. Exp. Biol. Med. 100: 853-855.

Weimer, H. E. and Nishihara, H.
1957 Effects of protein depletion and inanition on serum glycoprotein concentration in the rat.
Proc. Soc. Exp. Biol. Med. 95: 677-679.

Weimer, H. E., Nishihara, H., and Ling, S. H.
1959b Serial determinations of serum protein-bound carbohydrates and protein during protein depletion and repletion in the adult rat.
J. Nutrition 69: 151-157.

Wickham, N.
1958 Calcification in soft tissues associated with magnesium dietary deficiency in the guinea pig.
Austral. Vet. J. 34: 244-248.

Widdowson, E. M. and McCance, R. A.
1957 Effect of a low protein diet on the chemical composition of the bodies and tissues of young rats.
Brit. J. Nutrition 11: 198-206.

Wintrobe, M. M., Shumacker, H. B., Jr., and Schmitt, W. J.
1936 Values for number, size, and hemoglobin content of erythrocytes in normal dogs, rabbits and rats.
Amer. J. Physiol. 114: 502-507.

Wroblewski, F. and La Due
1956 Serum glutamic-pyruvic transaminase in cardiac and hepatic disease.
Proc. Soc. Exp. Biol. Med. 91: 569-571.

Yamane, G. M. and Singer, L.
1953 Effect of subminimal magnesium on Syrian hampsters.
J. Dent. Res. 32: 708-709.

Zawadzki, E. S. and Smith, A. H.
1941 The pattern of serum proteins during accelerated growth.
Amer. J. Physiol. 132: 362-367.

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APPENDIX

Table 27. Recovery of ammonium sulfate nitrogen of a standard solution from cages, simulating experimental conditions

Cage no.	Nitrogen in standard solution	Quantity of nitrogen recovered	
	(mg.)	(mg.)	(%)
I	14.03	13.48	96.1
		13.33	95.0
		13.53	96.4
II	14.03	13.53	96.4
		13.53	96.4
		13.64	97.2
	Average		96.2

Table 28. Recovery of nitrogen from creatinine by macrokjeldahl process

Creatinine nitrogen	Nitrogen recovered	
(mg.)	(mg.)	(%)
42.22	41.82	99.0
39.92	38.98	97.6
37.67	36.83	97.8
33.93	33.32	98.2
Average		98.2

Table 29. Recovery of nitrogen from creatinine solution by microkjeldahl procedure

Nitrogen in sample	Nitrogen recovered	Recovery
(mg.)	(mg.)	(%)
1.00	0.981	98.1
	0.994	99.4
	0.988	98.8
	0.986	98.6
	0.955	95.5
	Average	98.1
1.00	0.990	99.0
	0.966	96.6
	0.974	97.4
	0.990	99.0
	0.974	97.4
	0.991	99.1
	Average	98.1