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A Study of the Interrelationship of Magnesium Metabolism with that of Protein in the Human Adult

Sara McClanahan Hunt
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Sara McClanahan Hunt entitled "A Study of the Interrelationship of Magnesium Metabolism with that of Protein in the Human Adult." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Nutrition.

Frances A. Schofield, Major Professor

We have read this dissertation and recommend its acceptance:

Lura M. Odland, Bernadine Meyer, John T. Smith

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

July 15, 1963

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We have read this dissertation
and recommend its acceptance:

Sara M. Odland

Bernadine Meyer

John T. Smith

Torsten P. Salo

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

A STUDY OF THE INTERRELATIONSHIP OF MAGNESIUM METABOLISM WITH THAT OF PROTEIN IN THE HUMAN ADULT

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Sara McClanahan Hunt

August 1963

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CHAPTER I

INTRODUCTION

No dietary allowance for magnesium has been recommended although this mineral has long been recognized as indispensable to man. Since magnesium is widely distributed in food, it is generally believed that ingestion of foods furnishing calcium at the recommended level should assure an ample supply of magnesium. An average magnesium intake of the population of the United States with the exception of very young children approximates 300-350 milligrams (hereafter referred to as mg) daily. This amount of magnesium permits only small retentions of the mineral and is considered the approximate requirement of both children and adults.

As part of a cooperative study of metabolic patterns in preadolescent children, magnesium balances of 35 girls were estimated in the Nutrition Laboratory at The University of Tennessee. Diets furnishing 17 to 80 grams (hereafter referred to as g) protein daily supplied 121 to 250 mg of magnesium. Both nitrogen and magnesium balances were positive for all subjects; moreover, children fed the low-protein, low-magnesium diet stored as much magnesium as did those receiving twice as much of the mineral from the high-protein diet. The results suggested that either the magnesium requirement of children was not as great as had been previously supposed or the level of protein in the diet influenced the absorption and retention of the mineral.

The purpose of the work reported in this paper was to study the influence of level of dietary protein upon utilization of magnesium. The experiments consisted of a series of metabolic balance studies in which young adults were fed diets containing different, relatively low levels of protein and constant amounts of magnesium and other nutrients. Results will provide information concerning the influence of dietary protein upon magnesium metabolism in the adult and serve as a basis for planning a future similar study with preadolescent children.

CHAPTER II

REVIEW OF LITERATURE

No human dietary allowance for magnesium has been recommended although this mineral has long been recognized as indispensable to man. Stearns (1950) and Sherman (1952) pointed out that, since magnesium is widely distributed in foods, ingestion of foods furnishing calcium at the recommended level should assure an ample supply of magnesium. Duckworth and Warnock (1942), in a review of all reported studies on magnesium metabolism in man to that date, and Macy (1942) in growth studies found that children on generally good diets had magnesium intakes between 300 and 350 mg daily and retained only small quantities of the mineral. These findings are the basis for the assumption that approximately 300 mg magnesium daily represent the probable requirement for this age group. When Widdowson and McCance (1954) studied the effects of flour with different rates of extraction upon the growth and mineral metabolism of children in Germany, they found that, although children consuming white bread were receiving about 300 mg magnesium daily and those eating the wholemeal bread were getting approximately 800 mg, their retentions of the mineral were the same and almost identical with the magnesium retentions in 11- and 12-year-old children observed by Macy (1942).

Schofield and Morrell (1960), participating in a southern cooperative project devoted to the determination of metabolic patterns in preadolescent girls, studied magnesium retention in these girls fed diets supplying varying levels of protein. Magnesium intakes varied along with

the protein, so that girls receiving the smallest quantity of nitrogen were also ingesting the least amount of magnesium. Although the controlled diets were carefully planned to supply at recommended levels all nutrients, except protein, for which there are allowances, low intakes of nitrogen were accompanied by decreased quantities of certain B-vitamins and trace minerals which are associated with protein and magnesium in food sources and for which requirements are not established. Under these conditions children receiving as little as 0.1 mg nitrogen per kilogram body weight and 121 mg magnesium daily were in positive nitrogen balance and were retaining essentially the same amount of magnesium as were those on the diets supplying 0.39 to 0.46 mg nitrogen per kilogram body weight and up to 250 mg magnesium per day. The decreased excretion of magnesium and certain trace minerals in the feces was responsible for the increased percentage retention by children receiving restricted diets. Results of this study raise the question whether children have a lower magnesium requirement than previously supposed or whether the increased retention is an indication of interrelationships among nutrients.

In 1942 Duckworth and Warnock, taking the mean of the estimates of magnesium requirement for healthy adult males determined by Brull (1936) as approximately 200 mg per day and by Tibbets and Aub (1937) as being 300 mg daily, tentatively set the recommended intake for men at 250 mg daily and that of women at a 10 per cent lower level. These suggestions for magnesium requirement would seem to indicate that the American diet, which was estimated by Sherman (1952) to contain, on

the average, 350 mg magnesium per day, is adequate in this mineral.

Metabolic experiments conducted in other parts of the world where the dietary pattern is different from that of Britain and America produced results on magnesium utilization which did not agree with the tentative requirements for the mineral proposed by Duckworth and Warnock (1942). Basu and Malakar (1940) in India studied magnesium metabolism in three adult females who came from poor village families. The experimental diet was similar to the customary high-cereal diet of the subjects. These investigators, basing their conclusion upon the average of 20 metabolic experiments on the three female subjects, set the mean magnesium requirement for an adult at 429 mg daily. Normal Chinese adults of both sexes were studied in order to measure calcium, phosphorus, nitrogen and magnesium metabolism on varying levels of intake (Chu et al., 1941). No sex differences in the pattern of magnesium metabolism was found. Although no attempt was made to correlate magnesium retention with nitrogen intake, the authors believed that negative nitrogen balance caused by a low intake of protein had some relationship to the negative magnesium balances which occurred on an intake of 342 mg. These authors concluded that magnesium equilibrium could not be maintained on a daily intake of less than 300 mg.

More recent studies conducted for the purpose of determining the daily magnesium needs of the adult female have agreed no more closely in their results than have the earlier experiments. Leichsenring et al. (1951) studied magnesium metabolism in college women when they were maintained on a diet restricted in calcium and phosphorus but

supplying 261 mg magnesium along with 50 g protein and adequate amounts of other nutrients. This level of magnesium intake appeared to the authors to represent the approximate need of the subjects since they exhibited both positive and negative balances with a mean retention of 11.6±2.9 mg daily.

Meyer et al. (1955) investigated the response of college women to a standardized diet developed to serve as a basal diet suitable for a variety of human nutrition studies. In this particular study the diet was planned to be adequate in every respect during the first half of the experiment; during the last 20 days of observation the fat content was reduced for half of the subjects in order to determine the effect of a low-fat diet upon the metabolic pattern of the various nutrients. The mean daily nitrogen intake for all subjects for the entire study was 10.99 g, with 27 per cent derived from animal sources. About 69 per cent of the protein was supplied by gluten and cake flours served in the form of yeast rolls, cookies and cobblers. The calculated magnesium level was 220 mg, the amount suggested by Duckworth and Warnock (1942) as the daily need of adult females. Upon analysis, however, the average daily intake supplied by the diet was only 182 mg. On this intake of magnesium, 5 of the 6 subjects were in definitely negative balance. Decreasing the fat content of the diet from 76 to 24 g daily did not appear to alter magnesium metabolism in any way.

Leverton et al. (1961), using the same standardized diet adjusted to supply daily 11 g nitrogen furnished primarily from yeast rolls made with gluten flour and supplemented with casein, studied magnesium

metabolism in 30 young women on different dietary levels of the mineral. Magnesium intake was altered by varying the amount of magnesium gluconate incorporated into the rolls. Excretion and retention of magnesium increased significantly as intake increased, but only 22 per cent of the variation in excretion could be related to variation in intake. On the other hand, 44 per cent of the variation in retention could be related to level of intake. Positive magnesium balance was not attained on intakes lower than 274 to 282 mg daily, at which level the mean retention was 7 mg with 6 out of the 16 subjects in slightly negative balance. With a consumption of 320 mg daily all subjects were in positive magnesium balance with an average retention of 28 mg. On the lower intakes of 173 to 191 mg and 233 to 252 mg, the mean balances were -33 and -12 mg, respectively. From this experiment the authors concluded that no less than 280 mg magnesium daily was sufficient for equilibrium in adult females and that possibly an intake of 320 mg was necessary.

Calcium, phosphorus and magnesium balances of young college women consuming self-selected diets were reported by Scoular et al. (1957). Eighty-six students ranging in age from 17 to 27 years participated in this experiment, and determination of their mineral balances was based upon one 5-day metabolic balance period. The subjects were divided into two groups: group 1 was made up of girls ranging in age from 16 to 20 years; and group 2 consisted of those over 20 years of age. The total daily intake of magnesium provided by these self-selected diets ranged from 160 to 2550 mg in each group. For all subjects negative balances were more frequent when the magnesium intake

ranged from 160 to 300 mg daily, while the greater portion of the positive balances appeared when the average magnesium content of the diet was 950 mg and 1020 mg for groups 1 and 2, respectively. In general, more negative balances occurred among the taller girls of each group than among those below average height. From this study it would appear that a magnesium intake varying from 950 to 1020 mg might be recommended for young college women. Because calcium and phosphorus intakes, as well as magnesium, were high on these self-selected diets, it might be assumed that the amount of other nutrients ingested was above average also. The apparent requirement of approximately 1000 mg magnesium daily in these young women, in contrast to the much lower estimated needs of subjects on regulated diets, may indicate an interrelationship among nutrients. On the other hand, if each magnesium balance represented only one period of short duration, probably no interpretation is justified.

In spite of the relatively large magnesium requirement indicated by the studies reviewed, Fitzgerald and Fourman (1956) were unable to produce a magnesium deficiency in two men who were maintained for 20 days or longer on a diet containing only 13.4 mg magnesium daily. During the entire 20-day period Subject A excreted 823 mg, or an average of 41.1 mg per day, with approximately 45 per cent of the mineral appearing in the feces. In Subject B the magnesium excretion was greater because he was fed a cationic exchange resin which removed much of the mineral from the digestive tract. After the first 4 days of adjustment to the low-magnesium diet, urinary losses dropped to approximately 12 mg

daily, in agreement with the finding of McCance and Widdowson (1939) that the kidneys are exceedingly sensitive to variations in levels of the mineral in serum. The latter gave intravenous doses of magnesium to subjects in positive magnesium balance and found that the dose was almost completely excreted in the urine. When a dose was given orally, most of the magnesium appeared in the feces with the urinary magnesium reflecting only the extent of absorption of the excess mineral.

The magnesium conserving mechanisms in young female subjects receiving a liquid diet low in protein and calcium and containing no more than 1.5 mg magnesium daily have been studied recently (Barnes et al., 1958). Renal conservation prevented maximum losses in excess of 18 mg per day, and fecal excretion was negligible after a transition period. The quantity of magnesium lost in the feces was notably less than the amount of the mineral which would normally be carried by the digestive juices to the lumen of the bowel. The authors suggested that the magnesium requirement of adults may be considerably less than the usual estimate if the magnesium conserving mechanisms observed over a 10-day period in subjects receiving a low-calcium, low-protein diet are permanent.

Information on the effect of different levels of intake upon magnesium absorption and excretion in human adults was obtained by Graham et al. (1960) through the use of Mg^{28}. The subjects were fed diets containing three levels of magnesium. After adjustment to each particular level of intake, they were given orally a test dose of Mg^{28}. On a diet furnishing 23 mg magnesium and 18.1 g protein daily, the

subjects absorbed, on the average, 75.8 per cent of the ingested isotope. When a normal diet furnishing 243 mg magnesium and 74.2 g protein was consumed the subjects absorbed only 44.3 per cent of the administered isotope, and, following supplementation of the normal diet to provide 85 g protein and 572 mg magnesium daily, absorption of Mg^{28} was depressed to 23.7 per cent. To determine whether the extent of absorption was due to the load of magnesium presented to the intestinal mucosa or to the need of the body for the mineral, two subjects were maintained on the normal diet for 8 days and then were given a breakfast of different magnesium content along with the test dose of Mg^{28}. One subject received breakfast from the low-magnesium diet while the other was fed from the supplemented diet. The percentage absorption of the isotope indicated that absorption is not importantly affected by depletion or repletion of the body magnesium but is influenced by the load presented to the intestinal mucosa, since the pattern of absorption was almost identical to that obtained when the subjects were maintained on the high- or low-magnesium diet. The fact that each diet varied not only in its magnesium content but also in the amount of protein raises the question of the possible effect of the level of protein on percentage absorption. Children suffering with kwashiorkor have been found to absorb a higher percentage of a constant magnesium intake when maintained upon a low-protein diet than when receiving a high-protein diet (Linder et al., 1963). In all subjects studied by Graham et al. (1960) the rate of absorption had reached a steady level at two to three hours and continued more or less constant for the next 4 to 6 hours. This

period of time corresponds fairly well with that needed for the meal to traverse the small intestine and suggests not only slow absorption of the mineral but also a more or less uniform absorption along the length of the small intestine.

The transport of magnesium across the intestinal wall of the rat was found through in vitro studies to be significantly correlated with the concentration of the mineral being circulated through the intestinal lumen (Ross, 1962). The relationship between transport and concentration, however, was not linear. The absence of a linear relationship suggested that the absorption of magnesium was not a purely passive process but was achieved either through an active transport system or by means of facilitated diffusion of magnesium.

Studies with experimental animals have indicated that a relationship exists between the level of protein in the diet and the amount of magnesium required by the animal. Colby and Frye (1951) found that the severity of magnesium deficiency in young rats on a low-magnesium diet was increased by changing the casein content of the rations from 25 to 50 per cent. Amino acid supplementation of a 24 per cent casein diet caused weight loss in guinea pigs unless extra potassium and magnesium salts were added to the rations (Heinicke et al., 1956). Increasing the casein content of the diet from 24 to 30 per cent made amino acid supplementation even more harmful. Addition of either potassium acetate or magnesium oxide immediately reversed weight losses, but only through the inclusion of both salts was a satisfactory rate of gain maintained.

Menaker (1954) studied the effect on weight gain of different protein levels in magnesium-sufficient and deficient diets fed to adult female rats whose weights had been reduced 20 to 25 per cent by starvation. Casein was present in the amount of 7, 9 or 14 per cent of the diet. Little difference was found between gains of the rats on magnesium-sufficient and deficient diets at the 7 per cent protein level because, presumably, the amount of protein was the limiting factor. At the 9 per cent level, however, the difference between weight gain of the rats receiving the complete mineral supplement and those on the magnesium-deficient supplement was significant, and the greatest difference was observed at the 14 per cent level of protein. With the complete mineral supplement, on the 14 per cent casein diet gains were more than double those at the 7 per cent level. When magnesium was omitted at both levels of protein intake, however, the rats on the 7 per cent casein ration gained more than twice as much as those fed the highest level of protein.

The influence of the dietary level of protein on magnesium requirement was studied by Bunce et al. (1953) in both chicks and rats. Three groups of chicks were fed either 12, 24 or 36 per cent casein diets with a magnesium content of 100 parts per million (hereafter referred to as ppm), while each of three other groups received one of the same levels of protein with 600 ppm magnesium. In the rat experiment the animals were divided at weaning into 6 groups, three being fed a 12 per cent and the others a 36 per cent casein diet. At each protein level one group of rats received magnesium at 100, 200 or 600 ppm

of the diet. In both chicks and rats reduction of dietary magnesium to 100 ppm resulted in a severely restricted weight gain in the groups fed the higher levels of protein but was without effect in the 12 per cent protein group. Rats consuming 100 ppm magnesium and the 36 per cent protein ration had a significantly lower serum protein than did those receiving the same amount of magnesium but only 12 per cent casein. Moreover, the rats on the lower protein intake and the two lower levels of magnesium excreted significantly more free amino acids in the urine than did those receiving the same amount of protein but the highest level of magnesium. Bunce and his associates concluded that growth, appearance and rate of morbidity of the various groups together with the increased excretion of certain amino acids and decreased levels of serum protein in rats on reduced magnesium intake, support the belief that magnesium requirement of both chicks and young rats is directly related to the level of dietary protein.

Magnesium absorption was studied in human adults maintained on a diet in which the protein was supplied largely by vegetables and wholemeal bread (McCance _et al._, 1942). Daily protein and magnesium intakes were 45 to 70 g and 400 to 600 mg, respectively. When 100 g of several different protein supplements were added, magnesium absorption was increased from 32 to 41 per cent of the intake. In growing rats, however, an increase in the casein content of rations from 8 to 16 per cent had little effect upon magnesium absorption although the accelerated growth rate of animals fed the 16 per cent protein diet probably increased their magnesium requirement (Toothill, 1963).

Head and Rook (1955) suggested that hypomagnesaemia in cows grazing on new pasture in the spring months might be caused by the inability of the animals to absorb properly the marginal amounts of magnesium provided by the pastures because of the high nitrogen content of the young grass. Later Rook and Campling (1962) found that within any group of herbages the lowest "availability" of magnesium to cows occurred in the grasses cut at an early stage of growth and the highest in those cut at the mature stage. Supplementation of herbage with protein-rich oil cake also decreased the availability of dietary magnesium to the ruminant.

Lambs fed a purified ration simulating the high-protein, high-potassium composition of wheat pasture characteristic of "wheat pasture poisoning" excreted significantly more magnesium in the feces and retained less of the mineral than did the controls (Fontenot et al., 1960). The investigators concluded that the high-protein or the high-potassium content of the ration either interfered with magnesium absorption or promoted reexcretion of this ion into the gut.

The recently published data of O'Dell and Steel (Hathaway, 1962) showed that adult women could maintain magnesium equilibirum on a diet supplying daily 248 mg of magnesium and 8 g nitrogen provided primarily by amino acids in the FAO pattern. On the other hand, when Leverton et al. (1961) estimated the required magnesium intake for adult women to be 280 to 320 mg per day, the diet supplied 11 g nitrogen furnished primarily by gluten and casein. These results seem to indicate that magnesium requirement may be influenced by the level of nitrogen and

the amino acid pattern of the diet.

The research reviewed strongly suggests that the critical factor in magnesium metabolism is the absorption of the mineral from the digestive tract. Evidence is presented that absorption of magnesium is influenced by the amount of protein in the diet. Studies suggest also that the level and quality of dietary protein are related to magnesium requirements of humans as well as experimental animals.

CHAPTER III

PROCEDURE

Magnesium metabolism in the human adult and its possible interrelationship with that of protein was studied in three metabolic balance experiments. These studies were conducted within a two-year period (February 1961-November 1962), and each consisted of a preliminary interval followed by 5 balance periods of 6 days. The effects of 5 different diets upon magnesium and protein metabolism were investigated. With the exception of small supplements of "vitamin-free" casein, which were employed in Studies II and III, only natural foods were included in the diets.

The subjects were 12 graduate students and one instructor who were working in the areas of foods or nutrition in the College of Home Economics at The University of Tennessee. Two of the students took part in all three metabolic studies, while two others participated in two experiments. Ages of the 13 subjects ranged from 21 through 41 years. All were reported in normal health by the University physician before and following each study. Descriptive information on subjects appears in Tables I, II and III, pages 24, 25 and 26.

In the first balance study 5 young women were maintained on a controlled dietary regimen similar to the most restricted diet fed the preadolescent girls who were subjects of the southern cooperative project. Food intake furnished approximately 20 g protein and 100 mg magnesium. Recommended allowances of other nutrients were provided by the

inclusion of a supplement containing primary calcium phosphate, ferrous sulfate, niacin, riboflavin, thiamine and vitamin D. A sixth subject, a young man, received 1.5 times the amount of all foods fed the women with a corresponding decrease in the quantity of supplement.

On the basis of a two-week diet record kept by each subject prior to the experiment, the controlled diet of the women was planned to furnish 1800 Calories (calculated; Watt and Merrill, 1950) and that of the man, 2700 Calories daily. Additional calories necessary to maintain weight were supplied by sugar in beverages and by a fondant made from sucrose, corn syrup and artificial flavoring. Subjects were weighed routinely every fourth day before breakfast, and adjustments in sugar and fondant intake were made in an attempt to keep weight constant. Coffee prepared with demineralized water and the water itself were permitted ad libitum with the intake of each subject being recorded.

Only female subjects, 5 in number, participated in Study II. The protein and magnesium content of the food used in this experiment was essentially the same as that in Study I, but menus were changed to provide foods more appealing to adults and to increase the daily calories to approximately 2000. One daily addition which increased the variety of calorie sources was a 10 ounce coca-cola. If more than 2000 Calories were necessary to maintain the weight of the subjects, these were provided, as in the previous study, in the form of sugar and the special fondant. Because of its magnesium content, coffee was limited to 5 cups daily, but demineralized water was permitted ad libitum with the intake of each subject being recorded.

Beginning with day 4 of the second metabolic period, 5 g purified "vitamin-free" casein per subject per day were added to the diet, thus increasing the individual protein intakes to approximately 25 g. Because nitrogen balances still were no better than those in Study I, on day 1 of the third metabolic period, 50 g chopped cooked egg white were also included in the diet per subject per day, bringing the 24-hour calculated protein intake up to 30 g. Addition of casein and egg white to the diet changed the ratio of animal to plant protein from 1:2 to 1:1. With the inclusion of a vitamin and mineral supplement, as described for Study I, the diet provided recommended intakes of other nutrients. The daily magnesium content of the diet, including that from coffee, coca-cola and the mineral-vitamin supplement, amounted to approximately 183 mg per person.

In Study III, completed in November 1962, 8 female graduate students participated. In order to be at least partially adjusted to a restricted nitrogen intake at the onset of the experiment, the subjects agreed to reduce their protein consumption in self-chosen diets to approximately 50 g daily for two weeks just prior to the beginning date of the study. Diet records kept by the subjects indicated an average calculated protein consumption for the 14-day period of 44 to 49 g daily. Because the experimental plan was to maintain one group of subjects on a basal diet supplying 35 g protein while the other group received a supplemented diet containing 50 g daily, a 6-day preliminary period was employed rather than the 3-day one used in the other two studies when subjects were fed the same level of protein. During this

interval all subjects received a diet calculated to supply 42 g protein daily. The protein was supplied by the basal diet supplemented with 3.8 g "vitamin-free" casein and 38 g chopped cooked egg white. In this manner all subjects should have been in essentially the same state of protein nutrition at the beginning of the first experimental period. Individual protein and magnesium balances for this preliminary period appear in Table XX in the Appendix.

At the initiation of the experiment the 8 young women were randomly assigned, 4 to each of the two diets designated as Diet A and Diet B. Diet A was the basal diet planned to furnish 35 g protein (17.5 g of animal and 17.5 of plant origin) and, including coffee, coca-cola and mineral supplement, 183 mg magnesium daily. The foods in the diet were similar to those served in Study II except that breast of turkey, lean fish and small increases in ground round of beef, together with small amounts of skim milk and dry cottage cheese were used to provide the additional animal protein and to replace the casein and egg white used in the supplemented diet of Study II. Small increases in servings of vegetables gave the additional plant protein. The level of dietary fat was the same as in Study II and adjustments in quantities of the different fats were made in an attempt to avoid alteration in the lipid composition of the diet. As in previous studies demineralized water was allowed ad libitum, and sugar in coffee, along with the special fondant, furnished extra calories as needed. Supplements containing vitamin D, primary calcium phosphate, ferous sulfate and riboflavin brought the intake of all nutrients except protein up to their recommended levels.

Diet B was the same as Diet A except for its supplementation with 7.5 g "vitamin-free" casein and 75 g chopped cooked egg white daily. The calculated protein content was 50 g, 65 per cent of which was of animal origin. The supplemented diet necessarily contained more magnesium, but it was planned that both groups of subjects would ingest the same amount of mineral through regulation of coffee consumption.

The experimental procedures were the same in all studies. Natural foods including dry cereals, doughnuts, bread, cream soups, ground round of beef, turkey breast, halibut, cottage cheese, skim milk, green beans, asparagus, broccoli, squash, onions, cabbage, carrots, tomato, lettuce, a variety of fruits and juices, jelly, margarine and mayonnaise along with certain specially prepared desserts and cookies made up the basal diet of the subjects. Turkey breast, halibut and cottage cheese, however, appeared only in Study III. The selection of the foods employed in the diets was based upon their nutritive and caloric values with special attention focused upon their magnesium, fat and protein content. Variety in vegetables was limited because of the relatively large amounts of magnesium found in most of them. These carefully selected foods were incorporated into a set of 6 daily menus, which were served in the same sequence during each metabolic period. Calculated nutritive values and menus of Diet A employed in Study III are recorded in Tables XXIII and XXIV in the Appendix. In order that the eating habits of the subjects might remain as nearly normal as possible, suitable snacks were provided each day in addition to three regular meals. The subjects ate all their meals in

the metabolic unit of the Nutrition Department where the foods were carefully prepared, accurately weighed and served by nutrition staff members and graduate students working under supervision. Appropriate amounts of primary calcium phosphate and ferrous sulfate were weighed out and mixed in quantity. In an attempt to promote better absorption of the mineral supplement, as much of the mineral mix as possible was introduced into the foods during preparation. The remainder of the daily individual requirement for the supplement was weighed out, transferred to 6 capsules and packaged for each subject. Two capsules were ingested with each meal. A solution of vitamin B supplement was prepared and aliquots for the day were pipetted into the breakfast fruit juice. Vitamin D was supplied in a solution,* aliquots of which were pipetted into the breakfast cream mix.

At each serving period one-half portions of all foods and beverages (with the exception of water, coffee, coca-cola and margarine) were composited and frozen following the evening meal each day. At the end of each 6-day period, collections were thawed, homogenized in a stainless steel blendor, and made into period composites. Samples of each pound of margarine used during the entire study were pooled for separate analysis. Aliquots of the specially prepared coffee were collected, concentrated and frozen for period 1. Concentrates from each succeeding period were added to give a pooled composite. An identical procedure was followed for coca-cola. Aliquots of 24-hour urine samples were

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*Dristel, Winthrop Laboratories, New York 18, N. Y.

frozen with the daily addition of succeeding samples to furnish a 6-day period composite. In Studies I and II feces were marked by ingestion of carmine by capsule before breakfast on the first day of each period. During metabolic study III the carmine was taken on the last day of each period following the evening meal. The change in time at which carmine was taken was made in order to facilitate period separation of feces. Samples were frozen as collected, thawed for separation and homogenized in a stainless steel blendor for preparation of a period composite. Menses were collected on standard tampons (and in some instances, on Kotex) and frozen for subsequent analysis.

Portions of the period composites of food, feces and urine were frozen and stored for future analysis. Appropriate samples of these composites along with the pooled coffee and coca-cola concentrates were dry ashed in duplicate at 550° C for 24 hours for analysis. The tampons and Kotex containing menses, as well as an appropriate number of tampons and Kotex from the same lots to serve as blanks, were similarly ashed.

Aliquots of solutions of the ash from food, coca-cola and coffee, feces, urine and menses were analyzed for magnesium by a modification (Thomas, 1956, and Andrews, 1960) of the colorimetric method of Young and Gill (1951). The reproducibility and accuracy of this method is illustrated by data presented in Table XXV in the Appendix.

Nitrogen was determined on portions of all period and pooled composites (except menses) by the macro Kjeldahl method.

CHAPTER IV

RESULTS

The subjects, with one exception, remained in excellent health and followed their ordinary academic schedule throughout all experiments. One subject on Diet A of Study III was forced to drop out of the experiment during the final metabolic period because of a gastrointestinal upset. Hemoglobin levels were not influenced by the low-protein diets; changes appearing were no greater than variations noted under normal circumstances. Hemoglobin values together with caloric intakes, weight changes, and descriptive information about subjects are found in Tables I, II, and III.

During metabolic Study I when the diet provided approximately 1726 Calories and 3.194 g nitrogen from food sources* daily, the subjects complained of hunger often and had difficulty maintaining their initial weight despite the large quantities of fondant consumed daily. All subjects had an overall negative nitrogen balance, the mean being $-0.837 \pm .431$. Average balance data for all subjects are presented in Table IV and individual period balances in Table XVIII, Appendix. These data indicate that while the greater loss of nitrogen for all subjects appeared in the urine, magnesium excretion was larger in the feces for everyone with the exception of MAH.

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*Nitrogen from coffee and coca-cola was assumed to be unavailable and was not included in food nitrogen.

TABLE I

AGE, HEIGHT, WEIGHT, CALORIE INTAKE AND HEMOGLOBIN LEVELS OF SUBJECTS IN STUDY I

Subject	Age	Height	Calorie intake: Food	Calorie intake: Fondant and sugar	Calorie intake: Total	Weight: Initial	Weight: Final	Hemoglobin: Initial	Hemoglobin: Final
	yr	cm	kcal/24 hr			kg		g/100 ml	
GMB	24	158.8	1726	210	1936	55.7	55.3	13.3	13.3
BGB[a]	23	180.0	2589	587	3176	95.0	92.6	16.0	15.4
AC	34	170.6	1726	175	1901	52.2	51.5	12.0	12.8
SH	40	166.2	1726	223	1949	58.8	56.9	13.5	12.8
MAH	21	162.5	1726	218	1944	45.1	45.4	10.4	11.6
ES	23	162.5	1726	147	1873	70.1	69.1	12.4	12.8

[a]BGB was only male subject in group.

TABLE II

AGE, HEIGHT, WEIGHT, CALORIE INTAKE AND HEMOGLOBIN LEVELS OF SUBJECTS IN STUDY II

Subject	Age	Height	Calorie intake			Weight		Hemoglobin	
			Food	Fondant and sugar	Total	Initial	Final	Initial	Final
	yr	cm	Avg/24 hr			kg		g/100 ml	
GMB	25	158.8	1992	105	2097	55.9	55.9	12.5	12.8
ESE	35	170.0	1992	301	2293	65.2	63.6	14.2	12.4
SH	41	166.2	1992	105	2097	58.2	58.2	12.8	12.0
MN	22	165.6	1992	252	2244	80.7	79.0	12.0	11.6
ES	24	162.5	1992	178	2170	73.9	72.6	12.5	12.4

TABLE III

AGE, HEIGHT, WEIGHT, CALORIE INTAKE AND HEMOGLOBIN LEVELS OF SUBJECTS IN STUDY III

Diet	Subject	Age	Height	Calorie intake: Food	Calorie intake: Fondant and sugar	Calorie intake: Total	Weight: Initial	Weight: Final	Hemoglobin: Initial	Hemoglobin: Final
		yr	cm		Avg/24 hr		kg		g/100 ml	
Diet A	FG	25	158.8	1854	10	1864	47.5	47.2	13.3	13.8
	SH	41	166.2	2067	21	2088	59.4	59.3	12.8	10.9
	PM	33	157.5	2171	210	2381	63.0	62.3	12.0	12.4
	MR	27	156.2	2067	199	2266	64.8	65.0	12.8	12.0
Diet B	CE	22	157.5	1866	--	1866	51.5	51.2	12.4	13.8
	MN	22	165.0	2002	--	2002	78.9	76.8	12.4	12.4
	ES	24	162.5	2132	99	2231	77.4	77.0	11.2	10.9
	PT	28	158.8	2106	12	2118	55.6	55.2	11.2	11.2

TABLE IV

NITROGEN AND MAGNESIUM BALANCES
STUDY I

Subject	Nitrogen				Magnesium			
	Intake[b]	Urine	Feces	Balance	Intake[b]	Urine	Feces	Balance
	g/24 hr				mg/24 hr			
GMB	3.471±.042	3.529±.295	0.926±.044	-0.985±.288	171.4±9.0	93.1±11.4	129.2±14.9	-51.0±26.2
RGB[a]	5.163±.042	5.416±.704	1.299±.114	-1.553±.739	232.9±10.7	152.2±9.3	116.9±15.7	-36.2±30.3
AC	3.562±.042	3.568±.643	0.806±.118	-0.813±.658	186.5±8.9	102.4±7.6	141.6±19.1	-57.3±21.5
SH	3.364±.016	3.543±.318	0.985±.072	-0.964±.786	147.5±1.9	77.9±5.6	99.2±9.3	-29.6±10.4
MAH	3.365±0	2.691±.318	0.805±.099	-0.131±.330	147.6±4.6	95.1±6.6	72.7±5.9	-19.8±8.5
KS	3.505±.007	3.814±.540	0.981±.045	-1.290±.532	176.8±16.8	79.9±6.5	151.1±10.4	-54.2±22.6
Mean	3.453±.087	3.249±.481	0.901±.090	-0.837±.431	166.0±17.7	89.7±10.5	118.8±32.3	-42.4±16.7

[a]RGB is not included in mean values since he was the only male in the group.

[b]Intake includes the amount contained in coffee and supplements as well as that in food.

During the preliminary interval and the first period of Study II, the subjects received approximately the same amount of protein as had those on the previous experiment, but the energy content of the diet was increased from an average of 1726 Calories per day to 1975. It was hoped that the higher energy content of the diet, further increased as in Study I by fondant, would prevent weight losses in individual subjects and permit them to approach nitrogen equilibrium. After it became apparent, through analysis of urine and fecal samples as well as by observation of weight losses, that the subjects were still in negative energy and nitrogen balance, 5 g "vitamin-free" casein were added to the diet on day 4 of the second metabolic period. This addition accounted for the variation in protein intake during periods 1 and 2 of Study II (Table V). Although the increase in protein nitrogen was small and nitrogen balance did not improve, there was an improvement in magnesium balances resulting primarily from an increased absorption of the mineral. Magnesium intake remained at approximately the same level as in Study I.

Nitrogen losses in the urine indicated that a further increase of dietary protein was necessary to establish nitrogen equilibrium in these subjects; therefore, at the beginning of period 3, 50 g chopped cooked egg white were added to the daily regimen. With an average daily nitrogen intake of 4.874 g from food, equally divided between animal and vegetable sources, nitrogen balance improved from a mean of $-1.496 \pm .521$ to $-0.110 \pm .319$ (Tables V and VI). In the latter case the excretion exceeded intake by about 2 per cent and was within a range sometimes

TABLE V

NITROGEN AND MAGNESIUM BALANCES
STUDY II (PERIODS 1 AND 2)

Subject	Nitrogen				Magnesium			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
	g/24 hr				mg/24 hr			
GMB	3.510±.031	3.818±.045	1.180±0	-1.488±.070	166.3±1.7	81.8±5.0	103.2±8.4	-18.8±1.7
ESE	3.510±.031	4.558±.045	0.757±.122	-1.804±.109	166.3±1.7	85.9±2.4	92.0±5.0	-11.6±.9
SH	3.412±.031	3.466±.363	0.992±.195	-1.046±.571	145.3±1.7	62.6±.36	113.1±42.9	-30.4±41.5
MW	3.510±.031	3.544±.055	0.913±.070	-0.947±.130	166.3±1.7	90.6±3.2	93.9±20.2	-18.2±15.3
ES	3.510±.031	4.712±.095	0.993±.032	-2.194±.105	166.3±1.7	89.8±10.5	97.6±25.8	-21.0±13.6
Mean	3.490±.045	4.020±.579	0.967±.153	-1.496±.521	162.1±9.4	82.1±11.5	100.0±8.5	-20.0±6.8

[a]Intake includes the amount contained in coffee, coca-cola and supplements as well as that in food.

TABLE VI

NITROGEN AND MAGNESIUM BALANCES
STUDY II (PERIODS 3, 4, 5)

Subject[a]	Nitrogen				Magnesium			
	Intake[b]	Urine	Feces	Balance	Intake[b]	Urine	Feces	Balance
	g/24 hr				mg/24 hr			
GMB	5.146±0	4.133±.252	0.925±.050	0.087±.299	182.7±1.0	77.8±4.3	95.4±13.8	9.5±17.1
ESE	5.146±0	4.744±.161	0.885±.084	-0.483±.145	182.7±1.0	78.2±17.8	99.2±19.6	5.3±5.9
SH	5.047±0	4.253±.366	1.108±.118	-0.315±.465	161.7±1.0	60.9±10.7	101.9±5.5	-1.2±6.0
MN	5.146±0	4.111±.593	0.715±.110	0.320±.690	182.7±1.0	87.7±7.4	77.6±24.4	16.7±29.1
ES	5.146±0	4.336±.232	0.969±.077	-0.160±.290	182.7±1.0	71.3±3.6	105.4±23.8	5.9±22.9
Mean	5.126±.044	4.315±.257	0.920±.143	-0.110±.319	178.5±9.4	75.2±9.9	95.9±10.9	7.2±6.5

[a]Subjects are same as those for the first two periods of Study II.

[b]Intake includes the amount contained in coffee, coca-cola and supplements as well as that in food.

said to represent equilibrium (Leverton et al., 1956). Magnesium intake was unavoidably increased by approximately 16 mg when protein sources were added, but retention of the mineral improved in excess of the amount added. Magnesium balance changed from -20.0 ± 6.8 mg to 7.2 ± 6.5 mg as a result of increased absorption and decreased urinary loss of the mineral. Individual nitrogen and magnesium balances for all periods may be found in Table XIX, Appendix.

In Study III 4 subjects received basal Diet A and 4 were fed supplemented Diet B. A preliminary analysis of the nitrogen and magnesium content of foods in the amounts to be used in the experiment indicated that Diet A contained 5.600 g nitrogen and 142.8 mg magnesium per day while in Diet B the content of the two nutrients was 7.630 g and 160.8 mg, respectively. Using the figures for magnesium obtained from analysis of the coffee served during Study II (10.6 mg per 142 milliliter cup), it appeared that by the addition of 3.5 cups of coffee to Diet A and 2.0 cups to Diet B the magnesium intake on each diet would be approximately 180 mg per person per day. This amount was almost identical with the mean intake of magnesium during periods 3, 4 and 5 of Study II.

Unfortunately, when aliquots of the food actually consumed by the subjects during each period were analyzed, Diet A was found to supply, on the average, 154.9 mg magnesium daily while the content of Diet B averaged 179.8 mg. The mean nitrogen content for the two diets, however, was very close to the value determined prior to the beginning of the experiment. The 12 mg discrepancy between the magnesium content

of Diet A and that of the representative composite analyzed in advance could not be fully explained, but one possible explanation might be the use of chicken breast in the representative composite rather than the turkey breast which the subjects actually received during the experiment. The additional slight increase in magnesium content of Diet B might have been due to a difference in the mineral contained in the eggs and lot of casein used during the experiment from that of the eggs and casein analyzed prior to the initiation of the study.

A filter was used in preparation of the percolated coffee during Study III in order to eliminate some of the sediment which had proved objectionable to the subjects on previous studies. Presumably decreasing the amount of sedimentation also decreased the magnesium content of the coffee because analysis of that served during Study III showed a magnesium content of 8.6 mg per cup in contrast to 10.6 per cup found in coffee served in Study II.

An intake of 3.5 cups of coffee by all subjects fed Diet A, with the exception of SH, and only 2.0 cups per day by those receiving Diet B brought the average magnesium content of the two diets to 184.7$\pm$7.6 and 196.0$\pm$9.2 mg, respectively (Tables VII and VIII). Subject SH, receiving Diet A, was overstimulated by coffee; therefore, her intake was limited to 2.0 cups daily. This reduction in the coffee consumption of one subject accounts for the standard error of magnesium intake on the basal diet. Individual balance data for all periods appear in Tables XX and XXI, Appendix. In order to keep the weights of subjects at their initial values, it was necessary to decrease the food intake of one

TABLE VII

NITROGEN AND MAGNESIUM BALANCES
STUDY III (DIET A)

Subject	Nitrogen				Magnesium			
	Intake[b]	Urine	Feces	Balance	Intake[b]	Urine	Feces	Balance
	g/24 hr				mg/24 hr			
FG[a]	5.414±.082	4.479±.269	0.759±.092	0.176±.303	170.5±10.3	87.9±7.3	85.2±14.4	-2.7±24.1
SH	5.510±0	4.560±.156	0.927±.063	0.023±.184	175.9±0	78.5±4.3	94.4±3.5	3.0±6.1
PM	5.593±.016	4.515±.530	0.949±.151	0.130±.611	189.2±0.2	68.9±9.4	111.3±15.7	8.9±14.5
MR	5.574±0	4.142±.286	1.142±.032	0.290±.271	188.9±0	64.1±12.1	111.9±18.2	12.9±19.6
Mean	5.559±.044	4.406±.230	1.006±.118	0.148±.134	184.7±7.6	70.5±7.3	105.9±9.9	8.3±5.0

[a]Data for FG are not included in the mean values because her magnesium intake after period 1 was only 166 mg/day.

[b]Intake includes the amount contained in coffee, coca-cola and supplements as well as that in food.

TABLE VIII

NITROGEN AND MAGNESIUM BALANCES
STUDY III (DIET B)

Subject	Nitrogen				Magnesium			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
	g/24 hr				mg/24 hr			
CE	7.692±.077	6.170±.452	0.798±.141	0.702±.505	182.3±10.1	78.6±10.5	88.8±17.3	15.0±19.5
MN	7.830±0	5.774±.646	0.942±.079	1.114±.579	200.4±0	93.5±4.6	86.6±15.3	20.0±13.9
ES	7.854±0	6.467±.413	1.064±.142	0.323±.342	200.8±0	78.8±2.8	98.2±16.2	23.6±18.2
PT	7.849±.009	6.193±.405	0.970±.192	0.686±.408	200.7±.2	105.4±4.4	58.5±14.0	36.8±13.1
Mean	7.806±.077	6.151±.285	0.946±.110	0.706±.324	196.0±9.2	89.1±12.9	83.0±13.9	23.9±9.3

[a]Intakes include the amount contained in coffee, coca-cola and supplements as well as that in food.

subject on each of the two diets at the beginning of period 2. The foods removed along with their calorie, magnesium and nitrogen content are listed in Table IX. The necessity for calorie reduction was unexpected since the diets furnished approximately 2100 Calories, and since in the previous study, individual intakes of 2100 and 2300 Calories were not sufficient to prevent weight losses in some of the subjects. The only explanation for a decreased calorie requirement seemed to be the increase in dietary protein.

Because the decrease in foods supplied by Diet A reduced the magnesium intake of subject FG to only 166 mg per day, her balance data were not included in the mean values calculated for this study. The variation in the intake of magnesium by subject CE, who was receiving Diet B, accounts for the standard error of the mean magnesium intake on the supplemented diet. Since the magnesium intake of this subject (approximately 180 mg daily) remained close to that of subjects on Diet A, her balance data were included in the mean values. Subjects SH, PM and MR, who were fed Diet A, had an average magnesium intake of 184.7 ± 7.6 mg, and their excretion of the mineral was never more than 3 per cent above intake; slightly negative balances occurred in only 6 out of the 14 individual balance periods (Table XXI, Appendix). FG, who was not included in the overall mean because of her low magnesium intake beginning with the second metabolic period, did go into negative magnesium balance on an intake of 166 mg daily. She was, however, in strongly positive magnesium balance during the first period when her intake was 189 mg. On Diet B, which supplied 196.0 ± 9.2 mg magnesium,

TABLE IX

FOODS ELIMINATED FROM DIET AND DECREASES IN INTAKE OF CALORIES, NITROGEN AND MAGNESIUM OF SUBJECTS FG AND CZ DURING PERIODS 2, 3, 4, 5, STUDY III

Food	Weight	Calories (calculated)		Nitrogen (analyzed)		Magnesium (analyzed)	
	per period	per period	per 24 hr	per period	per 24 hr	per period	per 24 hr
	g			g		mg	
Margarine	60)						
Grape Juice	240)	815	136	.978	.163	135.6	22.6
Brown Sugar	60)						
Coca-cola	1800	780	130	.144	.024	2.4	.4
Total		1595	266	1.122	.187	138.0	23.0

slightly negative balances occurred in three out of the 20 individual balance periods, but negative balance was never found in the same subject more than once (Table XXII, Appendix). Mean balances for all subjects receiving as much as 176 mg magnesium daily were positive.

Menses collected from subjects participating in the three balance experiments were analysed for their magnesium content. Data reported in Table X show that magnesium losses in the menses were small as was expected because of the low magnesium concentration in blood. The higher mean excretion by subjects in Study III probably was not related to the slightly increased magnesium intake because subject ES lost more magnesium in Study II than in Study III, and subject SH excreted almost twice as much in Study III although her magnesium intake was the same in the two studies. Furthermore, the method of collection and correction for the mineral in Kotex and Tampons was subject to considerable error. Nevertheless, results indicate that magnesium losses in menses are too small to materially influence magnesium requirements.

Energy, nitrogen and magnesium contents of all the diets employed in the balance studies are summarized in Table XI, and the data obtained with the use of the 5 different diets in three successive experiments are summarized in Table XII. For the purpose of statistical analysis, it has been assumed that the subjects participating in the experiments, although small in number, represent a random sample from a population with a normal distribution.

Since three subjects, GMB, SH and ES, participated in Studies I and II, their balance data were analysed statistically to determine:

TABLE X

INDIVIDUAL MAGNESIUM LOSSES IN MENSES

(Avg per Menstrual Period)

Study I		Study II		Study III	
Subject	Loss	Subject	Loss	Subject	Loss
	mg		mg		mg
GMB	2.90	GMB	1.52	FG	2.16
AG	1.40	BSE	negligible	SH	4.63
MAH	negligible	SH	2.44	PM	7.50
SH	2.13	MN	2.20	MR	17.40
ES	2.46	ES	3.56	CE	5.17
				MN	3.69
				ES	2.70
				PT	8.77
Mean and standard deviation	2.22±.63		2.43±.85		6.50±4.95

TABLE XI

MAGNESIUM, NITROGEN AND ENERGY CONTENT OF DIETS EMPLOYED IN METABOLIC STUDIES (AVG)

Study	Energy (calculated)		Nitrogen (analyzed)		Magnesium (analyzed)		
	Food	Fondant and sugar	Food	Coffee and coca-cola	Food	Coffee and coca-cola	Mineral supplement
	Cal/24 hr		g/24 hr		mg/24 hr		
I	1726±0	196±36	3.194±0	0.261±.089[d]	105.1±0	54.7±18.6[d]	6.7
II							
Periods 1 & 2	1975±0	135±53	3.238±.032[c]	0.252±.054[d]	110.0±0	50.2±9.0[d]	2.0
Periods 3,4,5	2009±0	217±125	4.874±0	0.252±.041[d]	126.1±0	50.2±8.8[d]	2.0
III							
Diet A	2102±60[a]	94±94	5.401±0	0.155±.032[a]	154.9±0	26.3±6.5[a]	3.2
Diet B	2026±24[b]	30±25	7.710±.069[b]	0.096±.010[b]	175.3±9.3[b]	17.6±0.2[b]	3.2

[a]Variation due to differences in coca-cola and coffee consumption.

[b]Variation due to differences in coca-cola consumption and to reduced food intake of one subject.

[c]Variation due to addition of 5 g casein during last 3 days of Period 2.

[d]Variation due to differences in coffee consumption.

TABLE XII

MEAN BALANCE DATA OBTAINED ON 5 DIETS EMPLOYED IN THREE STUDIES DURING 1961-62

Study and diets	Nitrogen (g/24 hr)						Magnesium (mg/24 hr)			
	Intake									
	Food	Other sources	Total	Urine	Feces	Balance	Intake	Urine	Feces	Balance
Study I	3.194±0	0.261±.069	3.453±.087	3.429±.481	0.901±.090	-0.837±.431	166.0±17.7	89.7±10.5	118.8±32.3	-42.4±16.7
Study II										
Periods 1 & 2	3.238±.032	0.252±.054	3.490±.045	4.020±.579	0.967±.153	-1.496±.521	162.1±9.4	82.1±11.5	100.0±8.5	-20.0±6.8
Periods 3,4,5	4.874±0	0.252±.041	5.126±.044	4.315±.257	0.920±.143	-0.110±.319	178.5±9.4	75.2±9.9	95.9±10.9	7.2±6.5
Study III										
Diet A	5.401±0	0.155±.044	5.559±.044	4.406±.230	1.006±.118	0.148±.134	184.7±7.6	70.5±7.3	105.9±9.9	8.3±5.0
Diet B	7.710±.069	0.096±.010	7.806±.077	6.151±.285	0.946±.110	0.706±.324	196.0±9.2	89.1±12.9	83.0±13.9	23.9±9.3

(1) whether there were any significant differences among the nitrogen and magnesium balances obtained on the three diets which were employed during the two studies and (2) whether there was any interaction among periods. Analysis of variance of these data is given in Table XIII. A significant difference ($P < .01$) was observed in nitrogen balance when the subjects received the diet supplying 30 g protein rather than the one containing 20 g. The difference in nitrogen balance obtained with the feeding of diets varying in calorie content and the terminal addition of 5 g casein was not significant. Magnesium balance improved significantly ($P < .01$) with the feeding of the diet designated as High-Nitrogen (30 g protein), but there was also a significant increase ($P < .025$) in magnesium balance when the diet differing only in its higher calorie content and the terminal addition of 5 g casein was fed. Addition of the 5 g casein during the last three days of period 2 (Study II) had no apparent effect upon nitrogen retention, and interaction among periods was not evident in analysis of variance. In the study of magnesium retention, however, an interaction among periods was present and perhaps could be explained by the addition of the small amount of casein in the second period of the Low-Nitrogen, High-Calorie diet (Periods 1 and 2, Study II).

Nitrogen and magnesium balance data for all subjects were analyzed statistically for differences which might be present as a result of the variance in composition of Diets A and B employed throughout Study III and the diet fed during periods 3, 4 and 5 of Study II. Analysis of variance of these data is recorded in Table XIV. Using Duncan's

TABLE XIII

ANALYSIS OF VARIANCE OF NITROGEN AND MAGNESIUM BALANCES FOR SUBJECTS GMB, SH AND ES ON THREE DIETS EMPLOYED IN STUDIES I AND II

Source of variance	Nitrogen balance			Magnesium balance	
	df[b]	MS[c]	F[d]	MS[c]	F[d]
Blocks	2	.35		175.76	
Diets[a]					
LN-LC vs LN-HC	1	.79	2.17[NS] ($F_{.05}$ = 7.71)	1,991.52	7.71* ($F_{.025}$ = 5.98)
LN vs HN	1	8.19	22.4** ($F_{.01}$ = 21.2)	11,935.10	46.21** ($F_{.005}$ = 10.22)
Periods x Diets	7	.33	1.65[NS]	849.60	3.29* ($F_{.025}$ = 3.10)
Blocks x Diets	4	.36	1.82[NS]		
Error	14	.02		18 df (258.26) Pooled error term	

[a]Diet comparisons are orthoganal; LN-LC represents diet of Study I; LN-HC represents diet of periods 1 and 2 of Study II; LN represents diet of Study I and that used during periods 1 and 2 of Study II; HN represents the diet of periods 3, 4, 5 of Study II.

[b]df denotes degrees of freedom.

[c]MS represents mean square or variance.

[d]F is the variance ratio and indicates significance or lack of it.

TABLE XIV

ANALYSIS OF VARIANCE OF NITROGEN AND MAGNESIUM BALANCE
FOR STUDY II (PERIODS 3, 4, 5)
AND STUDY III (DIETS A AND B)

Source of variance	Nitrogen balance			Magnesium balance	
	df[a]	MS[c]	F[d]	MS[c]	F[d]
Diets	2	17.46	12.32** ($F_{.005}$=10.11)	12,403.98	9.41** ($F_{.01}$=8.02)
Individuals/ diets	9[b]	1.42		1,317.83	

[a]df denotes degrees of freedom.

[b]One degree of freedom was lost because of missing data calculation on one subject for one period.

[c]MS represents mean square or variance.

[d]F is the variance ratio and indicates significance or lack of it.

Multiple Range Test (Steel and Torrie, 1960, p. 114) to make comparisons among treatment means, the differences shown to exist by the F test were as follows:

Nitrogen Balance

($P < .01$)* Diet B Diet A Study II (periods 3, 4, 5)

Magnesium Balance

($P < .01$) Diet B Diet A Study II (periods 3, 4, 5)

($P < .05$) Diet B Diet A Study II (periods 3, 4, 5)

Analysis of variance using the split-plot design was performed on the nitrogen and magnesium data for subjects fed Diet A and Diet B to determine whether there were period interactions present. These data show that, although the differences in nitrogen and magnesium retentions were significant ($P < .05$) in the subjects fed these two diets, no significant interactions among periods were present. Analysis of variance of these data is presented in Table IV. These results can be interpreted to mean that the subjects had adjusted to a low-protein, low-magnesium diet by the end of the preliminary period.

In an effort to minimize the effect of the small excess of magnesium in Diet B over that in Diet A and to determine the cause of the increased magnesium retention on the supplemented diet, urinary and fecal magnesium excretion expressed as percentage of intake were studied. Mean urinary magnesium was 42.1 and 45.4 per cent of the intake while

*Any two treatments underscored by the same line are not significantly different from each other but are significantly different from those not underscored by the same line.

TABLE XV

ANALYSIS OF VARIANCE OF NITROGEN AND MAGNESIUM BALANCE
FOR DIETS A AND B (STUDY III)

Source of variance	df[a]	Nitrogen balance MS[c]	Nitrogen balance F[d]	Magnesium balance MS[c]	Magnesium balance F[d]
Diets	1	2.94	8.22* ($F_{.05}$=6.61)	3,288.78	7.96* ($F_{.05}$=6.61)
Subject/ Diet	5[b]	.36		413.10	
Periods	4	.26	1.41[ns]	522.90	1.98[ns]
Periods x Diets	4	.12		147.30	
Periods x Subjects/ Diet	23[b]	.18		264.64	

[a]df denotes degrees of freedom.

[b]One degree of freedom was lost because of missing data calculation on one subject for one period.

[c]MS represents mean square or variance

[d]F is the variance ratio and indicates significance or lack of it.

the fecal excretion averaged 55.3 and 42.5 per cent for Diets A and B, respectively. Analysis of variance is found in Table XVI.

These data indicate that there was no difference in urinary magnesium excretion of the subjects maintained on the two diets, but a difference, although not significant, existed in fecal magnesium. The lack of significance might be explained by the sample size. The results of this analysis of variance can be interpreted to suggest that increased magnesium absorption as well as a slightly larger intake of magnesium is responsible for the significant increase ($P < .05$) in magnesium retention in subjects fed Diet B over those receiving Diet A.

Estimates of the linear relationship between nitrogen and magnesium balance and between percentage apparent nitrogen and magnesium absorption were obtained by the standard product moment method (Steel and Torrie, 1960, p. 183) using the following formula:

$$r_{x_1y} = \frac{\sum_{i} x_1 y}{\sqrt{\sum_{i} x_1^2 \cdot \sum_{i} y^2}}$$

where:

x_1 = the independent variate (nitrogen balance; nitrogen absorption)

y = the dependent variate (magnesium balance; magnesium absorption)

r_{x_1y} = the estimate of the linear association of trait x_1 and trait y

$\sum x_1^2$ = the sum of squares of deviations from mean of trait x_1

$\sum y^2$ = the sum of squares of deviations from mean of trait y

TABLE XVI

ANALYSIS OF VARIANCE OF URINARY AND FECAL MAGNESIUM EXCRETIONS FOR DIETS A AND B (STUDY III)

Source of variance	Urinary excretion			Fecal excretion	
	df[a]	MS[c]	F[d]	MS[c]	F[d]
Diets	1	.0107	0	.1596	1.509[ns]
Subjects/Diets	5[b]	.0411		.1057	

[a]df denotes degrees of freedom.

[b]One degree of freedom was lost because of missing data calculation on one subject ~~for one period~~.

[c]MS represents mean square or variance.

[d]F is the variance ratio and indicates significance or lack of it.

$\sum x_1 y$ = the sum of the products of deviations from means of traits x_1 and y

$r^2_{x_1 y}$ = the estimate of per cent variation of trait y due to variation in trait x_1

The linear relationship between nitrogen balance and magnesium balance and between per cent apparent absorption of nitrogen and magnesium are given in Table XVII. From these data it can be observed that independence existed between nitrogen and magnesium balance in all subjects except in those fed Diet A during Study III. On this diet the subjects were only slightly on the positive side of nitrogen equilibrium while on the other diets the subjects were either in negative balance or in definitely positive balance.

Nitrogen absorption affected magnesium absorption significantly in subjects fed Diet B during Study III, and the dependence of magnesium absorption upon nitrogen absorption was evidenced also in the subjects receiving both diets employed in Study II. Very little importance, however, can be placed upon the results obtained during the first two periods of Study II since there were fewer observations available for statistical analysis and the diet composition was changed considerably during period 2.

That a relationship existed between the two variates (nitrogen and magnesium absorption) during periods 3, 4, and 5 in subjects participating in Study II and in the subjects fed Diet B during Study III is of particular interest since it was to these diets that the protein of excellent quality had been added.

TABLE XVII

RELATIONSHIP BETWEEN NITROGEN AND MAGNESIUM BALANCE AND BETWEEN NITROGEN AND MAGNESIUM APPARENT ABSORPTION ON ALL EXPERIMENTAL DIETS

Study and diet	Balance r_{x_1y} [a]	Balance $r^2_{x_1y}$ [b]	Apparent absorption r_{x_1y} [a]	Apparent absorption $r^2_{x_1y}$ [b]
Study I	.453	20.5%	.271	7.3%
Study II				
Periods 1 and 2	.163	2.7%	.790	62.4%
Periods 3, 4, 5	.342	11.7%	.750	56.2%
Study III				
Diet A	.787	61.9%	.410	16.8%
Diet B	.114	1.3%	.918	84.3%

[a] r_{x_1y} represents the estimate of the linear association between nitrogen and magnesium balance and between nitrogen and magnesium absorption.

[b] $r^2_{x_1y}$ denotes the estimate of per cent variation of magnesium balance due to variation in nitrogen balance and of magnesium absorption due to variation in nitrogen absorption.

CHAPTER V

DISCUSSION

Whenever metabolic balance studies have been used in an effort to determine requirements for some particular nutrient, the practice has been to supply in the diet all nutrients except the one under investigation in recommended amounts. Such a procedure more than likely masks any nutrient interrelationships in metabolism which may exist. It was only because no dietary allowance of magnesium has been recommended (National Academy of Sciences, 1958) that the mineral mix supplementing the low-protein diet fed preadolescent girls did not contain magnesium (Schofield and Morrell, 1960). This circumstance was responsible for the demonstration that when dietary magnesium as well as protein was decreased, retention of the mineral was independent of intake.

Preadolescent children were in positive nitrogen balance on diets supplying 17 to 22 g protein daily. Although a daily intake of 35 g protein had been reported minimal for nitrogen equilibrium in adults (Hegsted _et al_., 1946), the possibility could not be ignored that the relative amounts of several nutrients in the restricted diet of the children might have influenced utilization of nitrogen as well as minerals. Consequently, the diet used in the first experiment of the adult series was almost identical with the low-protein diet of the children except for the addition of coffee.

Failure of the adults to achieve nitrogen equilibrium on a daily intake of 3.124 g was in agreement with the work of Hegsted _et al_. (1946)

who found that 2.41$\pm$.04 g nitrogen per square meter (hereafter referred to as m^2) of body surface was necessary for equilibrium in adults when one-third of the nitrogen was supplied by meat. The mean surface area of the 5 young women participating in the first experiment was 1.63 m^2 and that of the male subject, 2.18 m^2. Average nitrogen intake of the 5 women was 1.91 g per m^2, while that of the man was 2.15 g per m^2. The one subject whose nitrogen balance approached equilibrium had a surface area of 1.49 m^2 as compared with the mean of 1.63 m^2; therefore, her protein intake more nearly agreed with the requirement proposed by Hegsted et al. (1948) than did that of the other female subjects. The children studied by Schofield and Morrell (1960), because of their smaller body size, were receiving on the average 3.45 g nitrogen per m^2 on the low-protein diet, which is substantially more than was being supplied the adults in the present study. Because children, however, require nitrogen not only for tissue repair but also for body growth, their ability to store nitrogen when the majority of the adults were unable to approach equilibrium may be an indication of better nutrient utilization by the growing organism.

Since weight loss and prolonged negative nitrogen balance are accompanied by tissue breakdown with loss of many components likely to occur, the negative magnesium balance observed in all subjects during the first experiment was not unexpected. If it can be assumed that the strongly negative nitrogen balance caused primarily by extensive urinary losses of nitrogen was indicative of breakdown of body tissue and that loss of magnesium was associated with this breakdown, then excretion of

magnesium in the urine might be expected to be high. Actually, however, urinary losses were only slightly higher than those of subjects in Study II who reached magnesium equilibrium. Instead of appearing in the urine, maximum losses of magnesium, except in one subject, occurred in the feces. This fact is difficult to reconcile with the usual concept of tissue breakdown. The origin of magnesium in the gastro-intestinal tract, however, cannot be traced entirely to food ingested since a fair amount of the mineral reaches the small intestine by means of the digestive juices. Possibly when protein intake is too low to allow for nitrogen equilibrium, absorption of magnesium regardless of source is adversely affected in some unexplained manner. Speculation regarding a possible cause for the increased fecal losses of magnesium when negative nitrogen balance exists leads to a consideration of the breakdown of protein stores as well as that of body tissue. Protein stores are generally assumed to be in the form of enzymes which can be easily hydrolyzed to supply the amino acids necessary for maintenance of essential body tissues when protein intake falls below requirement. Since magnesium ions are known to be necessary for activation of various enzymes and, therefore, are closely associated with these organic catalysts (MacIntyre, 1963), it is conceivable that as these enzymes are broken down to supply the required amino acids, the accompanying magnesium is secreted in some manner into the digestive tract and is lost in the feces due to poor absorption. Since so little is known about transport of the amino acids and magnesium across the intestinal wall, it is possible that the transport mechanisms could be the same for both

nutrients. If such a hypothesis were true, lack of optimal conditions for amino acid transport could prevent magnesium absorption because fecal nitrogen remains fairly constant regardless of level of protein intake.

Data for the first two periods of the second experiment indicated that subjects could not reach nitrogen equilibrium by an increase in calories and the addition of 5 g casein to the original diet. On an almost identical mean intake of magnesium, however, the balance improved significantly ($P < .025$) and seemed partially related to the addition of casein to the diet during the second period, since a significant period interaction ($P < .05$) was present when magnesium balance was compared in three subjects who participated in the first experiment and both sections of the second study. When mean balances for each period were studied, it appeared likely that the period interaction was due to marked decrease in magnesium losses for which increased apparent absorption during period 2 of the second study was responsible. The average apparent absorption improved from 28 to 38 per cent while urinary excretion remained close to 50 per cent of the intake. No period interactions at any time were observed for nitrogen balance nor for magnesium balance during the studies of the last experiment.

Increasing the daily protein content of the diet from 20 to 30 g by the addition of casein and cooked egg white changed the proportion of nitrogen furnished by animal sources from one-third to one-half. When the subjects were fed this diet, magnesium balance as well as nitrogen balance improved significantly ($P < .01$) although the mean magnesium

intake increased by only 17 mg daily. Despite the fact that nitrogen balance was still slightly negative with a mean of -0.110, the average apparent magnesium absorption improved from 38 to 46 per cent while the mean urinary excretion of the ion dropped from 51 to 42 per cent of the intake. On an average daily magnesium intake of 179 mg, 4 of the 5 subjects achieved an overall positive magnesium balance with a mean of 7.2 mg. SH, the subject who was in negative balance, was actually receiving only 162 mg magnesium in comparison with 183 mg for the others (Table VI, p. 30).

Meyer et al. (1955) found that 5 of 6 subjects receiving 182 mg magnesium were in negative balance on a diet furnishing 65 g protein. The average apparent magnesium absorption amounted to 40 per cent with the mean urinary excretion at 52 per cent of the intake. The highest and lowest retentions of the mineral in these subjects were associated with the highest and lowest fecal excretions. The young women studied by Leverton et al. (1961) received magnesium intakes of 173 to 320 mg from diets furnishing protein at a constant level of approximately 70 g daily. All subjects were in negative magnesium balance on an intake of 173-181 mg, and balance did not become positive for all subjects until the level reached 320 mg daily. As the dietary magnesium was increased, fecal losses also increased although the percentage loss decreased. When the intake was elevated from 277 to 320 mg, however, the fecal magnesium remained the same. At this particular protein and magnesium level, apparent absorption of the mineral was significantly improved and urinary excretion rose abruptly. Positive balance in all subjects

accompanied by a rise in the urinary excretion of magnesium suggests that absorption of the mineral was greater than the need. Results of magnesium metabolism experiments seem to indicate that the relative amounts of magnesium and protein fed as well as the level of the mineral itself affect magnesium absorption.

In Study III all subjects were fed approximately the same amount of magnesium daily, but protein was supplied at two different levels. To insure nitrogen equilibrium the low-protein diet, designated as Diet A, was planned to furnish approximately 35 g protein daily with equal amounts contributed by animal and vegetable sources. With a calorie content close to that of the diet employed in the second experiment and a protein increase of 5 g, all subjects achieved positive nitrogen balance with a mean of 0.148 as compared with -0.110 for the last three periods of the previous study. The difference in nitrogen balance was not significant, however. The magnesium content of the diet was only slightly higher than that in periods 3, 4, 5 of Study II and retentions were the same, +8.3 and 7.2 mg, respectively.

The 50 g protein level of Diet B was reached through the addition of 7.5 g purified casein and 75 g cooked egg white to the basal Diet A. The subjects fed Diet B attained a positive nitrogen balance of 0.706 mg which was significantly greater ($P < .01$) than that of subjects receiving Diet A. With an average magnesium intake of 196 mg, the subjects on Diet B had a mean magnesium balance of +23.9 mg which was significantly greater ($P < .05$) than that of those on the alternate diet. There was no difference between the percentage of magnesium intake excreted in

the urine of the two groups of subjects, the average being 42 and 45 per cent for Diets A and B, respectively. There was a difference, however, between the mean apparent absorption by the subjects fed Diets A and B although this difference was not significant, probably due to the sample size. The mean apparent absorption for subjects receiving Diet A was only 45 per cent as compared with 58 per cent for those fed Diet B. Although the subjects receiving Diet B ingested slightly more magnesium, the amount excreted in the feces was less. These results indicate that increased magnesium retention in subjects receiving Diet B was due to increased absorption as well as to a slightly larger intake of the mineral. Leverton et al. (1961) also found that magnesium intake and excretion are related, but the level of the mineral cannot be used to predict its excretion.

Since work with experimental animals has indicated that a high protein intake increases the need for magnesium (Bunce et al., 1963; Colby and Frye, 1951; Heinicke et al., 1956; Menaker, 1954) and perhaps decreases the absorption of the mineral from the gastro-intestinal tract (Fontenot et al., 1960; Head and Rook, 1955; Rook and Campling, 1962), magnesium absorption by subjects on Diet B might have been expected to decrease, perhaps resulting in a negative magnesium balance. On the other hand, McCance et al. (1942) found that increasing the protein intake of adult subjects by addition of various supplements brought about a significant improvement in magnesium absorption. They prefaced their report with the statement that phytin solubility had been found to be increased in solutions containing alpha amino acids

and suggested that calcium and magnesium absorption might be facilitated thereby. The authors presumably considered their experimental diet high in phytate and, consequently, high in unavailable magnesium. This assumption seems justified because, although the amounts were not specified, the diet contained bread of 92 per cent extraction in obviously considerable quantities, the regimen consisting chiefly of a fixed ration of milk along with bread, potatoes, and vegetables in amounts sufficient to supply 45 to 70 g protein daily. The relationship between improved magnesium absorption and protein intake in the present study could not have been related to a high phytate level in the diets employed since only refined breads and cereals were used and not in large quantities.

Because magnesium content of muscle cells is relatively high due to its important role as an activating ion in many enzymic reactions, particularly in those involving transphosphorylation (Fruton and Simmonds, 1958), it seems an attractive possibility that magnesium absorption is enhanced when the amino acids in the small intestine are in the ratio best suited for protein synthesis. Allison and Wannemacher (1963) have found in experimental animals that not only is muscle increase greatest when egg protein is fed but also that amino acids from this source are best for the acceleration of RNA and protein synthesis in the liver. Moreover, egg albumin has been shown to have amino acids in such a ratio and concentration that it is the protein most rapidly absorbed. The observation that young women, although in slightly negative nitrogen balance on a 30 g protein diet, one-third of which was contributed by egg white and casein, could attain positive magnesium balance on only

182 mg daily and that there was a positive correlation of .75 between apparent nitrogen and magnesium absorption during this second experiment strengthens the possibility that magnesium absorption is affected by the amino acid pattern. An even higher correlation of .92 was observed between apparent nitrogen and magnesium absorption in subjects fed Diet B in the last experiment. Assuming that the stated hypothesis of an interrelationship between the amino acid pattern and magnesium utilization is true, the higher concentration of egg white and casein in addition to 35 g protein, one-half of which was from animal sources, would account for this higher correlation. If protein of excellent quality, because of its rapidity of absorption, improves magnesium uptake and utilization by virtue of accelerated muscle and enzyme synthesis, then the quality of the protein in Diet B could have masked the depressing influence of a higher protein level upon magnesium absorption, an effect observed in experimental animals (Fontenot <u>et al</u>., 1960; Head and Rook, 1955; Rook and Campling, 1962) and suggested by the results of the studies with preadolescent children (Schofield and Morrell, 1960). It is also possible, of course, that better absorption of magnesium occurs within a certain range of nitrogen intake and that 50 g protein falls within this range. Leichsenring <u>et al</u>. (1951), however, found that on an intake of 50 g protein their subjects required 261 mg magnesium daily for a slightly positive mean balance of 11.6 mg. This amount of magnesium is considerably more than the 196 mg which were sufficient for an average balance of +23.9 mg attained by the subjects fed Diet B during the third study.

In a study of the influence of protein intake upon magnesium requirement of the rat, Menaker (1954) found that although starved adult females fed a 14 per cent casein diet containing adequate magnesium gained more than those on a 9 per cent level of protein, when the magnesium was removed from the rations, the rats receiving only 9 per cent casein gained approximately 4 times as much as those on the higher protein intake. The author concluded that a high protein intake must increase the magnesium requirement for some purpose other than the formation of part of the structure of new tissue; otherwise, at least as much weight would have been gained on a 14 per cent protein diet as on the 9 per cent. In view of this study and others in which symptoms of magnesium deficiency occurred much more rapidly when diets were not only low in magnesium but also high in protein (Bunce, et al., 1963; Heinicke et al., 1956; Colby and Frye, 1951), the possibility is suggested that a high amino acid level in the blood causes the magnesium pools to release part of their mineral stores, an appreciable amount of which is secreted into the digestive tract and lost through the feces. Aikawa et al. (1959), using Mg^{28}, have shown that an exchangeable magnesium pool exists in the bone and the skeletal muscles of humans as well as in connective tissue, skin, and soft tissues of the abdominal cavity. Experiments with growing rats on magnesium deficient diets (Duckworth et al., 1940; Duckworth and Godden, 1941) and the study of magnesium deficiency in man (Fitzgerald and Fourman, 1956) give evidence that magnesium deposits in the bone are the most labile and the ones which make the ion available to other sites in the body during time of need. Magnesium is

necessary for activation of enzymes involved in the formation of urea, in synthesis of glycogen and fat, and in release of energy from amino acids as well as being essential for tissue synthesis. The increased need for enzymes to catalyse the oxidation and conversion of excess amino acids in the blood stream, a result of high protein intake, could possibly cause the labile magnesium pool to release quantities of the mineral for activation of existing enzymes and synthesis of other catalysts in which magnesium forms an integral part.

If a high amino acid level in the blood does cause the magnesium pool or pools to release quantities of the ion, a large part of which is secreted into the intestinal tract in the digestive juices and lost in the feces if conditions are unfavorable for reabsorption, then this could explain the need for increased magnesium intake when the level of protein is high. That higher intake does increase the amount of magnesium absorbed has been shown by Ross (1962) with _in vitro_ studies on rats. He demonstrated a highly significant correlation between magnesium transported across the intestinal wall and the concentration of the circulating magnesium although the relationship is not linear. Graham _et al_. (1960) have illustrated in studies on humans, through the use of Mg^{28}, this same increased, non-linear absorption of the mineral with higher intakes.

No significant correlation between magnesium and nitrogen balance was observed except in the subjects fed Diet A in the third experiment. In these young women who had a mean nitrogen balance of +0.148 on the

35 g protein intake, the correlation between nitrogen and magnesium balances was 0.79. In other words, at this level of protein intake, 62 per cent of the variation in magnesium balance could be related to the variation in nitrogen balance. This association between variation in nitrogen and magnesium balances might indicate that the level of protein which is just adequate to produce nitrogen equilibrium is the amount of protein which promotes the most efficient utilization of magnesium. If this interpretation for the cause of correlation between magnesium and nitrogen balances for subjects fed Diet A is correct, then results of these experiments suggest that the adequacy of very low magnesium intakes for preadolescent girls fed low-protein diets could be explained by the relative amounts of magnesium and protein ingested (Schofield and Morrell, 1960). Thirty-five grams of protein furnished by a normal mixed diet may represent the level at which not only nitrogen but also magnesium can be most efficiently utilized by human adults.

The results from the last two experiments suggest that magnesium metabolism in the adult may be affected by the quality and the quantity of protein in the diet. Whatever the cause of the improved magnesium retention in subjects fed a diet restricted in protein, the data obtained from these present experiments make it clear that a population consuming protein in amounts just sufficient to assure equilibrium does not require as much magnesium for positive balance as do people regularly ingesting a high-protein diet. Since it appears evident that the need for magnesium does vary with the level of protein

in the diet, fortification of diets with protein-rich supplements, a popular practice at the present time, probably increases the amount of magnesium needed for positive balance in children and adults beyond the quantities previously estimated by means of balance studies employing controlled diets. It is possible that the work of Seoular _et al._ (1957) with young college women on self-selected diets gives evidence of this increased need.

Because it is apparent that the adequacy of magnesium intake influences the metabolism of experimental animals (Anstall _et al._, 1959; Krehl and Barberiak, 1958; Hellerstein _et al._, 1960; Nakamura _et al._, 1960; Tapley, 1955; Vitale _et al._, 1957a and 1957b) and since it is suggested that this mineral may have a similar effect in the human body, careful consideration needs to be given to any circumstance which might change the quantity of the mineral necessary for optimal physiological conditions.

CHAPTER VI

SUMMARY

The influence of level of dietary protein on utilization of magnesium in restricted diets was investigated in three metabolic balance experiments. Subjects were 12 young women and one young man; two subjects participated in all of the experiments and two others took part in two of the studies. The studies consisted of a short preliminary adjustment period followed by 5 metabolic balance periods of 6 days each. Diets were planned to supply protein at several low levels and magnesium in restricted amounts which were as nearly constant as possible. Other nutrients were provided to meet recommended allowances through the use of mineral and vitamin supplements. With the exception of small supplements of "vitamin-free" casein, diets were composed of natural foods only.

Study I: Subjects were maintained on a diet supplying on the average, 1726 Calories, 20 g protein (one-third from animal sources) and 166 mg magnesium daily. With these intakes all subjects were in strongly negative nitrogen and magnesium balances, the overall means being $-0.837 \pm .431$ g and -42.4 ± 16.7 mg, respectively.

Study II: Subjects received essentially the same diet as in Study I except that the food supplied an average of 1975 Calories. When weight loss and excessive urinary excretion of nitrogen by all the young women

indicated that the subjects were in negative nitrogen balance, 5 g "vitamin-free" casein and 50 g cooked egg white were added to the basal diet. The supplement raised the protein and magnesium levels to 30 g and 178 mg, respectively, while the calorie intake remained close to 2000 daily. When protein, one-half of which was of animal origin, and magnesium were ingested at these levels, nitrogen balance approached equilibrium with a mean of $-0.110 \pm .319$ g while magnesium balance became positive, the mean being 7.2 ± 6.5 mg.

Study III: Subjects were divided into two groups; one group received a basal diet supplying 34 g protein (one-half from animal sources), 185 mg magnesium and 2000 Calories daily, while the other was fed the same basal diet supplemented with "vitamin-free" casein and cooked egg white. The supplemented diet, increased only slightly in calorie content, supplied approximately 50 g protein and 196 mg magnesium daily. Subjects in both groups achieved positive nitrogen and magnesium balances. The group fed the basal diet had an overall nitrogen balance of $0.148 \pm .134$ g with a magnesium balance of 8.3 ± 5.0. The group receiving the supplemented diet achieved more positive balances of $0.706 \pm .324$ g and 23.9 ± 9.3 mg.

The fact that 185 mg magnesium daily was adequate for small storage in adults fed diets restricted in nitrogen, in contrast to numerous

reports in the literature that approximately 300 mg is necessary for equilibrium in subjects receiving recommended allowances of protein, strongly suggests that magnesium utilization is altered by the level of dietary protein. Evidence is also presented which suggests that the quality of protein ingested is an influencing factor in magnesium absorption and retention.

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APPENDIX

TABLE XVIII

INDIVIDUAL BALANCE DATA FOR STUDY I

Period	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject GMB				
1	3.492	3.960	0.927	-1.395	167.4	100.9	142.2	-75.7
2	3.448	3.625	0.944	-1.121	177.7	77.4	106.6	- 6.3
3	3.506	3.500	0.935	-0.929	178.1	93.5	143.2	-58.6
4	3.497	3.160	0.972	-0.635	176.3	106.5	128.3	-58.5
5	3.410	3.401	0.853	-0.844	157.2	87.4	125.7	-55.9
Mean and s d	3.471 ±.042	3.529 ±.295	0.926 ±.044	-0.985 ±.288	171.4 ±9.0	93.1 ±11.4	129.2 ±14.9	-51.0 ±26.2
				Subject RGB[b]				
1	5.185	6.585	1.316	-2.716	227.7	162.1	139.6	-74.0
2	5.214	4.894	1.131	-0.811	249.4	139.8	99.1	+10.5
3	5.176	4.834	1.368	-1.026	237.3	153.0	122.3	-38.0
4	5.128	5.354	1.428	-1.654	227.6	146.2	117.9	-36.5
5	5.110	5.414	1.253	-1.557	222.4	159.9	105.7	-43.2
Mean and s d	5.163 ±.042	5.416 ±.704	1.299 ±.114	-1.553 ±.739	232.9 ±10.7	152.2 ±9.3	116.9 ±15.7	-36.2 ±30.3

TABLE XVIII (continued)

Period	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject AC				
1	3.559	3.780	0.879	-1.100	181.4	97.5	162.1	-78.2
2	3.601	4.561	0.879	-1.839	191.5	95.0	162.1	-65.6
3	3.554	3.430	0.606	-0.482	188.0	100.8	123.7	-35.7
4	3.597	3.160	0.793	-0.356	197.4	104.2	126.2	-33.0
5	3.497	2.908	0.875	-0.286	174.4	114.6	134.0	-74.2
Mean and s d	3.562 ±.042	3.568 ±.643	0.806 ±.118	-0.813 ±.658	186.5 ±8.9	102.4 ±7.6	141.6 ±19.1	-53.7 ±21.5
				Subject SH				
1	3.388	4.012	0.962	-1.586	145.7	76.8	109.8	-40.9
2	3.355	3.734	0.952	-1.331	149.9	74.3	90.8	-15.2
3	3.350	3.322	0.997	-0.969	145.5	77.2	106.9	-38.6
4	3.364	3.335	1.103	-1.074	148.6	87.6	88.9	-27.9
5	3.364	3.311	0.913	-0.860	147.7	73.8	99.4	-25.5
Mean and s d	3.364 ±.016	3.543 ±.318	0.985 ±.072	-0.964 ±.786	147.5 ±1.9	77.9 ±5.6	99.2 ±9.3	-29.6 ±10.4

TABLE XVIII (continued)

Period	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject MAN				
1	3.360	3.230	0.752	-0.622	139.7	89.8	81.7	-31.8
2	3.364	2.565	0.749	+0.050	151.9	95.0	67.6	-10.7
3	3.364	2.514	0.901	-0.051	148.4	87.9	72.9	-12.4
4	3.364	2.713	0.922	-0.271	148.6	96.5	74.2	-22.1
5	3.374	2.434	0.700	+0.240	149.6	104.1	67.3	-21.8
Mean and s d	3.365 ±0	2.691 ±.318	0.805 ±.099	-0.131 ±.330	147.6 ±4.6	95.1 ±6.6	72.7 ±5.9	-19.8 ±8.5
				Subject ES				
1	3.521	4.724	0.975	-2.178	173.4	81.6	165.5	-73.7
2	3.559	3.675	0.988	-1.104	192.6	75.3	136.2	-18.9
3	3.572	3.541	1.039	-1.008	192.0	84.5	152.2	-44.7
4	3.488	3.315	0.988	-0.815	174.4	86.8	152.6	-65.0
5	3.383	3.816	0.914	-1.347	151.6	71.1	149.2	-68.7
Mean and s d	3.505 ±.077	3.814 ±.540	0.981 ±.045	-1.290 ±.532	176.8 ±16.8	79.9 ±6.5	151.1 ±10.4	-54.2 ±22.6

[a]Intake includes amounts obtained from coffee and supplements as well as that from food.

[b]BGB was only male subject participating in any of the three studies.

TABLE XIX

INDIVIDUAL BALANCE DATA FOR STUDY II

Period	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject GMB				
1	3.502	3.851	1.190	-1.539	167.5	78.3	109.2	-20.0
2	3.519	3.786	1.171	-1.438	165.1	85.4	97.3	-17.6
Mean and s d	3.510 ±.031	3.818 ±.045	1.180 ±0	-1.488 ±.070	166.3 ±1.7	81.8 ±5.0	103.2 ±8.4	-18.8 ±1.7
3	5.146	3.845	0.869	+0.432	181.7	79.6	88.0	14.1
4	5.146	4.312	0.934	-0.100	183.6	72.9	86.9	23.8
5	5.146	4.243	0.973	-0.070	182.7	80.8	111.3	- 9.4
Mean and s d	5.146 ±0	4.133 ±.252	0.925 ±.050	0.087 ±.299	182.7 ±1.0	77.8 ±4.3	95.4 ±13.8	9.5 ±17.1
				Subject ESE				
1	3.502	4.538	0.844	-1.880	167.5	84.2	95.4	-12.3
2	3.519	4.578	0.670	-1.729	165.1	87.6	88.5	-11.0
Mean and s d	3.510 ±.031	4.558 ±.045	0.757 ±.122	-1.804 ±.109	166.3 ±1.7	85.9 ±2.4	92.0 ±5.0	-11.6 ±0.9
3	5.146	4.684	0.818	-0.356	181.7	90.0	80.5	11.2
4	5.146	4.620	0.978	-0.452	183.6	86.9	97.4	- 0.7
5	5.146	4.929	0.858	-0.641	182.7	57.8	119.6	5.3
Mean and s d	5.146 ±0	4.744 ±.161	0.885 ±.084	-0.483 ±.145	182.7 ±1.0	78.2 ±17.8	99.2 ±19.6	5.3 ±5.9

TABLE XIX (continued)

Period	Nitrogen (g/24 hr) Intake[a]	Urine	Feces	Balance	Magnesium (mg/24 hr) Intake[a]	Urine	Feces	Balance
				Subject SH				
1	3.403	3.722	1.130	-1.449	146.5	62.8	143.4	-59.7
2	3.420	3.209	0.854	-0.643	144.1	62.3	82.8	- 1.0
Mean and s d	3.411 ±.031	3.466 ±.363	0.992 ±.195	-1.046 ±.571	145.3 ±1.7	62.6 ±0.4	113.1 ±42.9	-30.4 ±41.5
3	5.047	3.848	0.981	0.218	160.7	71.2	97.3	- 7.8
4	5.047	4.355	1.214	-0.522	162.6	61.8	100.4	0.4
5	5.047	4.557	1.130	-0.640	161.7	49.8	108.0	3.9
Mean and s d	5.047 ±0	4.253 ±.366	1.108 ±.118	-0.315 ±.465	161.7 ±1.0	60.9 ±10.7	101.9 ±5.5	- 1.2 ± 6.0
				Subject MH				
1	3.502	3.581	0.960	-1.039	167.5	88.3	108.2	-29.0
2	3.519	3.508	0.866	-0.855	165.1	92.8	79.6	- 7.3
Mean and s d	3.510 ±.031	3.544 ±.055	0.913 ±.070	-0.947 ±.130	166.3 ±1.7	90.6 ±3.2	93.9 ±20.2	-18.2 ±15.3
3	5.146	3.477	0.589	1.080	181.7	82.1	49.5	50.1
4	5.146	4.202	0.796	0.148	183.6	85.0	93.8	2.8
5	5.146	4.655	0.760	-0.269	182.7	96.1	89.5	- 2.9
Mean and s d	5.146 ±0	4.111 ±.593	0.715 ±.110	0.320 ±.690	182.7 ±1.0	87.7 ±7.4	77.6 ±24.4	16.7 ±29.1

TABLE XIX (continued)

Period	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject ES				
1	3.502	4.646	0.977	-2.121	167.5	82.3	115.8	-30.6
2	3.519	4.778	1.009	-2.268	165.1	97.2	79.3	-11.4
Mean and s d	3.510 ±.031	4.712 ±.095	0.993 ±.032	-2.194 ±.105	166.3 ±1.7	89.8 ±10.5	97.6 ±25.8	-21.0 ±13.6
3	5.146	4.284	1.009	-0.147	181.7	70.7	130.0	-19.0
4	5.146	4.135	0.884	0.127	183.6	75.2	82.5	25.9
5	5.146	4.590	1.015	-0.459	182.7	68.1	103.8	10.8
Mean and s d	5.146 ±0	4.336 ±.232	0.969 ±.077	-0.160 ±.290	182.7 ±1.0	71.3 ±3.6	105.4 ±23.8	5.9 ±22.9

[a]Intake includes amount obtained from coffee, coca-cola and supplements as well as that from food.

TABLE IX

NITROGEN AND MAGNESIUM BALANCES--PRELIMINARY PERIOD FOR STUDY III

Subject	Nitrogen (g/24 hr)[a]				Magnesium (mg/24 hr)[a]			
	Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance
CE	6.883	4.648	1.129	1.056	184.1	73.9	136.7	-26.5
MN	"	4.613	0.964	1.256	"	92.1	72.3	19.7
ES	"	7.102	1.110	-1.379	"	116.8	95.8	-28.5
PT	"	3.720	0.965	2.148	"	95.0	68.6	20.5
FG	"	4.255	0.861	1.717	"	89.1	117.0	-22.0
SH	"	5.423	1.101	0.309	"	66.8	121.6	- 4.3
PM	"	4.960	0.843	1.030	"	71.4	68.9	43.8
MR	"	4.018	0.990	1.885	"	54.6	110.0	19.5
Mean and s d	6.883 ±0	4.867 ±1.055	0.995 ±.111	1.135 ±1.116	184.1 ±0	95.7 ±19.6	113.0 ±26.5	3.2 ±26.9

[a]Intakes of nitrogen and magnesium include all that obtained from coffee, coca-cola, and mineral supplement as well as that contained in food.

TABLE XXI

INDIVIDUAL BALANCE DATA FOR STUDY III (DIET A)

Period	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject FG				
1	5.562	4.099	0.807	0.656	188.9	85.7	66.0	37.2
2	5.377	4.555	0.661	0.161	165.9	87.2	78.0	0.7
3	5.377	4.835	0.726	-0.184	165.9	77.2	102.1	-13.7
4	5.377	4.380	0.895	0.102	165.9	94.3	96.3	-24.7
5	5.377	4.524	0.707	0.146	165.9	95.2	83.8	-13.1
Mean and s d	5.414 ±.082	4.479 ±.269	0.759 ±.092	0.176 ±.303	170.5 ±10.3	87.9 ±7.3	85.2 ±14.4	- 2.7 ±24.1
				Subject SH				
1	5.510	4.528	0.913	0.069	175.9	78.4	89.2	8.3
2	5.510	4.682	1.000	-0.172	175.9	71.0	94.6	10.3
3	5.510	4.320	0.946	0.244	175.9	79.2	95.8	0.9
4	5.510	4.714	0.947	-0.151	175.9	81.6	98.7	- 4.4
5	5.510	4.554	0.831	0.125	175.9	82.4	93.6	- 0.1
Mean and s d	5.510 ±0	4.560 ±.156	0.927 ±.063	0.023 ±.184	175.9 ±0	78.5 ±4.3	94.4 ±3.5	3.0 ±6.1

TABLE XXI (continued)

Period[b]	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject PM				
1	5.574	5.393	1.014	-0.833	188.9	59.7	133.8	- 4.6
2	5.598	4.597	1.130	-0.129	189.3	72.8	115.0	1.5
3	5.598	4.074	0.958	0.566	189.3	60.6	111.1	17.4
4	5.598	4.164	0.922	0.512	189.3	82.4	106.7	0.2
5	5.598	4.346	0.719	0.533	189.3	69.0	90.1	30.2
Mean and s d	5.593 ±.016	4.515 ±.530	0.949 ±.151	0.130 ±.611	189.2 ±.2	68.9 ±9.4	111.3 ±15.7	8.9 ±14.5
				Subject MR				
1	5.574	4.128	1.175	0.271	188.9	61.0	95.6	32.3
2	5.574	3.900	1.124	0.550	188.9	59.1	102.6	27.2
3	5.574	4.547	1.106	-0.079	188.9	54.4	137.3	- 2.8
4	5.574	3.992	1.162	0.420	188.9	81.8	112.1	- 5.0
Mean and s d	5.574 ±0	4.142 ±.286	1.142 ±.032	0.290 ±.271	188.9 ±0	64.1 ±12.1	111.9 ±18.2	12.9 ±19.6

[a] Intake includes amount obtained from coffee, coca-cola and supplements as well as that from food.

[b] There are data for only 4 periods for subject MR since she became ill during period 5.

TABLE XXII

INDIVIDUAL BALANCE DATA FOR STUDY III (DIET B)

Period	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject CK				
1	7.830	5.868	0.712	1.250	200.4	85.0	83.9	31.5
2	7.657	6.004	0.592	0.976	177.8	83.2	62.2	32.4
3	7.657	6.825	0.929	-0.097	177.8	85.1	107.5	-14.8
4	7.657	6.017	0.891	0.749	177.8	60.2	99.6	18.0
5	7.657	6.158	0.866	0.633	177.8	79.6	90.7	7.5
Mean and s d	7.692 ±.077	6.170 ±.452	0.798 ±.141	0.702 ±.505	182.3 ±10.1	78.6 ±10.5	88.8 ±17.3	15.0 ±19.5
				Subject MN				
1	7.830	6.149	0.945	0.736	200.4	89.9	91.1	19.4
2	7.830	6.462	0.842	0.526	200.4	101.3	71.2	27.9
3	7.830	6.090	0.904	0.836	200.4	93.6	76.0	30.8
4	7.830	5.032	1.058	1.740	200.4	93.8	110.3	- 3.7
5	7.830	5.137	0.959	1.734	200.4	90.2	84.6	25.6
Mean and s d	7.830 ±0	5.774 ±.646	0.942 ±.079	1.114 ±.579	200.4 ±0	93.5 ±4.6	86.6 ±15.3	20.0 ±13.9

TABLE XXII (continued)

Period	Nitrogen (g/24 hr)				Magnesium (mg/24 hr)			
	Intake[a]	Urine	Feces	Balance	Intake[a]	Urine	Feces	Balance
				Subject PT				
1	7.830	6.878	0.828	0.124	200.4	99.0	53.4	48.0
2	7.854	6.166	1.191	0.497	200.8	107.0	62.2	31.6
3	7.854	6.116	1.074	0.664	200.8	103.1	75.6	22.1
4	7.854	5.974	0.720	1.160	200.8	110.0	37.8	53.0
5	7.854	5.830	1.038	0.986	200.8	107.8	63.7	29.3
Mean and s d	7.849 ±.009	6.193 ±.405	0.970 ±.192	0.686 ±.408	200.7 ±.2	105.4 ±4.4	58.5 ±14.0	36.8 ±13.1
				Subject ES				
1	7.854	6.140	0.996	0.718	200.8	76.0	91.7	33.1
2	7.854	6.429	1.115	0.310	200.8	78.6	88.4	33.8
3	7.854	7.181	0.873	-0.200	200.8	76.6	84.4	39.8
4	7.854	6.258	1.081	0.515	200.8	82.8	101.8	16.2
5	7.854	6.328	1.255	0.271	200.8	80.1	124.7	- 4.8
Mean and s d	7.854 ±0	6.467 ±.413	1.064 ±.142	0.323 ±.342	200.8 ±0	78.8 ±2.8	98.2 ±16.2	23.6 ±18.2

[a]Intake includes amount obtained from coffee, coca-cola and supplements as well as that from food.

TABLE XXIII

PERIOD MENUS FOR DIET A--STUDY III

Day 1

Breakfast

Food	g
Orange Juice	120
Puffed Rice	14
Brown Sugar	20
Doughnut	32
Margarine	10
Cream Mix[a]	120

Lunch

Food	g
Tomato Soup	150
Bread	40
Baked Turkey	56
Lettuce	15
Mayonnaise[a]	11
Canned Apricots	90
Cocoanut	10
Margarine	15

Dinner

Food	g
Browned Rice[a]	80
Baked Onion[a]	100
Lettuce	50
Fruit Dressing[a]	20
Broiled Pear[a]	92
Grape Juice	240

Snacks

Food	g
Coca-cola	300
Potato Chips	20
Molasses Cookies[a]	30
Raisins	20

Day 2

Breakfast

Food	g
Orange and Grape Juice Mix[a]	180
Rice Krispies	24
Brown Sugar	20
Doughnut	32
Margarine	10
Cream Mix[a]	120

Lunch

Food	g
Celery Soup[a]	150
Bread	40
Margarine	15
Cottage Cheese Mix[a]	45
Lettuce	15
Canned Peaches	90
Gingerbread[a]	50
Pineapple	100

TABLE XXIII (continued)

Dinner		Snacks	
Food	**g**	**Food**	**g**
Ground Beef Pattie (raw wt)	20	Coca-cola	300
Mashed Potatoes[a]	85	Graham Cracker	7
Canned Asparagus	100		
Lettuce	50		
Fruit Dressing[a]	20		
Margarine	10		
Stewed Prunes	60		
with Juice	10		
Coffee Cream	45		

Day 3

Breakfast		Lunch	
Orange and Pineapple Juice Mix[a]	120	Celery Soup[a]	150
Cornflakes	13	Bread	40
Brown Sugar	20	Margarine	10
Doughnut	32	Cottage Cheese Mix[a]	20
Margarine	10	Tomato	40
Cream Mix[a]	120	Lettuce	15
		Broiled Peach[a]	92

Dinner		Snacks	
Ground Beef Pattie (raw wt)	53	Coca-cola	300
Mashed Potatoes[a]	85	Potato Chips	20
Broccoli	50	Molasses Cookies	60
Margarine	5		
Cinnamon Applesauce[a]	55		
~~Lettuce~~	50		
Fruit Dressing[a]	20		
Banana Sherbet[a]	100		

TABLE XXIII (continued)

Day 4

Breakfast

Food	g
Orange and Pineapple Juice Mix[a]	120
Rice Chex	28
Brown Sugar	20
Doughnut	32
Margarine	10
Cream Mix[a]	120

Lunch

Food	g
Asparagus Soup[a]	150
Margarine	15
Bread	40
Baked Turkey	47
Lettuce	65
Mayonnaise[a]	11
Banana	50
Canned Plums	90
with Juice	10

Dinner

Food	g
Beef-rice Casserole[a]	100
Squash	100
Onions	50
Margarine	9
Bread	20
Slaw[a]	65
Canned Pears	90
with Juice	10
Cranberry Mousse[a]	60

Snacks

Food	g
Coca-cola	300
Graham Crackers	14
Jelly	20

Day 5

Breakfast

Food	g
Orange and Pineapple Juice Mix[a]	160
Rice Krispies	24
Brown Sugar	20
Doughnut	32
Margarine	10
Cream Mix[a]	120

Lunch

Food	g
Tomato Soup[a]	150
Margarine	15
Bread	40
Pineapple	50
Lettuce	15
Mayonnaise[a]	3
Canned Peaches	60
Coconut	10
~~Skim Milk~~	120

TABLE XXIII (continued)

Dinner		Snacks	
Food	g	Food	g
Ground Beef Pattie (raw wt)	55	Coca-cola	300
Mashed Potatoes[a]	129	Graham Crackers	14
Green Beans	75	Jelly	20
Margarine	5	Molasses Cookies	30
Lettuce	50		
Fruit Dressing[a]	20		
Rhubarb and Apples[a]	100		

Day 6

Breakfast		Lunch	
Orange and Pineapple Juice Mix[a]	120	Asparagus Soup[a]	150
Cornflakes	13	Margarine	15
Brown Sugar	20	Bread	40
Doughnut	32	Tomato	60
Margarine	10	Lettuce	15
Cream Mix[a]	120	Mayonnaise[a]	3
		Cherry Crisp[a]	104
		Coffee Cream	45

Dinner		Snacks	
Baked Halibut	48	Coca-cola	300
Noodles with Sauce[a]	80	Potato Chips	20
Canned Asparagus	100	Applesauce Cookies[a]	30
Margarine	5		
Lettuce	50		
Mayonnaise[a]	10		
Pineapple Tapioca[a]	150		
Whipped Cream[a]	20		

[a]These foods were prepared by recipes developed for Studies I, II and III.

TABLE XXIV

NUTRIENT CONTENT OF DIET A

Calories and nutrients	Day 1	2	3	4	5	6	Daily avg intake	Daily supplement	Daily avg total
Calories	2118	2051	2039	2093	2020	2080	2067	--	2067
Protein (g)	34.8	34.9	35.1	35.1	34.7	34.8	34.9	--	34.9
Fat (g)	102.0	106.2	109.4	97.8	92.5	105.4	102.0	--	102.0
Carbohydrate (g)	297.2	271.2	258.5	282.9	275.7	239.8	271	--	271
Calcium (mg)	454.0	577	578	451	554	445	501	505	1006
Phosphorus (mg)	764	820	845	770	797	748	791	869	1660
Iron (mg)	7.5	9.2	8.7	7.4	9.0	7.5	8.2	1.8	10.0
Vitamin A (I.U.)	4993	5841	5595	4936	4406	5651	5237	--	5237
Thiamine (mg)	1.28	0.75	1.80	0.69	1.36	0.76	1.1	--	1.1
Riboflavin (mg)	1.12	0.96	1.60	0.76	1.26	0.77	1.1	0.4	1.5
Niacin (mg)	10.5	9.5	8.0	13.6	10.2	11.0	10.7	--	10.7
Ascorbic Acid (mg)	86	78	97	80	78	86	84	--	84
Vitamin D (I.U.)								420	420

TABLE XXV

REPRODUCIBILITY AND ACCURACY OF MAGNESIUM DETERMINATION BY MODIFIED THIAZOLE YELLOW METHOD

Sample	Replicate analyses (mg per 24 hours)									Sample	Recovery added magnesium (%)		
	Food			Feces			Urine				Food	Feces	Urine
1	97.3	97.1	98.2	103.8	102.6	102.9	85.3	84.9	86.3	1	102	96	105
2	106.0	105.3	--	111.3	111.6	--	95.2	96.1	--	2	97	102	103
3	159.5	159.5	--	106.7	106.7	--	107.0	107.0	--	3	99	97	104
4	150.3	150.3	--	99.6	101.7	--	60.2	59.3	--	4	100	104	99
										5	103	103	96
										Mean and s d	100 ± 2.4	100 ± 3.6	101 ± 3.8