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To the Graduate Council:

I am submitting herewith a dissertation written by Polly Givler Martin entitled "The Influence of Protein on the Absorption of Iron, Copper, and Zinc in Adult Women." I 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:

James R. Savage

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Accepted for the Council:

Dean of the Graduate School

THE INFLUENCE OF PROTEIN ON THE ABSORPTION OF IRON, COPPER, AND ZINC IN ADULT WOMEN

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

Polly Givler Martin

December 1965

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CHAPTER I

INTRODUCTION

The distribution of iron in cellular materials has been studied extensively for about a century, whereas the biological significance of other trace minerals such as copper and zinc were considered of limited importance. During the earlier part of this century the important and essential role played by copper and zinc in animal metabolism came into focus. The three minerals are found in the blood components, as well as functioning as catalysts for many enzyme systems.

Recommended dietary allowances for iron have been established for men, women, and children. Ingestion of suboptimal amounts of iron coupled with growth, pregnancy, or menstrual loss can produce an anemia which is very prevalent throughout the world. Less research has been conducted on the dietary requirements for copper and zinc, as deficiency states produced by these minerals have not been established in man. Consumption of a normal mixed diet will allow sufficient intake for absorption of these minerals to meet the demands of the body.

Absorption of these three minerals is very low, approximately 90 per cent being excreted in the feces. Various factors appear to influence the amount of iron, copper, and zinc absorbed. Recent investigations with animals indicate that protein and amino acids may form complexes with certain minerals to facilitate uptake and transport.

The purpose of the studies undertaken in this laboratory was to investigate the possible relationship of protein level and protein quality on the absorption of these three minerals from natural diets. The two balance studies reported in this paper were part of a series of studies on college women. The protein levels of the diets used were 35 g. and 48 g. but the protein sources were altered to furnish different ratios of animal to plant protein.

CHAPTER II

REVIEW OF LITERATURE

I. IRON

While iron deficiencies and metabolism have been studied extensively for many years some of the factors regulating iron absorption are still undetermined (1). Age, sex, pregnancy, chemical form of the iron, protein content of the diet, phosphate, phytate, and anemia all exert an influence on the amount of iron absorbed from the diet. Of the many modifying factors influencing iron absorption, iron stores, rate of erythropoiesis, hemoglobin levels, and quantity of iron ingested appear to be of the greatest significance in both normal and abnormal circumstances (2, 3). Under normal conditions only 5 to 10 per cent of the dietary iron is absorbed, the remainder passes out of the body (4, 5). Once the iron is absorbed it is transported as transferrin which is in equilibrium with the storage forms of iron as well as the iron containing enzymes of the cell.

Although iron overload diseases are rare, there is a prevalence of iron deficiency states. Many common foods are either low in iron or contain other compounds that interfere with iron absorption. Milk diets of infants furnish subminimal quantities of iron while the foods and drink ingested by the Bantus contain excessive amounts of iron. Recommended dietary allowances for iron intakes were first made in 1943 by

the Food and Nutrition Board. Based on human studies in various normal age groups the 1964 revision of recommended daily dietary allowance for men is 10 mg. daily, for children 8 to 15 mg., and for women 15 mg. (6).

In an experiment on young women (7), ninety-nine one-week iron metabolism studies were made on sixty-nine women eating self-selected diets. As the daily intake of iron increased from 8 mg. to 16 mg. the percentage of negative balances decreased and the amount of iron stored increased. Two groups of subjects ingesting 7.2 mg. of iron daily were compared. One group, whose diets were generous in other nutrients, stored on an average 1.6 mg. of iron daily while the other group, who ate suboptimal diets, did not retain enough iron to replace body losses. Subjects ingesting adequate diets furnishing 8.0 to 10.0 mg. stored an average of 1.0 mg. of iron per day. Negative iron balances were observed on daily intakes up to 14.0 mg. and appeared to be associated with the quality of the diet as a whole rather than the amount of iron ingested. This study would indicate that level of iron intake cannot be considered alone but the general nutriture of the diet must be taken into account in any prediction of iron storage.

In an earlier study (8) college women fed diets furnishing 67 g. of protein but only 3.5 to 4.5 mg. of iron daily had iron losses of about 0.3 mg. when the intake was 3.5 mg. When the intake was increased to 6.6 mg. by replacing 750 ml. of milk in the basal diet with 116 g. of lean beef, fecal excretion of iron was approximately the same as on the lower iron intake. This low fecal excretion of iron did not continue as the iron content of the diet was further increased. In other women on

self-chosen diets fecal excretion rose to 7 and 12 mg. Contrary to these findings Ohlson and Daum (9) found that diets furnishing 13.7 mg. of iron were not sufficient to cover iron excretions of 14.9 mg. per day for three women. The self-chosen diets furnished liberal amounts of protein ranging from 61 to 79 g. daily and included generous amounts of other nutrients. Whether the diets included constituents that interferred with iron absorption was not determined.

Abernathy et al. (10) investigated the effect of amount and source of dietary protein on iron absorption in preadolescent girls. During six experimental periods in which the diet supplied 22 g. of protein from both plant and animal sources and 9.8 mg. of iron, an average of 1.2 mg. of iron was absorbed daily. Only one of the twelve subjects lost more iron through the feces than was ingested. When the same amount of protein was of plant origin exclusively, average iron absorption was 0.8 mg. from the daily intake of 10.2 mg. All subjects excreted less iron via the feces than was ingested. The difference in absorption was not statistically significant. An entire series of experiments in which the protein intake was varied from 17 to 88 g. failed to show any effect of protein level on iron absorption in seven- and nine-year-old girls under these experimental conditions. If iron intakes were below 7.9 mg. of iron per subject iron losses through the feces were greater than the amounts of iron ingested.

Five eighteen-year-old college women were maintained for ten weeks on a controlled diet furnishing two levels of iron (11). The basal diet included protein of animal and plant origin with adequate

nutrients furnished by natural foods. All bread was made from unenriched flour, no whole wheat cereals, shelled beans, or nuts were included in the diet; ground beef was provided once a day. During the first experimental period, 7 mg. of iron a day were ingested with an average retention of 0.6 mg. If menstrual losses of iron were included, the retentions were barely adequate to cover the needs of the body for four of the five girls and less than that needed by one subject. During the next part of the study (12), two beef patties were added to the diet so that beef was served at every meal. The iron intake was 10.4 mg. Average retentions ranging from 1.8 to 2.5 mg. a day more than covered the needs of the subjects. The absorption of iron increased from 11 per cent on the low iron intake to 21 per cent on the 10.4 mg. intake. Results were interpreted to mean that some factor other than increased iron content was responsible for increased absorption of iron.

Four young women served as subjects for a three-to-five-month study designed to determine the iron intake necessary to maintain balances including iron lost in menses (13). A basal diet was used which included a large variety of fruits and vegetables and animal sources of protein. Variation in amounts of certain foods provided calorie adjustments. The average daily intake of iron ranged from 10.0 to 13.6 mg. The corresponding balances for the four women between food and excreta were 0.72, 1.48, 0.71, and -0.20 mg. With menstrual losses averaging 11.1 to 22.8 mg. per cycle the subjects were in slight positive balance during nine of the sixteen menstrual cycles. If all iron losses were considered only two of the subjects were able to absorb

enough iron to cover their needs. Nevertheless hemoglobin levels were increased in all subjects by 1.0 g./100 ml. of blood by the end of the study.

The absorption of iron from spinach added to a basal diet providing 11.5 mg. of iron was investigated (14). When spinach containing 5.0 mg. of iron was added to the basal diet, iron absorption was increased from 0.76 mg. to 1.20 mg. The amount of iron absorbed from the spinach averaged 0.66 mg. or approximately 12 per cent of the amount supplied by the spinach. When beef was added to the diet furnishing 11.5 mg. of iron, there was a slight increase in absorption of iron which was not significant.

Many factors appear to influence iron absorption. The effect of seven test meals upon the absorption of either reduced ^{55}iron (hereafter referred to as ^{55}Fe) or reduced ^{59}iron (hereafter referred to as ^{59}Fe) was studied in adolescent boys (15). The test meals consisted of: I and VI, milk, rolled oats, tomato juice, white bread, and omelet; II, milk; III, milk and sodium phytate; IV and VII, milk and rolled oats; and V, water. Each meal contained approximately 8.3 mg. of iron furnished by natural foods, stable ferric chloride, and/or radioactive iron. Iron absorption was greatest from the water meal, V, with 27 per cent of the iron being absorbed. Absorption of iron from meal II, consisting of milk, was reduced to 17 per cent. Meals IV and VII, containing oatmeal and milk, reduced absorption to about 9 per cent. Although phytates have been implicated in reducing iron absorption, it was not affected when the quantity of oatmeal was reduced by 40 per cent as in meal IV.

The authors suggested that in view of the apparent lack of interference with iron absorption by the phytate occurring naturally in oats, the calcium in the milk might have been preferentially combined with the phytate making it unavailable for iron precipitation. However, when pure sodium phytate was used in meal III, iron absorption was decreased to 1.7 per cent even though the milk calcium present to combine with the phytate was nine times that in the other meal. It was noted that iron absorption progressively decreased with increasing quantities of food in the meal. This would suggest that the solid content of the meal may in itself interfere with iron absorption.

Contrary to the previous mentioned findings iron absorption was poor from Indian cereal diets containing 40 per cent natural phytate when the calcium content of the diet was low (16). The dietary level of calcium was altered by adding buffalo and skim milk. When the diet contained 360 to 408 mg. of calcium, the amount of dietary iron (16.6 mg.) was not sufficient to meet the needs of the men serving as subjects. When 1,000 mg. of calcium was provided with the same level of iron, 4 per cent of the iron was absorbed. Increasing the calcium to 1,500 mg. resulted in a 7 to 27 per cent iron absorption. It was suggested that the calcium counteracted the adverse effect of the high phosphate and phytate content of the cereal diet by reacting with these components thus making iron available for absorption. The varying amounts of calcium, iron, phytates, and phosphates in the diet appeared to affect their absorption patterns. The authors felt that this is a common situation in nutritional physiology where relative levels of nutrients

may determine the requirement of each other.

The interrelationship of phosphorus and iron on the absorption of iron has been shown (17). Iron absorption was compared in rats fed either Purina Chow or diets containing corn grits. Although the rats receiving a corn grit-lard diet supplemented with ferric citrate lost weight, the liver iron of these rats exceeded the total body iron of control rats. No correlation could be shown between the iron-phosphorus ratios of the diet and iron stored in the liver although the results indicated that the low phosphate of the corn grit diet was responsible for the accumulation of liver iron. When phosphate salts were added to the diet the iron content of the liver decreased. Not only did the absolute amounts of iron and phosphorus in the diet influence iron absorption but the iron-phosphorus ratio was a factor also.

McCance et al. (18) studied the influence of phytates on iron absorption by following serum iron levels in four normal men and five normal women. Sodium phytate was incorporated into the bread used and iron supplements were supplied as either ferrous or ferric salts. Serum iron levels were lower in the subjects receiving sodium phytate than in controls. The effect was more constant and depression was greater when ferric salts were used than ferrous salts. When disodium hydrogen phosphate was added to the bread, iron absorption was decreased from that of the controls but not to the same degree as with the subjects receiving the phytate. In vitro experiments showed that at the pH of the intestine both ferrous and ferric phytates appear to be more insoluble than the corresponding phosphates or hydroxides and probably

would not be available for absorption.

Incorporation of radioactive iron into several foods has been accomplished in order to measure the absorption of iron from a meal (19). Hens were injected with radioactive iron so the eggs produced contained the isotope. After several weeks the hens were killed and the liver and muscle were used as a source of radioactive iron for the subjects. Absorption was measured by two methods, the unabsorbed portion found in the feces and the amount incorporated into circulating hemoglobin. In most normal subjects the amount of iron absorbed from the eggs and liver was 10 per cent or less; the absorption from chicken muscle was slightly higher than other foods. When the eggs were incorporated either into cake or corn bread, or fed along with a meal of bacon, toast, and cereal, iron absorption was not influenced by the combinations. Greater iron assimilation could be achieved only when either relatively large amounts of grapefruit juice (200 to 250 ml.) or 1 g. crystalline ascorbic acid was fed with the meal.

In several studies on normal subjects the presence of eggs decreased absorption of iron salts. This decreased absorption in the meal may have been due to an increased solid content of the meal or to the formation of some insoluble compound of iron with the constituents of egg or other foods. Egg yolk iron is in the ferric state and is strongly complexed to the phosphate of yolk phosphoproteins which would account for the unavailability of the iron of the yolk but does not explain the effect produced on iron salts (20).

Iron absorption from different salts was studied by Steinkamp and coworkers (21). Ferrous sulfate, reduced iron, ferric orthophosphate, and sodium ferric pyrophosphate were fed, either baked into bread or as iron supplements. Iron absorption ranged from 1 to 12 per cent in twenty-eight of the thirty-two healthy men. In the remaining four subjects who were believed to have depleted iron stores, absorption ranged from 45 to 64 per cent. Additional studies on iron absorption from unenriched bread and from iron salts added to the bread indicate that the iron salts are more readily absorbed than the iron contained in the unenriched bread (22, 23).

The percentage of iron absorbed from ferrous chloride exceeded that of the mineral from vegetables (24). Chodos and coworkers administered a solution of ferrous chloride to fasting subjects to eliminate interference from other food components and found this iron to be more readily absorbed than iron contained in food.

Hemoglobin iron has been found to be absorbed as well or better than iron salts in normal subjects (25), but iron depleted or deficient subjects did not absorb as great a quantity. Food factors such as quantity of food eaten, phytate content or ascorbic acid appeared to have little effect on absorption.

The level of protein in the diet influenced iron absorption in rats, as well as erythropoiesis, passage of diet through the intestinal tract, and the chelating effect of the intestinal contents. Higginson et al. (26) found that rats fed diets containing either 4 per cent vitamin-free casein or Purina Chow absorbed less iron than rats fed a corn

meal diet. Klavins and coworkers (27) found that to promote adequate iron absorption the diets needed to contain approximately 15 to 18 per cent protein. Rats fed 5 or 10 per cent protein absorbed less iron than rats fed diets containing 15 per cent protein. Iron absorption further increased when the diet contained 25 per cent protein. In rats fed diets supplemented with 2 per cent ferric citrate, the same relationship between iron absorption and low level of protein in the diet was found but there was no significant difference between absorption on the higher levels of protein fed. The authors suggested that the higher level of protein furnished amino acids for chelation and transport, but this theory did not hold true when additional iron was added. When ferric citrate was added the amount of total body iron was greater at all levels of protein than when no supplement was given. The amino acid content may have played some preliminary role in iron uptake and transfer but with the addition of more total iron other factors apparently were also affecting utilization.

In order to study the relative effect of nine amino acids upon ^{59}Fe absorption from the gastrointestinal tract of rats, a section of the small intestine was ligated at the pylorus and about 4 cm. distal to the pylorus. A solution containing the amino acid, ^{59}Fe, and the buffer was administered directly into this segment of the intestine (28). These experiments showed that ^{59}Fe appeared more quickly and in greater amounts in the blood when administered with amino acids in a phosphate buffer than when administered with the phosphate buffer alone. The nine amino acids could be divided into three distinct groups on the basis of

their effect on the ^{59}Fe content in the blood. Injection of glutamine, glutamic acid, and asparagine resulted in the highest blood ^{59}Fe levels, whereas methionine, ethionine, proline, serine, and phenylalanine promoted only lower absorption. Iron absorption from a solution of histidine was slow at first but increased with time until at the end of the 60 minute period the amount of ^{59}Fe absorbed was greater than from the other eight amino acid solutions. After the 60 minute time period the rats were killed and the pH of the intestinal content was determined. The pH values of the contents were not correlated with the amount of blood ^{59}Fe activity. The amount of iron absorbed by the rats receiving the amino acids was five times as great as that absorbed by rats receiving the phosphate buffer complex. Amino acids are effective chelating agents for many metal ions but what role they play when associated with a mixture of materials such as found in the intestine is as yet unanswered (29).

Chelates of fructose, other reducing sugars and polyols have been shown to be extremely soluble at the pH encountered in the duodenum (30, 31). Stitt and coworkers found that ferrous sulfate and ferric sulfate plus reducing sugars were rapidly transferred across the intestinal membrane of rabbits. The build-up of iron in the liver of the animals was similar to that encountered in the Bantus.

The iron absorption of normal individuals fed a test bread was significantly greater than that of six untreated pernicious anemia patients (32). When normal gastric juice was added to the test meal of six patients with pernicious anemia, iron absorption was increased

two-fold. The authors believe that gastric secretion plays an important role in iron absorption from certain types of food but has little effect on absorption of large doses of iron salts. When neutralized gastric juice was substituted in the test meal the mean absorption was only 29 per cent, a figure not significantly different than the absorption obtained when bread was fed alone. These results suggest that the enhancing effect of gastric juice is related to pH. If the pH of the intestinal contents is kept low for a long period of time, insoluble complexes do not form to interfer with iron absorption.

Reports conflict as to whether ferrous iron is more readily absorbed from salts or foodstuffs than the ferric form (33, 34, 35). The amount of reduction of iron in various foods which occurs in the stomach of normal subjects was investigated by Bergeim and Kirch. The iron in some common foods such as breads, meats, and fruits was 50 to 90 per cent in the ferrous form while the iron reduced in milk and eggs was inconsistent. Ascorbic acid-containing foods reduced iron to the greatest extent. Orange juice caused 78 per cent reduction of the ferric salt, tomatoes 92 per cent, and boiled potatoes 52 per cent. White bread caused (59 per cent) reduction of the supplement whereas whole wheat bread caused 38 per cent reduction. Milk gave irregular results ranging from 0 to 39 per cent. The authors postulated that iron combines with the phosphates and phosphoproteins of milk and forms insoluble precipitates. Because milk exerts a strong buffering action the low acidity needed for solubility may not be reached in the gastric contents, with the result that the iron present is not available for absorption.

From the studies reviewed the food factors that may exert an influence on iron absorption are almost unlimited. Absorption of iron can be affected by quantity of iron ingested as well as availability in the diet. Iron salts appear to be more easily absorbed than iron contained in many foods, but in several investigations complex formation of iron with the phosphate of yolk proteins appeared to form insoluble compounds of iron which decreased absorption. In other studies proteins and amino acids enhanced uptake and transfer of iron contained in foods and in salts. Various minerals, such as calcium and phosphorus when present in varying ratios may also influence iron utilization.

II. COPPER

The occurrence of copper in plant and animal tissue has been known for over a century but it was not considered essential for growth and well-being until the 1920's. A few years later certain grazing diseases were attributed to a deficiency of copper in the soil. Copper was found to be vitally necessary for bone formation, keratinization of wool, reproduction, as well as hematopoiesis. The extent to which symptoms of deficiency are present depends on the species of animal as well as on age, environment, and other dietary factors (36). The question of copper deficiency in man has been investigated for many years without demonstration of any clinical symptoms (37, 38, 39). Wintrobe and coworkers (40) were unable to induce any signs of copper deficiency in two infants fed identical diets that had produced copper deficiency in piglets. Estimates of copper intakes in man vary over a

wide range based on the choice of foods. The poorer sources of copper are milk, cheese, margarine, white sugar, followed by non-leafy vegetables, most fruits, and refined cereal products (41).

A relationship between the level of copper ingested and retention was established in early work with college women on self-chosen diets (42). Twenty-four one-week metabolism periods were conducted in which daily copper intakes ranged from 1.4 to 3.6 mg. When copper intakes fell below 2.0 mg. per day, the subjects were in negative balance. Additional studies on sixty-five women on self-chosen diets showed daily intakes ranging from 1.0 to 4.9 mg. with an average intake of 2.6 mg. (43). Eight subjects were in negative balance. These eight subjects ingested an average of 1.8 mg. of copper daily and excreted 2.4 mg. daily. Nine subjects were in equilibrium on intakes of 2.2 mg. daily. Seventy-eight balances for fifty-six subjects were positive when the average intake was 2.8 mg. Four women on constant diets for an extended period of time showed similar retention patterns. They were in negative balance for eighteen periods when the intakes averaged 2.0 mg. and in equilibrium for ten balance periods on the same intake. Positive balances by the subjects were attained when the intake was increased to 2.6 mg. daily. In both groups (self-chosen and constant diets) increased intakes produced greater storage of copper. On three levels of copper, 1.6 to 2.5 mg., 2.5 to 3.4 mg., and 3.4 to 4.2 mg., the per cent of the increase retained was 84, 58, and 99, respectively. Protein intakes were 67 g. for seventy-eight of the ninety-five cases. It would appear from these two studies that at these levels of dietary copper each

additional amount of copper ingested was retained rather than unabsorbed or excreted.

In cooperation with the southern regional project on metabolism of nutrients in preadolescent girls the effect of different levels of nitrogen intake (2.9 to 14.1 g.) on copper metabolism was investigated (44). On nitrogen intakes of 7.7 to 14.1 g. daily, copper intakes and fecal excretions averaged 1.2 mg. On nitrogen intakes of 2.9 and 3.5 g. daily, copper intakes averaged 1.0 mg. a day with excretions slightly greater than intake. If other pathways of copper excretion such as urine, sweat, skin, and hair are to be considered even though the quantity eliminated is small, the girls were in negative copper balance on both of these levels of nitrogen and copper intake. All diets included protein of animal and vegetable origin. The authors concluded that since the copper intake was lower than the amount necessary to establish equilibrium no definite conclusions could be drawn as to the effect of nitrogen level on copper absorption.

On copper intakes ranging from 1.1 mg. to 1.5 mg. daily, three preschool-aged boys absorbed on an average 37 to 46 per cent of the dietary copper (45). The subjects were in positive balance throughout the thirty-five experimental periods regardless of copper intake. The boys had been maintained on the diets for an extended preliminary period, in order to allow sufficient time for adjustment to the copper content of each diet. This may have been the reason for retentions of copper on low levels of intake. Three slightly different diets were used. Diet I consisted of whole grain bread and cereals, ground lean beef, milk,

vegetables and fruits, and furnished an average daily intake of 1.1 mg. of copper. The average copper absorbed was 45 per cent of the intake. In Diet II white bread and cream of wheat were substituted for whole bread and cereal; in Diet III the amounts of meat and eggs were reduced and the quantity of vegetables and fruits increased. Diets II and III furnished daily an average of 1.5 mg. and 1.4 mg. of copper, respectively. The fecal losses averaged 0.6 mg. daily with absorptions of 37 and 41 per cent of the intakes from Diets II and III, respectively. The change in quantity of protein in the diet did not appear to affect the level of absorption when the quantity of copper furnished by the diet allowed for positive retentions.

Three normal women ingested 0.80 to 1.39 mg. of copper per day in diets containing 9.7 to 13.4 g. of nitrogen furnished from a mixture of animal and plant diet with liberal quantities of protective foods and calories (9). Fecal losses of copper greater than intakes were observed during all periods. Losses ranged from 0.02 to 0.40 mg. per day. Although the study was carried on for fifty-six days no trend towards adjustment to the low level of copper could be shown and different individual protein intakes appeared to exert no influence on copper absorption.

Equilibrium between copper intake and output which included both urinary and fecal losses of copper was established in half of the seventeen subjects fed diets furnishing from 2.2 to 2.7 mg. of copper (46). Half of the group was in slight negative balance. Absorption ranged from 4 to 27 per cent. Additional work on college women on

self-selected diets, in which copper was present in amounts ranging from 6.5 to 13.0 mg. daily with an average intake of 8.1 mg., showed an increased retention of copper ranging from 5.9 to 12.2 mg. (47).

Absorption studies were carried out on normal subjects and patients with Wilson's disease using ^{64}copper (hereafter referred to as ^{64}Cu) (48). Oral and intravenous injections were used to study the rate of attachment of the isotope to serum albumin and its incorporation into ceruloplasmin. Diminished fecal excretion of orally administered doses was observed in the patients with Wilson's disease as compared with the normal controls. At the end of twenty hours, circulating labeled ceruloplasmin in normal subjects represented almost all of the ^{64}Cu circulating in the blood, whereas at the end of the same time period the radiocopper in blood of patients with Wilson's disease was associated with the albumin fraction. Increased urinary excretion of copper occurred after both oral administration and intravenous injection in subjects with Wilson's disease while in normal subjects only a temporary rise was noted.

Additional work with normal subjects and four patients suffering from Wilson's disease showed that in normal subjects intake and output of copper was essentially the same (49). The patients maintained positive balance to the extent of 0.56 mg. of copper. Apparently absorption from the gastrointestinal tract was increased. It was suggested the increased absorption was an attempt to alleviate the subnormal amount of copper circulating as ceruloplasmin (50). Cartwright and coworkers tested compounds for their chelating effect with copper in

the intestine. Potassium sulfide and a casein hydrolysate were administered. One patient was given potassium sulfide orally with meals for ten days. The amount of copper excreted in the stool increased with the result that the overall copper balance for the patient with the liver disease changed from a storage of 0.43 to a loss of -0.14 mg. per day. No change was noted in urinary excretion. When casein hydrolysate was given orally, fecal and urinary copper excretion increased. The copper balance changed from 0.21 mg. per day to -0.75 mg. per day. The same result followed an intravenous injection of casein hydrolysate. Although these effects have been demonstrated with patients suffering from Wilson's disease, certain other factors in the intestine could influence copper absorption.

Pigs fed forms of sulfate or sulfide showed sulfide to be the more effective inhibitor of ^{64}Cu absorption than sulfate (51). The cupric sulfide at three levels of intake, 20, 127, and 145 mg., reduced absorption of ^{64}Cu by 20 to 70 per cent more than equi-molar quantities of copper sulfate.

Other dietary factors influencing copper absorption have been studied (52). Parchment thimbles were used in an *in vitro* experiment to investigate the effect of digests of egg white and egg yolk on copper absorption. The addition of this material which contains phosphoproteins and phosphatides did not greatly inhibit the transfer of copper across the membrane. Additional studies on absorption were undertaken using mice. The diets fed to the mice contained various amounts of calcium. Hydrochloric acid was added to diets high in calcium content.

Copper as copper sulfate was given orally. The total copper content of adult male and female mice was determined by ashing the carcasses. The amounts of copper absorbed by the control group and the group receiving a high calcium intake were comparable but when a low-calcium diet was used or the high-calcium diet with added hydrochloric acid was used, absorption was 75 per cent greater in both groups. Calcium content of the diet plus the acidity of the mixture appeared to play a role in copper absorption.

Copper may be absorbed through the intestinal wall in the form of soluble, stable organic complexes (53, 54). Rats receiving diets furnishing 3 µg. of copper from an aqueous extract of herbage had storage of copper in the liver comparable to that of rats receiving a basal diet with 10 and 15 µg. of added copper. It was suggested that copper may combine under physiological conditions with many biological materials. The solubility and stability of these products would vary greatly according to the pH of the intestine contents. Complex formation of copper with most amino acids and hydroxy acids yields soluble products whereas its combination with some proteins forms complexes that are not available for absorption. Mills also suggested that the complex formed in herbage extracts is available immediately whereas the copper which is ingested as the salt must complex with other available materials and the constituents of the intestine may interfer to make utilization impossible.

The effect of dietary protein and zinc on complex formation with copper was studied using ^{64}Cu (55). Copper absorption was determined by

analysis of total liver for copper or by the disappearance of radio-copper from the intestine of rats. Diets containing 10, 17.5, and 25 per cent protein were used with high levels of copper and/or zinc. Copper and/or zinc added at 0, 1,000, or 2,500 p.p.m. to 25 per cent protein diets had no effect on total liver copper or ^{64}Cu content. However, when the protein level was 10 or 17.5 per cent, copper intakes of 2,500 p.p.m. without added zinc produced accumulation of total copper and ^{64}Cu significantly greater than when no copper was added to the diet. Total copper storage on the 10 per cent protein diet was twice as much as on higher protein intakes. When 1,000 p.p.m. of copper was ingested the total copper in the liver was ten times higher than in animals receiving a 17.5 per cent protein diet. Added zinc had little effect on total copper absorbed except in rats fed the 10 per cent protein diet where 1,000 p.p.m. of zinc significantly decreased copper levels in the liver. Results of the study indicated that the accumulation of copper in the liver of rats was influenced by dietary protein. The mechanism of the effect of protein was more than just inhibition of absorption as rats fed normal levels of copper and high protein diets did not suffer from copper deficiency. Copper and zinc both form chelates with proteins, peptides, and amino acids and it is probable that the chemical and physical properties of the various chelates formed may affect their absorption from the intestinal tract.

From the studies reviewed, copper intakes from 1.0 to 2.0 mg. per day are minimal for all age groups when all other nutrients are ingested in the diet at recommended levels. No definite conclusions have been

reached as to the mechanisms involved in uptake and transfer of copper across the intestinal membrane although various dietary factors have been studied in their relationship to copper absorption.

III. ZINC

Zinc was shown to be an indispensable nutrient for the growth of Aspergillus niger almost 100 years ago (56). During the 1920's zinc was found necessary for the growth and well-being of rats and in the 50's it was shown to cure and prevent parakeratosis in pigs. Work at the cellular level has shown that zinc is a constituent of several enzymes including carbonic anhydrase and carboxypeptidase. In patients with anemia other than pernicious, zinc and carbonic anhydrase levels in blood decrease in proportion to the lower iron values although the decreased zinc intakes are not necessarily the cause. Dietary deficiencies of zinc in mice, rats, pigs, poultry, and cattle are characterized by retarded growth, loss of hair, changes in male sex characteristics as well as blood changes. Zinc deficiencies in man have only been noted in hepatic dysfunction and in Egyptian and Iranian boys. Studies with man in the laboratory have not produced deficiency states. Average daily intakes of zinc range from 10 to 15 mg. from a mixed diet and these amounts appear adequate for adult needs. As yet a recommended allowance has not been established for zinc.

Regression of zinc turnover with time determined for four species of mammals indicated the average effective half-time of ^{65}zinc (hereafter referred to as ^{65}Zn) in man was about 154 days (57). The authors

suggested a relationship existed between uptake and retention and body size. If the adult body contains approximately 1,925 mg. of zinc (41), with half of this being replaced every 154 days, the needs for replacement run between 5 and 6 mg. per day for an adult. Zinc deficiencies have been investigated in hepatic dysfunction where urinary excretion of zinc is elevated and liver levels and serum content of zinc indicate the presence of a deficiency state (58). Until recently, actual cases of zinc deficiency in humans had not been demonstrated.

Information has been gathered to indicate the presence of zinc deficiencies in adolescent boys in Egypt and Iran (59, 60, 61, 62). In comparing normal subjects with these boys, various biochemical standards such as plasma zinc, ^{65}Zn excretion in the stool, urinary excretion, and twenty-four hour exchange pools were used. ^{65}Zn was given intravenously to the subjects and samples were collected in order to determine disappearance of the isotope from plasma and its excretion in urine and stool. Clinical symptoms such as stunted growth, hypogonadism, and anemia found in these boys were similar to the conditions present in animals on zinc deficient diets. These dwarfed subjects exhibited depressed plasma zinc levels, excreted less of the injected ^{65}Zn in the urine and feces, and had a higher plasma turnover rate than normal controls. One group of Egyptian subjects suffered from parasitic infection as well as having a very poor diet which consisted of beans and bread with small amounts of vegetables. Since the cereals in these diets contain high amounts of phytates and phosphates, minerals may be complexed to render them unavailable for absorption. The drinking

water also contained very small amounts of zinc. Other subjects studied in Northern Egypt and Iran did not suffer from parasitic infection but displayed the same clinical symptoms and tests. No liver dysfunction was noted and could not be implicated in the various results.

Zinc retentions were studied in three preschool-aged boys fed controlled diets furnishing 3.9 to 5.9 mg. of zinc daily (63). A basic diet of whole-grain bread and cereal, butter, vegetables, eggs, ground lean beef, and milk provided an average of 4.4 mg. of zinc per day. The average zinc balance on this diet was 0.41 mg. per day with a range of -0.40 to 1.66 mg. When white bread and cream of wheat were substituted for the whole grain, tomatoes for green beans, and the orange juice was decreased, the average intake was 4.6 mg. with a retention of 0.2 mg. Individual balances ranged from -2.7 to 3.4 mg. Subjects were in negative balance during four of the eleven periods. The third change in the basal diet was to decrease the amount of meat and eggs and increase the fruit, vegetables, and butter. The average zinc intake was 4.8 mg. and retentions averaged 0.8 mg. per day (-0.9 to 2.1 mg.). There were no significant differences in zinc retention with changes in the diet. Fecal output varied greatly from 42 to 163 per cent of the ingested zinc and as much as 50 per cent from period to period in the same child.

The influence of protein quality and quantity on zinc absorption and retention in preadolescent girls was studied in order to estimate zinc requirements for this age group (64). Dietary intakes of zinc ranged from 4.6 mg. to 9.3 mg. per day during the three studies. Protein was furnished in two of the studies in ratios varying from (38:62) to

(70:30), animal to plant, and from plant sources alone in the third study. Diets providing 40 g. of vegetable protein daily furnished 4.6 and 7.2 mg. of zinc. Of the thirty-six individual balance periods, subjects were in negative balance during three periods when fed the low intake and for four periods when ingesting the higher intake. Retentions for the six girls fed the diets furnishing 7.2 mg. of zinc daily ranged from 0.93 mg. per day to 1.58 mg. with an average of 1.3 mg. (18 per cent). For the subjects ingesting 4.6 mg. per day of zinc, retentions ranged from 0.08 to 1.02 mg. with an average of 0.53 mg. (12 per cent). In the entire series of studies daily protein intakes of 18 to 88 g. were accompanied by zinc intakes of 6.5 to 9.2 mg. Retention of zinc ranged from 1.4 to 2.1 mg. and appeared to be related to level of zinc in the diet.

The absorption and excretion of zinc at different levels of zinc intake have been studied by McCance and Widdowson (65). The low intakes of zinc were obtained by providing 50 per cent of the total calories as white flour. The higher zinc diets were achieved by substituting 92 per cent extraction flour for the white flour. Three subjects, one woman and two men, ingesting 5.6, 4.9, and 6.1 mg. daily showed balances of 0.0, -0.8, and -0.2 mg. per day, respectively. Only after the levels of intake were above 19 mg. for a man and woman was a definite positive balance encountered. In further work with nine subjects, both male and female, three of the five on low zinc diets ranging from 5.7 mg. to 7.1 mg. were in negative balance. When zinc was injected intravenously into nine subjects on daily zinc intakes of 4.0 to 6.2 mg., the total

excretion of zinc was greater than the amount ingested by seven of the subjects. The greatest part of the zinc excreted was found in the feces with urinary zinc output remaining fairly stable. This study would seem to indicate the greatest differences in excretion between subjects was a reflection of their tissue stores.

College women on self-selected diets were shown by Tribble and Scoular (66) to have zinc intakes of 12 to 14 mg. daily. Retention of the mineral from the self-chosen diets or from these diets supplemented with soy grits, kelp, and a vitamin mixture averaged 6.6 mg. daily. Fecal excretion of zinc by the subjects on the self-chosen and supplemented diets averaged 38 and 47 per cent, respectively. This increased loss of zinc through the feces might result from the larger amount ingested or from interference with absorption by the components in the supplemented diet.

Several investigations have been undertaken to study the effect of zinc intake on zinc absorption. ^{65}Zn was administered orally to one male subject in order to determine the effect of previous zinc intake on zinc absorption (67). Analysis of fecal samples showed 11 per cent of the ^{65}Zn was excreted in a four-day period after the first ingestion of zinc. After a 700-day time lapse a second sample of ^{65}Zn was consumed. Prior to this dose the subject had been eating a self-chosen diet to which a 10 mg. supplement of zinc acetate had been added daily for thirty days. Zinc excretion was 44 per cent of the ingested ^{65}Zn after three days. Previous work with rats indicated zinc absorption decreased as amount of zinc in the diet increased. The authors suggested

the inhibition was caused by a mechanism affecting absorption which appeared to be functioning in humans as well as in rats.

Cotzias and coworkers (68) believe there are two mechanism which control zinc metabolism one at the site of absorption and one at the site of intestinal excretion. Decreased absorption of ^{65}Zn with increased body stores appears to be regulated at the site of absorption in mice. A second mechanism appears to increase the rate and amount of ^{65}Zn removed after a metabolic load of zinc salts was given. The excretion of excess zinc was shown to be almost exclusively a function of the intestine with only a small amount lost in the urine. The authors suggested the accelerated loss through the intestine would not have taken place without a homeostatic mechanism present to eliminate excess zinc.

The quantity of zinc needed in the diet does not appear to be altered by dietary calcium levels in man as indicated by zinc isotopic studies (69). Hospitalized men fed diets calculated to provide 12 to 15 mg. of zinc daily received calcium in stepwise increments ranging from 258 to 2,123 mg. daily. With each increase in dietary calcium a single dose of ^{65}Zn was given orally during breakfast so thorough mixing with the food could take place. Apparent absorption of ^{65}Zn averaged 32 per cent from diets containing the lowest calcium intakes. In diets containing the higher level of calcium (1,983 mg.) the apparent absorption ranged from 22.5 to 34.7 per cent with an average of 28.1 per cent. This slight difference was not significant. Urinary excretion of ^{65}Zn was low throughout the study and amounted to only 2 per cent of the

dose at the end of twenty-one days. The greatest portion of the dose was excreted through the feces and at the end of the time period 70 per cent of the ingested dose had passed with the stool leaving an average of 28 per cent retained by the body tissues.

When rats were fed casein or isolated soybean protein, the level of calcium, 0.8 and 1.6 per cent of the diet, did not alter zinc absorption (70). Later studies by Forbes (71) investigated the effect of various levels of calcium and zinc on rats fed diets containing either soy protein or egg protein. When calcium concentrations were increased from 0.4 per cent to 0.8 per cent on both levels of zinc in the egg protein diet, the percentage of zinc in the femur ash increased. When soybean protein was substituted a decrease in zinc concentration was evident on both levels of zinc intake. Soybean protein magnified the decrease in zinc absorption. Growth effects and absence of zinc deficiency symptoms on the low-zinc-soybean-protein diet in comparison to the egg white diet were probably due to the fact the total zinc in the soybean diet was greater. Apparent absorption from both diets was around 60 per cent. The author believed the depressing effect of calcium in the soybean diet was due to a third dietary constituent, phytic acid. In vitro studies have shown the amounts of zinc and of calcium precipitated in a phytic acid solution of calcium and zinc salts at the pH of the intestine are greater than when either is present alone (72). The amount of zinc thus removed would alter the quantity available for absorption.

In diets fed to chicks which contained either casein or amino acids as sources of nitrogen, the inclusion of 1.8 per cent phytic acid increased the amount of diet required for normal weight gain and decreased the concentration of zinc found in the femur (73). This is contrary to the report of O'Dell and Savage (74) who suggest that phytic acid combines with protein and makes the zinc unavailable. When calcium phytate was added to a casein-gelatin diet in the same quantity as found in the isolated soy-protein meal it had little or no effect on zinc availability.

Toxic levels of zinc were incorporated into diets containing eight different protein sources; three of these sources (distiller's dried solubles, soybean meal, and liver) alleviated the subnormal growth of zinc fed rats (75). Five purified proteins (casein, blood albumin, zein, egg albumin, and soy protein) failed to prevent the accumulation of zinc in the liver. Casein and commercial liver extract were investigated on three levels of intake. Accumulation of zinc (0.75 per cent of the diet) was less in rats fed 14 per cent casein diet than those fed diets containing 19 and 30 per cent casein. Liver zinc levels were lower in animals receiving diets in which soybean meal was substituted for the casein but exceeded those of the control animals; growth was suboptimal also. Brewer's yeast and commercial liver extract contained some growth stimulating factors that allowed for normal growth even with elevated zinc concentrations in the liver. Smith et al. (76) also found that the source of protein had an effect on zinc requirements of pigs. Pigs fed milk protein diets containing 6 to 18 p.p.m. of zinc showed

greater growth than pigs fed soybean oil meal which contained 16 to 22 p.p.m. The pigs receiving the soybean oil meal ration developed typical zinc deficiency symptoms.

The amount of zinc required to produce toxic symptoms is influenced also by source and level of protein (77). Rats receiving excessive zinc in 20 or 30 per cent soybean protein diets were healthier and had less total zinc accumulation in the livers than those animals receiving the same level of protein from casein. When 500 p.p.m. of copper or 500 p.p.m. of copper and 1,000 p.p.m. of iron was added to the 20 per cent casein diet accumulation of zinc in the liver was decreased and hemoglobin levels were increased. Decreased accumulation of zinc in the liver of animals fed the soybean protein may be due to the formation of stable chelates between zinc and a protein compound which inhibits absorption.

Further evidence of the beneficial effects of natural chelates and other chelating agents has been presented (78, 79, 80, 81). In work undertaken on zinc utilization by chicks and turkey poults, Scott and coworkers (78) studied three substances, liver extract, ethylenediaminetetraacetate, and corn distillers dried solubles. Addition of 1.5 per cent liver extract to the diet containing soybean protein or 3 per cent to the diet containing 10 mg. of added zinc produced growth comparable to that on diets containing 60 mg. of added zinc per kg. of diet. The authors suggested the liver extract contained a natural chelate. Corn distillers solubles and ethylenediaminetetraacetate also increased growth at low zinc intakes. When ashed samples of the natural materials

used were added to the diet, no greater growth increase was obtained than would be expected with the increase in zinc content. Work with radioactive ^{65}Zn indicated the beneficial effect of ethylenediaminetetraacetate and other chelating substances such as liver extract may be due to the increased availability of the zinc found in soybean meal diets.

When ^{65}Zn as the chloride in an acid solution was fed to rats either in combination with food and a liver extract, or as a homogenized suspension of bean leaves grown in a medium containing ^{65}Zn chloride, there was no difference in liver or total carcass zinc (82). The total zinc absorbed was greater by 50 and 82 per cent in the rats receiving liver extract than in those receiving zinc contained in homogenized bean leaf.

The literature reviewed suggests that absorption of iron, copper, and zinc is affected not only by level of intake of the individual nutrient, and body stores, but also by food factors which are present. Formation of insoluble complexes would decrease the amounts of the ions available for absorption and formation of chelates with protein and amino acids might enhance uptake and transfer by the intestinal wall.

With the possibility that the absorption of minerals may be enhanced by protein level and protein quality, a series of metabolic experiments were conducted on college women at the University of Tennessee in which levels of nutrients other than protein were maintained at constant intakes. The effect of varying protein level and protein quality on mineral absorption was studied.

CHAPTER III

PROCEDURE

Iron, copper, and zinc metabolism in the human adult and its possible interrelationship with that of protein was studied on two metabolic balance experiments. These two studies conducted during October-November, 1962 and 1964, were a continuation of investigations conducted in January-February, 1961, and January-February, 1962, on the influence of various low levels of protein on fat and magnesium metabolism. The studies consisted of a six-day preliminary adjustment period followed by five experimental periods of six days. The influence of level and quality of protein on the metabolism of iron, copper, and zinc was investigated. Natural foods were used with the exception of a small supplement of "vitamin free" casein in Study I.

The subjects were fourteen graduate students and two undergraduates 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 both metabolic studies. All participants were females with ages ranging from twenty to forty-two years. All were pronounced in good health after a physical examination. At the beginning of the 1964 study and at the end of Period 5, twenty milliliter samples of venous blood were taken from each subject. Ten milliliter samples were also taken during Period 3 and capillary blood was taken every three days during both studies. Hemoglobin and serum

proteins were determined on the venous blood samples at the beginning and end of Study II.

In Study I, completed in November, 1962, eight graduate students participated. The subjects agreed to restrict their protein intake to about 50 g. during the two-week period prior to the study in order to partially adjust to a restricted nitrogen intake. The average protein consumption for the fourteen-day period as calculated from the diet records kept by the subjects was 44 to 49 g. daily. In the second study where restricted protein intake was not requested diet records kept by the subjects for the two weeks prior to the experiment indicated protein intake ranged from 45 to 98 g. per day.

The six-day adjustment period included menus similar to the experimental diet. The diet furnished approximately 42 g. of protein during both studies. In Study I, Diet A contained 35 g. of protein. Fifty per cent was furnished by animal sources. Diet B consisted of the same foods with the addition of 7.5 g. of vitamin free casein and 75 g. of cooked white of egg per subject per day. The 48 g. of protein in the diet was furnished by 65 per cent animal protein.

The level of iron was calculated (83, 84, 85) to meet minimum adequate intakes for women but did not meet the National Research Council's (NRC) recommended levels. An intake of approximately 10 mg. was maintained during the two studies. For the first two studies, levels of nutrients had been patterned after the investigations on pre-adolescent girls by the Southern Regional Nutrition Committee (86). In order to investigate dietary influences other than the level of iron in

the diet on iron absorption, the level of iron intake was maintained as constant as possible. All other nutrients were planned to meet the NRC recommended allowances (87). Natural foods furnished 8.2 mg. of iron per day.

Primary calcium phosphate was added to special recipes of mashed potatoes, cream topping, and soups in an attempt to improve absorption. A supplement consisting of primary calcium phosphate, ferrous sulfate, and riboflavin supplied 505 mg. of calcium, 1.8 mg. of iron, and 0.4 mg. of riboflavin per day per subject. A mixture of the supplement was prepared, and the required daily amount was weighed out and distributed between six capsules. Two capsules were taken by the subject at each meal. Vitamin D was supplied by Drisdol* in a solution which was pipetted into the breakfast cream. Since no recommended levels existed for zinc and copper no attempt was made to control the intakes.

Study II was undertaken to investigate the possible influence of protein quality on mineral and fat metabolism. The diets fed were modifications of the basal diet of Study I. Protein foods of animal origin were substituted with plant protein foods such as bread, beans, and spaghetti in order to have 35 per cent of the protein supplied by animal foods. Intakes of protein, iron, magnesium, and fat were calculated to be similar to Study I. No iron supplement was administered to supply the 10 mg. As the calcium intake was low, primary calcium

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*Drisdol, calciferol in propylene glycol; 6.25 µg./drop, prepared by Winthrop Laboratories, New York, N. Y.

phosphate was added to special recipes as in Study I. Additional mineral supplement of 2.4493 g. of primary calcium phosphate and 0.0005 g. riboflavin prepared as in Study I supplied the remaining amounts necessary. No attempt was made to adjust the zinc and copper intakes to previous levels.

The experimental procedures were the same in both studies. At the beginning of the experiment the eight women were randomly assigned, four to each of the two diets. Diets A and B were planned to furnish 35 and 48 g. of protein, respectively. Caloric intakes on Diet A and B for Study I were calculated to furnish 2,067 and 2,133 calories per day. In Study II, Diets A and B were calculated to furnish 2,156 and 2,171 calories per day, respectively. The natural foods were incorporated into six daily menus for each group; the menus were served in the same sequence during the five periods. Calculated nutritive values and typical food groups for the two studies appear in Tables 15, 16, 17, and 18 in the Appendix. All meals were served in the metabolic laboratory kitchen of the Department of Nutrition. Snacks were provided each day in addition to the three regular meals. Caloric adjustments were made by supplementing the meals and snacks with Coca-Colas, fondant, and sugar cubes in order to maintain the weight of each subject. Tab, a one calorie cola bottled by the Coca-Cola Bottling Company, was permitted for those who did not need additional calories. Demineralized water was allowed ad libitum but coffee was restricted.

At each serving period one-half portions of all foods and beverages (with the exception of coffee, carbonated beverages, margarine, and

fondant) were composited. Coffee, carbonated beverages, margarine, and fondant were composited separately with portions removed during each day or in each lot. During Study I, the daily composites were frozen. In Study II, days 1, 2, and 3 were composited together and frozen. Foods from days 4, 5, and 6 of a period were composited together. At the end of the period in both Studies I and II, the two composites were made up to an equal weight with demineralized water and homogenized in a stainless steel Waring Blendor. Half portions from each composite were combined and homogenized to give a final composite for the period. Separate samples for various determinations were frozen in polyethylene containers.

Samples of 24-hour urine samples were frozen with the daily addition of succeeding samples to furnish a six-day period composite. Urine collections were made directly into polyethylene bottles containing 10-15 ml. of toluene. Feces were collected between dye markers for each period. Samples were frozen as collected. When period collections were completed the feces were transferred to a stainless steel Waring Blendor Jar, thawed, and homogenized with an equal weight of demineralized water to give the period composites. Small portions were frozen in polyethylene boxes. The marker taken in the first study was carmine. The capsule was taken before breakfast at the beginning of each period. In the second study the plan was to alternate brillant blue and carmine mixed with methyl cellulose (88). Carmine was used only once because separations were more precise with the brillant blue. Menses were collected on standard tampons and Kotex and frozen for subsequent analysis.

Appropriate samples of the composites were dry ashed in duplicate at 550°C. for 24 hours and treated for the removal of silica (89). The ash was dissolved with hydrochloric acid and made up to a specified volume. The entire menses sample for each subject was ashed and made up into one solution. Blanks were made from representative samples of the Kotex and tampons.

All glassware was cleaned with 1:1 hydrochloric acid in order to minimize contamination of the trace minerals being analyzed. The laboratory facilities were cleaned regularly.

Nitrogen balances were determined by other workers (90, 91), and are found in Tables 19, 20, 21, and 22 in the Appendix. Energy balances were determined in Study II (92). Aliquots of food, Coca-Cola, coffee, Tab, menses, urine, and feces were analyzed for iron, copper, and zinc.

Iron was determined by the Association of Official Agricultural Chemists method (89) using o-phenanthroline as the complexing agent. Copper determinations were made using sodium diethyldithiocarbamate. The original acidified samples were adjusted to a slightly alkaline pH for chelating the copper and extraction in carbon tetrachloride (89).

Acidified solutions of ash from food and feces containing between 1 to 5 p.p.m. of zinc were used for direct determination of zinc on a Perkin-Elmer Atomic Absorption Spectrophotometer.* Ashed samples of urine which contained between 0.1 p.p.m. and 0.3 p.p.m. were also

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*Perkin-Elmer Atomic Absorption Spectrophotometer 303. Perkin-Elmer Corporation, Norwalk, Connecticut.

analyzed directly using a tenfold multiplication. A hollow cathode lamp was the light source. The solution was aspirated into an air-acetylene flame. The aerosol was dried, melted, vaporized, and dissociated in order for absorption to occur. A decrease in energy of the cathode beam results from the absorption of zinc in the sample and the reading obtained gives the percentage absorption.

CHAPTER IV

RESULTS

During the two balance studies the subjects continued their ordinary academic schedule. All subjects remained in good health with one exception. One subject, MR, on Diet A in Study I was removed from the final metabolic period due to a temporary gastrointestinal upset. During Study I, only one subject, MN, lost weight. Subjects CE and FG had food removed from their diet in order to maintain weight (Table 23 in the Appendix). Caloric intakes for the eight subjects ranged from 1,864 to 2,381 with an average of 2,149 calories for the women receiving Diet A and 2,054 for those receiving Diet B. Descriptive information on the subjects is found in Table 24 and Table 25 in the Appendix. Hemoglobin fluctuations were within normal levels. During Study II weight changes of approximately 1 kg. was noticed in all subjects although added supplements of Coca-Colas, fondant, and sugar cubes were given. Weight changes of this size appear normal in people on self-chosen diets (92). Calculated average caloric intakes for Diets A and B were 2,343 and 2,269, respectively, with a range from 2,171 to 2,373 per day. Hemoglobin values for subjects receiving Diet A increased during the study from an average of 12.2 to 13.9 g./100 ml. Subjects receiving Diet B showed slightly lower final hemoglobin values. Absorption of iron from Diets A and B was about the same and the slight changes in hemoglobin levels of the subjects on the two diets did not appear to be realted to iron absorption.

Serum protein levels were determined for the subjects in Study II. No real changes were apparent between the initial and final determinations which would seem to indicate the level of protein furnished by the diets was adequate to maintain the pools of circulating protein within the normal range of 6 to 8 g./100 ml.

In an effort to minimize differences in intakes of the minerals on the different diets in evaluation of losses, absorption and retention are expressed as percentage of the intakes. The absorption of iron and copper were determined as follows:

$$\text{Apparent Absorption, \%} = \frac{\text{Mineral intake - fecal loss}}{\text{Mineral intake}} \times 100$$

(Apparent absorption will be referred to hereafter as absorption.) The retention of zinc was calculated as:

$$\text{Retention, \%} = \frac{\text{Zinc intake - (fecal zinc + urinary zinc)}}{\text{Zinc intake}} \times 100$$

I. IRON

In Study I basal Diet A furnished 34 g. of protein with an average intake of 9.3 mg. of iron daily. Addition of cooked white of egg and a small amount of vitamin free casein raised the protein to 47 g. and 9.9 mg. of iron daily. This was supplemented Diet B. Iron intakes ranged from 8.2 to 9.9 mg./day for Diet A (Table 1). Subject FG who had food removed from her diet averaged 0.5 mg. less than the other three subjects for four of the five periods, and averaged 9.0 mg./day for the five periods. Iron intakes for the remaining three subjects averaged

TABLE 1

IRON ABSORPTION BY SUBJECTS IN STUDY I

Period	Intake	Fecal loss	Absorption	Intake	Fecal loss	Absorption
	mg./24 hr.		%	mg./24 hr.		%
			Diet A			
	Subject FG			Subject SH		
1	9.6	7.9	18	9.6	9.0	6
2	8.6	8.7	- 1	9.1	8.6	6
3	9.3	8.8	5	9.8	8.4	15
4	8.2	10.4	-26	8.7	9.4	- 7
5	9.2	7.7	16	9.7	7.5	23
Mean	9.0	8.7	3	9.4	8.6	9
S.D.	±0.6	±0.3	±18	±0.4	±0.7	±11
	Subject PM			Subject MR		
1	9.6	9.8	- 2	9.6	10.7	-11
2	9.2	9.0	2	9.2	5.9	36
3	9.9	7.8	22	9.9	11.0	-12
4	8.8	8.2	7	8.8	12.4	-41
5	9.8	6.5	34	9.8	8.4	14
Mean	9.5	8.2	13	9.3	9.7	- 3
S.D.	±0.4	±1.3	±15	±0.4	±2.6	±29

TABLE 1 (continued)

Period	Intake	Fecal loss	Absorption	Intake	Fecal loss	Absorption
	mg./24 hr.		%	mg./24 hr.		%
			Diet B			
		Subject CE			Subject MN	
1	10.1	8.6	15	10.1	9.7	4
2	9.6	7.0	28	10.2	8.0	21
3	9.6	10.0	- 5	10.1	8.0	21
4	9.6	9.5	- 3	9.8	8.2	16
5	9.5	11.0	-16	10.0	9.9	1
Mean	9.6	9.2	4	10.0	8.8	13
S.D.	±0.3	±1.5	±17	±0.1	±1.0	±10
		Subject ESP			Subject PT	
1	10.1	9.5	6	10.1	8.0	21
2	10.2	9.8	4	10.2	10.1	2
3	10.2	8.0	21	10.2	9.9	2
4	9.8	9.9	- 1	9.8	4.3	56
5	10.0	11.9	-19	10.0	8.8	13
Mean	10.1	9.8	2	10.1	8.2	18
S.D.	±0.1	±1.4	±14	±0.1	±2.3	±22

9.4 mg./day. Iron intakes ranged from 9.5 to 10.2 mg./day for the four subjects on Diet B. One subject, CE, averaged 9.6 mg./day, while the other three subjects ingesting the higher protein diet averaged 10.1 mg./day of iron. Diet B provided an average of 0.6 mg./day more iron.

Fecal loss of iron by subjects receiving Diet IA averaged 8.9 mg./day with a range from 5.9 to 12.4 mg./day (Table 1, page 42). One subject, MR, lost more iron in the feces than was contained in the diet during three individual balance periods, with a slightly greater loss of iron than was ingested for the five periods. Subjects receiving Diet IA excreted more iron in the feces than they ingested during five of the twenty individual balance periods; in three individual periods the intake and output of iron were about equal (within ±5 per cent of the intake) and during the other twelve periods subjects excreted less in the feces than was ingested. Individual losses for subjects receiving Diet IB ranged from 7.0 to 11.9 mg./day with average losses for the four subjects of 9.2, 8.8, 9.8, and 8.2 mg./day (Table 1, page 43). During three of the individual balance periods, subjects lost more iron in the feces than was furnished by the diet, during seven periods subjects excreted about the same as was ingested, and for ten periods subjects lost less in the feces than was ingested. Average fecal loss of iron by subjects ingesting Diet IB was 9.0 mg./day.

Apparent absorption does not take into account the iron that was absorbed and reexcreted or the endogenous loss of iron from the lining of the stomach and intestine but merely considers the difference between the amount ingested and the iron lost in the feces. Average absorption of iron by subjects in Study I, Diets A and B, was 5 and 9 per cent,

respectively (Table 2). Three subjects receiving Diets IA absorbed an average of 3, 9, and 13 per cent of the intake. The other subject lost 3 per cent more iron in the feces than was ingested. The absorption ranged from -41 to 36 per cent with a wide individual variation. Absorption of iron by subjects receiving Diet IB ranged from -19 to 56 per cent. Variations in absorption were as great from subject to subject as variation in absorption by a subject.

During the second study in which 65 per cent of the protein was obtained from plant sources, variations in nitrogen intake were accomplished by varying the amount of protein containing foods in each diet. Diets A and B were calculated to furnish 35 and 48 g. of protein and 10.6 and 10.2 mg. of iron, respectively. When the final diets were analyzed, average daily iron intakes provided by Diets A and B were 9.9 and 10.3 mg./day, respectively (Table 3). Iron intakes ranged from 9.7 to 10.2 mg./day for those subjects receiving Diet IIA and for Diet IIB intakes ranged from 10.1 to 11.0 mg./day.

Subjects receiving Diet IIA lost on an average of 8.6 mg./day of iron via the feces. Fecal losses ranged from 5.3 to 12.2 mg./day. Subjects lost more iron than was ingested during three of the twenty individual balance periods, excreted approximately as much as was furnished by the diet during one period, and lost less iron in the feces during sixteen of the individual balance periods. Fecal losses by subjects ingesting Diet IIB averaged 8.8 mg./day with a range from 6.3 to 10.9 mg./day. Losses were approximately the same as those by subjects receiving Diet IIA. During one individual balance period more

TABLE 2

SUMMARY OF MEAN IRON ABSORPTION BY SUBJECTS
STUDY I AND II

Subject	Intake	Fecal loss	Absorption	Subject	Intake	Fecal loss	Absorption
	mg./24 hr.		%		mg./24 hr.		%
	Diet A - Study I				Diet A - Study II		
FG	9.0	8.7	3	RL	9.9	8.7	12
SH	9.4	8.6	9	MM	9.9	9.0	9
PM	9.5	8.2	13	CP	9.9	8.2	16
MR	9.4	10.0	- 3	RS	9.9	8.6	14
Mean	9.3	8.9	5		9.9	8.6	13
S.D.	± 0.2	±0.8	± 8		± 0.0	±0.3	± 3
	Diet B - Study I				Diet B - Study II		
CE	9.6	9.2	4	PM	10.3	9.0	13
MN	10.0	8.8	13	ESP	10.3	8.5	18
ESP	10.1	9.8	2	HR	10.3	9.0	12
PT	10.1	8.2	18	ET	10.3	8.6	16
Mean	9.9	9.0	9		10.3	8.8	15
S.D.	± 0.2	±0.7	± 7		± 0.0	±0.3	± 3

TABLE 3

IRON ABSORPTION BY SUBJECTS IN STUDY II

Period	Intake	Fecal loss	Absorption	Intake	Fecal loss	Absorption
	mg./24 hr.		%	mg./24 hr.		%
			Diet A			
	Subject RL			Subject MM		
1	9.7	6.6	33	9.7	12.2	-25
2	9.8	8.9	9	9.8	8.3	15
3	9.7	10.7	-10	9.7	8.5	12
4	10.2	8.3	19	10.2	8.9	13
5	10.2	9.3	8	10.2	7.2	29
Mean	9.9	8.7	12	9.9	9.0	9
S.D.	±0.2	±1.5	±16	±0.2	±1.9	±20
	Subject CP			Subject RS		
1	9.7	7.9	19	9.7	7.2	26
2	9.8	12.2	-25	9.8	9.0	8
3	9.7	5.3	45	9.7	8.0	18
4	10.2	8.1	20	10.2	10.2	0
5	10.2	7.8	23	10.2	8.4	18
Mean	9.9	8.2	17	9.9	8.6	14
S.D.	±0.2	±2.5	±26	±0.2	±1.1	±10

TABLE 3 (continued)

Period	Intake	Fecal loss	Absorption	Intake	Fecal loss	Absorption
	mg./24 hr.		%	mg./24 hr.		%
			Diet B			
		Subject PM			Subject ESP	
1	10.2	9.2	10	10.2	7.0	31
2	10.1	7.4	27	10.1	8.5	16
3	10.2	10.0	2	10.2	7.8	23
4	11.0	10.0	8	11.0	10.7	3
5	10.2	8.4	18	10.1	8.5	16
Mean	10.3	9.0	13	10.3	8.5	18
S.D.	±0.3	±1.1	±9	±0.3	±1.4	±11
		Subject HR			Subject ET	
1	10.2	10.5	- 3	10.2	10.9	- 7
2	10.1	6.7	34	10.1	8.6	15
3	10.2	9.9	3	10.2	8.2	20
4	11.0	8.4	23	11.0	9.1	16
5	10.2	9.7	5	10.2	6.3	38
Mean	10.3	9.0	12	10.3	8.6	16
S.D.	±0.3	±1.5	±16	±0.3	±1.7	±16

iron was excreted by one subject than was ingested. Iron intake and excretion by subjects was about equal during four balance periods and for fifteen balance periods less iron was lost via the feces than was ingested. Average fecal excretion by the eight subjects during Study II ranged from 8.2 to 9.0 mg./day. Seven subjects excreted between 8.6 to 9.0 mg./day.

Average absorption by all subjects receiving Diets IIA and IIB was 13 and 15 per cent, respectively (Table 2, page 46). Individual absorption of iron ranged from -25 to 45 per cent of the intake for subjects receiving Diet IIA. Variations in absorption were as great between individuals as within the individual periods investigated. Average absorption for the four subjects was 12, 9, 16, and 14 per cent. Average absorption for the four subjects ingesting Diet IIB was 13, 18, 12, and 16 per cent with a range of -7 to 38 per cent.

Menses collected during the two balance studies were analyzed for iron content. Iron lost in the menses ranged from 2.7 to 28.3 mg. per menstrual period (Table 4). Average losses of iron through the menses was 10.6 and 13.7 mg./cycle for Studies I and II, respectively. When the menses were ashed for Study I the samples were not treated for the removal of silica which may account for the slightly lower amounts of iron lost in Study I. Differences may also be accounted for in the method of collection which is subject to error with losses of sample in the urine and on clothing. However, the results indicate considerable loss of iron during a menstrual period which must be replaced by iron in the diet.

TABLE 4

INDIVIDUAL MENSTRUAL LOSSES OF IRON FOR STUDIES I AND II

Subject	Total iron per/cycle mg.	Subject	Total iron per/cycle mg.
	Study I		Study II
FG	2.7	RL	18.8
SH	6.3	MM	7.1
PM	9.5	CP	10.8
MR	16.1	RS	11.0
CE	10.5	ET	14.7
MN	13.7	ESP	16.3
ESP	4.9	HR	16.6
ESP	8.2	HR	10.5
PT	28.3		
Mean	10.6		13.7

Analysis of the variance using a split plot design with nested classification was used to indicate whether there was a difference between iron absorption by subjects on diets furnishing two levels of protein, 35 and 48 g., or between diets providing various ratios of plant to animal protein (93) (Table 5). Iron absorption was not affected significantly by level of protein in the diet although the average amount of iron absorbed by subjects on Diets IB and IIB was slightly greater (Table 2, page 46). Quality of protein in the diet appeared to have no affect on iron absorption. Average iron absorption was 7 and 14 per cent by subjects on Studies I and II, respectively. No difference in iron absorption was apparent during the different periods.

II. COPPER

Copper intakes and fecal losses by subjects were determined during Periods 1, 3, and 5 for Study I and II in order to investigate the effect of protein quality and level of protein on copper absorption. No attempt was made to calculate copper content of the diet for the two studies. Individual intakes for copper during Study I ranged from 0.98 to 1.46 mg./24 hr. (Table 6) for subjects receiving Diet A. Average ingestion for all subjects during the three periods was 1.27 mg./24 hr. (Table 7). Subjects receiving Diet IB received a mean intake of 1.18 mg./24 hr. with a range from 0.96 to 1.24 mg./24 hr. Copper intakes were lowest for two subjects, FG and CE, who had food removed from the diet during Periods 3 and 5. Average intakes for these two subjects was 1.2 and 1.0 mg./24 hr., respectively.

TABLE 5

ANALYSIS OF VARIANCE OF IRON ABSORPTION FROM DIETS A AND B (STUDIES I AND II)

Source of variance	df[a]	MS[c]	F[d]
Year	1	855.84	1.8
Level (Protein)	1	200.37	0.4
Level x Year	1	19.51	0.0
Subject/Level/Year	12	473.63	
Periods	4	136.50	0.7
Periods x Year	4	109.00	0.5
Periods x Levels	4	455.92	2.1
Periods x Levels x Years	4	508.18	2.47
Periods x Subjects/Level/Year	46[b]	201.50	

[a]df denotes degrees of freedom.

[b]Two degrees of freedom were lost because of missing value calculation on two subjects for two periods.

[c]MS represents mean square or variance.

[d]F is the variance ratio used to determine significance.

TABLE 6

COPPER ABSORPTION BY SUBJECTS IN STUDY I

Period	Intake	Fecal loss	Absorption	Intake	Fecal loss	Absorption
	mg./24 hr.		%	mg./24 hr.		%
			Diet A			
		Subject FG			Subject SH	
1	1.46	1.52	- 4	1.40	1.09	22
3	1.02	1.18	-16	1.21	1.12	7
5	0.98	1.04	- 6	1.17	0.96	18
Mean	1.15	1.25	- 9	1.26	1.06	16
S.D.	±0.3	±0.3	± 2	±0.1	±0.2	±8
		Subject PM			Subject MR	
1	1.46	1.42	3	1.46	1.38	6
3	1.29	1.11	14	1.29	1.36	- 5
5	1.25	0.93	26	1.25	1.29[a]	- 3
Mean	1.33	1.15	14	1.33	1.34	- 1
S.D.	±0.1	±0.2	±11	±0.1	±0.1	± 6

TABLE 6 (continued)

Period	Intake	Fecal loss	Absorption	Intake	Fecal loss	Absorption
	mg./24 hr.		%	mg./24 hr.		%
			Diet B			
		Subject CE			Subject MN	
1	1.24	1.24	0	1.24	1.70	-37
3	0.98	1.47	-50	1.22	1.05	14
5	0.96	1.16	-21	1.20	1.30	- 8
Mean	1.06	1.29	-24	1.22	1.35	-10
S.D.	±0.2	±0.2	±25	±0.1	±0.3	±26
		Subject ESP			Subject PT	
1	1.25	1.17	6	1.24	1.31	- 6
3	1.23	1.12	9	1.23	1.45	-18
5	1.21	1.51	-25	1.21	1.25	- 3
Mean	1.23	1.27	- 3	1.23	1.34	- 9
S.D.	±0.1	±0.2	±19	±0.1	±0.1	± 8

[a]Period 4 sample analyzed.

TABLE 7

SUMMARY OF MEAN COPPER ABSORPTION BY SUBJECTS
FOR STUDY I AND II

Subject	Intake	Fecal loss	Absorption	Subject	Intake	Fecal loss	Absorption
	mg./24 hr.		%		mg./24 hr.		%
	Diet A - Study I				Diet A - Study II		
FG	1.15	1.25	- 9	RL	1.45	1.45	0
SH	1.26	1.06	16	MM	1.45	1.40	3
PM	1.33	1.15	14	CP	1.45	1.25	8
MR	1.33	1.34	- 1	RS	1.45	1.25	13
Mean	1.27	1.20	5		1.45	1.36	6
S.D.	±0.1	±0.1	±12		±0.0	±0.1	± 7
	Diet B - Study I				Diet B - Study II		
CE	1.06	1.29	-24	PM	1.72	1.60	7
MN	1.22	1.35	-10	ESP	1.71	1.54	10
ESP	1.23	1.27	- 3	HR	1.72	1.77	- 3
PT	1.23	1.34	- 9	ET	1.71	1.41	18
Mean	1.18	1.31	-11		1.71	1.58	8
S.D.	±0.2	±0.2	± 9		±0.1	±0.2	± 9

Of the twelve individual balance periods studied, fecal losses were greater than intake during three periods by subjects on Diet IA, about equal to intake during three periods and less for six balance periods. Subjects on Diet IA lost on an average of 1.20 mg./24 hr. Excretion ranged from 1.06 to 1.34 mg./24 hr. Subjects receiving Diets IB lost on an average of 1.31 mg./24 hr. with losses from 1.27 to 1.35 mg./24 hr. Of the twelve individual balance periods investigated, fecal losses were greater than intake during seven periods, about equal to intake during two periods and were less than intake during three periods. Six of the subjects ingesting either Diets A and B excreted approximately the same amount of copper in the feces with a range of 1.27 to 1.35 mg./ 24 hr. Two subjects lost 1.15 and 1.06 mg./24 hr.

With copper intake at a low level, absorption by subjects showed wide variation. Absorption of copper ranged from -16 to 26 per cent by subjects on Diet IA with an average of 5 per cent. Variation in absorption was less in these four subjects than the four ingesting Diet IB. Absorption of copper ranged from -50 to 14 per cent with a mean of -11 per cent for subjects on the high protein diet, IB. Subject CE, whose average intake of copper was the smallest for the sixteen subjects during the two years, showed an absorption of -24 per cent. Fecal loss by this subject was 1.29 mg./24 hr.

Average copper intake of all subjects on the low protein diet during Study II was 1.4 mg./24 hr. (Table 7, page 55). Individual intakes of copper of subjects fed Diet IIA ranged from 1.33 to 1.59 mg./ 24 hr. (Table 8). Copper contents of the diets during Study II were

TABLE 8

COPPER ABSORPTION BY SUBJECTS IN STUDY II

Period	Intake	Fecal loss	Absorption	Intake	Fecal loss	Absorption
	mg./24 hr.		%	mg./24 hr.		%
			Diet A			
	Subject RL			Subject MM		
1	1.33	1.30	2	1.33	1.52	-14
3	1.44	1.56	- 8	1.44	1.36	6
5	1.58	1.48	6	1.59	1.33	16
Mean	1.45	1.45	0	1.45	1.40	2
S.D.	±0.1	±0.4	± 7	±0.1	±0.1	±16
	Subject CP			Subject RS		
1	1.33	1.42	- 7	1.33	1.17	12
3	1.44	1.01	30	1.44	1.30	10
5	1.58	1.57[a]	1	1.58	1.29	18
Mean	1.45	1.33	8	1.45	1.25	13
S.D.	±0.1	±0.3	±18	±0.1	±0.1	± 5

TABLE 8 (continued)

Period	Intake	Fecal loss	Absorption	Intake	Fecal loss	Absorption
	mg./24 hr.		%	mg./24 hr.		%
			Diet B			
		Subject PM			Subject ESP	
1	1.81	1.56	14	1.81	1.47	19
3	1.65	1.66	- 1	1.65	1.52	8
5	1.70	1.59	7	1.66	1.62	2
Mean	1.72	1.60	7	1.71	1.54	10
S.D.	±0.1	±0.1	± 7	±0.1	±0.1	± 8
		Subject HR			Subject ET	
1	1.81	1.98	-10	1.81	1.83	- 1
3	1.65	1.60	3	1.65	1.34	19
5	1.69	1.74	- 3	1.68	1.05	38
Mean	1.72	1.77	- 3	1.71	1.41	18
S.D.	±0.1	± 0.2	± 6	±0.1	±0.4	±19

[a]Period 4 sample analyzed.

slightly higher due to a higher intake of plant protein. Subjects ingesting Diet IIB averaged 1.71 mg./24 hr. with a range in intake of 1.65 to 1.81 mg./24 hr.

Average fecal losses of copper by subjects receiving Diet IIA was 1.25, 1.33, 1.40, and 1.45 mg./24 hr. with an individual range from 1.01 to 1.57 mg./24 hr. (Table 8, page 57). Average fecal losses by these four subjects was 1.36 mg./24 hr. Copper lost in the feces of subjects fed Diet IIB ranged from 1.05 to 1.98 mg./24 hr. with a mean loss of 1.58 mg./24 hr. The individual losses of copper by subjects averaged 1.60, 1.54, 1.77, and 1.41 mg./24 hr. A subject in one balance period showed a greater loss of copper than that ingested, whereas three subjects receiving Diet IIA lost more copper via the feces than was ingested during three individual balance periods.

Average absorption by all subjects on Diets IIA and IIB was 6 and 8 per cent, respectively (Table 7, page 55). Subjects ingesting Diet IIA, the low protein diet, showed absorptions ranging from -14 to 30 per cent of the intake. The average absorption by these subjects was positive during seven individual balance periods, reached equilibrium during two periods, and was negative during three periods. Average absorption by these four subjects was 0, 2, 8, and 13 per cent for the three periods. Average absorption for each of the subjects ingesting Diet IIB was 7, 10, -3, and 18 per cent. Absorption by these subjects ranged from -10 to 38 per cent. Of the twelve individual balance periods, subjects were in positive balance during six periods, reached a state of equilibrium during five periods, and were in a negative state

of absorption during one period. Variations in absorption by subjects was as great as variations in absorption between subjects.

Copper absorption was significantly influenced by nitrogen level ($P<.01$) (Table 9). Average absorption of copper by subjects receiving Diets IA and IIA was 6 but was only -2 per cent by subjects receiving Diets IB and IIB, the high protein diet. Absorption of copper was also significantly affected ($P<.01$) by nitrogen quality. Average absorption by subjects receiving Diets A and B for Study I was -3 per cent. The mean absorption by subjects ingesting Diets A and B for Study II was 7 per cent. The interaction between these two criteria, protein level and nitrogen quality, was also highly significant. This would indicate an interrelationship between these two sources of variation on copper absorption. As copper absorption is affected by both factors and the interaction is significant, the increased copper absorption cannot be interpreted on the basis of one or the other of these variables.

III. ZINC

Average zinc intakes and retentions are summarized in Table 10. Low zinc intakes in both Studies I and II were a result of a diet high in refined cereals and bread and low in foods containing zinc such as milk and meat. Although no attempt was made to keep intakes approximately the same, since the diets were similar in both studies, average intakes ranged from 4.1 to 4.8 mg./24 hr. Results obtained using the Perkin Elmer 303 Atomic Absorption Spectrophotometer showed good reproducibility (Table 26 in the Appendix).

TABLE 9

ANALYSIS OF VARIANCE OF COPPER ABSORPTION FOR DIETS A AND B (STUDIES I AND II)

Source of variance	df[a]	MS[b]	F[c]
Year	1	1246.44	23.38**
Level	1	633.65	11.88**
Level x Year	1	1045.34	19.60**
Subject/Level/Year	12	53.32	
Periods	2	54.48	0.16
Periods x Year	2	152.73	0.46
Periods x Levels	2	52.06	0.16
Periods x Levels x Year	2	60.66	0.18
Periods x Subjects/Levels/Year	24	334.91	

[a]df denotes degrees of freedom.

[b]MS represents mean square or variance.

[c]F is the variance ratio and determines significance.

**Significant at 1% level of probability.

TABLE 10

SUMMARY OF MEAN ZINC RETENTION BY SUBJECTS
FOR STUDY I AND II

Subject	Intake	Urine	Fecal loss	Retention	Subject	Intake	Urine	Fecal loss	Retention
	mg./24 hr.			%		mg./24 hr.			%
	Diet A - Study I					Diet A - Study II			
FG	4.6	0.4	5.3	-25	RL	4.2	0.2	5.2	-29
SH	4.7	0.3	4.4	- 1	MM	4.2	0.3	4.8	-22
PM	4.7	0.2	5.8	-27	CP	4.1	0.2	4.2	- 8
MR	4.7	0.2	5.7	-27	RS	4.1	0.2	4.5	-14
Mean	4.7	0.3	5.3	-20		4.2	0.2	4.7	-18
S.D.	±0.1	±0.1	±0.64	±13		±0.1	±0.0	±0.4	± 8
	Diet B - Study I					Diet B - Study II			
CE	4.4	0.3	5.4	-28	PM	4.8	0.2	5.4	-16
MN	4.6	0.2	4.4	- 1	ESP	4.8	0.3	5.0	-10
ESP	4.6	0.5	4.0	0	HR	4.8	0.2	4.8	- 1
PT	4.6	0.5	4.0	1	ET	4.8	0.2	4.5	4
Mean	4.5	0.4	4.4	- 7		4.8	0.2	4.9	- 6
S.D.	±0.1	±0.1	±0.7	±14		±0.0	±0.0	±0.4	± 9

Zinc intake of all subjects ingesting Diets IA averaged 4.7 mg./24 hr. with individual ranges from 4.4 to 4.9 mg./24 hr. (Table 11). All subjects receiving Diets IB had a mean intake of 4.5 mg./24 hr. with individual ranges from 4.2 to 4.8 mg./24 hr. Average intakes between the two diets, IA and IB, showed only slight differences.

Fecal losses of zinc by subjects on Diet IA averaged 5.3, 4.4, 5.8, and 5.7 mg./24 hr. with a range from 4.0 to 6.9 mg./24 hr. Average loss of zinc in the feces by the four subjects was 5.3 mg./24 hr. Variations in fecal losses were greater between individuals than in individual losses during the five periods. Losses of zinc in the feces by the four subjects receiving Diet IB was 5.4, 4.4, 4.1, and 4.0 mg./24 hr.; the average loss for all subjects was 4.4 mg./24 hr. Zinc losses ranged from 2.0 to 6.8 mg./24 hr. Two subjects, CE and PT, showed the greatest variation in fecal losses of zinc with losses of 3.7 to 6.8 and 2.0 to 5.6 mg./24 hr. Average zinc losses in the feces by the subjects receiving the higher protein diet was less than that lost by subjects on the low protein diet.

Urinary losses of zinc by subjects on Diet IA ranged from 0.1 to 0.6 mg./24 hr. with an overall average of 0.3 mg./24 hr. Individual variations were exceedingly small for three subjects but subject FG showed a range of excretion from 0.2 to 0.6 mg./24 hr. Urinary excretion did not appear to be affected by intake. Average urinary losses by subjects ingesting Diet IB ranged from 0.1 to 0.7 mg./24 hr. with an average of 0.4 mg./24 hr. Individual variations within periods were small but were slightly greater between individuals than periods.

TABLE 11

ZINC RETENTION BY SUBJECTS IN STUDY I

Period	Intake	Urine	Fecal loss	Retention	Intake	Urine	Fecal loss	Retention
	mg./24 hr.			%	mg./24 hr.			%
				Diet A				
	Subject FG				Subject SH			
1	4.9	0.5	4.0	10	4.9	0.4	3.9	13
2	4.5	0.2	5.2	-22	4.6	0.4	4.2	0
3	4.6	0.2	5.6	-27	4.7	0.4	4.9	-12
4	4.4	0.2	6.2	-46	4.6	0.3	4.9	-13
5	4.4	0.6	5.5	-38	4.6	0.1	4.2	7
Mean	4.6	0.4	5.3	-25	4.7	0.3	4.4	- 1
S.D.	±0.7	±0.2	±0.8	±18	±0.1	±0.1	±0.4	±11
	Subject PM				Subject MR			
1	4.9	0.2	6.9	-43	4.9	0.3	6.4	-35
2	4.7	0.2	6.6	-44	4.7	0.2	5.3	-19
3	4.8	0.2	5.4	-18	4.7	0.3	6.2	-36
4	4.6	0.2	6.0	-32	4.6	0.2	5.6	-26
5	4.6	0.1	4.4	4	4.6	0.2	5.2[a]	-18
Mean	4.7	0.2	5.8	-27	4.7	0.2	5.7	-27
S.D.	±0.1	±0.0	±1.0	±20	±0.1	±0.0	±0.5	± 8

TABLE 11 (continued)

Period	Intake	Urine	Fecal loss	Retention	Intake	Urine	Fecal loss	Retention
	mg./24 hr.			%	mg./24 hr.			%
				Diet B				
		Subject CE				Subject MN		
1	4.5	0.3	5.1	-19	4.5	0.2	3.9	9
2	4.6	0.3	3.7	12	4.8	0.1	4.5	3
3	4.3	0.4	6.8	-64	4.5	0.2	4.0	6
4	4.5	0.2	5.5	-26	4.6	0.1	4.8	- 5
5	4.2	0.3	5.7	-44	4.4	0.3	4.9	-17
Mean	4.4	0.3	5.4	-28	4.6	0.2	4.4	- 1
S.D.	±0.3	±0.1	±1.1	±28	±0.1	±0.1	±0.4	±11
		Subject ESP				Subject PT		
1	4.5	0.4	3.6	11	4.5	0.7	5.0	-27
2	4.8	0.4	3.8	12	4.8	0.4	5.6	-25
3	4.5	0.5	3.5	12	4.5	0.5	3.2	18
4	4.7	0.4	4.1	2	4.6	0.4	2.0	48
5	4.4	0.6	5.5	-38	4.4	0.6	4.2	- 8
Mean	4.6	0.5	4.1	0	4.6	0.5	4.0	1
S.D.	±0.1	±0.1	±0.8	±21	±0.1	±0.1	±1.4	±31

[a]Missing value calculation.

Two subjects (ESP and PT) excreted the greatest quantity of zinc via the urine and absorbed a larger percentage of the ingested zinc than did the other subjects.

Balance data for subjects on Study I and II indicated that zinc intakes were not sufficient to cover losses from the body via the feces and urine. Subjects receiving Diet IA retained on an average of -25, -1, -27, -27 per cent of the zinc intake. Balances per period ranged from 13 to -46 per cent for these subjects with a mean of -20 per cent. Variation in retentions was as great in individuals as between individuals. Of the twenty individual balance periods analyzed, a positive retention was achieved by the subjects during three of the periods. During two periods zinc intake and loss was about the same and during the remaining fifteen periods loss of zinc via the urine and feces was greater than intake. Subjects receiving Diet IB, the higher protein diet, had a positive retention of zinc during eight individual balance periods. Intake and excretion by subjects was about equal for three periods, and during nine periods individual losses were greater than intake. Average balances of these subjects was -28, -1, 0, and 1 per cent, with zinc balances ranging from 48 to -64 per cent with a mean of -7 per cent.

Average intakes of zinc by subjects receiving Diet IIA, the low protein diet, was 4.2 mg./24 hr. with a range from 4.0 to 4.4 mg./24 hr. (Table 12). Intakes of subjects on Study II, Diet B was 4.8 mg./24 hr. with intakes from 4.7 to 5.1 mg./24 hr.

TABLE 12

ZINC RETENTION BY SUBJECTS IN STUDY II

Period	Intake	Urine	Fecal loss	Retention	Intake	Urine	Fecal loss	Retention
	mg./24 hr.			%	mg./24 hr.			%
				Diet A				
	Subject RL				Subject MM			
1	4.0	0.2	4.3	-12	4.0	0.3	5.6	-50
2	4.0	0.2	5.1	-31	4.0	0.3	5.1	-33
3	4.4	0.3	5.5	-30	4.4	0.3	4.5	- 7
4	4.1	0.2	5.5	-39	4.1	0.3	4.5	-17
5	4.2	0.1	5.4	-32	4.2	0.2	4.1	- 4
Mean	4.2	0.2	5.2	-29	4.2	0.3	4.8	-22
S.D.	±0.2	±0.1	±0.5	±10	±0.2	±0.1	±0.6	±19
	Subject CP				Subject RS			
1	4.0	0.2	4.6	-22	4.0	0.2	3.9	- 4
2	4.0	0.3	6.4	-65	4.0	0.2	4.8	-24
3	4.4	0.2	3.3	20	4.4	0.2	4.2	1
4	4.1	0.2	2.7	28	4.1	0.2	5.1	-29
5	4.2	0.2	4.1[a]	- 3	4.2	0.3	4.4	-12
Mean	4.1	0.2	4.3	- 8	4.2	0.2	4.5	-14
S.D.	±0.2	±0.1	±1.4	±37	±0.2	±0.1	±0.3	±13

TABLE 12 (continued)

Period	Intake	Urine	Fecal loss	Retention	Intake	Urine	Fecal loss	Retention
	mg./24 hr.			%	mg./24 hr.			%
				Diet B				
		Subject PM				Subject ESP		
1	4.7	0.2	5.4	-20	4.7	0.4	5.0	-16
2	4.7	0.2	4.6	- 2	4.7	0.3	5.0	-13
3	4.8	0.3	6.1	-31	4.8	0.4	4.7	- 6
4	5.1	0.2	6.1	-25	5.1	0.4	5.7	-20
5	5.0	0.2	5.0	- 2	4.9	0.2	4.5	4
Mean	4.8	0.2	5.4	-16	4.8	0.3	5.0	-10
S.D.	±0.2	±0.1	±0.7	±13	±0.2	±0.1	±0.9	± 9
		Subject HR				Subject ET		
1	4.7	0.1	5.7	-26	4.7	0.1	5.7	-25
2	4.7	0.1	3.6	19	4.7	0.1	4.5	2
3	4.8	0.1	4.7	1	4.8	0.1	4.4	6
4	5.0	0.2	4.4	10	5.0	0.2	4.7	3
5	5.0	0.2	5.2	- 8	5.0	0.2	3.1	34
Mean	4.8	0.2	4.7	0	4.8	0.2	4.5	4
S.D.	±0.2	±0.1	±0.8	±17	±0.2	±0.1	±0.9	±21

[a]Missing value calculation.

Fecal losses of zinc by subjects ingesting Diet IIA averaged 4.7 mg./24 hr. with individual average losses of 5.2, 4.8, 4.3, and 4.5 mg./24 hr. Zinc losses for all periods ranged from 2.7 to 6.4 mg./24 hr. Losses of zinc by subjects ingesting Diet IIB averaged 4.9 mg./24 hr. with a range from 3.1 to 6.1 mg./24 hr. Individual losses averaged 5.4, 5.0, 4.7, and 4.5 mg./24 hr. Fecal losses were about the same for the two groups in Study II.

Urinary zinc losses averaged 0.2 mg./24 hr. for both Diets IIA and IIB. Losses ranged from 0.1 to 0.4 mg. daily for the study. No difference was noted in per cent of absorption and urinary excretion. All subjects excreted approximately the same amount of zinc via the urine.

Zinc intakes during Study II were not adequate to meet the demands of all subjects. Two subjects excreted approximately the same amount as was ingested. Balances of zinc by subjects receiving Diet IIA ranged from 28 to -65 per cent with a mean of -18 per cent. Average balances for the four subjects were -29, -22, -8, and -13 per cent. Subject CP showed greatest variation in balances ranging from 28 to -65 per cent of the intake. Balances by subjects on the high protein diet, Diet IIB, averaged -6 per cent with average retentions of -16, -10, 0, and 4 per cent.

Retentions of zinc for these four subjects ranged from 34 to -31 per cent. Variations in zinc retention were less between individuals than balance periods during the five periods studied. During four individual balance periods, subjects retained more zinc than was excreted;

during six periods subjects excreted approximately as much zinc as was ingested and during the remaining ten periods zinc excretion was greater than intake.

Analysis of variance to test the effect of nitrogen level and nitrogen quality on zinc retention was undertaken (Table 13). Retention of zinc was significantly influenced ($P<.05$) by level of nitrogen in the diet. When subjects were ingesting the lower level of protein, 35 g., the average balance was -20 and -18 per cent of the intake for Study I and II, respectively. Average retention by subjects ingesting the higher level of protein, 48 g., in Study I and II was -7 and -6 per cent. Quality of nitrogen did not appear to affect zinc absorption. No significant interaction was noted in zinc retention during various periods.

Menses were analyzed for zinc content. Zinc losses in the menses ranged from 0.15 mg./cycle to 0.72 mg./cycle (Table 14). Several samples from Study I were not available for analysis as the large amount of silica precipitate interferred with the determination. The precipitate was not present when the samples were first ashed and made up to a specified volume with demineralized water. On standing for a period of two years, silicate was formed in these samples.

TABLE 13

ANALYSIS OF VARIANCE OF ZINC RETENTION FOR DIETS A AND B (STUDIES I AND II)

Source of variance	df[a]	MS[c]	F[d]
Year	1	40.6	0.06
Level	1	3218.18	4.97*
Level x Year	1	5.2	0.01
Subject/Level/Year	12	647.37	
Periods	4	555.01	0.22
Period x Level	4	623.40	1.41
Period x Level x Year	4	678.31	1.58
Period x Subject/Level/Year	46[b]	394.78	

[a]df denotes degrees of freedom.

[b]Two degrees of freedom were lost because of missing value calculation on two subjects for two periods.

[c]MS represents mean square or variance.

[d]F is the variance ratio used to determine significance.

*Significant at 5% level of probability.

TABLE 14

INDIVIDUAL MENSTRUAL LOSSES OF ZINC FOR STUDIES I AND II

Subject	Total zinc lost/cycle mg.	Subject	Total zinc lost/cycle mg.
Study I		Study II	
PM	0.72	HR	0.39
ESP	0.40	HR	0.44
ESP	0.37	CP	0.20
MN	0.35	RL	0.16
SH	0.32	ESP	0.36
MR	0.45	RS	0.21
		MM	0.15
		ET	0.26
		PM	0.51

CHAPTER V

DISCUSSION

The absorption and retention of iron, copper, and zinc appear to be affected by many of the same factors: mineral content of the diet, protein quality and quantity, and general diet consumed. The absorption of these minerals is limited and in normal humans 90 to 95 per cent of the mineral passes through the intestine and is excreted in the feces.

I. IRON

Minimum adequate intakes of iron in the diets of women have been reported to be about 10.0 mg. daily (7, 8) when all other nutrients are supplied at recommended levels. In order to investigate the possible relationship of iron absorption with the level of protein intake and protein quality the amounts of iron ingested by the subjects in the two studies were kept as nearly constant as possible.

When balance studies are undertaken to determine absorption of various nutrients and quantities sufficient to meet the demands of the body, all pathways of excretion must be considered. Intestinal excretion of iron was calculated from studies undertaken on three college women to range from -0.12 to 0.27 mg. (94). Isotopic studies on normal humans showed the excretion of an injected dose through the intestine to vary from 0.4 to 2.0 mg. daily (95). Intestinal excretion of iron can originate from three sources, secretion of iron through the intestinal

wall as an excretory product, loss of desquamated cells containing iron from the lining of the stomach and intestine, and secretion of iron contained in digestive juices such as bile. These losses of iron are usually included as fecal iron along with the unabsorbed food iron.

Weintraub (96) has investigated iron losses through the skin of normal human subjects. Following intravenous doses of ^{59}Fe the loss of total body radioactivity was greater than could be accounted for by excretion through the stool or urine. Accumulation of iron in the epithilial tissues and subsequent loss through the skin was shown to occur. Iron in excess of physiological amounts needed by the cell can be stained. Comparisons of the iron deposition in the cells of normal subjects, a patient with hemochromatosis and an iron loaded subject demonstrated the accumulation of iron in the epithelial cells of the appendages and subsequent loss of this iron. This storage of iron by the cells of the skin would suggest that accumulation of iron in epithelial cells may represent an active excretory pathway. When temperatures are high enough to produce excessive sweating the dermal loss of iron in sweat must be considered (97, 98, 99). Analysis of the cell rich and cell free sweat showed an average iron content of 1.15 mg./liter and 0.34 mg./liter, respectively. The loss in sweat itself is negligible but loss from desquamated epithelial cells may be considerable. Since the women subjects for the two studies reported in this paper were not exposed to extremely high temperatures and hard labor, the loss of iron through the sweat was negligible.

Menstrual flow must also be considered a form of iron excretion. The amount of iron lost in normal menstruation varies widely in different individuals but usually falls between 16 and 32 mg. per period. Over a twenty-eight day cycle the daily loss would be about 0.5 to 1.0 mg. (13). Iron losses through the menses averaged 10.6 mg. for subjects in Study I with total losses of iron during a cycle ranging from 2.7 to 28.3 mg. Iron losses for subjects in Study II ranged from 7.1 to 18.8 mg. with a mean loss of 13.7 mg. Urinary excretion of iron usually is approximately 1.0 per cent of the daily intake in normal individuals (3). Urinary values for several subjects in the present studies averaged 0.3 mg. daily. When menstrual loss, urinary loss, and possible intestinal loss of iron were combined, two subjects on Study I, Diet A, did not absorb enough iron to cover excretion and two subjects on Diet B lost more iron from body stores than was ingested in the diet. All subjects in Study II absorbed enough iron to cover daily excretion from the body, exclusive of dermal losses.

Chemical balance studies carried out over a period of weeks have advantages over studies involving the absorption of iron from one food after one meal. Various physiological processes which affect absorption of iron would have a chance to adjust to the dietary change and absorption with longer dietary periods would be more similar to those experienced under normal conditions. In addition, the uptake of the mineral being studied is from an entire diet rather than just one food.

As the body has a very limited ability to excrete excess iron, the greatest portion of iron ingested merely passes through the

intestinal tract unabsorbed. Animal work has indicated that the content of iron in the diet affects uptake and transport of iron as well as body iron stores (100, 101, 102). This transport and storage system is the mechanism by which mucosal cells are able to put iron into temporary reserve until a larger than normal dose of iron is ingested, at which point a process suggestive of passive diffusion takes place.

Brown and coworkers (103) investigated the influence of large doses of inorganic iron fed to normal and iron deficient subjects several hours before a tracer dose of ^{59}Fe was administered. Extent of the blocking by the large doses varied with the individual. Absorption of the tracer dose was decreased when compared to the controls but assimilation was never blocked completely. Parenteral administration of iron did not cause decreased absorption. The authors emphasized the artificial nature of the block produced by large intakes of iron and the relatively small quantity of iron found in food which could not be expected to produce a block of this type. Experimental evidence does not justify the term "mucosal block" to describe physiologic regulation of iron absorption.

During Study I, food iron intake averaged 7.7 and 8.2 mg. for Diets A and B, respectively, with an additional 1.7 mg. furnished as part of a mineral supplement. With the change in protein quality in Study II all the iron was supplied by the food. The calculated amounts of iron furnished by iron supplements incorporated into food items such as bread, cereals, and spaghetti by the manufacturers was approximately

0.9 mg. Chodos and coworkers (23) have found that ferrous chloride is absorbed as well or better by normal subjects than iron in certain foods. Ferrous salts are much more readily absorbed than the ferric form but humans appear to have a mechanism for the efficient reduction of food iron (33).

It is generally believed that the amount of iron absorbed from food is dependent on the availability of the dietary iron and the person's capacity to absorb iron. Absorption of food iron may differ greatly according to the type of food in which it is found (14, 16, 18, 21, 22). Under ordinary circumstances iron is ingested in complex compounds.

In determining iron absorption individual foods are tested and not foods incorporated into a whole diet. Foods or supplements taken by fasting subjects (15) often show increased absorption. In animal studies where every mouthful is the same, food factors affecting iron absorption are often minimized. These situations are not typical of daily food habits.

The addition of 0.25 to 1 g. of ascorbic acid to foods has been reported to increase absorption of ferric iron (24). Whether this forms a complex that promotes transfer of the iron across the intestinal membrane is not known. The diets used in both Study I and II with college women furnished approximately 75 to 80 mg. daily of ascorbic acid. It would seem unlikely this amount of ascorbic acid in the diet would directly increase iron absorption. Phytates and phosphates also interfere with the absorption of iron by the formation of highly

insoluble salts (15, 16, 17, 18). Since the diets eaten during the two studies under investigation contained only refined cereal and bread products, interference by these radicals with iron absorption would not be a contributing factor to the slight differences in absorption. Primary calcium phosphate was added as a mineral supplement for Study I and II. Whether the phosphate interferred with iron absorption is not known but since the amounts of supplement added were approximately the same each year any effect of the supplement would probably be similar.

In Study I iron intakes averaged 9.3 and 9.9 mg. daily for Diets A and B. The protein was supplied at two different levels. Diet A furnished approximately 35 g. protein daily with equal amounts contributed by animal and vegetable sources. Diet B furnished 48 g. of protein obtained by adding 7.5 g. casein and 75 g. cooked egg white to the basal diet. Caloric contents of the diets were approximately equal. The mean iron absorption from these intakes was 5 and 9 per cent for Diets A and B, respectively. The slight difference in absorption was not significant. Fecal losses averaged 8.9 and 9.0 mg. for subjects receiving Diet A and B, respectively. As these losses were approximately the same it would seem to indicate absorption of iron was not affected by level of dietary nitrogen. Recent studies in rats have indicated that iron absorption was impaired by diets containing less than 15 per cent protein (26). In the present studies the low protein diet contained 35 g. of food protein, which furnished only 6 per cent of the total calories. Nine per cent of the total calories was furnished by the 48 g. of protein. In these studies the

two levels of protein investigated did not appear to impair iron absorption and there was no significant difference in absorption between the two levels. Abernathy and coworkers (10) also found that iron absorption in preadolescent girls was not affected by various levels of protein intake.

However, animal investigations have indicated a relationship between the level of protein in the diet and iron absorption (28). The way in which protein exerts its effect is not known but it could be due to the greater amount of amino acids present. Iron, amino acids, and proteins are capable of forming chelates which may enhance absorption. Study II was planned to furnish the same levels of nitrogen as in Study I but different ratios of animal to plant protein in order to investigate the influence of protein quality on iron absorption. In Diets A and B, 35 per cent of the protein was from animal sources. Mean absorption of the mineral was 13 and 15 per cent from Diets A and B, respectively. Fecal losses averaged 8.6 and 8.8 mg. on intakes of 9.9 and 10.3 mg., respectively. Level of protein appeared to exert no influence on apparent absorption of iron. Analysis of the variance indicated iron absorption was not different between the subjects in Study I and Study II although the average absorptions for the two studies were 7 and 14 per cent of the intake.

It is surprising that a greater amount of iron was absorbed from diets containing a higher portion of protein from plant sources. If the amino acid pattern in foods has an influence on iron absorption the variety of plant protein incorporated into the diet of Study II along

with the animal sources may have furnished amino acids in the right proportion to facilitate iron absorption to a degree comparable to a diet providing protein of a better quality at this level of iron intake. If the protein was provided from just a few sources, an imbalanced amino acid pattern may be present which could reduce iron absorption, even though sufficient iron was present in the diet.

Since the diets used in both studies furnished other nutrients at the recommended level, the diets could be considered of good quality even though minimal in iron and nitrogen. The level of protein or the quality of protein in the diet may not have exerted an all or nothing influence on iron absorption under these conditions. If adequate protein and amino acids are present with sufficient amounts of other essential nutrients the protein in both diets may have been adequate to allow for chelation and transfer of the iron across the membrane. If the other nutrients were not present in sufficient quantity to provide the atmosphere necessary in the intestine for chelation with amino acids at an optimum rate, then quantity and quality of protein present in the diet might influence the amount of iron absorbed. Absorption of iron appears to be influenced by many interrelated factors and as long as the diet contains liberal amounts of essential nutrients and protein of a mixed variety, iron will probably be efficiently absorbed and utilized by the individual.

II. COPPER

No recommended allowance has been established for daily copper intakes. Indications are that young women must ingest approximately 2.0 to 2.5 mg. of copper per day in order for copper balance to be attained (42, 43). On intakes in excess of this amount storage will take place in normal humans (47). No attempt was made in Studies I and II to regulate the copper content of the diet. Diets A and B furnished copper intakes of 1.31 and 1.20 mg. for Study I, and 1.36 and 1.58 mg. in Study II. The slightly higher copper content in diets used during Study II may be attributed to the larger amount of plant protein material. Plant material contains a greater amount of copper than animal sources.

Excretory pathways for copper are slightly different than those for iron and the total amounts of copper lost through these channels is usually smaller than that for iron. When ^{64}Cu was administered to normal humans either orally or intravenously the greatest rate of urinary excretion was noted in the first two hours following administration (48). Patients with Wilson's disease continued to excrete copper at an elevated level. Urinary excretion rate can be correlated to the copper-albumin fraction in the serum which is dissociated as it passes through the kidney with copper being lost. In normal humans copper is rapidly incorporated into ceruloplasmin, a globulin, but in person's with Wilson's disease globulin formation is depressed and copper circulates in the albumin fraction before removal into the tissues. Consequently urinary levels of copper are elevated. Considerable variation occurs

between the small amount of copper ingested and the quantity of copper excreted via the kidney. Porter (104) was unable to detect urinary copper in five of eleven normal adults. Six of the subjects excreted from less than 3.2 μg. to 14.7 μg./24 hr. Tompsett (105) found no correlation between copper intakes and urinary excretion in normal subjects. Average daily urinary excretion ranged from 220 to 520 μg./24 hr. Leverton (43) also found higher excretion levels by twenty-four college women ranging from 25 to 320 μg./24 hr. with an average excretion of 200 μg./24 hr. In patients with Wilson's disease the levels of urinary excretion may reach 1.5 mg./day. Copper excretion in the urine of a few subjects was determined in Study I. Values ranged from 30 to 135 μg./24 hr. Since the amounts of copper excreted via the urine are small and the determinations difficult to accomplish with such dilute solutions, urinary excretions were not included in Study I or Study II on college women.

Under ordinary dietary conditions 90 per cent or more of the ingested copper will appear in the feces. The greatest amount is unabsorbed copper but active excretion via the bile does occur. The importance of the liver, kidney, and intestinal wall in the excretion of intravenously administered ^{64}Cu has been shown in normal dogs, dogs with a complete biliary obstruction, and in dogs with the flow of bile diverted into the urinary bladder (106). Approximately 0.6 per cent of the administered activity was excreted into the urine, about 1.5 per cent passed directly through the intestinal wall, and about 7 to 10 per cent was excreted into the bile. When the biliary route was obstructed,

the excretion of copper through the kidney and intestinal wall was increased. Urinary copper excretion was increased when non-radioactive copper was ingested for thirty-two days by a dog with a bile duct ligation but was not increased in a normal dog. Gitlin (107) feels that tissue accumulation of copper in Wilson's disease is due to a defect in biliary copper excretion.

Negligible amounts of copper are lost in the sweat (108) and comparatively small amounts are lost in the menstrual flow (42). The copper content of sixteen menstrual periods for four subjects varied greatly from one period to another. Average copper lost was estimated at less than 0.5 mg. per period or 0.02 mg. per day. Analysis of menstrual loss of copper was undertaken for Study I and Study II. Due to the relatively large amounts of copper found in plant material the blanks made from the Kotex and tampons contained as much or more copper than was detected in the menstrual sample being analyzed. No accurate figures for copper losses through the menses could be obtained.

If one considers all avenues of excretion, the percentage of copper absorbed by the subjects in the two studies was not sufficient to meet the probable losses of eleven of the sixteen subjects. When intakes averaged 1.7 mg. on Study II, Diet B, average absorption of copper was 0.2 mg., only enough to cover minimum losses from the body.

Little is known about the mechanism of absorption of copper in higher animals. Acid seems to enhance the absorption of copper from the alimentary tract (52). High calcium levels appear to hinder absorption of copper from animal diets. Calcium contents of the diets in Studies I

and II on college women were not high. Supplements of primary calcium phosphate were added to the diets each year at 3.5350 g. and 2.4493 g. daily in Studies I and II, respectively. Whether the concentrated form of calcium in the diet affected copper absorption is not known.

Absorption of copper by the sixteen subjects in both Studies I and II ranged from an average of -24 to 18 per cent of the intake. Four subjects lost greater quantities of copper through the feces than was ingested, five subjects excreted approximately the same amount, and seven lost less in the feces than the intake. Average absorption of copper by subjects receiving the lower protein intakes in both studies was 6 per cent. Absorption of copper from diets furnishing 48 g. of protein was -2 per cent. The difference in absorption by subjects receiving the two levels of protein was significant.

Copper intakes averaged 1.3 and 1.2 mg. for Diets A and B, respectively, during Study I. Absorption of copper from the low protein diet averaged 5 per cent and from the higher protein diet -11 per cent. Average absorption by the subjects during Study II was 6 and 8 per cent of the intake from diets furnishing 35 g. and 48 g. of protein, respectively.

Preadolescent girls on low copper intakes of 1.1 to 1.3 mg. daily and nitrogen intakes of 7.7 to 14.1 g. daily excreted approximately the same amount of copper in the feces as was ingested (44). When the nitrogen intakes were reduced to 2.9 and 3.5 g. daily slightly negative balances resulted on copper intakes of 1.1 mg. to 1.5 mg. daily. Three preschool-aged boys ingesting diets providing 1.1 to 1.5 mg. daily

attained positive absorption levels (45). Each experimental period had been preceded by a long adjustment period and may have accounted for the relatively large absorption. Wintrobe and coworkers (40) feel that copper homeostasis is accomplished by an adjustment of the rate of excretion to that of absorption.

In herbages, copper exists as a neutral or anionic organic complex which is more easily absorbed by copper deficient rats than copper sulfate (53, 54). The author suggested the copper in herbage was utilized more rapidly than ionic copper because the organic complex was transported more easily through the intestinal wall. Complexing with amino acids or proteins may facilitate the movement of copper across the membrame. Some definite proportion of amino acids from the diet may provide the environment for enhanced absorption. Analysis of the variance showed that absorption of copper was affected by protein quality of the diet ($P<.01$). The average absorption by the subjects during Study I was -3 per cent when the diet furnished protein from 50 per cent animal sources for Diet A and 65 per cent animal sources for Diet B. In Study II, 35 per cent of the protein was from animal sources and absorption of copper averaged 7 per cent.

This effect of quality and level of protein on copper absorption did not result from a simple interrelationship between copper absorption and these dietary factors but a more complex interaction ($P<.01$) between these two effects and other possible factors that were not tested. One of these factors may have been copper intake which was low. Since intense competition for organic complexing agents such as proteins,

amino acids, and peptides by minerals and other compounds occurs in the intestine, the absorption of the limited quantity of copper could be affected by the total availability of sites on the protein. If sufficient sites are not available for complexing the copper, uptake and transfer of the mineral will be reduced and the copper will be excreted via the feces. Whether protein quality and quantity would still exert the same effect if copper levels were increased to 2.5 mg. or higher is not known, but when copper intake is not sufficient to establish equilibrium, absorption appears to be influenced by the quantity and quality of protein.

III. ZINC

Zinc, whether ingested or injected, leaves the body very largely by the way of the feces. Fecal zinc consists mainly of unabsorbed dietary zinc. A small amount of the zinc in the feces has been absorbed and re-excreted by the intestine. In studies on animals, injected ^{65}Zn was almost entirely excreted in the feces via the pancreatic juice, duodenal juice, and a small amount in the bile (109, 110). In man, zinc excretion via the bile is very low (69, 111). After a single intravenous injection of ^{65}Zn as the chloride the main pathway of excretion was the gastrointestinal tract. In forty-five days the cumulative fecal excretion averaged 19 per cent of the dose; ^{65}Zn uptake by the pancreas was not as high as the liver uptake. Zinc may be secreted in pancreatic juice in the form of the metallo-enzyme, carboxypeptidase. Calculated total secretion of ^{65}Zn in bile, gastric, and duodenal juice in man in

24 hours could not account for the entire ^{65}Zn content of the stool. The intestinal wall showed an uptake of ^{65}Zn and the results would indicate the fecal ^{65}Zn excretion is in part due to a release of ^{65}Zn from the intestinal mucosa (112).

In man, as in other animals (65, 67, 69), the amount of zinc excreted in the urine of normal subjects is very small and is independent of zinc intake. Excretions range from 0.1 to 0.9 mg./day with an average of 0.3 to 0.5 mg./day. When ^{65}Zn was injected into two human subjects the cumulative urinary excretion of ^{65}Zn in forty-five days was 0.9 per cent and 3.4 per cent of the dose. After two days the urinary excretion of the isotope became very low (113). Zinc lost in the urine of subjects on Study I and II ranged from 0.1 to 0.7 mg./day with an average of 0.3 mg./day. Urinary excretion of zinc during Study II averaged 0.2 mg./day with excretion ranging from 0.2 to 0.3 mg./day. Individual variations in zinc lost via the urine were small. The two subjects who absorbed the most zinc from the diet lost a greater amount in the urine; losses by other subjects did not reflect changes in absorption.

The amounts of zinc lost in the sweat are small (114). In tropical countries minerals lost through the skin may contribute to the problem of anemia. In normal subjects the zinc content of whole sweat was only slightly greater than cell-free sweat. The authors suggested that most of the zinc lost is in the cell-free or aqueous part of the sweat which is derived from the plasma. Since the subjects on Studies I and II were not subjected to excessive changes in temperature, losses of zinc through this avenue can be discounted.

Zinc is a normal constituent of erythrocytes associated with carbonic anhydrase. Zinc lost through the menses would probably be small in comparison to iron losses but no figures have been published showing zinc losses. Analysis of menses collected in the present studies showed small losses of zinc ranging from 0.2 to 0.7 mg. per menstrual period. Zinc excretion via this pathway was small and would not appear to be significant when considering replacement.

In normal adults, 5 to 6 mg. of zinc is needed daily to meet the losses by the body (57). Intakes of 9 to 12 mg. daily would be necessary to meet daily needs if absorption of zinc was 50 to 75 per cent of the amount ingested. The diets in Study I furnished an average of 4.7 and 4.5 mg. of zinc and in Study II, 4.2 and 4.8 mg./day. These intakes were not sufficient to meet the daily losses. Of the sixteen subjects, six excreted approximately as much as was ingested and ten subjects lost more via the feces and urine than was contained in the food.

The availability of zinc for absorption is increased or decreased by the presence of several compounds in the diet. Phytates reduce the availability of zinc to animals (70, 73, 74). The protein source did not seem to enhance zinc absorption when phytates were present. Since the diets ingested in both Studies I and II contained refined flours the influence of phytates from food sources would appear to be negligible. Calcium levels were adjusted to provide approximately 1 g. daily by adding primary calcium phosphate. Calcium content also may affect zinc absorption (71), and whether the calcium and phosphate contained in the

supplement influenced absorption of zinc by interference in the intestine cannot be determined.

Several studies have shown that as zinc intake increases retention increases even though fecal losses increase also (63, 65, 67). Subjects receiving Diet A (35 g. protein) in Studies I and II lost an average of 20 and 18 per cent more zinc, respectively, than was ingested. Subjects receiving Diets B (48 g. protein) lost only 7 and 6 per cent more zinc, respectively, than was furnished in the diets. The difference in the quantity of zinc lost on the two diets (A and B) was significant ($P<.05$). The level of protein in these diets appeared to exert an influence on the absorption of zinc.

Fecal excretion of zinc ranged from 2.0 to 6.4 mg./day in both studies. Average fecal excretions on Diets A and B were 5.3 and 4.4 mg./day in Study I and 4.7 and 4.9 mg./day on Diets A and B, respectively, in Study II. Average fecal losses ranged from 87 to 124 per cent of the average intake. Subjects receiving Diets A for Studies I and II lost 113 and 112 per cent of the ingested zinc in the feces, whereas subjects receiving Diets B lost 98 and 103 per cent of the ingested zinc. The greater apparent loss of zinc by subjects fed Diet A may have been due to a smaller quantity of amino acids and proteins available for chelation and transport. Since many of the metals appear to need amino acids for transport, competition for available sites may interfer with uptake and transfer of the zinc present in the diet when the supply of amino acids is minimal. It would seem possible that the quantity of protein would continue to exert an influence on uptake and transfer of

zinc even with increased zinc in the diet.

Evidence for the formation of natural chelates which aid in the utilization and absorption of zinc has been found in animals (78). Liver extracts and corn distillers' dried solubles enhanced the absorption of ^{65}Zn from soybean-containing diets. Diets having a high percentage of soybean protein depressed the accumulation of zinc in the liver of rats receiving large intakes of zinc, whereas casein diets were not effective (76, 77). The limiting action of the soybean protein diet would appear to be due to the combination of zinc with a specific compound within the protein and not to the simple formation of a chelate.

Protein quality did not appear to affect the absorption and retention of zinc in the present study. The mean apparent retention by subjects on Study I was--13 per cent and on Study II--12 per cent. Either the amino acid pattern furnished by the diets during both studies was similar or the quantity of amino acids present was sufficient to allow equal utilization of zinc. Zinc intakes were too low to allow for storage to occur and this may have masked the effect protein quality might exert on absorption.

From the experimental data presented, 4.2 to 4.8 mg. of zinc furnished daily in the diets of college women was not adequate to prevent losses of zinc from the body in excess of the amounts ingested. The quantity of protein furnished by the diet appeared to exert an effect on zinc retention, the higher protein intake of 48 g. preventing as great a loss of zinc from the body. This suggests that zinc may complex with proteins and amino acids for absorption. As the level of

protein increases more amino acids, peptides, and proteins are available for chelating with dietary zinc. At the lower protein intakes of 35 g., the competition between the various materials in the intestine for the sites available on the proteins reduces the quantity available for zinc uptake and reduces absorption.

Since the quality of protein did not appear to influence zinc absorption the two diets may have contained a satisfactory amino acid pattern for complexing or the proteins required for chelation are not limited to one or two and can be provided when the level of nitrogen intake is adequate.

CHAPTER VI

SUMMARY

The influence of protein quantity and protein quality on absorption of iron, copper, and zinc was investigated in two human metabolic balance studies at the University of Tennessee. The subjects were sixteen college women. The studies consisted of a six-day preliminary adjustment period followed by five six-day experimental periods during which two groups of four women ingested diets furnishing 35 g. (Diet A) and 48 g. (Diet B) of protein. During Study I, Diet A, 50 per cent of the protein was from animal sources and for Diet B 65 per cent from animal sources. In order to study the effect of quality on absorption, in Study II 35 per cent of the protein was from animal origin for Diets A and B. Other nutrients were provided to meet the recommended allowances through natural foods and vitamin and mineral supplementation.

I. IRON

Subjects received iron intakes of 9.3 and 9.9 mg./24 hr. during Study I, Diet A and B, respectively. Average absorption of iron by subjects on Diets A and B was 5 and 9 per cent, respectively. During Study II average iron intakes were 9.9 and 10.3 mg./24 hr. for subjects ingesting Diet A and B, respectively, with average absorptions of 13 and 15 per cent.

Average absorption by subjects ingesting Diets A (Studies I and II) was 9 per cent, and 12 per cent by subjects consuming Diets B (Studies I and II). The difference in absorption between these two groups on two levels of protein intake was not significant. Differences in absorption between Studies I and II, 7 and 14 per cent, were not significant.

It would appear that when the diet furnishes the essential nutrients in sufficient quantity, that the absorption of iron is not directly influenced by the level of protein in the diet. If the diet supplied suboptimal amounts of the protective foods or presented one nutrient in excessive quantities, protein quality and quantity might exert an influence on iron absorption.

II. COPPER

Copper intakes for subjects on Diet A and B for Study I were 1.27 and 1.18 mg./24 hr. Average absorption was 5 and -11 per cent for the subjects receiving Diet A and B, respectively. During Study II, subjects consuming Diet A ingested 1.45 mg./24 hr. and subjects on Diet B were furnished 1.58 mg./24 hr. Average absorption for the individuals on Diet A was 6 per cent and for Diet B, 8 per cent.

Although absorption of copper by subjects consuming different levels of protein was significant ($P<.01$), protein quality also exerted an influence ($P<.01$) on copper absorption. Since a significant interaction occurred the increased absorption of copper between subjects on different levels of protein and different quality of protein is not a

simple interrelationship but may be due to these two variables interacting. Another possibility is the level of copper was too low to allow for equilibrium to be established. Since the copper intake was low, the quantity of protein sites available for copper uptake and transfer could be critical. Whether this influence of protein quality and quantity would exert an influence together if copper intake was 2.5 mg. or higher would be difficult to determine in this study.

III. ZINC

During Study I, average zinc intakes for subjects on Diet A and Diet B were 4.7 and 4.5 mg./24 hr. Average balances were -20 and -7 per cent for subjects on Diet A and B, respectively. Zinc intakes by subjects for Study II were 4.2 and 4.8 mg./24 hr. Average balances were -18 and -6 per cent of the intake. Zinc absorption on the two different levels of protein, Diets A (35 g.) and Diet B (48 g.), was significantly different ($P<.05$). Average balances by the subjects on Diets A were -19 per cent and for subjects on Diets B was -6 per cent. Absorption was not affected by protein quality.

Although zinc intakes were not sufficient to allow equilibrium to be established, absorption appeared to be influenced by quantity of protein in the diet. Zinc, like the other two trace minerals investigated, forms chelates with proteins, peptides, and amino acids which could be involved in uptake and transfer across the intestinal membrane. If the level of protein is low in the diet, the competition for the available sites on the protein or amino acid molecule is intense and

absorption of zinc, in the limited quantity present in the two investigations on college women, would be reduced. It would appear possible that even with increased intakes of zinc, the level of protein would continue to exert an influence on zinc absorption.

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TABLE 15

REPRESENTATIVE FOODS FOR STUDY I AND II

Study I	Study II
Cereal	Cereal
Canned and frozen juices	Canned and frozen juices
White bread, doughnut	White bread, doughnut
Cream soups	Cream soups
Canned fruits	Canned fruits
Cottage cheese	Hamburger, turkey
Hamburger, turkey, fish	Rice
Rice, noodles	Spaghetti
Potatoes	Beans
Vegetables	Potatoes
Cookies (special recipes)	Vegetables
Deserts (special recipes)	Cookies (special recipes)
	Deserts (special recipes)

TABLE 16

CALCULATED NUTRIENT CONTENT OF DIET A AND B
STUDY I

	Cal.	Protein[a] A	Protein[a] V	Fat	Ca	P	Iron	Vit. A	Thia-mine	Ribo-flavin	Niacin	Ascorbic acid
		g.		g.	mg.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.
Day 1	2118	16.8	18.0	102	454	764	7.5	4993	1.28	1.12	10.5	86
Day 2	2051	18.1	16.8	106	577	820	9.2	5841	0.75	0.96	9.5	78
Day 3	2039	17.0	18.1	109	578	845	8.7	5595	1.80	1.60	8.0	97
Day 4	2093	18.4	16.7	98	451	770	7.4	4936	0.69	0.76	13.6	80
Day 5	2020	18.4	16.3	92	554	797	9.0	4406	1.36	1.26	10.2	78
Day 6	2080	17.2	17.9	105	445	748	7.5	5651	0.76	0.77	11.0	86
Ave./day basal diet	2067	17.6	17.3	102	501	791	8.2	5237	1.1	1.1	10.7	84
Mineral supplement[b]					505	869	1.8			0.4		
Casein and egg supplement	66	15.6	--	--	8	12	0.1	--	--	0.2	0.1	--
Total/day(Diet A)	2067	17.6	17.3	102	1006	1660	10.0	5237	1.1	1.5	10.7	84
Total/day(Diet B)	2133	33.2	17.3	102	1014	1672	10.1	5237	1.1	1.7	10.8	84

[a]Amounts of protein from animal and vegetable sources.

[b]420 I.U. of Vit. D/day were provided also by addition of Drisdol to the breakfast cream.

TABLE 17

CALCULATED NUTRIENT CONTENT OF DIET A
STUDY II

	Cal.	Protein[a] A	Protein[a] V	Fat	Ca	P	Iron	Vit. A	Thia-mine	Ribo-flavin	Niacin	Ascorbic acid
		g.		g.	mg.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.
Day 1	2238	14.2	24.5	102	514	900	9.5	5071	1.26	1.07	9.0	58
Day 2	2174	10.5	23.9	105	518	781	12.6	5862	1.02	1.15	10.3	95
Day 3	2180	12.8	24.4	96	536	843	9.4	4904	0.77	0.83	8.2	73
Day 4	2070	11.4	23.3	104	430	727	10.4	5675	1.09	1.14	10.7	94
Day 5	2039	11.3	22.6	93	473	779	10.8	4538	0.82	0.71	8.3	75
Day 6	2257	10.9	24.8	106	598	927	10.3	5936	0.96	1.11	10.8	110
Ave./day basal diet	2160	12	24	101	512	826	10.5	5331	0.99	1.00	9.6	84
Supplement[b]					510	869				0.5		
Total/day	2160	12	24	101	1022	1695	10.5	5331	0.99	1.50	9.6	84

[a]Amounts of protein from animal and vegetable sources.

[b]420 I.U. of Vit. D/day were provided also by addition of Drisdol to the breakfast cream.

TABLE 18

CALCULATED NUTRIENT CONTENT OF DIET B
STUDY II

	Cal.	Protein[a] A	Protein[a] V	Fat	Ca	P	Iron	Vit. A	Thia-mine	Ribo-flavin	Niacin	Ascorbic acid
		g.		g.	mg.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.
Day 1	2190	16.5	30.3	104	548	1003	9.3	5195	0.86	0.87	10.2	90
Day 2	2265	17.5	29.5	106	584	968	10.5	5701	1.28	1.17	10.0	87
Day 3	2147	18.0	30.9	98	550	934	9.8	4715	0.87	0.96	12.8	87
Day 4	2289	17.0	30.9	108	503	930	10.1	5959	1.36	1.20	10.8	95
Day 5	2107	18.2	29.5	94	556	929	11.5	4635	1.01	0.88	8.9	84
Day 6	2309	18.1	28.9	105	657	1094	8.9	6018	1.14	1.12	9.8	110
Ave./day basal diet	2218	18	30	103	566	976	10.0	5404	1.09	1.03	10.4	92
Supplement[b]					510	869				0.5		
Total/day	2218	18	30	103	1076	1845	10.0	5404	1.09	1.53	10.4	92

[a]Amounts of protein from animal and vegetable sources.

[b]420 I.U. of Vit. D/day were provided also by addition of Drisdol to the breakfast cream.

TABLE 19

INDIVIDUAL NITROGEN BALANCES, GRAMS PER TWENTY-FOUR HOUR[a]
DIET A, STUDY I

Period	Intake[b]	Urine	Feces	Balance	Intake	Urine	Feces	Balance
		Subject FG				Subject SH		
1	5.56	4.10	0.81	0.66	5.51	4.53	0.91	0.07
2	5.38	4.56	0.66	0.16	5.51	4.68	1.00	-0.17
3	5.38	4.84	0.73	-0.18	5.51	4.32	0.95	0.24
4	5.38	4.38	0.90	0.10	5.51	4.71	0.95	-0.15
5	5.38	4.53	0.71	0.15	5.51	4.55	0.83	0.12
Mean	5.41	4.48	0.76	0.18	5.51	4.56	0.93	0.02
S.D.	±0.08	±0.27	±0.09	±0.30	±0	±0.16	±0.06	±0.18
		Subject PM				Subject MR		
1	5.57	5.39	1.01	-0.83	5.58	4.13	1.18	0.27
2	5.60	4.60	1.13	-0.13	5.58	3.90	1.12	0.55
3	5.60	4.07	0.96	0.57	5.58	4.55	1.11	-0.08
4	5.60	4.35	0.72	0.53	5.58	3.99	1.16	0.42
5	5.60	4.35	0.72	0.53	--	--	--	--
Mean	5.60	4.52	0.95	0.13	5.57	4.14	1.14	0.29
S.D.	±0.02	±0.53	±0.15	±0.61	±0	±0.29	±0.03	±0.27

[a]Data reproduced from thesis of S. Hunt (90).

[b]Intake includes coffee, carbonated drinks, and supplements as well as from foods.

TABLE 20

INDIVIDUAL NITROGEN BALANCES, GRAMS PER TWENTY-FOUR HOUR[a]
DIET B, STUDY I

Period	Intake[b]	Urine	Feces	Balance	Intake	Urine	Feces	Balance
		Subject CE				Subject MN		
1	7.83	5.87	0.71	1.25	7.83	6.15	0.94	0.74
2	7.66	6.00	0.59	0.98	7.83	6.46	0.84	0.53
3	7.66	6.82	0.93	-0.10	7.83	6.09	0.90	0.84
4	7.66	6.02	0.89	0.75	7.83	5.03	1.06	1.74
5	7.66	6.16	0.87	0.63	7.83	5.14	0.96	1.73
Mean	7.69	6.17	0.80	0.70	7.83	5.77	0.94	1.11
S.D.	±0.08	±0.45	±0.14	±0.51	±0	±0.65	±0.08	±0.58
		Subject PT				Subject ES		
1	7.83	6.88	0.83	0.12	7.85	6.14	1.00	0.72
2	7.85	6.17	1.19	0.50	7.85	6.43	1.12	0.31
3	7.85	6.12	1.07	0.66	7.85	7.18	0.87	-0.20
4	7.85	5.97	0.72	1.16	7.85	6.26	1.08	0.52
5	7.85	5.83	1.04	0.99	7.85	6.33	1.26	0.27
Mean	7.85	6.19	0.97	0.69	7.85	6.47	1.06	0.32
S.D.	±0	±0.40	±0.19	±0.41	±0	±0.41	±0.14	±0.34

[a]Data obtained from dissertation of S. Hunt (90).

[b]Intake includes amount obtained from coffee, Coca-cola, and supplements as well as from foods.

TABLE 21

INDIVIDUAL NITROGEN BALANCES, GRAMS PER TWENTY-FOUR HOUR[a]
DIET A, STUDY II

Period	Intake[b]	Urine	Feces	Balance	Intake	Urine	Feces	Balance
		Subject RL				Subject MM		
1	5.68	5.20	1.37	-0.89	5.68	6.36	1.43	-2.11
2	5.84	4.74	1.14	-0.04	5.86	4.60	1.33	-0.07
3	5.76	5.84	1.36	-1.44	5.76	5.14	1.46	-0.84
4	5.85	4.32	1.62	-0.09	5.85	4.41	1.32	0.11
5	6.12	4.72	1.45	-0.06	6.12	4.34	1.33	0.45
Mean	5.85	4.96	1.39	-0.50	5.85	4.97	1.37	-0.49
S.D.	±0.08	±0.26	±0.24	±0.28	±0.08	±0.37	±0.03	±0.46
		Subject CP				Subject RS		
1	5.68	5.90	0.93	-0.98	5.68	4.95	0.92	-0.19
2	5.86	6.28	1.26	-1.68	5.84	5.30	1.17	-0.63
3	5.76	5.40	0.70	-0.33	5.76	3.97	1.42	0.37
4	5.85	4.91	0.91	0.03	5.85	5.90	1.30	-1.36
5	6.12	5.05	0.37	0.70	6.12	4.26	1.19	0.66
Mean	5.85	5.51	0.84	-0.46	5.85	4.78	1.20	-0.23
S.D.	±0.08	±0.08	±0.15	±0.41	±0.08	±0.35	±0.08	±0.36

[a]Reproduced by permission of V. T. B. Gomez (89).

[b]Intake includes coffee, carbonated drinks, and supplements as well as from foods.

TABLE 22

INDIVIDUAL NITROGEN BALANCES, GRAMS PER TWENTY-FOUR HOUR[a]
DIET B, STUDY II

Period	Intake[b]	Urine	Feces	Balance	Intake	Urine	Feces	Balance
		Subject PM				Subject EP		
1	7.57	5.93	1.30	0.34	7.59	6.95	1.11	-0.47
2	7.82	8.12	0.77	-1.07	7.84	5.81	1.36	0.67
3	7.66	6.29	1.17	0.19	7.66	6.37	1.23	0.05
4	7.83	6.29	1.47	0.08	7.83	5.91	1.25	0.68
5	7.99	5.75	1.25	1.00	7.89	5.94	1.53	0.41
Mean	7.78	6.48	1.19	0.11	7.76	6.19	1.30	0.27
S.D.	±0.07	±0.42	±0.12	±0.34	±0.06	±0.21	±0.17	±0.22
		Subject HR				Subject ET		
1	7.59	5.26	1.81	0.52	7.59	4.97	1.46	1.15
2	7.84	7.70	0.98	-0.84	7.84	5.74	1.11	1.00
3	7.66	6.27	1.06	0.33	7.66	5.94	1.18	0.54
4	7.82	5.92	1.14	0.76	7.81	5.87	1.22	0.72
5	7.98	5.85	1.24	0.89	7.98	6.13	0.78	1.06
Mean	7.79	6.20	1.25	0.33	7.77	5.73	1.15	0.90
S.D.	±0.07	±0.41	±0.15	±0.31	±0.07	±0.20	±0.11	±0.11

[a]Reproduced by permission of V. T. B. Gomez (89).

[b]Intake includes coffee, carbonated drinks, and supplements as well as from foods.

TABLE 23

FOODS ELIMINATED FROM DIET AND DECREASES IN INTAKE OF CALORIES, NITROGEN, IRON, COPPER, AND ZINC OF SUBJECTS FG AND CE DURING PERIODS 2, 3, 4, 5, STUDY I

Food	Weight per day	Calories (calc.)	Nitrogen (analyzed)	Minerals (analyzed)		
				Iron	Copper	Zinc
	g.	per 24 hr.	g./24 hr.	mg./24 hr.		
Margarine Grape Juice Brown Sugar	10) 40) 10)	136	.163	.52	.24	.15
Coca-cola	300	130	.024	.04	--	.01
Total		266	.187	.56	.24	.16

TABLE 24

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

Subject	Age	Height	Energy intake calc.	Weight		Hemoglobin	
				Initial	Final	Initial	Final
	yr.	cm.	calories/24 hr.	kg.		g./100 ml.	
			Diet A				
FG	25	158.8	1864	47.5	47.2	13.3	13.8
SH	41	166.2	2087	59.4	59.3	12.8	10.9
PM	33	157.5	2381	63.0	62.3	12.0	12.4
MR	27	156.2	2266	64.8	65.0	12.8	12.0
			Diet B				
CE	22	157.5	1866	51.5	51.2	12.4	13.8
MN	22	165.0	2002	78.9	76.8	12.4	12.4
ES	24	162.5	2231	77.4	77.0	11.2	10.9
PT	28	158.8	2118	55.6	55.2	11.2	11.2

TABLE 25

AGE, HEIGHT, ENERGY INTAKE, WEIGHT, HEMOGLOBIN LEVELS, AND SERUM PROTEINS OF SUBJECTS ON STUDY II

Subject	Age	Height	Energy intake calc.	Weight		Hemoglobin		Serum protein	
				Initial	Final	Initial	Final	Initial	Final
	yr.	cm.	calories/24 hr.	kg.		g./100 ml.		g./100 ml.	
				Diet A					
RL	24	164.5	2354	70.1	69.4	13.3	14.6	7.9	7.7
MM	20	158.1	2319	54.3	54.2	11.2	13.3	7.3	8.9
CP	21	170.8	2373	61.0	59.9	12.0	13.8	7.3	8.1
RS	25	162.6	2326	45.4	44.8	12.4	14.2	7.4	7.6
				Diet B					
PM	35	160.0	2333	62.6	62.7	12.9	12.4	7.4	8.0
ESP	26	165.1	2282	83.1	82.7	13.3	12.4	7.6	8.4
HR	29	168.2	2171	63.2	62.8	16.0	14.2	7.3	7.8
ET	21	168.3	2289	61.8	60.6	14.6	13.3	7.5	8.6

TABLE 26

REPRODUCIBILITY OF ZINC DETERMINATIONS USING A PERKIN-ELMER 303 ATOMIC ABSORPTION SPECTROPHOTOMETER

Sample	Date of determination	Zinc per 24/hr. sample
Food IA	5/4/65	4.70
Food IA	5/6/65	4.56
Food 2A	5/5/65	4.52
Food 2A	8/16/65	4.55
Food 2A (duplicate)	5/4/65	4.35
Food 2A (duplicate)	5/6/65	4.27
Food 2B	5/5/65	4.75
Food 2B	5/17/65	4.59
Food 3A	5/5/65	4.53
Food 3A	5/11/65	4.64
Coffee	5/11/65	0.0263/cup (142 ml.)
Coffee	5/17/65	0.0257/cup (142 ml.)
Feces SH 3	5/5/65	5.25
Feces SH 3	5/11/65	5.11
Feces SH 3 (duplicate)	5/5/65	4.68
Feces SH 3 (duplicate)	5/11/65	4.72
Feces MM 2	5/11/65	5.36
Feces MM 2	5/17/65	5.52
Feces ET 3	5/6/65	4.39
Feces ET 3	8/16/65	4.44
Feces RS 1	5/6/65	3.91
Feces RS 1	8/16/65	4.00
Feces MN 4	5/5/65	4.78
Feces MN 4	8/16/65	4.84