CALCIUM AND PHOSPHORUS RETENTION BY TWO 13-YEAR-OLD GIRLS

by

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INTRODUCTION

It is common knowledge that calcium is an important constituent of the body, particularly of the bones and teeth, but also of the soft tissues and body fluids. Nevertheless, calcium is one nutrient frequently found in short supply in the human diet. Phosphorus also is essential for normal bone and tooth development and has a role in most physiological functions. A deficiency of phosphorus, however, is uncommon and diets adequate in calcium usually are considered adequate in phosphorus, also.

Recent dietary surveys in various regions of the United States indicated that intakes of calcium by adolescent girls often were below present recommendations. Balance studies on calcium and phosphorus utilization in adolescent girls reported in the literature indicated that individuals vary widely in their ability to utilize these nutrients. Additional research is needed to supplement present available information. The purpose of this study was to determine the calcium and phosphorus retention of two 13-year-old girls ingesting approximately 1.0 g. calcium and 1.3 g. phosphorus daily.

REVIEW OF LITERATURE

Calcium and Phosphorus Metabolism

Absorption of Calcium. Hollinger and Pattee (1956) and Greenberg (1939) agreed that the duodenum or upper part of the small intestine especially is active in calcium absorption.

Greenberg (1939) attributed the decrease in absorption in the lower intestine to its lower acidity.

Harrison and Harrison (1951) investigated the rate of absorption of calcium. Young rachitic rats in the post-absorptive state received radioactive $Ca^{45}Cl_2$ by stomach tube. Animals were sacrificed at 2, 4, 24, or 72 hours following administration of the labeled ion. The investigators affirmed the belief that the most rapid rate of absorption of calcium was within four hours after administration. However, approximately one-third of the calcium entering the body was absorbed between 4 and 24 hours. Absorption at 72 hours was no greater than that at 24 hours.

Harrison and Harrison (1951) also studied the site of absorption of radiocalcium in the experiment described above. Four hours after administration, the amount of radioactive calcium in various divisions of the gastrointestinal tract of the animals was determined. The major portion of unabsorbed calcium was found in the distal one-third of the small intestine and the large intestine. This indicated that when calcium was absorbed four hours after administration, the absorption probably occurred at these sites.

Hathaway and Leverton (1959, p. 115) reported that the amount of calcium absorbed depended on the body's need for it, amount supplied by the diet, kind of food in which it occurred, and speed with which food passed through the gastrointestinal tract. Harrison (1959) regarded the following additional factors important: emotional disturbances, D vitamins present, and pH of the intestinal contents. Growth and hormones also have been

listed as factors affecting the absorption of calcium (Nicolaysen et al., 1953). Greenberg (1939) and Harrison (1959) agreed that the concentration of calcium in the small intestine also affected the amount of absorption.

pH. An acidic reaction in the intestine is one of the most important factors influencing absorption and utilization of calcium and phosphorus in the opinion of Elvehjem and Krehl (1947). According to Hollinger and Pattee (1956), calcium present in food is found in both the organic and inorganic state. It is assimilable in either state, but usually is absorbed in the inorganic forms as the acid phosphate, chloride, or carbonate which are soluble in dilute acids. With normal gastric acidity, compounds of calcium with weak organic acids such as gluconate and lactate are converted to the soluble chloride. If food is retained in the stomach for a sufficient time, even the less soluble phosphates may go into solution. However, adequate absorption also may occur without solution of the calcium salts by gastric juice.

The pH in the duodenum varies from 2.3 to 2.7. In the opinion of Hollinger and Pattee (1956), this range in pH determines whether calcium phosphate exists predominately in the more soluble acidic form or the less soluble basic form. Moderate quantities of dietary fat promote an acidic reaction in the intestine. Elvehjem and Krehl (1947), Hathaway and Leverton (1959, p. 115), and Stearns (1950) agreed that lactose also promotes an acidic reaction in the intestine. Elvehjem and Krehl (1947) explained that lactose establishes an aciduric

bacterial flora.

Vitamin D. Vitamin D is important in calcium utilization because it tends to favor the production of an acid pH. Harrison (1959) noted that vitamin D was particularly effective in increasing calcium absorption when a low concentration of calcium was present in the intestine. Possibly, vitamin D might act only when low concentrations were found. The mechanism of this phenomenon is not understood.

Greenberg (1945) tested the favored hypothesis that vitamin D increased the absorption of calcium from the gastrointestinal tract. He gave paired groups of young rachitic rats oral doses of 0.2 to 0.3 ml. irradiated ergosterol (10,000 U.S.P. XI units of vitamin D per g.) or cottonseed oil 72 hours and one hour prior to administration of radioactive materials. After sacrificing the animals, 70 to 110 percent of the radioactive material was recovered from their bodies. This worker concluded that vitamin D promoted calcium absorption from the intestinal tract. Also, vitamin D directly affected mineralization of bone. Bones from rats treated with vitamin D accumulated almost twice as much injected ion as bones from untreated animals.

Harrison and Harrison (1951) submitted evidence for the effect of vitamin D on calcium absorption that was contrary to that of Greenberg (1945). Young rachitic rats were divided into the following groups: (1) consumed 500 IU vitamin D_2 weekly, (2) received 3000 IU vitamin D_2 72 hours prior to the study, and (3) were given no additional treatment. Radioactive Ca^{45} was administered to the animals in the post-absorptive state.

The rats were sacrificed at 2, 4, 24, and 72 hours following administration of the ion. No significant effect of vitamin D upon the rate of calcium absorption could be detected at four hours. Later, absorption of calcium from the distal portion of the intestine was found in rats receiving vitamin D, but not in untreated ones unless the intestinal tract previously had been emptied of calcium by feeding a calcium-free diet. These workers interpreted their results to mean vitamin D was not an essential part of the transport mechanism involved in the passage of calcium through the intestinal mucosa into the body fluids. They suggested that vitamin D increased efficiency of absorption only when the calcium of the intestinal contents was poorly soluble.

Diet. Cohn et al. (1942) reported that any dietary constituent that affects intestinal secretion may affect net absorption of calcium. Further, the intestinal uptake can be diminished by agents that reduce the concentration of ionic calcium. Such agents as oxalate, phytate, phosphate, or possibly sulfate present in excess amounts can interfere with calcium absorption.

Davis (1959) asserted that most investigators have shown that as dietary calcium increased, the percent absorbed decreased. However, the total amount of absorption and retention might be increased. Harrison (1959) agreed that the level of calcium intake influenced the amount of calcium absorbed. Subjects maintained continuously on low calcium intakes absorbed a higher proportion of dietary calcium than subjects given higher levels, and then changed to low calcium diets. When large intakes of calcium were

given, the percentage of intake rejected by the intestine was increased, so that the amount absorbed was not proportional to the intake. Harrison thought this rejection might represent a mechanism to prevent an excessive calcium load for the kidneys. The tubules in the kidneys reabsorb most of the filtered calcium load. In patients with a nutritional calcium deficiency, this reabsorption can be essentially complete.

Davis (1959) reported calcium intakes from 0.5 to 0.8 percent of the diet and a high level of protein favorably influenced the utilization of calcium up to a maximum. However, the maximum appeared to vary considerably with the different species. Elvehjem and Krehl (1947), Stearns (1950), and Harrison (1959) concurred that diets high in protein favored the absorption of calcium. The amino acids, lysine and arginine, particularly were active in promoting calcium absorption (Harrison, 1959).

An American Institute of Nutrition symposium on the effects of high calcium intakes defined a calcium intake above 1.0 g./day as high (Mickelsen, 1959). Davis (1959) suggested high levels of ingested calcium frequently reduced digestibility of food and food nutrients. However, he thought experimental data supporting this statement were limited. Furthermore, the mechanism whereby calcium might have reduced digestibility was poorly evaluated. How excess calcium exerted its influence was not clear. Davis (1959) concluded that high levels of calcium, especially those over one percent of the diet, might depress sharply utilization of nutrients such as protein, fats, vitamins, phosphorus, magnesium, iron, zinc, and manganese.

Hegsted (1959) thought a substantial proportion of the population of the United States was influenced adversely by their habitual high intakes. Better methods of determining intake and more information about requirements might modify this conclusion. He believed current information did not justify the great emphasis nutritionists place upon calcium and saw no benefits from further increasing the calcium intake. Davis (1959) agreed in part with Hegsted (1959). He thought calcium requirement figures reflected the dietary pattern more than absolute requirements.

Two girls, ages 10 and 11 years, participated in a study on the effect of orange juice on the retention of calcium and phosphorus (Chaney and Blunt, 1925). The girls were considered normal but had some indications of malnutrition. Supplementing a basal diet with 600 to 700 cc. of unstrained fresh orange juice increased the retention of calcium and phosphorus. This increase came chiefly from a lessened excretion in the feces. Phosphorus had a slightly negative balance before supplementation; but with orange juice, a positive balance was noted. The authors ascribed this favorable retention to one or more of these facts: (1) vitamins promoted economical use of the elements present; (2) additional calcium and phosphorus induced retention of these minerals; (3) some factor that stimulated the flow of hydrochloric acid in the stomach caused greater acidity in the upper part of the small intestine; or (4) the basic residue favored normal activities during growth.

McKay et al. (1942) observed that the type of diet

influenced the retention of calcium in 124 college women on self-chosen diets. The mean daily calcium intake was 0.941 g. (range 0.322 to 2.323 g.). Variations in calcium balance in the same individual were less significant than variations among individuals. The calcium retention was related directly to calcium intake. Losses of calcium were more frequent than retentions at mean intake levels of less than 0.8 g. However, the amount of calcium retention by individuals varied at some mean daily intake levels.

The utilization of calcium from milk by four women and three men was reported by Breiter et al. (1941). The women habitually consumed one pint of milk daily, whereas two of the men had existed on a low calcium diet for several years. The basal series was separated from the supplemented diet by a 12-day, self-chosen diet that included either large quantities of milk or calcium acid phosphate. The total calcium intake on the basal diet varied from 231 to 309 mg., the average was 270 mg. The supplemented diet - basal diet plus fluid milk - contained 451 to 873 mg. The quantity for each subject was based on losses during the basal diet. Both levels of calcium, designed to provide less than the maintenance requirement, induced negative balances. Utilization was based on the theory that if a calcium deficit existed, calcium from a test substance would be utilized and its utilization could be measured quantitatively. The average utilization of calcium from milk was 24.2 percent, and ranged from 15.3 to 35.1 percent. Two fairly distinct groups were found: those who utilized 20 percent or

less, and those who utilized 30 percent or more. Neither sex, age, nor nutritional status was related to the utilization group. The wide range of values showed it was not the form in which calcium occurred in a given food that determined availability.

Willard and Blunt (1927) compared evaporated and pasteurized milks as sources of calcium and phosphorus for four children. Experimental periods of 13 and 12 days were divided into two periods of six or seven days. Milk supplied 90 to 95 percent of the calcium intake. The retention of calcium was approximately 0.01 g./kg. body weight. Evaporated milk proved to be as satisfactory as or slightly better than pasteurized milk as a source of calcium for children. Evaporated milk also gave a greater phosphorus retention.

Steggerda and Mitchell (1946) studied 19 men to determine utilization of calcium from diets containing milk products or equally available calcium salts in amounts to furnish about 60 percent of the total calcium intake. The average length of the 75 diet periods was 20 days. Mean utilization of calcium in the experimental diets was about 32 percent. The coefficient of variation was 23.3 percent. None of the treatments--heating milk to 160°F. for 30 minutes; homogenization; addition of sodium alginate, citric acid, or citrates in moderate amounts; or precipitation of a soft curd milk by base exchange--produced any change in the utilization of calcium.

The effect of oxalic acid on calcium absorption was studied by Johnston et al. (1952). Six healthy college women, ages 20 to 31 years, who habitually consumed at least two cups of milk

daily were subjects. The diet met the NRC allowances for all nutrients except iron and calcium, and minimum levels of these were used. The experiment was divided into three four-week periods. During the first period, the basal diet contained 820 ± 3.7 mg. calcium per day. Spinach increased the calcium intake to 975 ± 5.4 mg./day in period two and 982 ± 3.9 mg. daily in period three. On a dry weight basis, the spinach contained 4.1 percent oxalic acid. All milk and cheese were eaten at noon, and spinach was eaten either for breakfast or dinner. Spinach did not alter the calcium balance significantly. The mean daily balance was -26 mg. during the basal diet, -30 mg., and -51 mg. during periods two and three, respectively. Apparently, an amount of calcium equivalent to that in spinach was not retained. The absorption of calcium might have been favored if milk had been served in the same meal with spinach. However, the oxalic acid content of the spinach may not have been as high as average for cooked spinach. Johnston et al. (1952) thought most research workers in the field of calcium metabolism would agree that young adults with normal stores required one-half to one g. calcium daily.

The effect of cocoa upon calcium utilization and requirements was investigated by Bricker et al. (1949). Seven healthy young college women were subjects. The low-calcium diet contained 225 mg. calcium and 70 to 80 g. protein. Five levels of medium-cost, American process cocoa (5.6, 21.0, 28.5, 34.8 or 56.2 g.) were used. Milk was the major source of calcium except for the 21.0 g. cocoa diet, that was milk-free. The average daily

calcium balance was -12.85 ± 11.0 mg. and -8.70 ± 9.7 mg. for the non-cocoa and cocoa diets, respectively. The difference between average balances was not statistically significant. A significant effect on the pathway of calcium excretion was noted. Urinary calcium fell and fecal calcium rose when the cocoa diet was compared to the cocoa-free diet.

Fuqua and Patton (1953) employed nine college women to study the effect of three levels of fat intake on calcium metabolism. The basal metabolism rates of these women fell into three groups that averaged 3.6, 8.3, and 13.3 percent below the Aub-DuBois standard. One subject from each group was placed on each of three levels of fat. The diets contained 45, 91, or 135 g. fat as 19, 39, or 58 percent of the calories, respectively. Food energy and calcium intakes totaled 2100 calories and 600 mg., respectively. Fat had no influence on utilization of calcium. Neither did calcium balance seem to be related to the basal metabolism rate. Calcium equilibrium required 630 mg. calcium per day.

Calcium-to-Phosphorus Ratio. The relationship of the calcium-to-phosphorus ratio and utilization of these minerals in young college women has been investigated by Patton et al. (1953). The calcium intake levels were 344, 944, and 1544 mg.; and phosphorus levels were 766, 1066, and 1366 mg. The calcium-to-phosphorus ratios varied from 1:0.05 to 1:3.97. Calcium retentions were greater and were related to the ratio of calcium-to-phosphorus, when the ratio was less than one. However, the amount of calcium appeared to have greater significance in

determining calcium balance than the calcium-to-phosphorus ratio. At each level of calcium intake, an increase in phosphorus intake had no significant effect on calcium balance. At a constant phosphorus intake, phosphorus balances were not affected by an increase in the calcium intake. At each level of phosphorus intake, an increase in calcium resulted in a significant increase in calcium storage. However, an increase in phosphorus intake from 766 to 1366 mg. gave higher phosphorus balances only if the calcium level was as high as 944 mg. For equilibrium, 674 mg. calcium and 1100 mg. phosphorus were necessary as judged by regression equation. Urinary calcium, as percent of the calcium intake in mg./kg., increased with an increased calcium-to-phosphorus ratio and constant calcium intake.

Absorption of Phosphorus. Phosphorus, according to Hollinger and Pattee (1956) is absorbed later and further down the gastrointestinal tract than calcium. The organic phosphate esters are split by the pancreatic and intestinal juices, whereas the sodium, potassium, and calcium acid salts probably are absorbed as such by diffusion. These workers believed the role of phosphorylation in the process of phosphorus absorption had not been elucidated. The absorption of phosphorus depends partly on the absorption of calcium (Hathaway and Leverton, 1959, p. 134). Two theories (Hollinger and Pattee, 1956) were advanced to explain this: (1) Absorption of phosphorus apparently varies with the concentration of ultraviolet light. Ultraviolet rays increase the absorption of calcium, allowing more free soluble phosphorus to be absorbed. (2) Vitamin D favors the absorption of calcium,

and less calcium remains to precipitate phosphorus. Indirectly, therefore, vitamin D would enhance the absorption of phosphorus. Vitamin D also has a favorable influence on utilizing the phosphorus in phytin (Hollinger and Pattee, 1956). Minerals that interfere with phosphorus utilization are certain cations such as iron and manganese which form insoluble phosphate compounds in the intestine if fed in sufficient concentration (Elvehjem and Krehl, 1947; Cohn et al., 1942).

Cohn et al. (1942) reviewed studies on radioactive phosphorus in rats. Only a small amount (two percent) of injected radioactive phosphorus (P^{32}) as disodium hydrogen phosphate appeared in the feces within two months. This demonstrated the small part that blood plays in the origin of fecal phosphorus. When the same amount of P^{32} was given orally as a salt, 93 percent remained in the body for over two months. If ingested as a food, one-third of the ion appeared promptly in the feces. The uptake of P^{32} by tissues is rapid and transitory in functional compounds such as adenosine triphosphate, and slower and more lasting in structural compounds such as bone. The greatest relative uptake is seen in rapidly growing and metabolizing tissue.

Utilization of Calcium and Phosphorus. Puberty, estrogens, thyroid, and growth are factors that affect mineral retention, particularly in the adolescent. Harrison (1959) pointed out that the efficiency of calcium absorption was much greater in young growing animals and human beings than in older ones. Stearns (1950) asserted the most important factor in mineral

utilization by adolescents is maintenance of a good nutritional state for several years preceding puberty. She noted that the period of greatest retention frequently may precede rapid growth by two or more years.

Puberty. Puberty is one factor believed to influence absorption and utilization of calcium and phosphorus. The basal metabolism rate of 38 children including 28 girls 10 to 16 years old was studied from one to four years at six months to one year intervals (Topper and Mulier, 1932). A definite increase in metabolism rate was noted before and during early puberty. In girls, the increase began one to eight months prior to the menarche and was maintained for one to six months following it.

Johnston (1940) studied the influence of puberty on the retention of calcium. He associated puberty in girls with a decreased calcium retention. Three normal girls were subjects on continuous balance studies covering a range of 96 to 300 days which included the onset of menstruation. The calcium intakes varied from 1.2 to 1.4 g./day. When calcium retentions for pre- and post-menarcheal periods were compared, a decreased retention was observed following the menarche. A high dietary intake of calcium was needed to prevent negative balance during the period of diminished retention.

Estrogens. Johnston (1941) administered estrogen to five apparently normal girls at puberty. Since it was presumed that all subjects were secreting normal amounts of estrogen, this study involved excess amounts of estrogen. Estrogen depressed calcium balance and increased the calcium excreted in both urine

and feces for one week following administration.

Manunta et al. (1957a) studied the effect of estradiol on the retention of radioactive calcium in adult male albino rats. A daily subcutaneous injection of estradiol benzoate and an intraperitoneal injection of $Ca^{45}Cl_2$ were given for four days. Considering radioactive decay, each rat received 20 to 28 mg. Ca^{45}/100 g. body weight. Two days after the last injection, the animals were killed and the radioactivity in bone and serum determined. Estrogen-treated animals showed increased radioactivity of blood serum when compared with the untreated group. However, bone radioactivity for both groups was similar.

Manunta et al. (1957b) also investigated the metabolism of Ca^{45} in lactating rats treated with estradiol. Twelve pregnant albino rats were divided into two groups. At parturition, the litters were reduced to six. All mothers were injected intraperitoneally with radioactive Ca^{45} over a three-day period starting at parturition. The experimental group received subcutaneous injections of estradiol benzoate daily for four days. Five days post-partum, the animals were killed, and radioactivity of serum and bone determined. Radioactivity of both serum and bone in the treated rats was significantly higher than the controls. This occurred despite the withdrawal of calcium in milk. The mechanism for estrogen action is not known. Observations by these workers suggested the possibility of a higher renal or fecal threshold for calcium. The authors concluded from this and the previous study that estrogen has a role in

retention of calcium in the animal body.

Thyroid. The thyroid gland also affects the retention of calcium. Four children having abnormal metabolism had optimal retentions of calcium when their metabolism approached normal (Johnston and Maroney, 1939). It was proposed that small doses of thyroid that did not stimulate basal metabolism would increase retention of calcium during growth. However, additional amounts of thyroid would diminish retention.

Bone Metabolism. Hollinger and Pattee (1956) discussed bone formation, reabsorption, and mineral balance. To maintain normal balance between bone formation and reabsorption, normal tissue and plasma concentrations of calcium and phosphorus were needed. Normal concentration required adequate dietary intakes of calcium, phosphorus, vitamins A and D, ascorbic acid, and normal functioning of the parathyroid, renal, and respiratory organs. The composition and structure of bone salts appeared to vary greatly in different areas and under different circumstances. This was attributed in part to a large and active surface of bone mineral crystal to which carbonate, phosphate, bicarbonate, fluoride, sodium, potassium, magnesium, citrate and other ions were attached interchangeably.

The bone trabeculae act as a reserve supply of calcium. Five cats on a high calcium diet and four on a low calcium diet each had one leg amputated (Bauer et al., 1929). The bone removed after the high calcium intake contained many trabeculae, and the bone removed after the low intake had fewer trabeculae. No gross change in cortical thickness was noted. There appeared

to be a marked reduction of trabeculae in kittens as a result of bone growth. It was stated that the calcium in bone was partly structural and partly a readily available reserve supply. Negative calcium balance decreased and positive balance increased the number of trabeculae. When the trabeculae were depleted, the structural part of the skeleton probably gave up calcium.

Neuman and Neuman (1957) formulated a new concept of calcium and bone metabolism. They postulated that bone salts existed in the serum in a supersaturated state in respect to apatite. Previously, it was believed that plasma was undersaturated in respect to bone minerals. The supersaturated solution permitted spontaneous and continuous calcification of the matrix. Apparently, the maintenance of the supersaturated condition of plasma and extracellular fluid must be an active process.

In a normal animal or human being, the mechanisms for maintaining blood calcium levels within the relatively narrow normal range readily manage the amounts of calcium from the intestine (Whedon, 1959). Wide variations in calcium intake are not reflected in the analytical values for soft tissues. Tissue deposits of calcium have been produced experimentally by alkali, vitamin D, and parathormone; but not by high calcium intake alone. In animals, experiments indicated that high calcium intake increased bone mass. Bones from rats on high calcium intakes for many months had a higher calcium content than bones from rats on a lower, but theoretically sufficient, calcium intake. If positive balances continued over many weeks

or months, the mineral had nowhere to go except to bone. Clinically, it was postulated that long continued calcium storage might ultimately mean an increase in human bone strength.

Distribution of Calcium and Phosphorus in the Body

Calcium is the most abundant mineral of the body and comprises between 1.5 and 2.0 percent of the adult body weight (Hathaway and Leverton, 1959, p. 112). In the fully-developed body, the percent of phosphorus is one-half as large as the percent of calcium (Sherman, 1947, p. 51). Phosphorus composes from 0.8 to 1.1 percent of the body weight (Hathaway and Leverton, 1959, p. 112).

Bones and Teeth. Approximately 99 percent of the body calcium is found in the bones and teeth. Of this amount, 90 percent is present as calcium phosphate and calcium carbonate. Hathaway and Leverton (1959, p. 113) pointed out that a 13- to 14-year-old who weighs 110 pounds may deposit as much as 90 g. of calcium yearly. Hollinger and Pattee (1956) stated that bone from the time it ceases to grow until death gradually becomes richer in carbonate and poorer in phosphate, thus making it more brittle. The calcium-to-phosphorus ratio in bone is 2.15:1.00. Bones can accumulate calcium and phosphorus when intakes of these minerals are generous (Hathaway and Leverton, 1959, p. 114). This reserve in bone can be used in times of stress. However, if no reserve is available, calcium is taken from the bone itself.

From 80 to 90 percent of the phosphorus is located in the

bones and teeth, the remainder being in the soft tissues and body fluids (Hathaway and Leverton, 1959, p. 112). Stearns (1950), however, stated that more body phosphorus is found in tissues than in bone. All body phosphorus exists as orthophosphate, either organic or inorganic. Phosphorylation is an essential step for the absorption of many nutrients.

Plasma. Plasma contains about 9.0 to 11.5 mg. percent (4.5 to 5.5 mEq/liter) of calcium according to Hollinger and Pattee (1956). Stearns (1950) set this level as 10 mg. percent. The red blood cells, on the other hand, contain only minute amounts of calcium. The plasma calcium is found in two portions--a diffusible portion of 5.0 to 6.5 mg. percent and a non-diffusible portion of 4.0 to 5.0 mg. percent. The non-diffusible fraction depends on the plasma protein concentration, especially the albumins, in the proportion of about 0.84 mg. per g. protein. Hollinger and Pattee (1956) stated that the plasma calcium level varied directly with the concentration of plasma proteins and the hydrogen ion concentration and, inversely, with the concentration of plasma phosphorus.

The parathyroid hormone is responsible for keeping serum calcium at the normal level (Hathaway and Leverton, 1959, p. 115). This hormone can shift calcium and phosphorus from bone to blood (Stearns, 1950). If the calcium blood level is too high, this hormone increases excretion of these minerals. When calcium is ingested orally, the calcium plasma level reaches a maximum in two to three hours and is normal in four hours. With intravenous injection of calcium, the maximum level is reached in a

few minutes and returns to normal in approximately one-half hour (Hollinger and Pattee, 1956).

The diffusible plasma calcium may be subdivided into a non-ionized portion (about 0.25 mg. percent) which consists of insoluble colloidal calcium salts of citrate and phosphate and an ionized portion (4.75 to 6.25 mg. percent) which is physiologically active (Stearns, 1950). Ionized calcium is required during blood coagulation for the formation of thrombin from its inactive precursors (Hollinger and Pattee, 1956).

Hollinger and Pattee (1956) stated that inorganic phosphorus exists as an electrolyte component of the intracellular fluid and urine. It is the chief inorganic ion of the intracellular fluids. The level of plasma phosphorus is influenced by the parathyroid hormone.

Muscle. Muscle contains about eight mg. calcium per 100 g. of wet weight (Hollinger and Pattee, 1956). Muscle irritability is increased by decreasing the calcium concentration (Hollinger and Pattee, 1956; Hathaway and Leverton, 1959, p. 112). The exact site of action of these ions is questionable, but peripheral nerves seem to be involved according to Hollinger and Pattee (1956). Raised calcium ion levels tend to inhibit skeletal, unstriated muscle and voluntary and autonomic nervous systems. A decrease in plasma calcium causes increased neuromuscular irritability with twitchings and, in advanced stages, convulsions. The autonomic ganglion is stimulated by this decrease. If the calcium drop is sufficient, the heart will stop beating in diastole.

Requirements for Calcium and Phosphorus

Sherman (1947, p. 29) believed passably normal health could be maintained for a lifetime or through successive generations on very different levels of calcium intake and output and on somewhat different levels of calcium content of the body. Ohlson and Stearns (1959) concurred that individuals have existed on minimum amounts of calcium. They suggested adaptation to minimum levels probably occurred in the bone structure during growth and in the adult size. Another opinion expressed by Steele et al. (undated) was that while a human could adapt to a poor or mediocre diet without showing undue stress, the abundance of food in this country made adaptation unnecessary.

Holmes (1945) listed two major determinants of calcium requirement during growth as the rate of growth and extent of utilizing dietary calcium. During the pubertal spurt, differences in growth rate significantly modify calcium requirement. Leitch (1937) also regarded growth rate as one factor that determines calcium requirement. Other factors were the composition of the skeleton in children and miscellaneous demands for calcium. This investigator thought a definite difference in the supply of bone-forming material existed between fast and slow growing children. When calcium intake was above the minimum maintenance level, retention increased to a maximum which depended on growth rate. Leitch (1937) placed the calcium requirement of adolescents between 1.0 and 2.0 g. daily. Other workers (Ohlson and Stearns, 1959) maintained that a mean calcium

retention of 400 mg. daily was necessary for several years to fully calcify a girl's skeleton by age 18. This retention was found only with daily intakes of 1.5 to 1.6 g. calcium and 400 IU vitamin D.

Recommended Allowances. The recommended dietary allowance of the Food and Nutrition Board, National Research Council (1958) for calcium is 1.3 g. daily for girls 13 to 15 years old. The recommended allowances are designed to maintain good nutrition in healthy persons and to afford a margin of sufficiency above minimal requirements, but are not designated as optimal intakes. No allowance is stated for phosphorus because a phosphorus deficiency is uncommon and diets adequate in calcium are considered adequate in this mineral, also. However, the Food and Nutrition Board suggested allowances for phosphorus in children's diets should be at least equal to those for calcium.

Dietary Intake Surveys. From 1947 to 1958, over 200 nutritionists investigated the nutritional status, including the amount of nutrients eaten, of population groups within the United States. The state Agricultural Experiment Stations, the Institute of Home Economics of the U.S. Department of Agriculture, and several state departments of public health sponsored the studies. The country was divided into four regions: Northeast, North Central, South, and West. Eleven groups of 13- to 15-year-old girls were studied--eight in the West, three in the Northeast, and one in the North Central region.

Morgan (1959) summarized the nutritional status surveys. Girls were more deficient in calcium intake than boys.

Adolescent girls presented the least favorable picture of all the groups. Their average calcium intake was termed seriously low. Survey results indicated calcium was ingested in increasingly low amounts starting at age 11 years. Teen-age girls had a wide gap between the recommended allowance and the average intake. It was suggested that the large number of women who could not or did not nurse their babies and the prevalence of dental caries resulted from low intakes. However, no objective evidence of harmful effects from low intakes was found. Morgan (1959) theorized the allowance for calcium might be excessive because it was based on balance studies that were affected strikingly by previous diet.

The mean daily intakes of calcium and phosphorus for 13- to 15-year-old girls by state are shown in Table 1. For calcium, the state mean intakes ranged from 0.77 to 1.42 g./day. The mean daily calcium intakes in the various states, with the exception of New York, were slightly less than the NRC recommended allowances. Only two state groups, Colorado and the Spanish-American group in New Mexico, had mean calcium intakes below two-thirds of the NRC allowance. However, there were individual intakes from two to four times the recommended allowance, and at least an equal number with the lowest amount of calcium consistent with adequate calories. Ohlson and Stearns (1959) estimated approximately 150 mg. calcium per 1000 calories as the lowest practical intake in American dietary patterns.

Three states (Maine, New York, and Rhode Island) reported mean daily phosphorus intakes. All of the means met the 1.3 g.

Table 1. Mean daily intakes of calcium and phosphorus for girls 13 to 15 years old by state.

State	No. of subjects	Calcium Mean	Calcium Standard error	Phosphorus Mean	Phosphorus Standard deviation
		g.	g.	g.	g.
Western Region[1]					
Colorado	63	0.77	0.04		
Idaho	70	1.11	0.05		
Montana	111	0.90	0.03		
New Mexico					
Anglo American	19	0.97	0.11		
Spanish American	33	0.78	0.07		
Utah	25	1.08	0.08		
Washington	107	1.02	0.03		
North Central Region[2]					
Iowa					
13 yrs	44	0.99	0.06		
14 yrs	37	0.99	0.05		
15 yrs	39	0.90	0.05		
Northeast Region[3]					
Maine	123	1.09	0.43[4]	1.46	0.40
New York	113	1.42	0.52[4]	1.72	0.58
Rhode Island	45	0.96	0.31[4]	1.32	0.27

[1]Wilcox et al. (Undated)
[2]Eppright et al. (1954)
[3]Tucker et al. (1952)
[4]Standard deviation

suggested by the NRC.

Western Region. Wilcox et al. (undated) compiled data on 428 adolescent girls in seven western states. Seven-day food intake records and dietary histories were evaluated. Vitamin and mineral supplements were recorded but not included in the nutrient value of the diets. The cumulative frequency curves indicated many individuals were not well fed although average intakes were adequate. Adolescent girls were low in calcium. Of the 13- to 15-year-old girls, 46 percent had less than 67

percent of the NRC recommended allowance, 35 percent had from 67 to 100 percent, and 10 percent had 100 percent or above. It was suggested that diets of girls and women could be improved by greater use of milk and milk products.

North Central Region. In Iowa, the nutrient intakes were studied from seven-day dietary records (Eppright and Roderuck, 1955). Among the 12- to 14-year-old girls, 29.7 percent had diets with calcium levels less than 67 percent of the NRC allowance. This inadequate intake stemmed from low consumption of milk and vitamin-rich fruits and vegetables.

Northeast Region. In the Northeast region, the dietary intakes of 281 adolescent girls were evaluated from seven-day dietary records, dietary histories, and four-day dietary records (Tucker et al., 1952). In comparing the calcium intake with the NRC allowance for girls 13 to 15 years old, 39 percent had 66 percent or less of the allowance, 30 percent had from 67 to 100 percent, and 31 percent had 100 percent or above. For phosphorus, 10 percent of the girls in this age group had 66 percent or less of the amount suggested by the NRC, 31 percent had 67 to 100 percent, and 59 percent had 100 percent or above.

Balance Studies. Balance studies provide another method of judging calcium and phosphorus requirement. However, only a limited number of balance studies of adolescent girls have been reported in the literature. A wide range of calcium intakes--0.002 to 0.020 g. per kg. body weight--was found as the daily requirement to maintain balance in these girls. Intakes of 0.004 to 0.008 g. phosphorus per kg. body weight were reported

as necessary for balance.

One early study on calcium requirement was done by Wang et al. (1928). These workers had 41 girls, ages four to 13 years. Part of the subjects were underweight, the others had normal weight. The children ate a weighed diet for at least six days. Excretions were collected during the last three days of the period. Absorption ranged from 0.009 to 0.033 g. calcium oxide per kg. of body weight or 13.4 to 42.1 percent of the calcium intake. Calcium absorption and retention showed wide individual variations among normal and undernourished children. The underweight children showed a slight tendency toward increased calcium storage.

Wang et al. (1936) studied mineral metabolism in 23 12- to 15-year-old girls. Daily mean intake and range were 1604 mg. (1186 to 1806 mg.) and 1708 mg. (1276 to 2081 mg.) for calcium and phosphorus, respectively. Calcium retention ranged from 79 to 823 mg., and the mean was 417 mg. per day. No close relationship was demonstrated between retention and either intake or body weight as shown by low coefficients of correlation. Phosphorus retention varied from -11 mg. to 480 mg. daily, the average was 198 mg.

Sherman and Hawley (1922) determined the rate of calcium storage and the nature and amount of intake to support optimum calcium storage in the growing child. In the first phase, calcium retention and age were investigated. Twelve normal children, ages three to 13 years, were studied for nine days preceded by one preliminary day. The daily diet contained

750 g. milk, and the total calcium intake was approximately one g. The intakes were about the same as the children received before the study. Calcium retention varied from 0.15 to 0.62 g. per day and showed a closer relationship to size and age than did phosphorus retention. Phosphorus storage was related to intake and body weight.

In the second phase of their experiment, Sherman and Hawley (1922) studied one 12-year-old girl for eight periods of six days each. Daily calcium intake was varied in each period by changing the amount of milk from 250 to 1500 g. The subject required 1000 g. milk daily for optimum storage of calcium. A total of 139 three-day balance periods showed optimum storage of calcium occurred when the diet contained one quart of milk daily.

During two other portions of their study, Sherman and Hawley (1922) compared calcium and phosphorus retention from milk and vegetables. In the first and third periods, milk was the only calcium-rich food. During the second period, vegetables (carrots and spinach) furnished half of the calcium intake. Calcium retention was lower on vegetable-rich diets than on diets with the same or smaller amounts of calcium supplied by milk. In some cases, vegetables also had an unfavorable effect on phosphorus storage. Hathaway and Leverton (1959, p. 115) stated the lower absorption of calcium from vegetables was attributed to the high fiber content. Fiber caused it to move quickly through the gastrointestinal tract and thereby reduced absorption.

The calcium requirement of seven 12- to 13-year-old girls was investigated by McKay et al. (1948). Four levels of calcium were eaten during consecutive 20-day periods. The basal diet contained 0.183 g. calcium. Ice cream and milk increased the calcium content of the basal diet to 1.994, 2.303, 0.935, or 0.455 g. The percent utilization of calcium from milk and ice cream for each subject was 23.1, 39.0, 40.1, 30.3, 34.1, 40.0, and 54.2. Four subjects had maximum calcium retention and their calculated calcium requirements were 1.009, 1.654, and 1.113, and 0.700 g. daily.

Six 13- to 14-year-old girls who habitually drank three glasses of milk daily were subjects on an eight week study by Johnston et al. (1950). Approximately 0.9 of the 1.0 g. of dietary calcium was from milk or milk products. The mean retention was 281 mg. daily or 26 percent of the mean intake, and ranged from 16 to 41 percent for the various subjects. These investigators suggested rate of calcification as the most important factor influencing retention. Other factors proposed were the need as affected by storage and rate of calcification, supply of vitamin D, and amount of glandular secretions.

Bone Density. Another criterion of mineral status and requirements is bone density. Williams and Samson (1960) thought bone density extended the information about calcium requirement derived from balances. Odland et al. (1958) found little relationship between bone density and nutrient intake as calculated from seven-day dietary records of children in the Western region, with one exception. In Arizona, Papago Indian

girls eating a limited lunch consistently had lower bone density coefficients than girls receiving ample lunches. Odland et al. (1958) and Williams et al. (1957) agreed that bone density coefficients indicate cumulative nutritional status, whereas nutrient intake estimates current dietary trends.

MATERIALS AND METHODS

During a study primarily concerned with iron utilization by six adolescent girls, samples of the diet and body excretions were collected and preserved. In the present study, all samples of the diet and samples of the body excretions were analyzed for calcium and phosphorus to determine the balance of these two minerals by two of the subjects.

Subjects

The subjects were healthy 13-year-old girls who had reached puberty. They lived in a home management house on the campus and continued their usual activities during the 50-day experiment. They were supervised by a faculty member of the Department of Foods and Nutrition.

Diet

A five-day adjustment period preceded the main study. The main study was divided into three parts, each part having three five-day periods. Daily menus were planned to contain approximately 1.0 g. of calcium and 1.3 g. phosphorus and to meet the NRC recommended dietary allowances (1953) for all other

nutrients except iron. The most recent NRC allowances (1958) differed from the 1953 revision only in calories and niacin. Dietary calcium, phosphorus, food energy, protein, carbohydrate, fat, vitamin A, thiamine, riboflavin, niacin, iron, and ascorbic acid were estimated from food value tables. Food value tables used were USDA Handbook No. 8 (Watt and Merrill, 1950) supplemented by Bowes and Church (1951).

Two levels of iron were used--11 mg./day during Part I and 13 mg./day during Parts II and III. Part III differed from Part II in that it contained a large quantity of milk chocolate candy. Unenriched flour products were used in Parts I and III, and enriched products in Part II. Skim milk replaced whole milk in Part III. Also, other foods were substituted and serving size of various foods adjusted to maintain approximately the same level of nutrient intake in all parts.

Distilled, demineralized water was used for cooking and drinking. Sodium chloride, C. P., served as a dentifrice. At the beginning of the experiment, sufficient quantities of the non-perishable and frozen foods were obtained for the duration of the study. All baked products were made in the laboratory. Edible portions of all foods were weighed to the nearest 0.1 g. All subjects were served the basal diet, but were allowed butter cookies ad libitum and a maximum of two soft drinks per day for between-meal snacks.

Collection, Preservation, and Analysis of Samples

Methods used for collection and preservation of food and excretory products were described by Leichsenring et al. (1958). Aliquot portions (one-fifth) of the daily diet were collected and combined for each five-day period. The liquid food, solid food, butter cookies, chocolate, and soft drinks were placed each in separate containers. Urine and feces were collected separately in waxed paper cartons. All of the foods and excretory products were wet ashed and placed in paraffin-sealed glass bottles. Further details about sampling, preservation, and wet ash methods are found in the Appendix, pp. 61-65.

Triplicate samples of all foods and excretions of the two girls were wet ashed. Triplicate samples from each ashing were then analyzed for calcium using the method of McCrudden (1911). Duplicate samples from each ashing were analyzed for phosphorus using the method of Fiske and Subbarow (1925). Details of the analytical procedures are given in the Appendix, pp. 65-68.

Analysis of Data

Calcium and phosphorus absorption was calculated as the difference between dietary intake and fecal loss. Percent absorption = $\frac{\text{Absorption}}{\text{Intake}} \times 100.$

Retention of calcium and phosphorus was defined as the difference between dietary intake and fecal and urinary excretion. Percent retention = $\frac{\text{Retention}}{\text{Intake}} \times 100.$

Data were tabulated by period and by individual girl. Retentions of calcium and phosphorus were calculated for each girl for each period and for the entire study. The calcium and phosphorus retentions of each subject were compared with those of the other subject and with retentions reported in the literature.

RESULTS AND DISCUSSION

Subjects

Age, weight, height, and body surface area of the subjects at the beginning of the study are shown in Table 2. A physician, who examined the girls, found them healthy.

Table 2. Description of subjects.

Subject	Age	Weight	Height	Body surface area	Age of menarche
	years	kg.	cm.	sq. m.	years
A	13	60.4	166.4	1.68	12
C	13	64.0	171.4	1.75	11

Calculated Dietary Intakes of Calcium and Phosphorus

The mean daily nutrient intake for each period is shown in Table 3. Daily menus and the calculated mean nutrient intake for each day are included in the Appendix, pp. 69-75. The plan was to provide 11 mg./day iron in Part I and 13 mg./day iron in Parts II and III. A pilot study revealed that values for iron

Table 3. Mean daily nutrient content of basal diet calculated from food value tables.[1]

Part	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus
	Cal.	g.	g.	g.	mg.	mg.
I	2249	82.4	105.0	213.9	1011	1354
II	2281	84.1	107.3	255.8	996	1376
III	2328	84.3	109.5	261.7	1079	1412

	Iron	Vitamin A	Thiamine	Riboflavin	Niacin	Ascorbic acid
	mg.	IU	mg.	mg.	mg.	mg.
I	9.6	6498	1.22	1.80	13.4	112
II	12.5	6512	2.94	1.94	15.8	118
III	12.4	5445	1.16	2.17	13.2	109

[1] Based on Watt and Merrill (1950) supplemented by Bowes and Church (1951).

calculated from food value charts were higher than analyzed values. Therefore, the diets were calculated for nine and 12 mg./day iron to obtain the desired amount. The amount of thiamine during Part II was more than twice either of the other parts because enriched flour products were substituted for unenriched ones. Use of skim milk and smaller amounts of butter reduced the vitamin A content in Part III. Calcium intake fell in Part II because of decreases in milk and milk products. Additional phosphorus in Part III was a result of substituting milk chocolate candy for part of the milk. This candy had more phosphorus in proportion to calcium than milk. Chocolate was responsible also for increased riboflavin in Part III.

Enriched flour and additional meat increased the niacin intake in Part II.

Analyzed Values for Dietary Calcium and Phosphorus

Chemical analyses showed the basal diet contained considerably less calcium and phosphorus than the amounts calculated from food value tables (Table 4). Analyzed values for calcium ranged from 0.5 to 34.8 (mean 16.0) percent less than the calculated values. The extremes occurred in Part II when the menus were identical. Apparently, variation in nutritive content was greater during this part. Analyzed values for phosphorus ranged from 10.8 to 30.4 (mean 24.4) percent below the calculated values.

Thomas et al. (1950) compared analyzed and calculated values of a diet. Wide variation in analyzed and calculated values for calcium was attributed to methodology and techniques of sampling, preservation, and measurement. Smaller relative variations were found in duplicate analyses for phosphorus for one day and among days for one week.

In the present investigation, a pilot study included chemical analyses of the diet for iron, but the other nutrients were determined by calculation only. Before future balance studies are undertaken, the calcium and phosphorus content of the diets should be determined by chemical analyses. This would be necessary because the calcium and phosphorus content of the diets calculated from food value tables were found to

Table 4. Mean daily content of calcium and phosphorus in the basal diet.

Period	Calcium			Phosphorus		
	Analysis[1]	Calculation[2]	Difference	Analysis[3]	Calculation[2]	Difference
	g.	g.	%	g.	g.	%
Part I						
1	0.843	1.011	16.6	1.000	1.354	26.1
2	0.858	1.011	15.1	0.949	1.354	29.9
3	0.986	1.011	2.5	1.208	1.354	10.8
Part II						
4	0.991	0.996	0.5	0.996	1.376	27.6
5	0.854	0.996	14.2	0.957	1.376	30.4
6	0.649	0.996	34.8	1.081	1.376	21.4
Part III						
7	0.773	1.079	28.4	1.147	1.412	18.8
8	0.886	1.079	17.9	1.072	1.412	24.1
9	0.926	1.079	14.2	0.982	1.412	30.4
Mean			16.0			24.4

[1]Method of McCrudden (1911).
[2]Based on Watt and Merrill (1950) supplemented by Bowes and Church (1951).
[3]Method of Fiske and Subbarow (1925).

vary widely from the analyzed values.

Calcium

Dietary Intakes. Intake of the subjects (Table 5) varied because of soft drinks and butter cookies. The data showed that subjects A and C had mean daily calcium intakes of 0.869 and 0.875 g. for the entire study. During various periods, calcium intakes of subjects A and C ranged from 0.654 to 1.000 g. and 0.662 to 1.004 g., respectively. The total intake fell below the NRC allowances of 1.3 g. in all periods. The smallest intake (Period 6) was approximately half the recommended amount. In Period 7, 0.271 g. and in Periods 8 and 9, 0.299 g. of the calcium was from milk chocolate candy.

Urinary Excretion. The daily urinary excretion of calcium averaged 0.132 and 0.178 g. for subjects A and C, respectively (Table 5). These losses ranged from 0.108 to 0.186 g. in subject A and from 0.111 to 0.251 g. in subject C, which were within the ranges found in other studies of adolescent girls (Table 6). However, losses in the urine of the two subjects were always in the upper region of reported ranges. Urinary losses of 0.150 g. daily were considered average by Hollinger and Pattee (1956). In Table 5, the mean urinary calcium levels for the nine periods varied greatly for each as well as between the two girls. Boyce and King (1959) and Leichsenring et al. (1951) found urinary calcium excretion was more constant for an individual than between individuals.

Table 5. Mean daily intake, excretion, absorption, and retention of calcium.

Period	Intake	Excretion		Absorption		Retention	
		Urine	Feces				
	g.	g.	g.	g.	%	g.	%
Subject A							
Part I							
1	0.851	0.128	0.844	0.007	0.8	-0.121	-14.2
2	0.861	0.123	0.883	-0.022	- 2.6	-0.145	-16.8
3	0.989	0.142	0.782	0.207	20.9	0.065	6.6
Part II							
4	1.000	0.186	0.536	0.464	46.4	0.278	27.8
5	0.863	0.159	0.808	0.055	6.4	-0.104	-12.0
6	0.654	0.109	0.715	-0.061	- 9.3	-0.170	-26.0
Part III							
7	0.783	0.108	0.963	-0.180	-23.0	-0.288	-36.8
8	0.891	0.124	1.049	-0.158	-17.7	-0.282	-31.6
9	0.929	0.112	0.853	0.076	8.2	0.036	3.9
Mean	0.869	0.132	0.826	0.043	3.3	-0.081	-11.0
Subject C							
Part I							
1	0.855	0.185	0.730	0.125	14.6	-0.060	- 7.0
2	0.873	0.164	0.632	0.241	27.6	0.077	8.8
3	0.998	0.187	0.650	0.348	34.9	0.161	16.1
Part II							
4	1.004	0.176	0.779	0.225	22.4	0.049	4.9
5	0.874	0.251	0.766	0.108	12.4	-0.143	-16.4
6	0.662	0.189	0.765	-0.103	-15.6	-0.292	-44.1
Part III							
7	0.779	0.160	0.792	-0.013	- 1.7	-0.173	-22.2
8	0.895	0.177	0.703	0.192	21.4	0.015	1.7
9	0.934	0.111	0.763	0.171	18.3	0.060	6.4
Mean	0.875	0.178	0.731	0.144	14.9	-0.034	- 5.8

Table 6. Daily calcium intake, excretion, and retention by adolescent girls as reported in the literature.

Investigator	No. subjects	Intake		Excretion				Retention			
				Urine		Fecal					
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
		g.	g.	g.	g.	g.	g.	g.	g.	g./kg.	g./kg.
Sherman and Hawley (1922)	1	0.979 - 1.039	1.017	0.033 - 0.116	--	0.237 - 0.470	--	0.476 - 0.710	0.622	---	0.011
	1	0.425 - 1.794	--	---	--	---	--	0.222 - 0.782	--	0.007 - 0.023	--
Willard and Blunt (1927)	1	1.12 - 1.24	--	---	0.02	0.46 - 0.89	--	0.21 - 0.64	--	0.026 - 0.028	--
Wang et al. (1936)	16	1.186 - 1.799	1.604	0.015 - 0.323	0.136	0.559 - 1.444	1.052	0.079 - 0.823	0.417	---	0.011
Johnston (1941)	6	1.125 - 1.488	--	0.019 - 0.221	--	0.761 - 1.003	--	0.316 - 0.570	--	---	--
Johnston et al. (1950)	6	1.050 - 1.131	1.079	0.077 - 0.196	--	---	--	0.174 - 0.444	0.281	---	--

Urinary excretion did not appear to be related to calcium intake as also reported by Hegsted et al. (1952) and Nicolaysen et al. (1953). On the other hand, Wang et al. (1936) did find a relationship between urinary excretion and calcium intake.

The mean urinary calcium excretion was greater in the heavier subject (C). Wang et al. (1936) found urinary calcium to be independent of body weight.

The amount of calcium in urine according to Boyce and King (1959) was related to the presence of unidentified complexing agents and to factors which influenced tubular transport mechanisms. Nicolaysen et al. (1953) postulated an endogenous factor or factors were responsible for the amount excreted, whereas Henneman (1959) considered urinary excretion of calcium to vary directly with the level of serum calcium.

Fecal Excretion. The mean daily fecal calcium for each period ranged from 0.536 to 1.049 g. and 0.632 to 0.792 g. for subjects A and C, respectively (Table 5). Mean fecal excretion for all periods for subject C (0.731 g./day) was lower than for subject A (0.826 g./day). This larger excretion and greater variation would appear to indicate subject A had poorer absorption. Personal observation of this subject would suggest that she might fit into a category proposed by Harrison (1959). He noted that emotional disturbances would decrease absorption of calcium even without obvious diarrhea. Subject A appeared to be less stable emotionally than the other girls. Carmine capsules which she ingested before breakfast would appear in the feces in the afternoon of the same day.

Both subjects had greater mean fecal excretions of calcium than Sherman and Hawley's (1922) subject (Table 6). The ranges for both subjects were within the ranges reported by other investigators (Wang et al., 1936; Johnston, 1941; and Willard and Blunt, 1927). Hollinger and Pattee (1956) considered 0.4 to 0.8 g. as the average range for fecal excretion. They attributed most of this loss to unabsorbable food calcium. Johnston et al. (1950) observed little variation in fecal calcium from one individual to another, with one exception. Wang et al. (1936) obtained a close correlation between intake and fecal calcium loss. This was not verified by these data.

Subject A had the greatest fecal excretion during Part III when milk chocolate candy was consumed (Table 5). The diet during this part of the study could be compared with diets containing large amounts of cocoa. Bricker et al. (1949) found the calcium requirement was lower on a diet without cocoa than on one with cocoa, but the difference was not statistically significant. They did observe a significant effect of cocoa on the pathway of calcium excretion. When comparing periods with and without cocoa, the former caused urinary calcium to decrease and fecal calcium to increase. This effect also was noted in subject A. The greatest amounts of fecal calcium were found during Part III when chocolate was ingested. These losses did not seem to be related to the mean intakes of calcium. In addition, urinary excretion in subject A was lower in Part III when chocolate was fed. Subject C exhibited these same trends to a lesser degree.

Absorption. The mean percent and ranges in percent for absorption of calcium for the nine periods appear in Table 5. These values were 3.3 (-23.0 to +46.4) percent and 14.9 (-15.6 to +34.9) percent for subjects A and C, respectively. The percent absorption was much lower than that reported by Johnston et al. (1950). She found 38.2 (range 26.3 to 51.1) percent absorption as the mean for six subjects on calcium intakes of 1.051 to 1.131 (Table 6).

Howard (1957) indicated that under normal circumstances, little more than 0.100 g. calcium would be absorbed daily. Mean absorption in the subjects in the present study was 0.043 and 0.144 g. daily. Boyce and King (1959) observed that absorption of calcium was related more closely to need than to intake.

Brine and Johnston (1955) stated that the endogenous calcium in feces was composed mainly of calcium from digestive juices. After an extensive study of the literature, these workers agreed with Howard (1957) that about 0.5 g. calcium would be excreted into the gastrointestinal tract daily. However, part of this endogenous calcium is reabsorbed and would not appear in the feces.

Retention. Daily calcium retentions for all periods ranged from -0.288 to +0.278 g. for subject A and from -0.292 to +0.161 g. for subject C as seen in Table 5. Mean daily retentions were -0.081 g. and -0.034 g. for subjects A and C, respectively. A calcium intake of approximately 0.9 g. was needed to achieve positive balance in these two girls. Losses for both girls

were larger than any reported in the literature (Table 6). McKay et al. (1942) stated that with a mean calcium intake of less than 0.8 g., losses of calcium were more frequent than retentions.

Mean daily calcium retentions of -11.0 (range -36.8 to +27.8) percent and -5.8 (range -44.1 to +16.1) percent were found for subjects A and C, respectively (Table 5). These were much lower than the 26.0 (16.0 to 41.0 range) percent reported by Johnston et al. (1950) and the 45.0 to 52.0 percent reported by Willard and Blunt (1927) (Table 6).

Steggerda and Mitchell (1946) investigated the relationship of body size and adult calcium requirement. Within a moderate range, body size had little effect on requirement as compared with other causes of variation. They found the daily requirement was 0.343 g./sq.m. Subject A retained -0.048 g. calcium/sq.m. of body surface (Table 7), and subject C retained -0.019 g. calcium/sq.m. Thus, the subject with the larger surface area retained more calcium.

In regard to retention per unit of body weight, the subjects varied considerably. Subject A had a mean daily retention for all periods of -0.002 g./kg. body weight, and subject C had a mean retention of -0.0005 g./kg. body weight (Table 7). The ranges for various periods were -0.005 to +0.004 g./kg. and -0.004 to +0.002 g./kg. body weight for subjects A and C, respectively. Data in Tables 5 and 6 show the highest retentions were less than the lowest values reported in the literature. This may be because of the higher dietary intakes for subjects reported in

Table 7. Mean daily retention of calcium.

Period	Retention per unit: Body weight g./kg.	Retention per unit: Surface area g./sq. m.
	Subject A	
Part I		
1	-0.002	-0.072
2	-0.002	-0.086
3	0.001	0.039
Part II		
4	0.004	0.165
5	-0.002	-0.062
6	-0.003	-0.101
Part III		
7	-0.005	-0.171
8	-0.005	-0.168
9	-0.000	0.021
Mean	-0.002	-0.048
	Subject C	
Part I		
1	-0.001	-0.034
2	0.001	0.044
3	0.002	0.092
Part II		
4	0.001	0.028
5	-0.002	-0.081
6	-0.004	-0.166
Part III		
7	-0.003	-0.098
8	0.000	0.008
9	0.001	0.034
Mean	-0.0005	-0.019

the literature. Wang et al. (1936) observed no close relationship between retention and intake or retention and body weight as shown by low coefficients of correlation. Sherman and Hawley (1922) claimed calcium retention showed a closer relationship to size than phosphorus.

Another means of judging calcium retention was advocated by Johnston et al. (1950). They studied the total increment of calcium from birth to adulthood. Increments during growth were correlated with weight increases. The age when accelerated growth occurred was related to the age at menarche. Therefore, the daily increment for adolescent girls was estimated for early, middle, and late menarche. Johnston et al. indicated their subjects retained more than the estimated requirement. However, the subject, who needed the least amount by this estimation, retained the least, and the one who needed the most, retained most. Using the table these workers prepared, subjects A (medium menarche) and C (early menarche) needed to retain 0.189 and 0.090 g. calcium daily. Both subjects retained far less than this estimate. In contrast to the findings of Johnston et al. (1950), the girl in the present study who needed more calcium retained less and the one who needed less, retained more.

Calcium-to-Phosphorus Ratio. Patton et al. (1953) related greater calcium retention with a calcium to phosphorus ratio of less than one. They found the absolute amounts of dietary calcium to be more significant in determining balance than the ratio. The calcium-to-phosphorus ratio in different periods of the present study varied from 0.57 to 0.93 for subject A and from 0.56 to 0.92 for subject C (Table 8). Since all of these ratios were less than one, positive calcium retentions should have occurred. In actuality, both girls exhibited negative retentions, which probably were caused by their low dietary intakes.

Table 8. The calcium-to-phosphorus ratios of mean daily intakes.

Period	Ca:P	Ca:P
	Subject A	Subject C
Part I		
1	0.80	0.78
2	0.85	0.84
3	0.78	0.77
Part II		
4	0.93	0.92
5	0.83	0.82
6	0.57	0.56
Part III		
7	0.64	0.64
8	0.78	0.77
9	0.89	0.88

Requirement. An intake of approximately 0.9 g. calcium was necessary to maintain positive balance in the two subjects (Table 5). This study affirmed the NRC recommended daily allowance (1.3 g.) to maintain positive balance and to allow for a safety margin and individual variation.

Phosphorus

Dietary Intakes. Mean daily phosphorus intakes for the entire study of 1.114 g. for subject A and 1.133 g. for subject C are shown in Table 9. These intakes were below the amount (1.3 g.) suggested by the NRC (1958). The intakes during the nine periods ranged from 1.039 to 1.272 g. for subject A and from 1.044 to 1.297 g. for subject C. Intakes varied because foods (butter cookies and soft drinks) that provided additional calories contained phosphorus. During Period 7, 0.222 g., and during Periods 8 and 9, 0.253 g. phosphorus were from milk

Table 9. Mean daily intake, excretion, absorption and retention of phosphorus.

Period	Intake	Excretion: Urine	Excretion: Feces	Absorption		Retention	
	g.	g.	g.	g.	%	g.	%
			Subject A				
Part I							
1	1.066	0.789	0.543	0.523	49.1	-0.266	-25.0
2	1.013	0.783	0.555	0.458	45.2	-0.325	-32.1
3	1.272	0.802	0.554	0.718	56.4	-0.084	- 6.6
Part II							
4	1.072	0.826	0.558	0.514	47.9	-0.312	-29.1
5	1.040	0.812	0.556	0.484	46.5	-0.328	-31.5
6	1.155	0.535	0.484	0.671	58.1	0.136	11.8
Part III							
7	1.233	0.631	0.542	0.691	56.0	0.060	4.9
8	1.139	0.917	0.567	0.572	50.2	-0.345	-30.3
9	1.039	0.738	0.527	0.512	49.3	-0.226	-21.8
Mean	1.114	0.759	0.543	--	51.0	-0.188	-17.7
			Subject C				
Part I							
1	1.089	0.882	0.523	0.566	52.0	-0.316	-29.0
2	1.044	0.739	0.469	0.575	55.1	0.164	15.7
3	1.297	0.863	0.459	0.838	64.6	-0.025	- 1.9
Part II							
4	1.088	0.960	0.527	0.561	51.6	-0.399	-36.7
5	1.064	0.704	0.461	0.603	56.7	-0.101	- 9.5
6	1.173	0.844	0.539	0.634	54.0	-0.210	-17.9
Part III							
7	1.224	0.866	0.476	0.748	61.1	-0.118	- 9.6
8	1.155	0.869	0.446	0.709	61.4	-0.160	-13.8
9	1.062	0.562	0.460	0.602	56.7	0.040	3.8
Mean	1.133	0.810	0.484	--	57.0	-0.121	-11.0

chocolate candy.

Urinary Excretion. Daily urinary phosphorus means for all periods were 0.759 g. (A) and 0.810 g. (C). During the various periods, subjects A and C ranged from 0.535 to 0.917 g. and from 0.562 to 0.960 g., respectively (Table 9). These ranges approximate those of Willard and Blunt (1927) and of Wang et al. (1936) (Table 10). Hollinger and Pattee (1956) estimated daily urinary excretion of phosphorus would be 1.0 g.

Hollinger and Pattee (1956) proposed that phosphorus intake was low if 70 percent of the total phosphorus excretion was in the urine. The mean percent of total phosphorus excretion for all periods found in the urine of subject A was 58 and subject C was 63. Using this criterion, the phosphorus intake was not low.

Fecal Excretion. Mean fecal excretions of phosphorus for the study were 0.543 g. for subject A and 0.484 g. for subject C (Table 9). Ranges for the different periods were from 0.484 to 0.567 g. and from 0.446 to 0.539 g. for subjects A and C, respectively. The amount in the feces was relatively constant for both girls. These losses were greatly above the losses reported by Willard and Blunt (1927) and Sherman and Hawley (1922), but within the range found by Wang et al. (1936) (Table 10). When fecal losses and intake were compared, no relationship was noted. This agreed with the work of Wang et al. (1936).

Absorption. In contrast to calcium, the girls exhibited positive absorption of phosphorus in all nine periods (Table 9). Mean percent absorptions were 51.0 for subject A and 57.0 for

Table 10. Daily phosphorus intake, excretion, and retention by a dolescent girls as reported in the literature.

Investigator	No. subjects	Intake		Excretion				Retention			
				Urine		Fecal					
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
		g.	g.	g.	g.	g.	g.	g.	g.	g./kg.	g./kg.
Sherman and Hawley (1922)	1	1.311 - 1.547	1.460	0.448 - 0.992	--	0.175 - 0.355	--	0.355 - 0.719	0.527	---	0.010
	1	0.886 - 2.009	--	---	--	---	--	0.165 - 0.665	--	0.005 - 0.019	--
Willard and Blunt (1927)	1	---	1.18	0.68 - 0.73	--	0.17 - 0.30	--	0.15 - 0.33	--	0.007 - 0.015	--
Wang et al. (1936)	16	1.276 - 2.081	1.708	0.684 - 1.130	0.981	0.359 - 0.894	0.530	-0.011 - 0.480	0.198	---	0.005

subject C.

Retention. Mean daily phosphorus retention for subject A was -0.188 g. (range -0.345 to +0.060 g.) and for subject C was -0.121 g. (range -0.399 to +0.164 g.). Data in Table 9 reveal that each girl achieved positive balance in only two periods. As with calcium, these retentions were far below most reports (Table 10). Wang et al. (1936) did have one subject in negative balance -0.011 g., but the group mean was 0.198 g. Sherman and Hawley (1922) and McKay et al. (1942) both reported a direct relationship between intake and retention. Wang et al. (1936) contradicted this belief. One of the subjects in the present study, had retentions which followed intakes in general. Since the highest retention was not linked with the highest intake, other factors apparently were affecting retention. The other subject (C) showed no apparent relationship between retention and intake.

The mean percents of phosphorus intake retained were -17.7 (A) and -11.0 (C) as stated in Table 9. The ranges in phosphorus retention for the various periods (-32.1 to +11.8 and -36.7 to +15.7 percent for subjects A and C, respectively) indicated great variation. These percent retentions were below the 13 and 29 percent reported by Willard and Blunt (1927) (Table 10).

No reports were found concerning intake of phosphorus per sq. m. of body surface. The subjects in the present study (Table 11) had mean daily retentions of -0.112 and -0.071 g./sq.m. body surface for the study. The smaller amount was retained by the larger subject (C). This contradicted Sherman and Hawley's

(1922) conclusion that phosphorus retention and body size were related directly.

Mean retention of phosphorus per unit of body weight for all periods was -0.003 g. for subject A and -0.002 g. for subject C (Table 11). The range from -0.006 to +0.002 g. was

Table 11. Mean daily retention of phosphorus.

Period	Retention per unit	
	Body weight	Surface area
	g./kg.	g./sq. m.
	Subject A	
Part I		
1	-0.004	-0.158
2	-0.005	-0.193
3	-0.001	-0.050
Part II		
4	-0.005	-0.186
5	-0.005	-0.195
6	0.002	0.081
Part III		
7	0.001	0.036
8	-0.006	-0.205
9	-0.004	-0.134
Mean	-0.003	-0.112
	Subject C	
Part I		
1	-0.005	-0.180
2	0.002	0.093
3	0.000	-0.014
Part II		
4	-0.006	-0.228
5	-0.002	-0.057
6	-0.003	-0.119
Part III		
7	-0.002	-0.067
8	-0.002	-0.091
9	0.001	0.023
Mean	-0.002	-0.071

identical for both subjects. Maximum mean daily retentions for these subjects were below the lowest retentions reported in the literature (Wang et al., 1936; Sherman and Hawley, 1922), Table 10.

Requirement. The largest dietary phosphorus intake in each subject was not associated with a positive balance. However, positive balance was obtained on lower intakes. Since high positive absorptions were associated with negative retentions, the phosphorus intakes were apparently larger than required for these subjects.

SUMMARY

Previous reports in the literature indicated individual adolescent girls varied widely in their ability to utilize calcium and phosphorus. In order to supplement present information, the calcium and phosphorus retentions of two 13-year-old girls were determined. Retention values were based on chemical analyses of the diet and excretory materials.

The balance study contained nine five-day periods. Food value tables were used to plan daily menus that contained approximately 1.0 g. calcium and 1.3 g. phosphorus and met the NRC recommended allowances for all other nutrients except iron. Two levels of iron were used-- 11 mg./day during Part I and 13 mg./day in Parts II and III. During Part III, the basic menus were adjusted to include a large amount of chocolate candy. Both subjects ate the basal diet but consumed different amounts of butter cookies and soft drinks to provide additional calories. Aliquot samples of food and excretory products were

preserved for analyses by wet ashing. Chemical analyses of the basal diet revealed it contained a mean of 16 percent less calcium and 24 percent less phosphorus than calculated from food value tables.

The analyses showed the two subjects had mean daily calcium intakes of 0.869 and 0.875 g. which were both below the NRC recommended allowance of 1.3 g. The interpretation of the data from this study were hampered by the low levels of intake as well as the variations in the levels. Mean urinary calcium losses were not related to intake as reported in the literature. The ranges for urine and fecal calcium were within the ranges reported in other studies of adolescent girls. Milk chocolate candy seemed to exert an adverse effect on calcium absorption. The negative mean daily calcium retentions of the subjects were probably caused by their low dietary intakes. Approximately 0.9 g. calcium was required to maintain calcium balance in the two subjects. This requirement confirmed the adequacy of the NRC allowance (1.3 g.) to maintain positive balance and allow for a safety margin and individual variation.

Mean daily dietary intakes of phosphorus were 1.114 and 1.133 g. which were below the amount suggested by the NRC. Urinary excretion was within the ranges reported for other adolescent girls, but fecal excretion was higher than other reports. The subjects were in negative balance during seven of the nine periods. The larger fecal excretion and high positive absorption of phosphorus were linked with negative retentions. This probably indicated that phosphorus intakes were larger than required for these subjects.

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LITERATURE CITED

Bauer, W., J. C. Aub, and F. Albright.
Studies of calcium and phosphorus metabolism. V. A study of the bone trabeculae as a readily available reserve supply of calcium. J. Exptl. Med. 49:145-162. 1929.

Bowes, A. deP., and C. F. Church.
Food values of portions commonly used. 7th ed., Philadelphia: College Offset Press, 1951.

Boyce, W. H., and J. S. King, Jr.
Effects of high calcium intakes on urine in human beings. Federation Proc. 18:1102-1111. 1959.

Breiter, H., R. Mills, J. Dwight, B. McKey, W. Armstrong, and J. Outhouse. The utilization of the calcium of milk by adults. J. Nutrition. 21:351-362. 1941.

Bricker, M. L., J. M. Smith, T. S. Hamilton, and H. H. Mitchell.
The effect of cocoa upon calcium utilization and requirements, nitrogen retention and fecal composition of women. J. Nutrition. 39:445-461. 1949.

Brine, C. L., and F. A. Johnston.
Endogenous calcium in the feces of adult man and the amount of calcium absorbed from food. Am. J. Clin. Nutrition. 3:418-420. 1955.

Chaney, M. S., and K. Blunt.
The effect of orange juice on the calcium, phosphorus, magnesium, and nitrogen retention and urinary organic acids of growing children. J. Biol. Chem. 66:829-845. 1925.

Cohn, W. E., E. T. Cohn, and J. C. Aub.
Calcium and phosphorus metabolism: Clinical aspects. Ann. Rev. Biochem. 11:415-440. 1942.

Davis, G. K.
Effects of high calcium intakes on the absorption of other nutrients. Federation Proc. 18:1119-1123. 1959.

Elvehjem, C. A., and W. H. Krehl.
Inbalance and dietary interrelationships in nutrition. J. Am. Med. Assoc. 135:279-287. 1947.

Eppright, E. S., and C. Roderuck.
Diet and nutritional status of Iowa school children. Am. J. Public Health. 45:464-471. 1955.

Eppright, E. S., V. D. Sidwell, and P. P. Swanson.
Nutritive value of the diets of Iowa school children.
J. Nutrition. 54:371-388. 1954.

Fiske, C. H., and Y. Subbarow.
The colorimetric determination of phosphorus. J. Biol.
Chem. 66:375-400. 1925.

Food and Nutrition Board, National Research Council.
Recommended dietary allowances. Washington, D. C.:
National Academy of Sciences. Publication 589, 1958.

Fuqua, M. E., and M. B. Patton.
Effect of three levels of fat intake on calcium metabolism.
J. Am. Dietet. Assoc. 29:1010-1013. 1953.

Greenberg, D. M.
Mineral metabolism. Calcium, magnesium, and phosphorus.
Ann. Rev. Biochem. 8:269-300. 1939.

Greenberg, D. M.
Studies in mineral metabolism with the aid of artificial
radioactive isotopes. VI. Tracer experiments with radio-
active calcium and strontium on the mechanism of vitamin D
action in rachitic rats. J. Biol. Chem. 157:99-104.
1945.

Harrison, H. E.
Factors influencing calcium absorption. Federation Proc.
18:1025-1092. 1959.

Harrison, H. E., and H. C. Harrison.
Studies with radiocalcium: the intestinal absorption of
calcium. J. Biol. Chem. 188:83-90. 1951.

Hathaway, M. L., and R. M. Leverton.
Calcium and phosphorus. Food: The Yearbook of Agriculture.
U.S.D.A. Washington: Government Printing Office, 1959.

Hegsted, D. M., I. Moscoso, and C. Collazos Ch.
A study of the minimum calcium requirements of adult man.
J. Nutrition. 46:181-201. 1952.

Henneman, P. H.
Effects of high calcium intakes on renal function.
Federation Proc. 18:1093-1096. 1959.

Hollinger, H. Z., and C. J. Pattee.
A review of normal calcium and phosphorus metabolism.
Can. Med. Assoc. J. 74:912-921. 1956.

Holmes, J. O.
The requirement for calcium during growth. Nutrition Abstr. Revs. 14:597-612. 1945.

Howard, J. E.
Calcium metabolism, bones, and calcium homeostasis. A review of certain current concepts. J. Clin. Endocrino. and Metabolism. 17:1105-1123. 1957.

Johnston, F. A., T. J. McMillan, and G. D. Falconer.
Calcium retained by young women before and after adding spinach to the diet. J. Am. Dietet. Assoc. 28:933-938. 1952.

Johnston, F. A., D. Schlaphoff, and T. McMillan.
Calcium retained from one level of intake by six adolescent girls. J. Nutrition. 41:137-147. 1950.

Johnston, J. A.
Factors influencing retention of nitrogen and calcium in period of growth: III. Puberty in the normal girl and in the girl with minimal reinfection type of tuberculosis. Am. J. Diseases Children. 59:287-309. 1940.

Johnston, J. A.
Factors influencing retention of nitrogen and calcium in period of growth: IV. Effect of estrogen. Am. J. Diseases Children. 62:708-715. 1941.

Johnston, J. A., and J. W. Maroney.
Factors affecting retention of nitrogen and calcium in period of growth. II. Effect of thyroid on calcium retention. Am. J. Diseases Children. 58:1186-1198. 1939.

Leichsenring, J. M., A. Biester, H. Roberts, P. P. Swanson, M. R. Gram, R. M. Leverton, W. Brewer, and L. M. Burrill.
Methods used for human metabolic studies in the North Central region. Minn. Agr. Exp. Sta. Tech. Bull. 225. 1958.

Leichsenring, J. M., L. M. Norris, S. A. Lamison, E. D. Wilson, and M. B. Patton. The effect of level of intake on calcium and phosphorus metabolism in college women. J. Nutrition. 45:407-418. 1951.

Leitch, I.
The determination of the calcium requirement of man. Nutrition Abstr. Revs. 6:553-578. 1937.

Manunta, G., J. Saroff, and C. W. Turner.
Relationship between estradiol and parathyroid on retention of Ca^{45} in bone and blood serum of rats. Soc. Proc. Exp. Biology Med. 94:785-787. 1957a.

Manunta, G., J. Saroff, and C. W. Turner.
Metabolism of Ca^{45} in blood, bones, and young of lactating rats treated with estradiol. Soc. Proc. Exp. Biology Med. 94:788-789. 1957b.

McCrudden, F. H.
The determination of calcium in the presence of magnesium and phosphates: the determination of calcium in urine. J. Biol. Chem. 10:187-199. 1911.

McKay, H., M. B. Patton, M. A. Ohlson, M. S. Pittman, R. M. Leverton, A. G. Marsh, G. Stearns, and G. Cox. Calcium, phosphorus, and nitrogen metabolism of young college women. J. Nutrition. 24:367-384. 1942.

McKay, B. V., E. Smith, and J. M. Smith.
Calcium requirement of seven adolescent girls. Federation Proc. 7:293-294. 1948.

Mickelsen, O.
Symposium on effects of high calcium intakes. Federation Proc. 18:1075-1076. 1959.

Morgan, A. F.
Nutritional status U.S.A. An interregional research publication. Calif. Agr. Exp. Sta. Bull. 769. 1959.

Neuman, W. F., and M. W. Neuman.
Emerging concepts of the structure and metabolic functions of bone. Am. J. Med. 22:123-131. 1957.

Nicolaysen, R., N. Eeg-Larsen, and O. J. Malm.
Physiology of calcium metabolism. Physiol. Revs. 33:424-444. 1953.

Odland, L. M., K. P. Warnick, and N. C. Esselbaugh.
Cooperative nutritional status studies in the Western region. II. Bone density. Mont. Agr. Exp. Sta. Tech. Bull. 534. 1958.

Ohlson, M. A., and G. Stearns.
Calcium intake of children and adults. Federation Proc. 18:1076-1085. 1959.

Patton, M. B., E. D. Wilson, J. M. Leichsenring, L. M. Norris, and C. M. Dienhart. The relation of calcium-to-phosphorus ratio to the utilization of these minerals by 18 young college women. J. Nutrition. 50:373-382. 1953.

Sherman, H. C.
Calcium and phosphorus in foods and nutrition. New York: Columbia University Press, 1947.

Sherman, H. C., and E. Hawley.
Calcium and phosphorus metabolism in childhood. J. Biol. Chem. 53:375-399. 1922.

Stearns, G.
Human requirement of calcium, phosphorus, and magnesium. J. Am. Med. Assoc. 142:478-485. 1950.

Steggerda, F. R., and H. H. Mitchell.
Variability in calcium metabolism and calcium requirements of adult human subjects. J. Nutrition. 31:407-422. 1946.

Thomas, R. V., M. M. Rutledge, E. B. Beach, E. Z. Moyer, M. C. Drummond, I. Miller, A. R. Robinson, O. N. Miller, M. N. Coryell, and I. G. Macy. Nutritional status of children. XIII. Accuracy of calculated intakes of food components with respect to analytical values. J. Am. Dietet. Assoc. 26:889-896. 1950.

Topper, A., and H. Mulier.
Basal metabolism of normal children. The puberty reaction. Am. J. Diseases Children. 43:327-336. 1932.

Tucker, R. E., F. W. Chalmers, H. N. Church, M. M. Clayton, W. D. Foster, L. O. Gates, G. C. Hagan, B. F. Steele, A. W. Wertz, and C. M. Young. Cooperative nutritional status studies in the Northeast region. IV. Dietary findings. Rhode Island Agr. Exp. Sta. Bull. 319. 1952.

Wang, C. C., M. Kaucher, and M. Frank.
Metabolism of undernourished children: IV. Calcium metabolism. Am. J. Diseases Children. 35:856-861. 1928.

Wang, C. C., M. Kaucher, and M. Wing.
Metabolism of adolescent girls: IV. Mineral metabolism. Am. J. Diseases Children. 52:41-53. 1936.

Watt, B. K., and A. L. Merrill.
Composition of foods--raw, processed, prepared. Bur. Human Nutr. and Home Econ., Agr. Res. Admin., U.S.D.A. Handbook No. 8, 1950.

Whedon, G. D.
Effects of high calcium intakes on bones, blood and soft tissue; relationship of calcium intake to balance in osteoporosis. Federation Proc. 18:1112-1118. 1959.

Wilcox, E. B., H. L. Gillum, and M. M. Hard.
Cooperative nutritional status studies in the Western region. I. Nutrient intake. Utah Agr. Exp. Sta. Bull. 383. Undated.

Willard, A. C., and K. Blunt.
A comparison of evaporated with pasteurized milk as a source of calcium, phosphorus, and nitrogen. J. Biol. Chem. 75:251-262. 1927.

Williams, D. E., B. B. McDonald, E. Morrell, F. A. Schofield, and F. L. MacLeod. Influence of mineral intake on bone density in humans and in rats. J. Nutrition. 61:489-505. 1957.

Williams, D. E., and A. Samson.
Bone density of East Indian and American students. J. Am. Dietet. Assoc. 36:462-465. 1960.

APPENDIX

Collection and Preservation of Samples

Unless otherwise stated, all reagents conformed in purity to Recommended Specifications for Analytical Reagent Chemicals of the American Chemical Society, and acids were concentrated. Water was distilled and further purified in a demineralizer (Crystalab Deeminizer Model C1-5, Crystal Research Laboratories, Inc., Hartford, Connecticut). Samples were stored in narrow mouth, 300 cc. glass bottles with plastic screw lids and sealed with paraffin. A paraffin coating prevented gummed labels from drying and falling off the bottles.

Food. Food was weighed in the form in which it was served, e.g., boiled, baked, broiled, steamed, or raw. Inedible parts (bones, rinds, or egg shells) were removed before weighing. Five-day composites of several foods with similar physical and chemical properties were prepared and converted into digests. Food samples were divided into liquid, fatty, and solid composites. A brown (acid) digest was used to preserve the composites for analysis. A Harvard trip balance (Ohaus Scale Corp., Union, New Jersey) with a sensitivity of 0.1 g. was used for weighing.

Preparation of Liquid Food Composite. Aliquot portions (one-fifth) of all milk, fruit and vegetable juices, ice cream, sherbets, and soft custard consumed in one day were weighed. Water and tea were omitted. Soft drinks were analyzed separately. A record was kept of the weight of each food added to the composite.

Preparation of Fatty Food Composite. Aliquots (one-fifth)

of all butter, cream, mayonnaise, and French dressing were weighed and weights recorded. Butter cookies and chocolate each formed a separate digest. Since fat is difficult to handle in a digest, it was separated from the fatty foods. The foods were heated with 50 to 100 ml. water until the fat melted. Upon cooling, the solidified layer of fat was removed. The liquid was added quantitatively to the liquid food composite. Solid particles which appeared in this composite were separated by straining and then added to the solid food composite.

Preparation of Solid Food Composite. This composite included all foods not covered in the other composites. The composites were stored in beakers at refrigerator temperature until a five-day period was completed.

Preparation of Brown (Acid) Digest. Brown digests of each food composite were made with HCl. The food composite was transferred quantitatively to an electric blender (Waring). If the composite was too thick to permit thorough blending, water was added. A known weight of the total composite was transferred to an Erlenmeyer flask. From 100 to 150 ml. HCl per one liter of blended solid food was added. The flask was heated on a hot plate set at low heat. To prevent charring and to mix the contents, the flask was swirled occasionally. The heat treatment continued until the mixture was uniform in consistency, brown in color, and evaporated to an amount that could be diluted to the desired volume. The contents and water rinsings from the Erlenmeyer flask were added to a weighed volumetric flask before diluting to volume with water. The volumetric flask plus its

contents was weighed. Contents of the flask were mixed by inverting and rotating 50 times. Two 300 cc. glass bottles were filled. The flask was inverted and rotated 25 times before filling the second bottle. The digests were stored at room temperature in paraffin-sealed glass bottles.

Feces. On the first day and on the morning following the last day of the balance period, a carmine capsule was taken before breakfast. To allow for any fecal lag at the end of the experiment, the subjects continued on the weighed diet until the carmine was obtained. This additional time was not considered part of the balance period but was used to avoid drastic diet changes which might alter gastrointestinal action.

Preparation of Fecal Composite. The composite contained all feces from the first appearance of the carmine, up to, but not including, those colored by the next capsule. From the first fecal collection, that part which preceded the carmine was discarded. During the collection period, the feces were rinsed with water from the cartons into a two liter wide-mouth Erlenmeyer flask containing enough HCl so that the final concentration of acid in the brown digest was approximately 10 percent. The covered flask was kept in a refrigerator until the collection was completed.

Preservation for Analysis. The fecal composite was preserved for analysis by preparing a brown digest. Directions previously given for food were followed omitting the steps using an electric blender.

Urine. On the first day of the collection period, the bladder was emptied immediately after arising. The urine collected upon rising was considered a part of the urine for the preceding day.

Preparation of Urine Composite. The 24-hour sample was mixed by inverting and rotating the bottle 20 times. A graduated cylinder was used to measure volume and to obtain an aliquot (one-fifth) of each day's sample. Volume was recorded. The daily aliquots were combined in a stoppered container and stored at refrigerator temperature. A composite covered a five-day period.

Preservation for Analysis. The urine composite was transferred to an Erlenmeyer flask and 100 ml. HCl added. The composite was preserved for analysis by preparing a brown digest. Directions previously given for food were followed omitting the steps using an electric blender.

Ashing Procedure for Calcium and Phosphorus Determinations

Samples were pipetted into weighed porcelain crucibles and weighed. Crucibles were heated on a hot plate until the sample was charred (three to five hours). Then, the crucibles were placed in a cold muffle, and the temperature gradually raised to 550°C. The samples were left in the muffle until the ash was white. If the sample did not turn white, it was cooled and a few drops of dilute HCl (1:3) were added before repeating the ashing. Samples were cooled. The residue was dissolved in 20 ml. concentrated HCl, heated, and filtered through moistened

filter paper into a 100 ml. volumetric flask. After washing the filter paper with water, the filtrate was made to volume.

Calcium Determination

A modification of McCrudden's (1911) method was used.

Special Reagents.

0.04 Percent Bromcresol Purple. A solution of 3.7 ml. 95 percent alcohol and 0.1 g. bromcresol purple was diluted to 25 ml.

20 Percent Sodium Acetate. A 20 percent solution of the hydrated salt was prepared.

Dilute NH_4OH. Each liter contained 20 ml. concentrated reagent.

Standard Permanganate. From 0.31 to 0.32 g. $KMnO_4$ was weighed and diluted to one liter. This solution was stored in a brown bottle at room temperature for three to five days before use. Permanganate solutions were standardized with 0.01 N sodium oxalate solution at least once a week.

Standard Sodium Oxalate Solution. Sodium oxalate was dried at 110°C. for two hours and cooled in a desiccator over H_2SO_4. Exactly 0.6700 g. of the dry salt was dissolved in approximately normal H_2SO_4 and diluted to a liter with normal H_2SO_4. This solution was stable for two months. To titrate, five ml. of this solution was pipetted into a beaker and heated to 90°C. Permanganate titrations began and ended slowly. The liquid was kept at approximately 90°C. during titration.

Procedure. Sample size was regulated to have titration values between two and 10 ml. $KMnO_4$. The sample was pipetted into a 250 ml. Pyrex beaker and neutralized with concentrated NH_4OH using bromcresol purple as an indicator. Then, concentrated HCl was added until the color just changed to a definite yellow. Constant stirring was required when 10 ml. of 2.5 percent oxalic acid and 10 ml. 20 percent sodium acetate were added.

The beakers were covered with watch glasses and allowed to precipitate overnight (12 to 18 hours). The calcium oxalate was obtained by filtering with filter crucibles. The beaker and filter were washed two to three times with dilute NH_4OH. After the outside of the crucible was rinsed with distilled water, the crucible was placed in the beaker. The precipitate was dissolved in 50 ml. of approximately normal H_2SO_4. The beaker was heated in boiling water for several minutes. The temperature was maintained between 80 and 90°C. while titrating with 0.01 N $KMnO_4$.

Calculations.

$$(\text{Titration value} - \text{Blank}) \times \frac{\text{Normality } KMnO_4}{0.01} \times 0.2$$

$$\times \frac{\text{Weight of brown digest}}{\text{Weight of sample of brown digest}} \times \frac{\text{Volume of wet ash}}{\text{sample of wet ash}}$$

$$\times K = \text{mg. of calcium/day.}$$

For diets and urine $K = \frac{1}{\text{Aliquot portion}} \times \frac{1}{\text{Days in period}}$

For feces $K = \frac{1}{\text{Days in period}}$.

Phosphorus Determination

The method of Fiske and Subbarow (1925) was used.

Special Reagents.

2.5 Percent Ammonium Molybdate in 5 N H_2SO_4. Water (200 ml.) was used to dissolve 25 g. of the salt. This solution was rinsed into a liter volumetric flask containing 500 ml. 10 N H_2SO_4 and made to volume with distilled water.

10 N H_2SO_4. This solution contained 450 ml. concentrated acid poured into 1500 ml. water.

Standard Phosphate Solution. The phosphorus content of this solution was 0.4 mg./5 ml. Exactly 0.3509 g. pure KH_2PO_4 was dissolved in water. After quantitative transfer to a liter volumetric flask, 10 ml. of 10 N H_2SO_4 were added before diluting to volume.

Phenolphtalein. This indicator consisted of 1.0 g. phenolphtalein in 100 ml. 95 percent alcohol.

0.25 Percent Aminonaptholsulfonic Acid. A graduated cylinder was used to dissolve 40 g. $Na_2S_2O_5$ and 2 g. Na_2SO_3 in 250 ml. water. Aminonaptholsulfonic acid (1.0 g.) was added before diluting to 400 ml. The cylinder was shaken until all reagents dissolved. After filtering, the solution was stored in a brown bottle. New reagent was required at least every two weeks.

Procedure. An amount of ash solution which gave the most accurate reading was placed in a 100 ml. volumetric flask. One drop of phenolphthalein served as an indicator. Concentrated NH_4OH was added until the solution was faintly pink. After each addition of reagent--10 ml. 2.5 percent ammonium molybdate in 0.5 N H_2SO_4 and four ml. 2.5 percent aminonaptholsulfonic acid--the contents were mixed gently. The sample was diluted to volume with distilled water.

Six minutes later, the samples were read in an Evelyn Colorimeter with a No. 660 filter, and the reagent blank set at 100. A curve using standard phosphate solution was used to compare samples.

Calculations.

$$\text{Reading on curve} \times \frac{\text{Weight of brown digest}}{\text{Weight of sample of brown digest}}$$

$$\times \frac{\text{Volume of wet ash}}{\text{Sample of wet ash}} = \text{mg. phosphorus/digest.}$$

Menus for Part I

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 1	Peaches, frozen	100	Frankfurter	60	Ham	100	Coca Cola	199
	Egg	54	Bun, unenriched	40	Potatoes, boiled	84		
	Biscuits, unenriched	40	Catsup	17	Beets, canned	42		
	Butter	7	Celery, raw	20	Bread, unenriched	25		
	Strawberry Jelly	10	Carrots, raw	50	Butter	5		
	Milk, whole	183	Potato Chips	25	Angel Cake	45		
			Orange Sections	97	Raspberries, frozen	80		
			Coconut	3.9	Sugar	10		
			Milk, whole	244	Milk, whole	244		
Day 2	Apricots, canned, sirup	125	Tuna	60	Roast Beef, rolled rib	75	Coca Cola	199
	Oatmeal, cooked	177	Noodles, cooked	100	Green Beans, frozen	85	Popcorn	28
	Cream, 20%	60	Green Pepper, raw	10	Butter	10	Butter	10
	Sugar	4	Ritz Cracker	9.3	Minted Pear, canned	75	Iced Tea	
	Toast, unenriched	25	Milk, whole	30	Lettuce	25		
	Butter	7	French Bread, unenriched	40	Bread, unenriched	25		
	Milk, whole	244	Butter	7	Butter	7		
			Orange Slices	150	Blanc Mange	125		
			Coconut	3.9				
			Milk, whole	244				
Day 3	Orange Juice, frozen	308	Ground Round Steak	70	Tenderloin Steak	60	Coca Cola	199
	Ground Pork Shoulder	40	Bun, unenriched	40	Corn, frozen	100	Potato Chips	20
	Toast , unenriched	25	Catsup	17	Lettuce	50	Butter	5
	Raspberry Jelly	10	Potato Chips	20	French Dressing	10	Sugar	10
	Milk, whole	244	Carrots, raw	25	Bread, unenriched	25	Iced Tea	
			Celery, raw	40	Butter	7		
			Apple, A P	150	Orange Sherbet	192		
			Milk, whole	244	Milk, whole	100		

Menus for Part I (concl.)

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 4	Applesauce, canned	100	Salmon, red, drained	50	Ground Round Steak, raw	125	Coca Cola	199
	Biscuits, unenriched	40	Cheddar Cheese	33	Oyster Crackers	10	Brazil Nuts	36
	Grape Jelly	20	Sweet Pickle	5	Tomato Juice, canned	25	Iced Tea	
	Milk, whole	100	Bread, unenriched	50	Peas, frozen	100		
			Butter	5	Potato, boiled	75		
			Mayonnaise	10	Carrots, raw	25		
			Cabbage, Slaw	50	Raisins	3.3		
			Dressing	18	Bread, unenriched	25		
			Pears, canned	100	Butter	20		
			Sugar Cookies	26	Jello, dry	21.7		
			Milk, whole	244	Sugar	20		
Day 5	Grapefruit Sections	100	Bologna	80	Tomato Juice, canned	125	Coca Cola	199
	Egg	54	Bread, unenriched	50	Pork Chop	90	Hershey Goodbar	18
	Toast, unenriched	25	Butter	7	Rice	84	Sugar	10
	Butter	5	Lettuce	50	Broccoli, frozen	70		
	Honey	20	French Dressing	15	Cranberry Jelly	30		
	Milk, whole	244	Cherries, canned	100	Lettuce	25		
			Milk, whole	229	French Bread, unenriched	40		
					Butter	5		
					Vanilla Ice Cream	124		

Menus for Part II

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 1	Peaches, frozen	100	Frankfurter	60	Ham	125	Coca Cola	199
	Egg	54	Bun, enriched	40	Potatoes, boiled	84		
	Biscuit, enriched	40	Catsup	17	Beets, canned	42		
	Butter	2	Celery, raw	20	Green Olives	39		
	Strawberry Jelly	10	Carrots, raw	50	Bread, enriched	25		
	Milk, whole	83	Potato Chips	25	Angel Cake	45		
			Orange Sections	97	Strawberries, frozen	100		
			Coconut	11.7	Sugar	10		
			Milk, whole	244	Milk, whole	244		
Day 2	Apricots, dried	75	Tuna	70	Roast Beef, rolled rib	75	Coca Cola	199
	Oatmeal, cooked	115	Noodles, cooked	100	Green Beans, frozen	85	Popcorn	28
	Cream, 20%	60	Green Pepper, raw	30	Butter	10	Butter	10
	Sugar	4	Ritz Crackers	18.6	Minted Pear, canned	75	Iced Tea	
	Toast, enriched	25	Milk, whole	30	Lettuce	25		
	Butter	7	French Bread, enriched	40	Raisins	13		
	Milk, whole	244	Butter	7	Bread, enriched	25		
			Orange Slices	150	Butter	7		
			Coconut	7.8	Blanc Mange	100		
			Milk, whole	244				
Day 3	Orange Juice, frozen	308	Ground Round Steak	70	Apple Juice, canned	187	Coca Cola	199
	Ground Pork Shoulder	40	Bun, enriched	40	Tenderloin Steak	70	Potato Chips	20
	Toast, enriched	25	Catsup	17	Corn, frozen	100	Butter	5
	Raspberry Jelly	10	Potato Chips	25	Lettuce	50	Iced Tea	
	Milk, whole	244	Carrots, raw	25	French Dressing	10		
			Celery, raw	40	Sweet Pickle	30		
			Apple, A P	150	Bread, enriched	25		
			Milk, whole	244	Butter	7		
					Orange Sherbet	96		
					Milk, whole	100		

Menus for Part II (concl.)

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 4	Applesauce, canned	100	Salmon, red, drained	50	Ground Round Steak, raw	125	Coca Cola	199
	Biscuits, enriched	40	Cheddar Cheese	33	Oyster Crackers	10	Brazil Nuts	36
	Grape Jelly	10	Sweet Pickle	5	Tomato Juice, canned	25	Iced Tea	
	Milk, whole	100	Bread, enriched	50	Egg Yolk	17		
			Butter	5	Peas, frozen	100		
			Mayonnaise	10	Potato, boiled	75		
			Cabbage Slaw	50	Carrots, raw	25		
			Dressing	18	Raisins	13.3		
			Pears, canned	100	Bread, enriched	25		
			Sugar Cookies	26	Butter	10		
			Milk, whole	244	Jello, dry	21.7		
					Sugar	20		
Day 5	Grapefruit Sections	100	Bologna	80	Tomato Juice, canned	125	Hershey Goodbar	18
	Egg	54	Bread, enriched	50	Pork Chop	90		
	Toast, enriched	25	Butter	7	Rice	84		
	Butter	5	Lettuce	50	Broccoli, frozen	70		
	Honey	20	French Dressing	15	Cranberry Jelly	30		
	Milk, whole	244	Cherries, canned	100	Lettuce	25		
			Milk, whole	229	French Bread, enriched	40		
					Vanilla Ice Cream	124		
					Butterscotch Sauce	37		
					Sugar	5		

Menus for Part III

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 1	Peaches, frozen	80	Frankfurter	60	Ham	85	Hershey Bar	56.7
	Egg	54	Bun, unenriched	40	Potatoes, boiled	84	Hershey Kisses	60
	Biscuits, unenriched	40	Catsup	17	Beets, canned	42		
	Butter	5	Celery, raw	40	Bread, unenriched	25		
	Milk, skim	256	Carrots, raw	50	Butter	2		
			Green Olives	39	Angel Cake	45		
			Potato Chips	20	Strawberries, frozen	50		
			Coconut	11.7	Milk, skim	244		
			Orange Sections	97				
Day 2	Apricots, canned, sirup	75	Tuna	70	Roast Beef, rolled rib	75	Popcorn	14
	Oatmeal, cooked	84	Noodles, cooked	100	Green Beans, frozen	85	Hershey Bar	56.7
	Cream, 20%	60	Green Pepper, raw	10	Minted Pear, canned	75	Hershey Kisses	60
	Sugar	4	Ritz Crackers	18.6	Lettuce	25		
	Toast, unenriched	25	Milk, whole	30	Bread, unenriched	25		
	Butter	7	French Bread, unenriched	40	Butter	7		
	Milk, skim	200	Butter	7	Blanc Mange	50		
			Orange Slices	150				
			Coconut	3.9				
			Milk, skim	200				
Day 3	Orange Juice, frozen	308	Ground Round Steak	70	Tenderloin Steak	70	Hershey Bar	56.7
	Ground Pork Shoulder	40	Bun, unenriched	40	Corn, frozen	100	Hershey Kisses	60
	Toast, unenriched	25	Catsup	17	Lettuce	50	Iced Tea	
	Milk, skim	244	Carrots, raw	25	French Dressing	10		
			Celery, raw	40	Bread, unenriched	25		
			Potato Chips	25	Butter	10		
			Apple, A P	150	Orange Sherbet	96		
			Milk, skim	244	Sugar	5		

Menus for Part III (concl.)

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 4	Applesauce, canned	100	Salmon, red, drained	50	Ground Round Steak, raw	125	Hershey Bar	56.7
	Biscuits, unenriched	40	Cheddar Cheese	16	Oyster Crackers	10	Hershey Kisses	60
	Milk, skim	100	Sweet Pickle	5	Tomato Juice, canned	25	Iced Tea	
			Bread, unenriched	50	Egg yolk	17		
			Mayonnaise	10	Potato, boiled	75		
			Cabbage Slaw	50	Peas, frozen	80		
			Dressing	18	Carrots, raw	25		
			Pears, canned	100	Raisins	13.3		
			Milk, skim	244	Butter	15		
					Bread, unenriched	25		
					Jello, dry	21.7		
					Sugar	20		
Day 5	Grapefruit Sections	80	Bologna	80	Tomato Juice, canned	125	Hershey Goodbar	9
	Sugar	5	Bread, unenriched	50	Pork Chop	90	Hershey Bar	56.7
	Egg	54	Butter	2	Rice	84	Hershey Kisses	60
	Toast, unenriched	25	Lettuce	50	Broccoli, frozen	70		
	Milk, skim	100	French Dressing	5	Cranberry Jelly	6.5		
			Cherries, canned	100	Lettuce	25		
			Milk, skim	200	French Bread, unenriched	40		
					Butter	5		
					Vanilla Ice Cream	124		
					Butterscotch Sauce	19		

Table 12. Calculated nutrient intake of daily menus.[1]

Day	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A	Thiamine	Riboflavin	Niacin	Ascorbic acid
	Cal.	g.	g.	g.	mg.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.
Part I												
1	2256	79.8	106.9	250.6	1023	1226	9.6	9211	1.27	2.03	10.8	94
2	2257	82.6	104.5	256.5	1028	1437	9.4	5532	1.01	1.87	15.9	112
3	2224	87.8	95.9	269.3	1022	1370	9.4	5623	1.25	1.90	14.1	158
4	2259	82.0	111.4	242.0	1017	1467	10.0	6077	1.13	1.36	14.1	66
5	2250	80.0	106.3	250.9	966	1272	9.7	6046	1.46	1.84	12.2	128
Mean	2249	82.4	105.0	213.9	1011	1354	9.6	6498	1.22	1.80	13.4	112
Part II												
1	2337	83.0	111.6	258.3	942	1196	12.5	8817	2.26	2.04	13.3	124
2	2275	84.9	107.7	251.5	1017	1456	12.1	5634	2.52	1.91	19.2	109
3	2255	87.2	100.6	266.7	962	1362	12.2	5678	2.79	2.04	16.1	160
4	2250	85.0	108.6	242.8	1057	1577	13.0	6298	3.40	1.56	16.2	66
5	2288	80.4	108.2	259.6	1000	1290	12.6	6131	3.74	2.17	14.3	129
Mean	2281	84.1	107.3	255.8	996	1376	12.5	6512	2.94	1.94	15.8	118
Part III												
1	2392	80.0	117.2	264.1	1110	1308	12.6	8138	1.23	2.32	9.9	102
2	2291	85.4	106.8	256.7	1079	1475	11.5	3503	0.91	2.13	16.2	107
3	2318	91.2	107.4	261.8	1082	1471	12.1	4750	1.25	2.30	14.2	154
4	2275	82.9	101.3	265.4	1111	1466	13.0	5566	0.91	1.96	14.2	63
5	2365	81.9	114.7	260.3	1011	1339	13.0	5267	1.48	2.14	11.3	118
Mean	2328	84.3	109.5	261.7	1079	1412	12.4	5445	1.16	2.17	13.2	109

[1]Based on Watt and Merrill (1950) supplemented by Bowes and Church (1951).

Table 13. Calcium content of the solid food composites.[1]

Ashing	Part I Period 1	Part I Period 2	Part I Period 3	Part II Period 4	Part II Period 5	Part II Period 6	Part III Period 7	Part III Period 8	Part III Period 9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
A	0.184	0.119	--	0.201	0.148	0.289	0.246	0.106	0.147
	0.181	0.134	--	0.180	0.157	0.298	0.240	0.105	0.140
	0.178	0.113	--	0.214	--	0.282	0.249	0.126	0.155
Mean	0.181	0.122	--	0.198	0.152	0.290	0.245	0.112	0.147
B	0.174	0.148	0.237	0.199	--	0.273	0.247	0.106	0.153
	0.192	0.127	0.218	0.195	--	0.298	0.252	0.135	0.151
	0.171	0.112	0.219	--	--	0.263	0.251	0.122	0.168
Mean	0.179	0.129	0.225	0.197	--	0.278	0.250	0.121	0.157
C	0.150	0.099	0.279	0.176	0.200	0.267	0.283	0.122	0.155
	0.136	0.156	0.264	0.177	0.162	0.268	0.277	0.107	0.154
	0.151	0.147	0.300	0.206	0.163	0.288	0.245	0.101	0.158
Mean	0.146	0.134	0.281	0.186	0.175	0.274	0.268	0.110	0.156
Grand Mean	0.169	0.128	0.253	0.194	0.164	0.281	0.254	0.114	0.153

[1]Method of McCrudden (1911).

Table 14. Calcium content of the liquid food composites.[1]

Ashing	Part I Period 1	Part I Period 2	Part I Period 3	Part II Period 4	Part II Period 5	Part II Period 6	Part III Period 7	Part III Period 8	Part III Period 9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
A	0.799	0.756	0.705	0.816	0.694	0.368	--	0.466	0.444
	0.754	0.858	0.762	0.807	0.676	0.422	0.240	--	0.494
	0.690	0.750	0.696	0.812	0.707	0.373	0.218	0.430	0.495
Mean	0.748	0.788	0.721	0.812	0.692	0.388	0.229	0.448	0.478
B	0.573	--	0.719	0.781	0.669	0.335	0.228	0.449	0.463
	0.630	--	0.732	0.786	0.715	0.364	0.218	0.433	0.465
	0.601	--	0.801	0.790	0.668	0.349	0.311	0.473	--
Mean	0.601	--	0.751	0.786	0.684	0.349	0.252	0.452	0.464
C	--	0.672	0.732	0.773	0.693	0.361	0.279	0.534	0.528
	--	0.679	0.713	0.789	0.691	0.375	--	0.505	0.439
	--	0.663	0.736	0.821	0.702	0.365	0.248	--	0.471
Mean	--	0.671	0.727	0.794	0.695	0.367	0.264	0.520	0.479
Grand Mean	0.674	0.730	0.733	0.797	0.690	0.368	0.248	0.473	0.474

[1] Method of McCrudden (1911).

Table 15. Phosphorus content of the solid and liquid food composites.[1,2]

Ashing	Part I Period 1	Part I Period 2	Part I Period 3	Part II Period 4	Part II Period 5	Part II Period 6	Part III Period 7	Part III Period 8	Part III Period 9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
Solid Food Composite									
A	0.424	0.365	0.668	0.463	0.425	0.803	0.696	0.369	0.342
B	0.414	0.360	0.632	0.449	0.444	0.813	0.700	0.386	0.341
C	0.411	0.362	0.639	0.466	--	0.800	0.694	0.371	0.340
Mean	0.416	0.362	0.646	0.453	0.434	0.805	0.697	0.375	0.341
Liquid Food Composite									
A	0.583	0.594	0.566	0.552	0.528	0.276	0.227	0.446	0.389
B	0.592	0.580	0.552	0.536	0.519	0.273	0.230	0.449	0.384
C	0.578	--	0.567	0.542	0.523	0.279	0.226	0.437	0.390
Mean	0.584	0.587	0.562	0.543	0.523	0.276	0.228	0.444	0.388

[1] Each figure is a mean of two analyses.
[2] Method of Fiske and Subbarow (1925).

Table 16. Calcium and phosphorus contents of chocolate candy, butter cookies, and soft drinks.[1]

Ashing	Chocolate[2]	Chocolate[3]	Chocolate[4]	Butter cookies	Soft drink
	g.	g.	g.	g.	g.
			Calcium		
A	0.125	0.183	0.139	0.000	0.003
	0.137	0.173	0.142	0.012	0.000
	0.139	0.161	0.143	0.009	0.004
Mean	0.134	0.172	0.141	0.007	0.002
B	0.136	0.151	0.141	0.005	0.002
	0.125	0.153	0.138	0.003	0.000
	0.132	0.149	0.132	0.010	0.001
Mean	0.131	0.151	0.137	0.006	0.001
C	0.129	0.156	0.134	0.008	0.002
	0.129	0.156	0.139	0.002	0.000
	0.132	0.161	0.145	0.014	0.001
Mean	0.130	0.158	0.139	0.008	0.001
Grand Mean	0.132	0.160	0.139	0.007	0.001
			Phosphorus[5]		
A	0.103	0.132	0.116	0.015	0.035
B	0.104	0.139	0.119	0.014	0.034
C	0.106	0.134	0.119	0.015	0.034
Mean	0.104	0.135	0.118	0.015	0.034

[1] Methods of McCrudden (1911) for calcium and of Fiske and Subbarow (1925) for phosphorus.
[2] Chocolate kisses, Period 7.
[3] Chocolate kisses, Periods 8 and 9.
[4] Chocolate bars, Periods 7, 8, and 9.
[5] Each figure is a mean of two analyses.

Table 17. Calcium excreted in the urine by subject A.[1]

Ashing	Part I Period 1	Part I Period 2	Part I Period 3	Part II Period 4	Part II Period 5	Part II Period 6	Part III Period 7	Part III Period 8	Part III Period 9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
A	0.133	0.130	0.131	0.189	0.154	0.109	0.112	0.130	0.110
	0.128	0.122	0.167	0.190	0.156	0.113	0.107	0.128	0.120
	--	0.129	--	0.193	0.165	0.113	0.102	0.123	0.112
Mean	0.130	0.127	0.149	0.191	0.158	0.112	0.107	0.127	0.114
B	0.124	0.117	0.122	0.176	0.155	0.109	0.106	0.131	0.102
	0.120	0.125	0.145	0.174	0.164	0.108	0.099	0.129	0.101
	0.118	0.117	0.138	0.175	0.169	0.108	0.101	0.126	0.108
Mean	0.121	0.120	0.135	0.175	0.163	0.108	0.102	0.129	0.104
C	0.125	0.128	0.119	0.194	0.156	0.109	0.124	0.116	0.117
	0.143	0.113	0.171	0.190	0.158	0.106	0.108	0.115	0.116
	0.130	0.125	0.136	0.188	0.158	0.108	0.117	0.119	0.123
Mean	0.133	0.122	0,142	0.191	0.157	0.108	0.116	0.117	0.119
Grand Mean	0.128	0.123	0.142	0.186	0.159	0.109	0.108	0.124	0.112

[1] Method of McCrudden (1911).

Table 18. Calcium excreted in the urine by subject C.[1]

Ashing	Part I Period 1	Part I Period 2	Part I Period 3	Part II Period 4	Part II Period 5	Part II Period 6	Part III Period 7	Part III Period 8	Part III Period 9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
A	0.188	0.175	0.184	0.189	0.236	0.188	0.153	0.178	0.117
	0.190	0.166	0.186	0.212	0.254	0.212	0.155	0.177	0.110
	0.177	0.172	0.184	0.186	0.235	0.184	0.136	0.186	0.109
Mean	0.185	0.171	0.185	0.196	0.242	0.195	0.148	0.180	0.112
B	--	0.162	0.186	0.194	0.249	0.187	0.159	0.181	0.110
	--	0.160	0.190	--	0.245	0.187	0.157	0.180	0.110
	--	0.163	0.197	0.222	0.257	0.186	0.167	0.176	0.112
Mean	--	0.162	0.191	0.139	0.250	0.187	0.161	0.179	0.111
C	0.197	0.163	0.192	0.189	0.266	0.177	0.174	0.174	0.110
	0.178	0.155	0.183	0.196	0.262	--	0.170	0.174	0.110
	0.181	0.157	0.179	0.191	0.259	0.196	0.170	0.172	0.111
Mean	0.185	0.158	0.185	0.192	0.262	0.186	0.171	0.173	0.110
Grand Mean	0.185	0.164	0.187	0.176	0.251	0.189	0.160	0.177	0.111

[1]Method of McCrudden (1911).

Table 19. Calcium excreted in the feces by subject A.[1]

Ashing	Part I Period 1	Part I Period 2	Part I Period 3	Part II Period 4	Part II Period 5	Part II Period 6	Part III Period 7	Part III Period 8	Part III Period 9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
A	0.852	0.841	0.744	0.795	0.800	0.675	0.977	1.085	0.847
	0.888	0.850	0.782	0.810	0.760	0.684	0.974	1.064	0.845
	0.832	0.841	0.787	0.813	0.786	0.689	0.989	1.074	0.866
Mean	0.857	0.844	0.771	0.806	0.849	0.683	0.980	1.074	0.853
B	--	0.870	0.788	--	0.776	0.724	0.942	1.020	0.832
	0.835	0.875	0.744	--	0.812	0.733	0.942	1.030	0.871
	0.826	0.878	0.766	--	0.764	0.736	0.960	1.017	0.855
Mean	0.830	0.874	0.766	--	0.784	0.731	0.948	1.022	0.853
C	0.843	0.857	0.793	0.786	0.797	0.740	0.963	1.064	--
	0.846	1.055	0.825	0.825	0.795	0.734	0.935	1.066	--
	0.849	0.878	0.812	0.792	0.784	0.719	0.987	1.024	--
Mean	0.846	0.930	0.810	0.801	0.792	0.731	0.962	1.051	--
Grand Mean	0.844	0.883	0.782	0.536	0.808	0.715	0.963	1.049	0.853

[1] Method of McCrudden (1911).

Table 20. Calcium excreted in the feces by subject C.[1]

Ashing	Part I Period 1	Part I Period 2	Part I Period 3	Part II Period 4	Part II Period 5	Part II Period 6	Part III Period 7	Part III Period 8	Part III Period 9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
A	0.739	0.619	0.635	0.781	0.796	0.743	0.795	0.650	0.829
	0.732	0.635	0.618	0.764	0.734	0.767	0.781	0.646	0.812
	0.711	0.629	0.633	0.770	0.834	0.754	0.799	0.656	0.804
Mean	0.727	0.628	0.629	0.772	0.788	0.755	0.792	0.651	0.815
B	0.736	0.622	0.644	--	0.733	0.781	0.808	0.717	0.725
	0.726	0.617	0.644	--	0.858	0.766	0.764	0.745	0.738
	0.734	0.630	0.636	--	0.729	0.760	0.792	0.776	0.727
Mean	0.732	0.623	0.641	--	0.773	0.769	0.788	0.746	0.730
C	0.730	0.627	0.687	0.801	0.682	0.771	0.767	0.722	0.755
	0.742	0.658	0.687	0.802	0.793	0.779	0.826	0.718	0.765
	0.725	0.649	0.670	0.754	0.740	0.761	0.791	0.697	0.813
Mean	0.732	0.645	0.681	0.786	0.738	0.770	0.795	0.712	0.744
Grand Mean	0.730	0.632	0.650	0.779	0.766	0.765	0.792	0.703	0.763

[1]Method of McCrudden (1911).

Table 21. Phosphorus excreted in the urine and feces by subjects and by periods.[1, 2]

Ashing	Part I Period 1	Part I Period 2	Part I Period 3	Part II Period 4	Part II Period 5	Part II Period 6	Part III Period 7	Part III Period 8	Part III Period 9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
URINE									
Subject A									
A	0.795	0.779	0.811	0.838	0.818	0.545	0.631	0.922	0.730
B	0.776	0.785	0.785	--	0.801	0.545	0.626	0.922	0.739
C	0.795	0.785	0.811	0.814	0.818	0.514	0.635	0.907	0.744
Mean	0.789	0.783	0.802	0.826	0.812	0.535	0.631	0.917	0.738
Subject C									
A	0.884	0.739	0.863	0.970	0.699	0.830	0.878	0.864	0.568
B	0.879	0.739	0.863	0.955	0.692	0.846	0.857	0.858	0.555
C	--	0.739	0.863	0.955	0.720	0.855	0.864	0.864	0.564
Mean	0.882	0.739	0.863	0.960	0.704	0.844	0.866	0.866	0.562
FECES									
Subject A									
A	0.540	0.557	0.552	0.559	0.554	0.470	0.552	0.565	0.529
B	0.538	0.545	0.558	0.556	0.564	0.590	0.535	0.569	0.517
C	0.550	0.564	0.553	--	0.550	0.493	0.539	0.567	0.536
Mean	0.543	0.555	0.554	0.558	0.556	0.484	0.542	0.567	0.527
Subject C									
A	0.527	0.464	0.454	0.525	0.458	0.536	0.476	0.457	0.463
B	0.525	0.467	0.468	0.529	--	0.546	0.475	0.441	0.461
C	0.518	0.475	0.454	--	0.464	0.536	0.478	0.440	0.457
Mean	0.523	0.469	0.459	0.527	0.461	0.539	0.476	0.446	0.460

[1]Each figure is a mean of two analyses.
[2]Method of Fiske and Subbarow (1925).

CALCIUM AND PHOSPHORUS RETENTION BY TWO 13-YEAR-OLD GIRLS

by

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Previous reports in the literature indicated individual adolescent girls varied widely in their ability to utilize calcium and phosphorus. In order to supplement present information, the calcium and phosphorus retentions of two 13-year-old girls were determined. Retention values were based on chemical analyses of the diet and excretory materials.

The 50-day study was divided into three parts preceded by a five-day adjustment period. Each part contained three five-day periods. Food value tables were used to plan daily menus that contained approximately 1.0 g. calcium and 1.3 g. phosphorus and met the NRC recommended allowances for all other nutrients except iron. Two levels of iron were used--11 mg./day during Part I and 13 mg./day in Parts II and III. During Part III, the basic menus were adjusted to include a large amount of chocolate candy. Both subjects ate the same basal diet but consumed different amounts of butter cookies and soft drinks.

Aliquot samples of food and excretory products were preserved for analysis by wet ashing. Chemical analyses of the basal diet showed it contained a mean of 16 percent less calcium and 24 percent less phosphorus than calculated from food value tables.

The analyses showed the two subjects had mean daily calcium intakes of 0.869 and 0.875 g. which were both below the NRC recommended allowance of 1.3 g. Mean urinary calcium losses were not related to intake. This finding agreed with studies reported in the literature. The ranges for urine and fecal calcium were within the ranges reported in other studies

of adolescent girls. Milk chocolate candy seemed to exert an adverse effect on calcium absorption. The negative mean daily calcium retentions of the subjects probably were caused by their low dietary intakes. Approximately 0.9 g. calcium was required to maintain calcium balance in the two subjects. This requirement confirmed the adequacy of the NRC allowance (1.3 g.) to maintain positive balance, and to allow for a safety margin and individual variation.

Mean daily dietary intakes of phosphorus were 1.114 and 1.133 g., which were below the amount suggested by the NRC. Urinary excretion was within the ranges reported for other adolescent girls, but fecal excretion was higher than that in other reports. The subjects were in negative phosphorus balance during seven of the nine periods. The large fecal excretion and high positive absorption of phosphorus were linked with negative retentions. This probably indicated that phosphorus intakes were larger than required for these subjects.