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To the Graduate Council:

I am submitting herewith a dissertation written by Jessie Russell Ashe entitled "Utilization of Calcium, Phosphorus, Magnesium, Iron, and Protein by Pregnant Women Consuming Self-Chosen Diets With or Without Vitamin, Mineral Supplements." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Nutrition.

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Major Professor

We have read this dissertation
and recommend its acceptance:

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Vice Chancellor for
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UTILIZATION OF CALCIUM, PHOSPHORUS, MAGNESIUM, IRON, AND PROTEIN BY PREGNANT WOMEN CONSUMING SELF-CHOSEN DIETS WITH OR WITHOUT VITAMIN, MINERAL SUPPLEMENTS

A Dissertation

Presented to

the Graduate Council of

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In Partial Fulfillment

of the Requirement for the Degree

Doctor of Philosophy

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by

Jessie Russell Ashe

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ABSTRACT

The utilization of calcium, phosphorus, magnesium, iron, and nitrogen in 10 healthy pregnant women consuming self-selected diets was investigated. Seven-day balance experiments, conducted under ordinary home conditions, were spaced periodically throughout the duration of the pregnancy. A multiple vitamin-mineral supplement provided part of the calcium and iron intake of some subjects; others received their total mineral intake from food sources. Seven of the 10 subjects also completed a seven-day balance period within three to 11 weeks post partum.

The calcium intake of pregnant women with a balanced diet including dairy products was sufficient without supplementation of calcium. The magnesium intake of all 10 subjects was below that recommended for pregnant women, and balances were generally negative. An adaptation of the fecal loss of calcium and iron was suggested during the latter half of pregnancy, but the increased retention was not significant.

The retention of both calcium and phosphorus when calcium carbonate supplied 20% of the calcium intake was usually less than the retention of the minerals when calcium was provided entirely by food sources. The retention of iron whether partially provided by ferrous fumerate or ferrous sulfate or provided entirely by food sources appeared to be more dependent on the iron intake than the source of the mineral.

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CHAPTER I

INTRODUCTION

Pregnancy is a period of increased nutritional requirement. In determining the requirements of the mother, the practice has been to add the requirement for fetal development and accessory tissues to the basic requirements of the nonpregnant woman. It has now been realized that pregnancy involves physiological changes which result in a more efficient utilization of various nutrients. This nutritional adaptation is operative in helping to meet the increased nutritional demands of pregnancy.

It is also important that the available supply of nutrients should be increased. Whereas the importance of a good diet including adequate intakes of dairy products was once advised, multiple vitamin-mineral supplements are increasingly relied on to meet the additional nutritional requirements of pregnancy. Consequently, less emphasis has been placed on the importance of a good prenatal diet.

The utilization of calcium and iron salts, two minerals generally provided by a prenatal supplement, has been variable. The effectiveness appears to depend on the mineral salt used and the nutritional needs of the subject. The pregnant woman has received limited attention in these investigations of mineral salt utilization.

In order to ascertain the need of a prenatal supplement, and to determine its effectiveness in meeting the calcium and iron requirements of pregnancy, metabolic balance experiments have been conducted on normal pregnant women periodically throughout the duration of the pregnancy.

Self-selected diets were used to determine the nutrient intakes of women during pregnancy under ordinary home conditions. The utilization of calcium, phosphorus, magnesium, and iron was investigated in women receiving a vitamin-mineral supplement or only ordinary foods. Because of the importance of adequate protein in the diet and its relationship to other nutrients, nitrogen utilization was investigated also.

CHAPTER II

REVIEW OF THE LITERATURE

Calcium, phosphorus, and magnesium are three of the major mineral constituents of the body (1). As a component of hemoglobin and various enzymes iron, although present in smaller amounts than calcium, phosphorus, and magnesium, is also a nutrient essential to life (2). The absorption of these minerals from the intestine is relatively inefficient, the utilization being influenced by various dietary and physiological factors. During pregnancy, when additional nutrients are needed for the developing fetus and accessory tissues, the utilization of calcium and iron as well as protein has been reported to increase.

I. NATIONAL RESEARCH COUNCIL'S RECOMMENDED ALLOWANCES

Adult Males and Females

The Food and Nutrition Board of the National Research Council established their first recommended dietary allowances for protein, calcium, and iron in 1943 (3). The 1968 revised recommendations (4) for men and women 18 to 35 years old are presented in Table 1. Specific allowances for phosphorus and magnesium were recommended for the first time in the 1968 revised recommendations. Based on the ratio of phosphorus to calcium in the tissues of the body and consideration of the phosphorus intake with ordinary diets, the recommended dietary allowance for phosphorus is equal to that of calcium (Table 1). The recommended allowance for magnesium (Table 1) was based on balance studies which

TABLE 1

1968 RECOMMENDED DIETARY ALLOWANCES

	Protein	Calcium	Phosphorus	Magnesium	Iron
	g	g	g	mg	mg
Adult Male					
18-22 yr	60	0.8	0.8	400	10
22-35 yr	65	0.8	0.8	350	10
Adult Female					
18-22 yr	55	0.8	0.8	350	18
22-35 yr	55	0.8	0.8	300	18
Adult Pregnant Female	65	1.2	1.2	450	18

estimated that a magnesium intake of approximately 300 mg daily was necessary to maintain positive balance in a wide age group of adults (5,6).

Adult Pregnant Females

The customary practice for determining dietary standards for the pregnant woman has been on the basis of the requirement of the non-pregnant woman plus the calculated requirements for the growth and development of the fetus and accessory tissues. During pregnancy, however, a number of physiological alterations occur resulting in a change in the metabolism of some nutrients (7,8). Despite the physiological adaptations, additional daily allowances for protein, calcium, phosphorus, and magnesium are recommended during pregnancy. The recommendation for iron is the same as that for the nonpregnant woman (Table 1).

II. UTILIZATION OF CALCIUM, PHOSPHORUS, MAGNESIUM, AND NITROGEN

Because of the interrelationships of calcium, phosphorus, and magnesium, these minerals will be considered simultaneously in this chapter. Nitrogen utilization will be reviewed primarily as it relates to these minerals.

Absorption and Retention

Although there is considerable individual variation, the utilization of calcium by humans is generally inefficient; only about 20 to 30% of the dietary calcium is absorbed (1). Fecal losses of the mineral are large representing unabsorbed dietary calcium as well as endogenous calcium (9,10). Renal calcium excretions are variable, but the tubular reabsorption of calcium is generally high (11).

Dietary phosphorus is absorbed better than calcium; the fecal loss represents approximately 30% of the dietary intake under ordinary conditions. Consequently, renal excretions are larger for phosphorus than for calcium, and the phosphorus balance is more dependent on variations in renal excretion (11).

The percent of the intake of magnesium lost in the feces and urine is relatively constant, the amount lost varying with the intake. Fecal losses may represent 55 to 65% of the magnesium intake, while urinary excretions may represent 25 to 45% (12).

In contrast to the minerals, the absorption of nitrogen from dietary protein is fairly efficient. Some is taken up by the stomach wall, but

approximately 90% is absorbed from the intestines (13). Consequently, the fecal losses of nitrogen are smaller than the amounts excreted in the urine.

Although utilization of protein is dependent on the quality of the protein, dietary factors have little influence on its absorption. On the other hand the absorption and utilization of calcium, phosphorus, and magnesium are influenced by numerous factors.

Some Influencing Factors

Various dietary components have been shown to influence mineral utilization in animals. Lactose stimulated the absorption and retention of calcium (14,15) and favored bone calcification in growing rats (15,16). High levels of certain poorly absorbed triglycerides depressed the absorption and retention of calcium and magnesium in rats (17). Phosphorus retention was inhibited in chicks following a large injection of ascorbic acid (18).

Calcium absorption in human subjects was found to be independent of the time of day the calcium was ingested (19), but a decreased urinary excretion of calcium and magnesium has been reported to occur at night (20). Although calcium absorption was not related to the time of day it was ingested, dividing a calcium dose throughout the day resulted in an absorption rate which was 20% greater than the absorption rate when the calcium was given as a single dose. The investigators suggested that the greater absorption may have resulted from a less saturated intestinal absorption mechanism which allowed the divided calcium dose to be absorbed with greater efficiency (21).

The effect of the dietary level of calcium on its utilization has been demonstrated both in animals and man. As the calcium level of diets increased, the percent of calcium absorbed and retained decreased, although the absolute amount of calcium absorbed and retained was greater (9, 14, 15, 22, 23). The increased calcium intake in rats was accompanied by an increase in fecal and urinary calcium losses (15). By use of a tracer it was established that in the rat skeleton a high calcium diet resulted in a decrease in the resorption rate of calcium from bone and an increase in net deposition of calcium (22).

Adaptation to low dietary levels of calcium resulted in more efficient utilization of the mineral. Four Ceylonese children with calcium intakes as low as 0.2 g of calcium per day were found to absorb and retain from 34 to 89% of the calcium available, and all four children were in positive calcium balance (24).

Mechanisms of Absorption

The development of the everted intestinal sac technique has stimulated studies to elucidate the mechanisms for intestinal absorption of minerals. Everted intestinal sacs prepared from segments of the proximal small intestine of rabbits, rats, and guinea pigs transported ^{45}calcium (hereafter referred to as ^{45}Ca) across the mucosal membrane against a concentration gradient. Most of the calcium transferred was ionized calcium suggesting an active cation transport mechanism. The active transport process was dependent on oxidative phosphorylation and was relatively specific for the calcium ion (25,26).

The mechanism of adaptation of calcium absorption to dietary levels was investigated in rats following a dietary calcium restriction. The rats adapted to the calcium intake by a greater rate of active transport of the calcium ion across the intestinal wall (27).

Under conditions in which oxidative metabolism was inhibited, ^{45}Ca was transferred across the intestinal sacs by a diffusion process. Unlike the active transport system which was demonstrated only in the proximal portion of the intestine, the diffusion process occurred over the entire length of the small intestine (28). In both the diffusion process, which was independent of oxidative metabolism, and the energy-dependent active transport system, the transfer of ^{45}Ca through sacs prepared from animals fed vitamin D-deficient diets was greatly depressed.

Recent investigations of vitamin D have been directed toward the metabolic transformations it undergoes in its role of facilitating calcium absorption in the intestine and calcium resorption from bone. DeLuca (29) identified the predominant vitamin D_3 metabolite present in the blood as 25-hydroxycholecalciferol. This compound was produced by the liver from cholecalciferol and acted more rapidly than the latter in stimulating intestinal calcium absorption and increasing bone calcium resorption in rats. More recently, Norman (30) identified a metabolite produced by the kidney as 1, 25-dihydroxycholecalciferol. This compound was over four times as effective as cholecalciferol and over twice as effective as 25-hydroxycholecalciferol in stimulating intestinal calcium absorption. Norman proposed that cholecalciferol and 25-hydroxycholecalciferol were precursors of this biologically active substance produced by the kidney.

Using everted intestinal sacs prepared from the small intestine of rats, Harrison and Harrison (31) demonstrated the active transport of inorganic phosphate across the intestinal wall. The energy-dependent in vitro system required the presence of calcium and was enhanced by an increased potassium concentration. Furthermore, the transfer of inorganic phosphate was greatly diminished in the preparations from vitamin D-deficient animals. Since the effect of vitamin D on the transport of calcium across the intestinal wall had previously been shown, it was suggested that the vitamin D effect on the transport of inorganic phosphate could be secondary to that of calcium transport. The complete removal of calcium from the in vitro system inhibited the active transport of phosphate across the intestinal wall and eliminated the vitamin D effect.

Parathyroid hormone functions to maintain extracellular ionized calcium within physiologic limits. This hypercalcemic agent directs its action on the kidneys, the bones, and probably the gastrointestinal tract. Vitamin D appears to be necessary for the action of the hormone in promoting bone resorption, but is not necesary for its action on the kidney (11,32). The administration of bovine parathyroid extract to human subjects resulted in a decrease in the tubular reabsorption of phosphate, and a decrease in the serum phosphate. On the other hand, the serum calcium was elevated (33).

Vitamin D also appears to be necessary for the action of the hormone in promoting the intestinal absorption of calcium (11). Measured by a radio-isotope counting technique, six normal subjects showed an

increase in intestinal calcium absorption after the administration of parathyroid extract (34). A 50% decrease in calcium absorption was reported after parathyroidectomy of rats (35). The hormone has also been shown to increase phosphate transport by 70% in in vitro studies using everted intestinal sacs of rats. This increased phosphate uptake could not be attributed to a secondary effect due to the increased calcium absorption (36).

Interrelationships of Calcium and Phosphorus

In addition to the influence of dietary and physiological factors previously discussed, the utilization of calcium, phosphorus, and magnesium is dependent on their interrelationships with each other and with nitrogen. The interrelationship of calcium and phosphorus has not been completely established, and much data are conflicting. The importance of the calcium-phosphorus ratio has been questioned. Rickets was produced in vitamin D deprived rats with diets providing various ratios of calcium to phosphorus. It was suggested that absolute amounts of calcium and phosphorus were more important than the ratio of one to the other (37). However, many of the more recent investigations have indicated that the relative amount of calcium and phosphorus is an influencing factor in their utilization.

Dietary phosphorus appears to be necessary for the optimal retention of calcium in animals. Young et al. (38) reported that the calcium absorption of lambs was depressed when dietary phosphorus was restricted and increased when phosphorus intake was increased to an adequate level. Clark (39) found that rats given a diet containing adequate levels of

calcium, phosphorus, and magnesium had calcium and phosphorus retentions of 50% and 46% respectively. However, a diet devoid of phosphorus decreased calcium retention to 20%. Conversely, calcium was necessary for the optimal retention of phosphorus. The absence of calcium reduced the phosphorus retention to 17%.

High dietary levels of phosphorus in animals have been shown to have an adverse effect on calcium metabolism. Both in rats (40) and ponies (41), calcium absorption and retention were depressed when dietary phosphorus was elevated. Although urinary calcium excretion was decreased, both dietary and endogenous fecal losses were increased so that less calcium was retained (41). On the other hand, absorption and retention of phosphorus increased as the phosphorus intake increased (41,42).

The reports from human studies have been less consistent and not always in agreement with the results reported on animals. In an investigation by Patton and Wilson (43), 18 healthy young women received diets having varying ratios of calcium to phosphorus, approximately 1:0.5 to 1:4. Calcium intakes varied from 344 to 1544 mg, while the phosphorus intakes were 766 to 1366 mg. Decreasing the calcium-phosphorus ratio by increasing the phosphorus intake had no effect on the calcium retention. However, phosphorus retention increased as the phosphorus intake was elevated if the calcium intake was as much as 944 mg.

Leichsenring et al. (44) maintained 17 college women on a basal diet providing 300 mg of calcium and 800 mg of phosphorus for four weeks. During the next four weeks, six subjects received a supplement of calcium

carbonate to provide a calcium intake of 1500 mg, and six subjects received supplements of calcium carbonate, dibasic calcium phosphate, and dibasic sodium phosphate to provide an intake of 1500 mg and 1400 mg of calcium and phosphorus respectively. Although there was an increase in calcium retention when both the calcium and phosphorus intakes were increased, the elevated retention was less than when only the calcium intake was increased. The high phosphorus intake appeared to influence the calcium retention. Phosphorus retention was unchanged by the addition of phosphorus to the diet.

A similar investigation using college women was reported by Schofield et al. (45). Basal diets providing 0.3 g of calcium and 0.8 g of phosphorus were supplemented with dicalcium phosphate or natural foods to provide daily intakes of 1.0 to 1.4 g of calcium and 1.3 to 1.7 g of phosphorus. When the addition of calcium and phosphorus to the diet was provided by a mineral salt, the calcium retention was similar to that reported by Leichsenring et al. (44). However, when essentially the same amounts of calcium and phosphorus were provided by ordinary foods, calcium retention was much better. The investigators suggested that the source of calcium and phosphorus rather than the level of dietary phosphorus may influence calcium utilization.

High dietary levels of calcium were found to inhibit phosphorus absorption in rats (42). Patton and Wilson (43) reported that increasing the calcium-phosphorus ratio in young women by increasing the calcium intake from 344 to 1544 mg had no effect on the retention of phosphorus.

However, calcium retention increased as the amount of calcium ingested increased. Similar results were reported by Leichsenring et al. (44) in the investigations with young college women previously described. On the other hand, Spencer et al. (46) reported that phosphorus absorption of adult men and women, 39 to 77 years old, increased when the phosphorus intake was increased from 656 to 1628 mg. However, the elevated phosphorus absorption was greater when the subjects were receiving a low calcium diet, 202 mg, than when receiving a high calcium diet, 1522 mg.

Interrelationships of Calcium, Phosphorus, and Magnesium

The influence of magnesium on calcium and phosphorus metabolism has been investigated in an effort to elucidate the interrelationships of these minerals. Using the everted intestinal sac technique with rats, Schachter and Rosen (25) found that the active transport of ^{45}Ca in vitro was depressed by magnesium ions. The effect of the divalent cation was attributed to a possible competitive inhibition of calcium transport. In vivo studies using rats showed that more calcium was absorbed by the animals which were magnesium deficient than by the animals receiving the control diet (39,47). Again a common intestinal transport mechanism for the absorption of calcium and magnesium was suggested.

Phosphorus retention was also affected by the dietary magnesium. Rats receiving a diet adequate in phosphorus and magnesium but lacking calcium retained 17.2% of the phosphorus. Phosphorus retention was 38.9% when both calcium and magnesium were absent (39).

On the other hand, a high magnesium intake in the presence of adequate intakes of calcium and phosphorus was shown to improve the

utilization of the minerals. Introduction of small amounts of magnesium to an otherwise adequate diet resulted in an initial fall in calcium retention (48), but if the magnesium content of the diet was adequate (0.05%) or higher (up to 1%), there was a decrease in fecal calcium and phosphorus resulting in an increased retention of the minerals (48-50). Fecal magnesium losses were also depressed and magnesium retention was elevated as the magnesium intake increased (48).

Reports from the literature appear conflicting; magnesium has been shown both to increase and decrease the absorption and retention of calcium and phosphorus. Clark (49) pointed out that the antagonistic action of magnesium in vivo had been shown in rats receiving abnormally low magnesium intakes, an unnatural condition since magnesium is an integral part of normal diets. The investigator emphasized that this could have affected change in the intestinal mucosa allowing the increased absorption of calcium and phosphorus in the absence of magnesium.

The influence of calcium and phosphorus intake on the metabolism of magnesium has received attention also. Magnesium deficient guinea pigs were maintained for eight weeks on diets supplying various intakes of calcium, 0.9 to 2.5%, and phosphorus, 0.4 to 1.7%. High calcium and phosphorus intakes precipitated the magnesium deficiency in the guinea pigs causing depressed growth and high mortality. The amount of magnesium needed for a maximum rate of gain was increased. Eighty mg of magnesium per 100 g of diet were required when the dietary phosphorus level was 0.4%, whereas 240 mg of magnesium were required when the dietary phosphorus level was 1.7%. It is possible that both calcium and phosphorus are metabolic antagonists of magnesium (51).

Nine healthy college women received a basal diet supplying 260 mg of magnesium, 300 mg of calcium, and 800 mg of phosphorus for four weeks. During the next four weeks, three subjects received a calcium supplement, increasing the calcium intake to 1500 mg; three subjects received both a calcium and phosphorus supplement, increasing the intake to 1500 mg of calcium and 1400 mg of phosphorus. No significant relationship was observed between calcium and phosphorus intake and either the fecal loss or retention of magnesium. However, urinary excretion of magnesium increased as the intake of calcium and phosphorus increased (52).

Interrelationships with Nitrogen

The addition of protein to a low protein diet has been shown to increase the amount of calcium and magnesium absorbed from the intestine, but the overall effect of a high protein intake on the balance and retention of these minerals is less certain. The low protein basal diet of three adult subjects was supplemented with 100 to 130 g of protein while keeping the calcium, magnesium, and phosphorus intakes relatively constant. The increase in the dietary protein was accompanied by an increase in the apparent absorption (referring to the mineral intake minus the loss of the mineral in the feces) of the dietary calcium and magnesium. An increased urinary excretion of these minerals was noted also (53).

Similar results were reported in young men when the dietary protein was increased from 48 to 141 g daily. However, this increased calcium absorption was not comparable to the two-fold increase in urinary calcium excretion. Calcium balance was 10 mg and -84 mg on the low and high protein intakes respectively (54).

Hunt and Schofield (55) reported an improved magnesium balance in adult women as the protein intake was increased from 34 to 48 g while the limited magnesium intake was held relatively constant. With these limited protein intakes, the young women were in magnesium equilibrium or positive balance, although magnesium intakes were only 178 to 196 mg daily. Earlier investigations had reported that a magnesium intake of approximately 300 mg was necessary to maintain positive balance in women ingesting recommended intakes of protein (5,6). Hunt and Schofield (55) suggested that the utilization of magnesium was dependent on the protein intake.

Rats fed experimental diets of 5% and 20% protein were injected intraperitoneally with ^{45}Ca after five weeks. Although rats fed the low protein diet had higher levels of fecal calcium, by use of the radioactivity measurements it was determined that the excessive fecal loss of calcium was of endogenous rather than of dietary origin. When the endogenous loss was considered there was little difference in the percent absorption of dietary calcium in the rats fed low or high levels of protein (56).

Changes Observed in Pregnancy

During periods of growth and pregnancy when nutrient requirements are increased, the body responds with a more efficient utilization of some materials (1,2). Hansard and Crowder (57) reported that calcium absorption, retention, and excretion were greatest in young rats. These factors decreased rapidly to sexual maturity and then decreased more slowly to maturity and old age.

In vitro studies using everted intestinal sacs of rats showed that the rate of active transport of calcium with sacs from growing rats was greater than the rate of transport in older rats. Furthermore, the rate of active transport of calcium was greater for the pregnant rats than for the nonpregnant controls (26).

Chemical analyses of feti performed at different fetal ages have shown that little calcium is deposited in the fetus during the first trimester of pregnancy. The calcium deposition increased during the second trimester with the greatest accumulation during the third trimester (58). Shenolikar (59) reported that the amount of calcium absorbed and retained by 12 low income pregnant Indian women increased during the second trimester of pregnancy; this elevated absorption and retention was maintained during the third trimester. The habitual dietary intake of calcium for low income Indian groups is about 0.400 g per day. The calcium intake of these women ranged from 0.420 to 0.460 g daily during the study. Apparent calcium absorption increased from 42% in the first trimester to 53% in the second and third trimesters. The increased retention of calcium during the second trimester was attributed to an increase in the absorption of calcium from the intestine, while the increase in retention from the second to the third trimester was the result of a reduced urinary calcium excretion.

An increase in calcium retention in late pregnancy has been reported by Coons and Blunt (60). Twenty-three four- to six-day balance experiments were performed on nine women between the eleventh and thirty-ninth weeks of gestation. Remaining in their own homes under ordinary living

conditions, the women consumed self-chosen unsupplemented diets. Calcium, phosphorus, and magnesium intakes varied from 0.7 to 1.7 g, 0.9 to 2.1 g, and 0.2 to 0.8 g respectively. Calcium retention was irregular, but there was a tendency for the retention to rise toward the end of the gestation period. On the other hand, the retention of magnesium was characterized by a tendency toward larger negative balances in the latter part of pregnancy.

Similar "at home" balance studies using self-selected diets were carried out by Macy et al. (61) on three housewives. The pregnancy was in close succession to the last pregnancy, and all three subjects had just completed a year of heavy lactation. A large milk consumption contributed to a high calcium intake, 1.5 to 2.7 g, and a high phosphorus intake, 1.5 to 3.0 g. Balance experiments done at monthly intervals during the last half of pregnancy revealed a high retention of phosphorus. However, calcium retention was irregular and was not related to the period of gestation. In spite of the large consumption of calcium and phosphorus, there were periods of calcium losses in various weeks of gestation by all three subjects.

A continuous metabolic balance study on a woman in her fourth reproductive cycle was begun at approximately the one hundred thirty-fifth day of gestation and continued throughout the pregnancy. After a 10 day post partum interruption, the study continued through the first two months of lactation. A voluntary diet provided a generous food intake of all nutrients including average daily intakes of approximately 3.0 g of calcium, 0.6 g of magnesium, and 2.7 g of phosphorus. The retention of

the elements fluctuated widely and was found to have no relationship to either the needs of gestation or the intake. However, in the final 145 days of pregnancy, the subject had retained 53 g of calcium, 15 g of magnesium, and 37 g of phosphorus. Based on a comparison of these figures with rough approximations of fetal needs, a maternal retention of minerals had occurred during pregnancy in excess of that necessary for fetal growth (62).

Lactation, in contrast to pregnancy, was a period of lowered retention with a negative balance of calcium, magnesium, and phosphorus. During 43 days of lactation in which mineral losses, including that in expressed breast milk, were considered, the negative balances represented a loss of 21 g of calcium, 1.6 g of magnesium, and 15.8 g of phosphorus (62). Large negative calcium and phosphorus balances during early lactation had been reported previously by Hunscher (63). In later lactation, the fiftieth to sixtieth weeks, milk secretion decreased and calcium and phosphorus balances were again positive (62). The investigators concluded that the maternal retention of minerals was a means of preparing the body to carry out lactation without undue stress to the mother.

Goss (64) reported a renal conservation of calcium during pregnancy. Urinary and serum calcium levels were determined at regular intervals between the tenth and forty-first weeks of gestation on 205 clinic patients. An average daily calcium intake of 650 mg was estimated from diet histories. The urinary calcium excretion of 180 unsupplemented patients was significantly lower during the latter weeks of gestation,

the time of maximal fetal calcium requirement. However, 25 patients taking various self-prescribed calcium supplements, usually calcium phosphate or lactate in combination with multi-vitamins, exhibited high urinary calcium excretions which persisted throughout the pregnancy. It was suggested that because intakes were in excess of requirements, the supplement was obscuring the adaptive mechanism.

Kerr et al. (65) reported that renal conservation of calcium was not found in 24 women given 2 g of calcium orally in the sixth through the eighth month of pregnancy. The preparations given were dicalcium phosphate, calcium sulfate, calcium lactate, and reconstituted nonfat dry milk. When compared to the values for nongravid subjects, neither calcium nor phosphorus reabsorption were significantly altered in the pregnant women. However, the effect of the orally administered calcium varied with the medication used. Calcium sulfate and dicalcium phosphate resulted in no change in either the serum or urine calcium values, while calcium lactate and nonfat dry milk elevated urinary calcium excretion. Only calcium lactate produced a rise in the serum calcium level. On the other hand, all the oral calcium preparations raised the phosphorus levels of serum and decreased phosphaturia. As was suggested by Goss (64), it is possible that the high intake of calcium was concealing a renal conservation of this mineral. The metabolism of calcium ingested as a single oral medication or as part of a normal dietary regime may also have been a factor.

As early as 1916, Wilson (66) reported that pregnant women had the capacity to retain nitrogen in excess of the needs of the developing fetus

analogous to that described by Hummel et al. (62) for various minerals. Numerous metabolic balance studies have been conducted on women during the last half of pregnancy to determine nitrogen utilization. Large nitrogen intakes, ranging from 12 to 24 g daily, resulted in retentions which exceeded the estimated fetal needs by as much as 2.5 g daily (67-69). Nitrogen losses via sweat, hair, nail, and skin were not considered. Zuspan and Goodrich (69) reported that one subject with a nitrogen intake of 6 g per day had nitrogen retentions which fell short of conceptual product needs. The investigators concluded that maternal reserves were accumulated only if nitrogen intake was adequate.

The concept of maternal nitrogen storage beyond that explained by the developing fetus and accessory tissues has been reevaluated in the light of current knowledge. The early studies reporting large daily nitrogen retentions in pregnant women (67-69) were based on balance studies which failed to take into account any nitrogen loss beyond those in urine and feces, and the data may be in considerable error. Recent studies of gain in total body water in relation to increases in body weight failed to indicate any large increases in body water which accompanies nitrogen retention in tissues (70). These findings led to the conclusion that maternal stores were not protein, but fat.

Naismith (71) reported that the retention of nitrogen by pregnant rats was greater than that which could be accounted for by the products of conception. At the termination of pregnancy, rats on diets providing 20% casein showed no gain or loss of protein from the carcass, but fat reserves had increased almost 45%. Animals fed a low protein diet, 10%

casein, had a loss of protein from the maternal carcass; nevertheless, a 20% increase in fat had occurred. A two phase physiological mechanism was hypothesized for nitrogen. The first phase is a period of accumulation of protein when the demands of the fetus are minimal. The second phase is a period of catabolism of protein as the fetal demands increase. This catabolism occurs regardless of the protein intake.

Utilization of Calcium Salts by Young Men and Women

With the introduction of food enrichment and fortification, and a growing awareness and concern for nutrient requirements, the effectiveness and utilization of mineral salts was investigated. The utilization of various calcium salts was compared both to one another and to food sources of the mineral.

The absorption and utilization of calcium added to the diet of adult human subjects as calcium citrate (72) or calcium carbonate (73) was comparable to that given as calcium gluconate. Calcium given as calcium lactate was better absorbed and utilized than that from calcium gluconate (74). In contrast to these investigations, Patton and Sutton (75) reported that no significant difference in the utilization of calcium from four salts, gluconate, lactate, carbonate, and sulfate, was found in young college women. However, the order in which the salts were taken was influential, the salt taken first being utilized to the greatest extent. The average utilization from the salts was approximately 18%.

Of the foods commonly used in the American diet, dairy products, and especially milk, have been recognized as the main dietary source of

calcium and a rich source of phosphorus (76). The availability of calcium from milk has often been used as a standard of reference in determining the degree of utilization and effectiveness of mineral salts.

Thirteen young male medical students ingested a low calcium basal diet supplemented with evaporated milk or calcium sulfate to provide the estimated body requirements for calcium. The calcium sulfate was almost as well utilized as the milk, the average utilization being 24% and 29% respectively (77). A single adult man studied over a period of 172 days received a diet in which three-fourths of the calcium intake was supplied by either milk solids or calcium gluconate. Calcium from both sources was approximately 20% utilized (78).

Young college women consumed diets in which one-half of the calcium was supplied from either milk, calcium carbonate, or calcium gluconate. Although no significant difference was indicated under the experimental conditions used, the utilization of calcium from the three sources indicated some discrepancy. The average utilization of calcium from milk and calcium carbonate was comparable, 24% and 19% respectively, but the utilization of calcium from calcium gluconate was only 12% (79).

Schofield et al. (45) reported a significantly greater difference in the utilization of calcium from food sources compared to a mineral salt. Young college women ingesting low calcium and phosphorus diets received mineral supplementation to the diet from foods or dicalcium phosphate. Utilization of the calcium from food averaged 28%, while the utilization of the calcium from dicalcium phosphate was only 6%. The absorption of both calcium and phosphorus was less efficient when the source of the minerals added to the diet was the mineral salt.

Utilization of Calcium Salts by Children

Studies with children indicated that the utilization of calcium from dicalcium phosphate may be related to age. The percent utilization of the calcium in milk by six preschool boys and five preschool girls (76) was approximately the same as the utilization of the calcium from dicalcium phosphate determined in a following study using the six preschool boys (80). Approximately 20% of the calcium from both milk and dicalcium phosphate was utilized by the young boys. In an investigation by Schofield and Morrell (81,82), retention of calcium and phosphorus in seven- to nine-year-old children was similar whether part of the mineral source was dicalcium phosphate or all minerals were from ordinary food sources.

Stearns and Jeans (83) found that the calcium and phosphorus retentions of children fed dicalcium and tricalcium phosphates were approximately the same as the mineral retentions from milk. The retention of calcium from calcium gluconate, lactate, and carbonate was less consistent than the retention from the calcium phosphates. Generally, calcium was well retained, but phosphorus was retained well only if the intake was approximately equal to the intake of calcium.

Because of differences in experimental conditions, age and sex of subjects, and calcium salts used, comparison of the investigations was difficult. However, the data suggested that the utilization of calcium salts depended not only on the salt used, but also on the influential role of the age and nutritional need of the subjects.

Utilization of Calcium Salts by Pregnant Women

Traditionally, the increased nutrient requirements of pregnant women were stressed by advising the patient of the importance of consuming a nutritionally adequate diet rich in high quality protein foods and dairy products. In recent years there has been a developing trend toward including a multiple vitamin-mineral prenatal supplement with a decreasing emphasis on the importance of the prenatal diet.

Page and Page (84) observed a higher incidence of leg cramps in pregnant women consuming a high protein, milk-rich diet. They concluded that the phosphorus level of such diets was responsible for a lowered ionizable serum calcium level producing muscular tetany. On this basis it was recommended that leg cramps could be prevented or relieved by a decreased milk intake, use of phosphorus-free calcium salts, and the addition of aluminum hydroxide gel to the diet to hinder the absorption of dietary phosphorus from the intestine.

In support of these findings, Hardy (85) reported an increase in blood phosphorus with a decrease in ionized calcium in patients receiving dicalcium phosphate, while the converse was true for patients receiving calcium lactate and calcium carbonate. Leg cramps were relieved when the blood calcium levels were maintained.

In another study, however, patients treated with calcium citrate, carbonate, or lactate experienced an increase in the levels of ionic phosphorus as well as calcium. Furthermore, the ionic calcium change was not influenced by the changes in the phosphorus level (86).

Although leg cramps have been reported to occur in about one-half of the women in the last trimester of pregnancy, the incidence is only

about 33% in indigent patients. A nutritionally adequate diet generally relieved ordinary symptoms so that only 6% of the patients required calcium treatment. If calcium therapy failed to relieve leg cramps, patients were treated with thiamine (87).

The cause of leg cramps in pregnancy is not clear; neither is the method of treatment unanimous. The conclusions of Page and Page (84) have received criticism. Kerr et al. (65) supplemented the diets of pregnant women with calcium as calcium lactate, calcium sulfate, dicalcium phosphate, or reconstituted nonfat dry milk. Despite the high phosphorus content, subjects ingesting nonfat dry milk and dicalcium phosphate did not experience a depressed serum calcium level or tetany.

Abrams and Aponte (88) found no evidence to support the concept of a correlation between ingestion of dairy products and leg cramps. In their study calcium lactate was no more effective than dicalcium phosphate in preventing or relieving cramps. In spite of leg cramps pregnant women were encouraged to consume ordinary foods to meet recommended intakes of calcium, phosphorus, and other essential nutrients.

Most investigations concerning the effectiveness of calcium salts have focused attention on the ability of the various salts to influence calcium and phosphorus serum levels. The effects of various calcium salts on the retention of calcium and phosphorus by pregnant women, and a comparison of the utilization of calcium from salts and food sources by this group have been ignored. As obstetricians increasingly depend on supplements to meet the requirements of pregnant patients, the absorption and retention of mineral salts become of concern.

Utilization of Calcium Salts by Animals

The superiority of the availability of calcium from milk has varied widely in rat studies. Henry and Kon (89) reported a retention of 98% of milk calcium as compared to 96% of the calcium from dicalcium phosphate. Drake et al. (90) observed that rats retained 93% of the calcium from dried whole milk and only 85% of the calcium from calcium carbonate. Lengemann et al. (91) found that when the calcium sources used were milk or calcium chloride solutions, both young and old rats absorbed one and one-half times as much calcium from milk.

Ground beef enriched with calcium gluconate was compared with milk as a source of calcium and phosphorus for small experimental animals. As determined by analysis of the carcasses, the calcium-supplemented meat diet was as effective as the diet supplemented with milk as a source of the minerals (92).

The much greater retention of calcium by the rat as compared to the human was noted in these experiments. Kinsman et al. (76) pointed out that the disparity in the utilization values of humans and rats illustrated that these two species do not necessarily react similarly. The need for more extensive human studies in establishing human dietary requirements is indicated.

III. UTILIZATION OF IRON

Absorption and Retention

The primary site of intestinal absorption of iron is the duodenum with decreasing amounts absorbed from the jejunum and ileum respectively (93).

Although the ability of a healthy individual to absorb dietary iron is only about 9 to 14% (94-97), the absorption of available iron is responsive to the body's requirement for iron.

Mechanism of Absorption

Charley and Saltman (98) reported that the rate of iron transport in the rabbit intestine was controlled by the degree of unsaturation of transferrin. Iron absorption decreased as the iron binding capacity of transferrin decreased.

Crosby (99) proposed a control mechanism wherein a requirement for iron accelerates the turnover of plasma iron and thus hastens the removal of iron from the intestinal mucosa. After two to three days the intestinal epithelial cells formed in the iron-poor environment cover the intestine. These cells lack the ability to refuse the absorption of available iron, and the iron absorption is increased until the restoration of body iron reverses the chain of events.

In studies of iron absorption in both men and women, no significant correlations were found between iron absorption and hemoglobin, serum iron, total iron binding capacity, plasma clearance of iron and packed cell volume (100,101). After giving oral treatments of ferrous fumerate for four weeks to women observed to have elevated iron absorptions, Höglund and Reizenstein (101) reported that absorption decreased significantly, while no substantial changes occurred in serum iron or plasma clearance of iron, and total iron binding capacity was surprisingly elevated.

Further investigations by Höglund (102) involved iron absorption in male blood donors before and at various times after four weeks of oral

treatment with ferrous fumerate. The decreased iron absorption resulting from the oral ingestion of ferrous fumerate was transitory, lasting up to three days after treatment had ended. Iron absorption values then rapidly returned to high pretreatment levels. This time lag correlated well with the life span of the intestinal mucosal cells loaded with iron from the oral administration of ferrous fumerate. When these iron-rich cells died, the absorption of iron increased.

The modification of iron uptake induced by alterations in iron stores also suggested the role of mucosal cells in the regulation of intestinal iron absorption. Iron-loaded and iron-depleted female rats were used to collect intestinal mucosal preparations. The binding of ferrous iron to the absorption cells was not an energy requiring process, but rather appeared to result from adsorption. The uptake of iron by the proximal mucosal cells of iron-loaded rats was depressed. On the other hand, there was an elevation in the uptake of iron by the ileal mucosal cells of iron-depleted animals (103). Similarly, Thomson et al. (93) reported a depressed intestinal uptake of iron by the duodenum of iron-loaded animals, and an increased uptake in iron-depleted animals.

Changes Observed in Pregnancy

In order to meet the iron requirements of the developing fetus and the increased red cell mass of the mother, the efficiency of iron absorption of the pregnant woman may increase. The demand of the fetus and placenta alone may average 300 to 400 mg of iron (104).

An early study by Coons (105) concerned with dietary iron retention of women during pregnancy revealed that during the second and third

trimesters the retention of dietary iron was elevated with a tendency toward lower retentions during the final month of pregnancy. More recently Apte and Iyengar (106) also reported an enhancement in the absorption of dietary iron during the second and third trimesters of pregnancy. The average absorption of iron by normal Indian women subsisting on predominantly cereal-based diets was 7% during the eighth to sixteenth weeks of gestation, increasing to 26% during the twenty-fourth to twenty-eighth weeks and 31% during the thirty-sixth to thirty-ninth weeks. The enhancement of iron absorption in iron-deficient pregnant women was even greater, increasing to 38% and 35% in the second and third balance periods respectively.

Hahn et al. (107), using ferrous chloride tagged with ^{59}iron, found the quantity of iron absorbed to increase as the pregnancy progressed in normal healthy women. The median uptake of iron in the group of women at 20 weeks of gestation or less was 10%, increasing to 35% in the group past the twentieth week of gestation. However, as the dosage of iron increased beyond 9 mg, the percent of iron taken up decreased. Even when iron was administered during the period of maximum absorption, a six- to seven-fold increase in the amount of iron administered resulted in an average two-fold increase in the quantity of iron taken up. This suggested an inefficiency of absorption of large doses of iron by pregnant women.

The enhancement of iron absorption in pregnant rats was shown to be influenced by iron stores. A quantity of iron thought to be equivalent to the amount of iron in a newborn litter of rats was injected in female rats as dextran five weeks before mating. The injection

depressed the absorption of iron during pregnancy to the nonpregnant level. This suggested that the increased absorption of iron in the latter part of pregnancy was dependent on the maternal iron demands created by the products of conception and the maternal iron stores (108). The extent that the maternal iron stores contribute to the fetal iron was also investigated. It was determined that 72% of the fetal iron had originated in maternal iron stores (109).

Holly and Grund (110) showed that in humans storage iron is available for increased requirements during pregnancy. Storage iron estimated by bone marrow biopsies, hemoglobin, and serum iron was compared in five patients in early and late pregnancy. Women who had some iron in reserve maintained normal hemoglobin and serum iron values, while women with only a trace of stainable iron early in pregnancy showed greater decreases in hemoglobin. In every case iron stores were decreased, and in women with only traces of iron stores in early pregnancy all iron reserves had been depleted. A second group of four patients received iron and cobalt supplementation during pregnancy. By late pregnancy three of these four patients had absorbed enough iron to correct anemia or to maintain a normal hemoglobin, while storage iron had increased. Unfortunately, little attention was given to dietary intakes, but the findings indicated the importance of adequate iron reserves in maintaining the woman's hematologic status during pregnancy in addition to the role of dietary intake of iron during this period.

Utilization of Iron Salts

The absorption of dietary iron is generally inefficient, although there is large variation between individuals and in the availability of

iron from various sources. In normal adult subjects, isotopic studies have indicated that usually less than 10% of the labeled iron was absorbed from individual foods. With the exception of eggs, iron from various meat sources was more efficiently utilized than that supplied by leafy vegetables or wheat (111-113). Determining iron absorption from labeled wheat, chickpea, broad bran and okra in iron-deficient men, Mameesh et al. (114) reported that whole wheat iron was significantly less available than the other plant sources of iron.

Because of the indication of inadequate iron stores with which many women enter pregnancy and because of the sufficiently high incidence of iron deficiency during pregnancy, the Council on Foods and Nutrition of the American Medical Association (115) stated that prophylactic iron administration was justified. The availability of some iron salts appears comparable to that of dietary iron. The average absorption of ferrous ascorbate in normal adult subjects was approximately equivalent to the absorption of iron from chicken muscle (112) or iron from hemoglobin and ferritin of rabbits (111). In adult patients with iron deficiency anemia, food iron both from animal and plant sources was less available than ferrous ascorbate in spite of an increase in the average absorption of food iron accompanying the deficiency (111,114).

In an effort to raise hematocrit levels of preschool low socio-economic children with milk anemia, dietary and medicinal iron were provided for five weeks. Two meals per day prepared and fed in the school cafeteria furnished 5 mg of iron. There was a small significant increase in hematocrit levels when either diet alone or diet plus

30 mg of iron given as ferrous gluconate was provided. In correcting the anemia of the preschool children, dietary iron was as effective as dietary iron plus medicinal iron (116).

The enrichment of wheat flour with iron salts could contribute to our iron intake provided it was available for absorption. Indian women consumed chapattis made with whole wheat or white flour, with or without the addition of ferric ammonium citrate. The absorption of the iron from unenriched whole wheat flour was 2%; the absorption from unenriched white flour was 4%. Nevertheless, the amount of iron absorbed from the unenriched flour was greater than the amount absorbed from flour with added iron salt (117).

A comparison was made of the absorption of iron from white and bran bread with that from white bread enriched with ferric ammonium citrate. The percent absorption of the iron from the bread enriched with the iron salt was comparable to the percent absorption of the natural iron in the white bread, and greater than the percent absorption of the iron in bran bread. However, the absolute amount of iron absorbed from the enriched white bread was equivalent to the amount absorbed from the bran bread (118).

The measurement of absorption in adults of ferric ammonium citrate incorporated in white bread compared with the absorption from a standard dose of ferrous sulfate indicated that the iron absorption from bread was only about one-tenth of that absorbed from the iron dose (119). The absorption of iron salts added to bread appeared to be less efficient than iron salts administered directly.

Ascorbic acid administered with the iron was found to enhance its absorption from bread (112,119), meat and leafy vegetables (113) and various iron salts (120). Gastric juices, succinic acid, and monosodium succinate were also found to increase the iron absorption from ferrous fumerate and ferrous sulfate when added to these oral iron preparations. The use of ferrogradument, a slow-release type of oral iron preparation, has shown no advantage in iron absorption (120).

IV. SUMMARY

The literature reviewed has shown that the absorption of calcium, phosphorus, magnesium, and iron is influenced by numerous factors including dietary components and the physiological state of the individual. The utilization of these minerals by the human organism is remarkably uneconomical. Comparison of the data suggests the possibility that the source of calcium and iron may be influential in the utilization of these minerals. Several studies also indicate the influential role of the age and nutritional needs of the subject in mineral utilization. An adaptive mechanism response resulting in a more efficient utilization of minerals, regardless of the source, may be operative during periods of increased nutrient requirement.

Because of a growing trend to meet increased nutritional requirements of pregnancy through the use of prenatal vitamin-mineral supplements, and because of a concern for the need of further investigation of mineral utilization during normal conditions of pregnancy, metabolic balance studies have been conducted on pregnant women consuming self-selected diets. The utilization of calcium, phosphorus, magnesium, and

iron was investigated in normal pregnant women receiving a vitamin-mineral supplement or consuming only ordinary foods. Because of the importance of adequate protein in the diet, its relationship to other nutrients, and as an indication of the general adequacy of the diet, nitrogen utilization was also investigated.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. GENERAL PLAN

Calcium, phosphorus, magnesium, and iron metabolism in healthy pregnant women were studied during metabolic balance experiments spaced periodically throughout the duration of the pregnancy. In order to investigate the mineral utilization of women during pregnancy under ordinary conditions, the balance experiments were conducted with the subjects remaining in their homes and consuming self-selected diets. While part of the mineral intake of some subjects was provided by a vitamin-mineral supplement, other subjects received their total mineral intake from usual food sources. Because of its importance in the diet and its relationship to calcium, phosphorus, and magnesium, nitrogen metabolism was also investigated. The balance experiments were begun in July of 1969 and continued through April of 1971. Laboratory analyses were completed in October of 1971.

Ten women participated in the experiment. A maximum of six seven-day balance periods, spaced periodically throughout the pregnancy, were completed on the subjects, two balance periods per trimester of pregnancy. Since the week of gestation was only an estimation based on the calculated date of conception, the final balance period was planned not later than the thirty-sixth week of gestation to insure completing this experimental period. The number of balance periods completed on a subject was determined by the week of gestation in which

the subject was begun on the experiment. Six seven-day balance periods were completed on two subjects, five on four subjects, and four on four subjects. In addition to these balance periods conducted during pregnancy, seven of the 10 subjects completed a seven-day balance period within three to 11 weeks post partum.

Subjects

The subjects were private patients of Knoxville obstetricians, two of whom referred most of the women to the study. The obstetricians also provided the records of weight gain throughout the pregnancy and the hemoglobin or hematocrit which was determined in early pregnancy. With the exception of Subject 1, further hemoglobin or hematocrit determinations were not available.

Upon referral to the study the woman was visited in her home with the husband present if possible. The purpose of the study was explained, the responsibilities of the subject were emphasized, and inconveniences and possible difficulties associated with participating in the study were discussed. Informed consent in writing was obtained from the couple if they agreed to participate in the experiment.

The subjects included three graduate students and two undergraduate students at the University of Tennessee, the wife of a University of Tennessee student, a registered nurse, two secretaries, and a former elementary school teacher. The ages of the women ranged from 19 to 29 years, and all 10 subjects were nulliparous.

In partial compensation for the food samples provided for analysis and for the inconveniences and time required of the couple, a nominal

amount was paid for their participation. Participants showed interest and concern in the study and cooperated fully during the investigation.

Diets

The diets of the subjects were self-selected to determine mineral intakes under ordinary home conditions. The subjects were encouraged to continue their usual routines while participating in the experiment. A vitamin and/or mineral supplement was taken if it had been initially prescribed by the obstetrician or if the need became evident as the pregnancy progressed. Two subjects received no supplementation; two received an iron supplement only; five received a multiple vitamin-mineral supplement; one received both a multiple vitamin-mineral supplement and an iron preparation. A summary of when the supplements were taken and the content of the supplements is presented in Tables 2 and 3.

A short questionnaire (Appendix A) completed by the subjects provided information concerning food intakes of the subjects prior to pregnancy. How the pregnancy had caused change in intakes was also indicated.

Collection of Samples

Subjects were instructed in the weighing, recording, and collection of food samples and in the collection of feces, urine, and vomitus. Written instructions were also provided. All equipment needed for weighing and collecting samples was furnished by the laboratory. With the exception of water and some beverages, and very small quantities of solids, all food consumed during a collection period was weighed to the

TABLE 2

DISTRIBUTION OF THE SEVEN-DAY BALANCE PERIODS AND DIETARY SUPPLEMENTATION OF SUBJECTS DURING THESE PERIODS

Subject	Estimated Week of Gestation						Week
	First Trimester		Second Trimester		Third Trimester		Post Partum
1	-	-	20[a]	24[a]	29[a,b]	36[a,b]	3
2	8	13	19[c]	25	30	36	11
3	-	-	16[d]	22[d]	29[d]	35[d]	-
4	-	10	16[d]	22[d]	29[d]	35[d]	6
5	-	-	16[d]	22[d]	29[d]	35[d]	-
6	-	9	16[e]	22[e]	29[e]	35[e]	8
7*	5	9[d]	16[d]	21[d]	-	-	-
8	6	12[f]	16[f]	22[g]	27[g]	35[g]	7[g]
9	-	11	16	22	29	35	6
10	-	11	16	22	29	34	7

[a]Filibon F. A., Lederle Laboratories, Pearl River, New York.

[b]Ferro-Sequels, Lederle Laboratories, Pearl River, New York.

[c]Fero-Folic-500, Abbott Laboratories, North Chicago, Illinois.

[d]Natalins, Mead Johnson Laboratories, Evansville, Indiana.

[e]Fero-Gradumet, Abbott Laboratories, North Chicago, Illinois.

[f]Pramilet F. A. (121), Ross Laboratories, Columbus, Ohio.

[g]Pramet F. A. (147), Ross Laboratories, Columbus, Ohio.

*Subject moved away from Knoxville and balance experiments could not be continued in the third trimester of pregnancy.

TABLE 3

VITAMIN AND MINERAL CONTENT OF DIETARY SUPPLEMENTS*

Supplement**	Fe^{++}	Ca^{++}	Folic Acid	Ascorbic Acid	Vitamin D	Vitamin A
	mg	g	mg	mg	units	units
a	30 (as fumerate)	0.230 (as carbonate)	1	50	400	4000
b	50 (as fumerate)	-----	-----	---	---	----
c	105 (as sulfate)	-----	0.350	500	---	----
d	40 (as fumerate)	0.250 (as carbonate)	0.1	100	400	6000
e	105 (as sulfate)	-----	-----	---	---	----
f	40 (as fumerate)	0.250 (as carbonate)	0.350	60	400	4000
g	60 (as sulfate)	0.250 (as carbonate)	1	100	400	4000

*Medical Economics Incorporated 1972 Physician's Desk Reference to Pharmaceutical Specialties and Biologicals, ed. 26. Oradell, New Jersey. Various supplements also contained thiamine, riboflavin, and niacinamide with small amounts of magnesium, zinc, copper, manganese, potassium, iodate, Vitamin B_{12}, pyridoxine hydrochloride, and calcium pantothenate.

**Supplements correspond with those identified in Table 2.

nearest gram on Hanson scales and recorded on the record sheets provided for each day. Water, tea, coffee, cola, and small quantities of solids were measured using standard kitchen measuring cups and spoons supplied by the laboratory. The kind and quantity of any medications and dietary supplements taken during the period were also recorded. A similar and approximately equal sized sample of all food, medications, and supplements which had been ingested were collected by the subject at each meal and saved for analysis. Each liquid food was collected in polyethylene cartons and bottles of various sizes. Each solid food was placed in small disposable plastic weighing trays and sealed in plastic bags. Each individual container was labeled as to subject, date, meal, and the food contained therein. Although the quantity of water consumed each day was recorded, only one cup of water was collected daily. The instructions provided to the subjects and the charts for recording food, beverages, medications, and dietary supplements are presented in Appendix A.

Each 24-hour urine collection was made in polyethylene bottles containing approximately 10 ml of toluene. The containers used for the 24-hour urine collection were labeled as to the subject and the date.

Fecal samples were collected between dye markers for each seven-day balance period. Each fecal sample was collected separately in a polyethylene carton and labeled as to the subject, date, and hour of collection. Subjects collected all samples during the seven-day experimental period and continued to collect all fecal samples following the experimental period until informed that the collection was complete.

The subjects stored all food, urine, and fecal samples in an ice chest which was picked up daily and taken to the laboratory.

II. LABORATORY COMPOSITING OF SAMPLES

Intake

One-fifth of the recorded weight of all food, medications, and supplements were weighed to 0.1 g, composited together, and frozen for each seven-day period. Tea and coffee, if consumed in large amounts, were composited separately. These liquids were concentrated by boiling and frozen to be analyzed separately. An aliquot of the water consumed by each subject during the week was analyzed separately also.

The seven-day intake composite of food, medications, and supplements was transferred to a weighed stainless steel Waring Blendor jar and thawed. Demineralized water, used for the complete transfer of the composite to the blendor jar, was added to the composite, and the final composite weight was recorded. The composite was homogenized and two small portions of the homogenate were frozen in polyethylene boxes and stored until analyzed.

Feces

Feces were collected between brilliant blue markers for the seven-day experimental period (121). The marker capsule containing brilliant blue dye mixed with methyl cellulose was taken before breakfast at the beginning of each period and before breakfast the first day after the balance period had ended. Carmine dye was used at the end of the balance period if the fecal samples were not completely clear of the

brilliant blue which had been used to mark the beginning of the fecal collection. Fecal samples were separated when necessary and then frozen as they were collected. The date and time of collection on each fecal box aided in the separation of the feces.

The fecal samples collected between the dye markers and corresponding to the seven-day experimental period were weighed, transferred to a 5 liter stainless steel Waring Blendor jar, thawed, and homogenized with a weighed amount of demineralized water. Any vomitus which had been collected during this seven-day balance period was treated in the same manner as a fecal sample and homogenized with the feces as part of the fecal composite. The amount of demineralized water added to the fecal composite and used for the complete transfer of the fecal and vomitus samples to the blendor jar was one-half to one and one-half the weight of the samples. Two small portions of the fecal homogenate were frozen in polyethylene boxes and stored until analyzed.

Urine

The volume, pH, and creatinine content of each 24-hour urine sample were measured. A urine composite was prepared during the seven-day period by the addition of 5% of the volume of each 24-hour urine sample. The composite was kept frozen in a large polyethylene container to which the daily additions were made if the creatinine determination indicated a complete urine collection.

Vestergaard and Leveerett (122) reported very large individual differences in the variability of creatinine excretion. While some individuals showed little variability in daily creatinine excretions,

others had variations greater than 20% in one out of 10 24-hour urine samples. The variability was attributed to possible variations in the glomerular filtration rate. Smith (123) concluded that a 24-hour creatinine excretion deviating more than ± 25% from the mean may be due to error in collection.

The subjects of this study were instructed to begin the collection of each 24-hour urine sample with the first voiding after breakfast and end with the last voiding before breakfast the following morning. Because the subjects were being investigated under ordinary home conditions, it was found that creatinine levels were varying on weekends when breakfast was eaten at a later hour. However, if problems such as this were compensated for by averaging weekend days, the daily creatinine levels were within ± 25% of the mean creatinine level for the seven-day experimental period. When urine losses were reported by the subjects and the creatinine level indicated a significant loss in the collection, the aliquot of urine for that day was omitted from the period composite.

III. METHODS OF ANALYSIS

All glassware and polyethylene containers used for collection, storage, compositing, and analysis of the samples were washed, rinsed in tap water, then rinsed in distilled water with final thorough rinsings in demineralized water. Precaution was taken to minimize contamination of the samples during all stages of analysis.

Creatinine

The micro-modification of Folin's method was used for the colorimetric determination of the creatinine content of each 24-hour urine

collection (124). By reference to a calibration curve, the creatinine content of the urine samples was compared against standard creatinine solutions containing 0.0 to 1.0 mg of creatinine.

Nitrogen Determination

The nitrogen balance of the pregnant women was determined by analyzing in duplicate the total nitrogen content of each seven-day composite of the intake, tea and coffee (if composited separately), feces, and urine. A modification of the macro-Kjeldahl method described by Hawk (124) was used to determine total nitrogen in samples estimated to contain 10 to 15 mg of nitrogen. Weighed samples of the intake, tea and coffee, and feces composites and pipetted samples of the urine composite were placed in 500 ml Kjeldahl flasks to which were added 5 g sodium sulfate, approximately 0.3 g copper sulfate, concentrated sulfuric acid (20 ml for intake, tea and coffee, and feces; 10 ml for urine), a selenized Hengar crystal, and a few glass beads. Blanks were prepared in a similar manner. All samples were digested for 20 minutes after the mixture became almost colorless to insure the complete oxidation of the samples.

The flasks were allowed to cool, diluted with 200 ml of distilled water and mixed well. Then 50 ml of a concentrated solution of sodium hydroxide was poured down the side of the flask so that the alkali formed a layer beneath the solution and did not mix with it. The flask was connected to the condenser with the delivery tube immersed in 50 ml of a 4% boric acid solution and a few drops of methyl red-methyl blue contained in a receiving flask. The contents of the flasks were mixed

and heating was begun. The distillation process was continued until approximately 150 ml of the distillate had been collected in the receiving flask. The distillate was titrated with 0.1 N hydrochloric acid, and the nitrogen content of the sample was calculated:

$$\frac{(\text{ml of acid})(\text{N of acid})(0.014)(\text{daily composite weight})}{\text{g of sample analyzed}} = \text{g nitrogen/24 hr}$$

Ashing

Duplicate weighed samples of each seven-day composite of intake, tea and coffee, and feces and pipetted samples of the urine composite were put in silica dishes and dried slowly under an infrared lamp, then charred over a Bunsen burner to prevent spattering before being dry ashed at 550° in a muffle furnace for at least 24 hours. Intake and fecal samples were treated for the removal of silica by the AOAC method (125). The ashed samples were wet with demineralized water and dissolved in hydrochloric acid upon heating. More water was added, and the ash solutions were filtered through ashless hardened filter paper into volumetric flasks, transferring quantitatively from the silica dishes. The filters were washed with demineralized water until acid-free and the flasks were made up to volume.

The Perkin-Elmer Atomic Absorption Spectrophotometer 303*

The calcium, magnesium, and iron contents of the acidified ash solutions were determined with a Perkin-Elmer Atomic Absorption Spectrophotometer 303. All calcium, magnesium, and iron determinations were performed in duplicate. For calcium and iron, the duplicate samples

*Perkin-Elmer Corporation, Norwalk, Connecticut.

represented the two separate ashings. Because of the greater sensitivity of the method for magnesium determination, both magnesium duplicates represented a single ashing.

With only slight daily modification in the instrument settings to obtain maximum sensitivity, the standard operating conditions for calcium, magnesium, and iron were used (126). The energy source was a hollow cathode tube for each of the respective minerals, and an acetylene and air flame was used with a Boling burner head.

Because of daily modifications in the operating conditions of the instrument as well as fluctuations in line current, it was necessary to determine a standard curve with each set of determinations. Fluctuations in line current as well as changes during prolonged use of the instrument also necessitated rezeroing of the Atomic Absorption Spectrophotometer at intervals during use with the establishment of a new standard curve when necessary.

Preparation of the samples for reading on the Perkin-Elmer Atomic Absorption Spectrophotometer 303 is presented in the following pages. The percent absorption reading which was obtained directly was converted to absorbance and the mineral content calculated from a standard curve.

Calcium Determination

Preliminary to the determination of all minerals, standard solutions and diluted samples were prepared and analyzed in order to determine the best range of linearity and the dilutions necessary to provide samples with concentrations falling within this range. Aliquots of the ash solutions of intake, tea and coffee, feces, and urine, in amounts estimated

to provide 2 to 10 ppm of calcium after dilution, were pipetted into volumetric flasks. Into these flasks was also pipetted a volume of 5% lanthanum oxide solution necessary to provide a final concentration of 1% lanthanum oxide. The addition of lanthanum oxide was necessary to prevent the interference of phosphates in the determination of calcium.

The calcium content of the drinking water was determined directly on the untreated samples collected. The water samples were diluted with demineralized water to a calcium concentration of 2 to 10 ppm. Because of the low concentration of phosphates, the addition of lanthanum oxide to the water samples was unnecessary.

Working standard calcium solutions, prepared from a stock calcium chloride solution, contained 2 to 10 ppm of calcium. Standard solutions used for the standard curve for intake, tea and coffee, feces, and urine determinations had lanthanum oxide added before diluting to volume. Standard solutions used for the standard curve for water determinations had no lanthanum oxide added. The standard curve was linear between 2 and 10 ppm of calcium. However, concentrations less than 2 ppm or more than 10 ppm resulted in a loss of linearity. If the readings fell on the outer limits of this range, the solutions were rediluted and the readings repeated.

Magnesium Determination

For the determination of magnesium, aliquots of the ash solutions of intake, tea and coffee, feces, and urine were diluted with demineralized water to a final concentration between 0.1 and 1.0 ppm of magnesium. The magnesium content of the drinking water was determined directly on the

untreated water samples after diluting with demineralized water to a magnesium concentration of 0.1 to 1.0 ppm. These very low concentrations of magnesium could be determined because of the great sensitivity of the method for magnesium; because of the low concentrations of the solutions analyzed, the addition of lanthanum oxide was not necessary. Without the addition of lanthanum oxide, there were less problems of aspiration of the samples with the Spectrophotometer.

Working standard magnesium solutions, prepared from a stock magnesium sulfate solution, contained 0.1 to 1.0 ppm of magnesium. The standard conditions for magnesium determination using the Atomic Absorption Spectrophotometer (126) suggested an optimum working range of 0.2 ppm to 2 ppm of magnesium. However, by using lower concentrations of magnesium, the addition of lanthanum oxide could be avoided; the standard curve was linear within the lower range of 0.1 to 1.0 ppm.

Iron Determination

The ash solutions of intake and fecal samples were analyzed for iron; the iron content of tea and coffee and water was found to be less than 2 ppm. The iron content of the urine of subjects receiving the largest supplementations of iron was determined directly on undiluted ash solutions and also found to be less than 2 ppm. Extraction of the iron in urine followed by the formation of a color complex with 2:2'-dipyridyl allowed Man and Wadsworth (127) to determine the urinary losses of iron. In both men and nonpregnant women, losses were 100 µg or less per 24 hours. Using the thiocynate colorimetric determination, Coons (105) found that in balance experiments on pregnant women the

urinary loss of iron was usually less than 2% of that in the diet. She concluded that the amounts were within the limits of experimental error, and did not affect the final balance appreciably. Because of the small urinary losses of iron, the excretion of iron in the urine was not further investigated.

For the determination of iron, the ash solutions of intake and feces were read directly on the Atomic Absorption Spectrophotometer without diluting. However, solutions of low iron concentration changed rapidly on standing. Similar difficulties reported by others were attributed to adsorption of the iron on the container (126). For this reason, it was necessary to reash larger samples of the intake and fecal composites while making the ash solutions up to the same volume as the original ash solutions. In this way ash solutions with a greater iron concentration were provided. As soon as possible after ashing, the more concentrated reashed solutions of intake and feces were diluted with demineralized water to provide final sample concentrations of 2 to 8 ppm of iron and the iron levels determined on the Atomic Absorption Spectrophotometer immediately. The addition of lanthanum oxide was not necessary for iron determinations.

Working standard iron solutions, prepared from a concentrated stock iron solution, were prepared fresh with each group of samples read. Although the optimum working range suggested for iron determinations on the Atomic Absorption Spectrophotometer was 2 to 20 ppm of iron (126), linearity was lost above 10 ppm. The range of linearity was between 2 and 8 ppm, and if readings fell on the outer limits of this range, the solutions were rediluted and the readings repeated.

Phosphorus Determination

The original acidified ash solutions of intake, tea and coffee, feces, and urine were analyzed colorimetrically for phosphorus with the Beckman B Spectrophotometer using a modification of the micro-method of the AOAC (125). The phosphorus content of water was found to be too low for determination by this method.

Samples of the ash solutions containing 0.01 to 0.05 mg of phosphorus were pipetted into 50 ml volumetric flasks and approximately 30 ml of demineralized water was added. To this was added 1 ml each of sulfuric acid (one volume concentrated sulfuric acid + two volumes water), ammonium molybdate, sodium sulfite, and hydroquinone, in that order, shaking well after each addition. Standard phosphorus solutions were prepared from potassium phosphate to contain 0.0 to 0.05 mg of phosphorus per ml. Then 1 ml of each standard phosphorus solution was pipetted into 50 ml flasks and treated in the same manner as the samples. Blanks were also prepared in the same manner as the samples.

The samples were stoppered, mixed well, and allowed to stand for an hour. The flasks were then made to volume and again thoroughly mixed. Approximately 10 ml of the solution was transferred to a colorimeter tube and the optical density read at a wavelength of 650 mμ. The phosphorus content of the samples was calculated by comparing the optical density readings to the optical density readings of the standard phosphorus solutions.

Recovery

Recoveries of nitrogen, calcium, phosphorus, and iron using the methods of analysis described in this chapter are presented in Table 4.

TABLE 4

RECOVERY OF NITROGEN, CALCIUM, PHOSPHORUS, MAGNESIUM, AND IRON

Determination	Intake	Feces	Urine
	%	%	%
Nitrogen	100	97	98
Calcium	107	103	--
Phosphorus	101	94	102
Magnesium*	104	106	104
Iron	96	98	--

*Values obtained previously in this laboratory.

Weighed amounts of urea for nitrogen and aliquots of standard solutions of each mineral were added to composite samples of intake, feces, and urine and analyzed simultaneously with similar samples of composites to which no additional nutrients had been added. Because of the unavailability of a cathode tube for magnesium at the time recoveries were determined, the magnesium recoveries presented in Table 4 are values obtained previously in this laboratory using the same method of analysis as was used in this investigation. The ash solutions of intake, feces, and urine were diluted to a final concentration of 0.1 to 1.0 ppm of magnesium, and the magnesium determined on the Atomic Absorption Spectrophotometer without the addition of lanthanum oxide.

IV. STATISTICAL METHODS

The means of the data for each individual subject and the means of the data for all 10 subjects were calculated by:

$$\frac{\text{Data for each seven-day experimental period}}{\text{Number of seven-day experimental periods}}$$

The mineral intakes of the individual subjects were compared using analysis of variance: the one-way classification, and Duncan's new multiple-range test for groups with unequal replication. The relationship of nitrogen apparent absorption to nitrogen intake, phosphorus apparent absorption and balance to phosphorus intake, and the fecal loss of iron to iron intake was analyzed using simple linear regression. Analysis of variance: the one-way classification was performed to analyze the utilization of minerals throughout the weeks of gestation. The mineral retentions of supplemented and unsupplemented groups were compared using the Student t test for unpaired comparisons. These tests are described by Steel and Torrie (128).

CHAPTER IV

RESULTS

I. GENERAL

Subjects

Of 13 subjects begun on the study, data were collected for 10. Three subjects did not complete the study. One moved from the Knoxville area early in the experiment; another experienced severe and continuing nausea; the third suffered a miscarriage in early pregnancy.

Subject 7 moved from the area during the third trimester of pregnancy. However, four balance periods had been completed during the first and second trimesters of pregnancy and the data for this subject were included.

The balance period conducted during the sixteenth week of gestation for Subject 6 was deleted from all calculations. Diarrhea occurring at the end of the seven-day experimental period made separation of the final fecal sample for this period impossible.

With the exception of Subject 10 who was a housewife, all subjects continued their regular academic and employment schedules outside the home throughout most of the pregnancy. Although some subjects experienced slight nausea in early pregnancy, all remained in good health and delivered normal healthy children at the termination of the gestational period. Information concerning the mothers and their children is presented in Table 5.

TABLE 5

AGE, WEIGHT, AND HEMOGLOBIN OR HEMATOCRIT OF MOTHER AND BIRTH WEIGHT AND SEX OF CHILD

Subject	Age	Weight		Hemoglobin***	Hematocrit***	Birth Wt. of Child	Sex of Child
		Initial*	Final**				
	yr	kg	kg	g/100 ml	%	kg	
1	23	57.4	67.7	13.4		3.1	Female
2	25	51.3	62.6		38	3.5	Male
3	23	44.4	55.3		40	2.7	Male
4	29	59.0	71.7		39	2.9	Female
5	19	45.4	58.1		40	2.9	Male
6	26	54.4	65.3		39	3.3	Male
7	23	64.0	72.1		42	3.2	Male
8	23	47.6	60.3		39	2.9	Male
9	25	52.2	58.9	13.6		2.9	Female
10	28	59.9	66.2	11.9		3.6	Female

*Weight of mother on initial visit to obstetrician.

**Weight of mother on final visit to obstetrician prior to delivery.

***Hemoglobin or hematocrit of mother in early pregnancy.

Weight Gain

The average weight gain during pregnancy was 10.3 kg representing 22.6 pounds (Table 5). Except for three subjects, the weight gain fell within a range of 10.3 to 12.7 kg (23 to 28 pounds). Three subjects had a mean weight gain of 7.0 kg (15.7 pounds).

The report of the Committee on Maternal Nutrition of the National Academy of Sciences (129) suggested a weight gain of 9.1 to 11.3 kg (20 to 25 pounds) with emphasis that the pattern of weight gain was of greater importance than the total amount. A gain of 0.7 to 1.4 kg (1.5 to 3 pounds) during the first trimester and a continuing gain of 0.4 kg (0.8 pound) per week during the remainder of the gestation was the recommendation. The weight gains of the subjects of this study were relatively constant with no sudden, sharp increases.

Questionnaire

A questionnaire designed to indicate food intakes previous to pregnancy and how pregnancy had altered dietary intakes was completed by nine of the subjects. A description of a typical day's meals and snacks prior to pregnancy indicated that the milk group and the vegetable group, especially dark green leafy vegetables, had been most often deficient. Beef and pork had been most frequently consumed. Fish and lamb had been included once or less per week.

Only Subject 7 took a dietary supplement previous to pregnancy, a non-prescription multiple vitamin preparation plus iron. With the exception of one subject who had been given a weight gaining diet, no special diets had ever been prescribed by a physician for these subjects prior to pregnancy.

All subjects except one indicated that pregnancy had caused some change in their dietary intakes. Five subjects indicated a greater concern toward weight gain, being more conscious of snacks and the consumption of sweets and starchy foods. A change in the variety of milk commonly used was also evident. Before pregnancy, seven of the subjects had used whole milk. During pregnancy, only one subject continued to use whole milk, the other subjects changing to skim, dry skim, and 2% milks.

II. NITROGEN

Intake

The mean nitrogen intake of the 10 subjects on self-selected diets (Table 6) was 9.2 $\pm$ 2.2 g daily (58.3 g of protein). Three subjects had mean nitrogen intakes less than 9.0 g (56.2 g of protein). However, the mean nitrogen intake of the other seven subjects was 10.1 g (62.8 g of protein), an intake comparable to the recommended daily allowance of 10.4 g of nitrogen (65 g of protein) for pregnant women (4). Subjects 3 and 5 had the lowest nitrogen intakes; however, these two subjects were also the smallest women on the study.

Nitrogen Balance

Nitrogen balance was calculated as the intake minus the sum of the fecal and urinary losses. The mean fecal loss of nitrogen for the 10 subjects (Table 6) was 1.0 $\pm$ 0.4 g per day. A test for simple linear regression (128) revealed that the apparent absorption of nitrogen (the nitrogen intake minus the fecal loss) was significantly dependent on the

TABLE 6

NITROGEN UTILIZATION BY INDIVIDUAL SUBJECTS

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		g/24 hr		
		Subject 1		
20	9.4	1.1	9.2	-0.9
24	8.9	1.1	7.7	0.1
29	11.6	1.3	7.8	2.5
36	10.6	1.7	5.8	3.1
Mean	10.1	1.3	7.6	1.2
SD	±1.2	±0.3	±1.4	±1.9
Post Partum*	10.5	1.3	8.1	1.1
		Subject 2		
8	11.7	0.8	12.0	-1.1
13	8.0	1.3	11.0	-4.3
19	10.2	0.9	8.7	0.6
25	10.0	0.8	8.8	0.4
30	12.2	1.1	8.8	2.3
36	11.2	0.8	6.2	4.2
Mean	10.6	1.0	9.3	0.4
SD	±1.5	±0.2	±2.0	±2.9
Post Partum	10.5	0.7	9.0	0.8
		Subject 3		
16	6.7	2.7	9.9	-5.9
22	6.6	2.0	6.6	-2.0
29	6.9	1.0	7.0	-1.1
35	9.1	1.4	6.5	1.2
Mean	7.3	1.8	7.5	-2.0
SD	±1.2	±0.7	±1.6	±3.0

TABLE 6 (continued)

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		g/24 hr		
		Subject 4		
10	10.5	0.7	9.7	0.1
16	9.5	0.7	8.2	0.6
22	7.6	0.6	6.0	1.0
29	9.6	0.8	6.2	2.6
35	8.8	0.7	6.1	2.0
Mean	9.2	0.7	7.2	1.3
SD	±1.1	±0.0	±1.6	±1.0
Post Partum	9.0	0.4	7.1	1.5
		Subject 5		
16	7.2	0.9	7.3	-1.0
22	10.4	1.2	5.0	4.2
29	5.7	0.8	5.8	-0.9
35	5.3	1.0	5.2	-0.9
Mean	7.2	1.0	5.8	0.4
SD	±2.3	±0.2	±1.0	±2.6
		Subject 6		
9	11.7	1.0	9.6	1.1
22	11.2	1.1	8.3	1.8
29	10.7	0.8	8.4	1.5
35	12.1	0.6	9.2	2.3
Mean	11.4	0.9	8.9	1.7
SD	±0.6	±0.2	±0.6	±0.5
Post Partum	8.9	0.5	7.2	1.2
		Subject 7		
5	9.8	1.0	7.2	1.6
9	8.2	0.6	6.7	0.9
16	9.5	1.0	6.6	1.9
21	10.6	1.1	7.6	1.9
Mean	9.5	0.9	7.0	1.6
SD	±1.0	±0.2	±0.4	±0.5

TABLE 6 (continued)

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		g/24 hr		
		Subject 8		
6	6.7	0.8	5.9	0.0
12	9.5	0.6	7.8	1.1
16	8.9	0.8	7.7	0.4
22	10.3	0.7	9.2	0.4
27	15.0	0.7	9.4	4.9
35	11.2	0.5	9.1	1.6
Mean	10.3	0.7	8.2	1.4
SD	±2.8	±0.1	±1.3	±1.8
Post Partum*	9.1	0.8	5.8	2.5
		Subject 9		
11	9.3	0.8	6.7	1.8
16	8.2	1.0	8.0	-0.8
22	7.8	0.9	8.4	-1.5
29	10.0	1.2	7.7	1.1
35	11.4	1.3	7.7	2.4
Mean	9.3	1.0	7.7	0.6
SD	±1.4	±0.2	±0.6	±1.7
Post Partum	8.5	1.1	6.9	0.5
		Subject 10		
11	8.9	0.9	7.2	0.8
16	9.2	1.0	6.9	1.3
22	8.2	0.9	5.1	2.2
29	9.6	0.9	6.9	1.8
34	7.5	0.9	5.0	1.6
Mean	8.7	0.9	6.2	1.5
SD	±0.8	±0.0	±1.1	±0.5
Post Partum	6.5	0.9	6.7	-1.1

*Subject was lactating during the post partum experimental period.

intake ($P<0.001$, Table 13, Appendix B). The amount of nitrogen absorbed increased as the intake increased. There was no indication that the fecal loss varied with the level of nitrogen intake. Apparent absorption as defined here does not take into consideration endogenous losses of nitrogen.

Primary losses of nitrogen occurred through the urinary excretion of the nutrient. The mean urinary loss of nitrogen by the 10 subjects (Table 6) was 7.6 $\pm$ 1.6 g daily. With the exception of Subject 3, all subjects had a positive mean nitrogen balance (Table 6), although only one balance was positive for Subject 5. The mean balance for the 10 subjects was 0.8 $\pm$ 2.0 g daily.

Post Partum

The post partum nitrogen data of Subjects 1, 2, 4, 6, 8, 9, and 10 are presented in Table 6. Subjects 1 and 8 were lactating at the time of the post partum experimental period and were considered separately in this report. The mean nitrogen intake of the five subjects not lactating was 8.7 $\pm$ 1.4 g daily (54.2 g of protein), and the mean nitrogen balance was 0.6 $\pm$ 1.0 g daily. Only Subject 10 with an inadequate nitrogen intake was in negative nitrogen balance.

Both Subject 1 and 8 had nitrogen intakes below the recommended daily allowance of 12.0 g (75.0 g of protein) for lactating women (4). Both women appeared to be in positive nitrogen balance, but nitrogen loss in breast milk was not determined.

III. CALCIUM

Intake

Subjects 1, 3, 4, 5, 7, and 8 received a multiple vitamin-mineral supplement containing calcium as calcium carbonate (Tables 2 and 3, pages 39 and 40). The mean daily calcium supplement actually ingested by the subjects for each seven-day experimental period is indicated in Table 7. Subjects 4, 7, and 8 began supplementation after the first experimental period. Subjects 2, 6, 9, and 10 received no multiple vitamin-mineral supplement containing calcium.

The mean calcium intake of the 10 subjects (Table 7) was 1.372 $\pm$ 0.287 g daily. Only Subject 5 with a mean intake of 0.994 g failed to meet the recommended daily allowance of 1.2 g for pregnant women (4). An analysis of variance (128) established that the calcium intakes were not significantly different (Table 14, Appendix B) from subject to subject regardless of the calcium supplementation of six subjects.

Calcium Balance

The mineral balances of calcium, phosphorus, and magnesium were calculated as the intake minus the sum of the fecal and urinary losses. The mean fecal loss of calcium for the 10 subjects (Table 7) was 1.193 $\pm$ 0.523 g daily. The urinary loss of calcium for an individual subject was relatively constant throughout the pregnancy (Table 7). The mean urinary loss of calcium for the 10 subjects was 0.257 $\pm$ 0.106 g daily.

The mean calcium balance for the 10 subjects (Table 7) was -0.079 $\pm$ 0.517 g daily. No subject was in positive calcium balance in every

TABLE 7

CALCIUM UTILIZATION BY INDIVIDUAL SUBJECTS

Week of Gestation	Supp. of Ca	Intake*	Fecal Loss	Urinary Loss	Balance
			g/24 hr		
		Subject 1			
20	0.230	0.905	0.659	0.354	-0.108
24	0.230	1.274	0.795	0.301	0.178
29	0.230	1.269	0.772	0.534	-0.037
36	0.230	1.274	0.967	0.485	-0.178
Mean		1.180	0.798	0.418	-0.036
SD		±0.183	±0.127	±0.109	±0.154
Post Partum**	--	1.024	0.807	0.098	0.119
		Subject 2			
8	--	1.329	1.027	0.261	0.041
13	--	1.183	0.761	0.312	0.110
19	--	1.132	0.348	0.289	0.495
25	--	1.153	1.012	0.307	-0.166
30	--	1.519	0.949	0.276	0.294
36	--	1.234	0.802	0.062	0.370
Mean		1.258	0.816	0.251	0.191
SD		±0.146	±0.254	±0.094	±0.241
Post Partum	--	0.604	0.641	0.106	-0.143
		Subject 3			
16	0.250	1.810	1.647	0.331	-0.168
22	0.250	0.733	2.962	0.181	-2.410
29	0.250	1.337	1.461	0.177	-0.301
35	0.250	1.836	1.712	0.132	-0.008
Mean		1.429	1.946	0.205	-0.722
SD		±0.517	±0.686	±0.086	±1.132

TABLE 7 (continued)

Week of Gestation	Supp. of Ca	Intake*	Fecal Loss	Urinary Loss	Balance
			g/24 hr		
		Subject 4			
10	--	1.274	0.828	0.350	0.096
16	0.250	1.444	0.841	0.278	0.325
22	0.250	1.379	1.109	0.109	0.161
29	0.250	1.810	1.657	0.119	0.034
35	0.250	1.170	1.353	0.067	-0.250
Mean		1.415	1.158	0.185	0.073
SD		±0.244	±0.353	±0.122	±0.211
Post Partum	--	1.345	1.198	0.127	0.020
		Subject 5			
16	0.214	0.955	1.272	0.202	-0.519
22	0.250	1.163	1.223	0.165	-0.225
29	0.250	0.953	1.118	0.158	-0.323
35	0.250	0.904	1.402	0.115	-0.613
Mean		0.994	1.254	0.160	-0.420
SD		±0.115	±0.117	±0.035	±0.177
		Subject 6			
9	--	1.327	1.794	0.270	-0.737
22	--	1.671	2.735	0.248	-1.312
29	--	1.470	2.134	0.245	-0.909
35	--	1.754	1.651	0.275	-0.172
Mean		1.556	2.078	0.260	-0.782
SD		±0.193	±0.482	±0.014	±0.473
Post Partum	--	0.646	1.209	0.125	-0.688
		Subject 7			
5	--	1.385	0.684	0.230	0.471
9	0.250	1.321	0.944	0.298	0.079
16	0.250	1.329	1.253	0.334	-0.258
21	0.250	1.566	1.407	0.430	-0.271
Mean		1.400	1.072	0.323	0.005
SD		±0.114	±0.322	±0.083	±0.350

TABLE 7 (continued)

Week of Gestation	Supp. of Ca	Intake*	Fecal Loss	Urinary Loss	Balance
			g/24 hr		
		Subject 8			
6	--	0.771	0.538	0.233	0.000
12	0.250	1.603	0.622	0.330	0.651
16	0.250	1.369	1.174	0.289	-0.094
22	0.250	1.453	0.862	0.320	0.271
27	0.250	1.626	1.325	0.338	-0.037
35	0.250	1.909	1.231	0.141	0.537
Mean		1.455	0.959	0.275	0.221
SD		±0.383	±0.333	±0.075	±0.317
Post Partum**	0.250	1.212	1.071	0.144	-0.003
		Subject 9			
11	--	1.296	0.873	0.163	0.260
16	--	1.262	1.024	0.186	0.052
22	--	1.609	1.758	0.178	-0.327
29	--	1.764	1.343	0.217	0.204
35	--	1.910	1.467	0.195	0.248
Mean		1.568	1.293	0.188	0.087
SD		±0.285	±0.352	±0.017	±0.246
Post Partum	--	0.819	0.898	0.104	-0.183
		Subject 10			
11	--	1.652	0.916	0.228	0.508
16	--	1.442	1.487	0.283	-0.328
22	--	1.255	0.714	0.256	0.285
29	--	1.375	0.885	0.313	0.177
34	--	1.303	0.569	0.528	0.206
Mean		1.405	0.914	0.322	0.170
SD		±0.155	±0.349	±0.119	±0.307
Post Partum	--	0.886	0.733	0.096	0.057

*Intake includes supplement.

**Subject was lactating during the post partum experimental period.

seven-day experimental period, and Subjects 3, 5, and 6 were not in positive balance during any of the experimental periods. However, the mean calcium balance of five of the subjects indicated a retention of calcium, whereas two other subjects were in equilibrium (± 5% of the intake).

Calcium Utilization by Week of Gestation

The week of gestation in which the calcium balance was determined was also considered as a potential influence on calcium utilization. Calcium data were divided into six groups as to week of gestation in the same manner as is shown for calcium balance (Table 8). Calcium intakes generally increased as pregnancy progressed except for a decreased intake at approximately the twenty-second week of gestation. The urinary excretion of calcium was relatively constant. The fecal excretion increased as the intake increased until approximately the twenty-second week of gestation followed by a slight decrease which continued throughout the remaining experimental periods. A decreasing calcium balance continued through the latter part of the second trimester of pregnancy followed by an increasing calcium balance which continued throughout the duration of the experimental periods (Figure 1). However, an analysis of variance (128) established that the calcium balance did not significantly differ (Table 15, Appendix B) over the weeks of gestation.

Utilization of Supplemental Calcium

The calcium balance data were grouped as 23 seven-day experimental periods in which a calcium supplement was not part of the calcium intake

TABLE 8

CALCIUM BALANCE BY WEEK OF GESTATION

Subject	Week of Gestation	Ca Balance Ca Unsupp.	Ca Balance Ca Supp.	Subject	Week of Gestation	Ca Balance Ca Unsupp.	Ca Balance Ca Supp.
		g/24 hr				g/24 hr	
			First Trimester				
				2	8	0.041	
				7	9		0.079
				6	9	-0.737	
				4	10	0.096	
				9	11	0.260	
				10	11	0.508	
7	5	0.471		8	12		0.651
8	6	0.000		2	13	0.110	
Mean		0.236	--	Mean		0.046	0.365
Total Mean		0.236		Total Mean		0.126	
SD		±0.333		SD		±0.413	
			Second Trimester				
				2	19	0.495	
				1	20		-0.108
				7	21		-0.271
				8	22		0.271
				5	22		-0.225
9	16	0.052		4	22		0.161
10	16	-0.328		3	22		-2.410
3	16		-0.168	10	22	0.285	
4	16		0.325	9	22	-0.327	
5	16		-0.519	6	22	-1.312	
7	16		-0.258	1	24		0.178
8	16		-0.094	2	25	-0.166	
Mean		-0.138	-0.143	Mean		-0.205	-0.343
Total Mean		-0.141		Total Mean		-0.286	
SD		±0.273		SD		±0.813	

TABLE 8 (continued)

Subject	Week of Gestation	Ca Balance Ca Unsupp.	Ca Balance Ca Supp.	Subject	Week of Gestation	Ca Balance Ca Unsupp.	Ca Balance Ca Supp.
		g/24 hr				g/24 hr	
			Third Trimester				
8	27		-0.037	10	34	0.206	
6	29	-0.909		6	35	-0.172	
9	29	0.204		9	35	0.248	
10	29	0.177		3	35		-0.008
1	29		-0.037	4	35		-0.250
3	29		-0.301	5	35		-0.613
4	29		0.034	8	35		0.537
5	29		-0.323	2	36	0.370	
2	30	0.294		1	36		-0.178
Mean		-0.058	-0.133	Mean		0.163	-0.102
Total Mean		-0.100		Total Mean		0.016	
SD		±0.370		SD		±0.358	

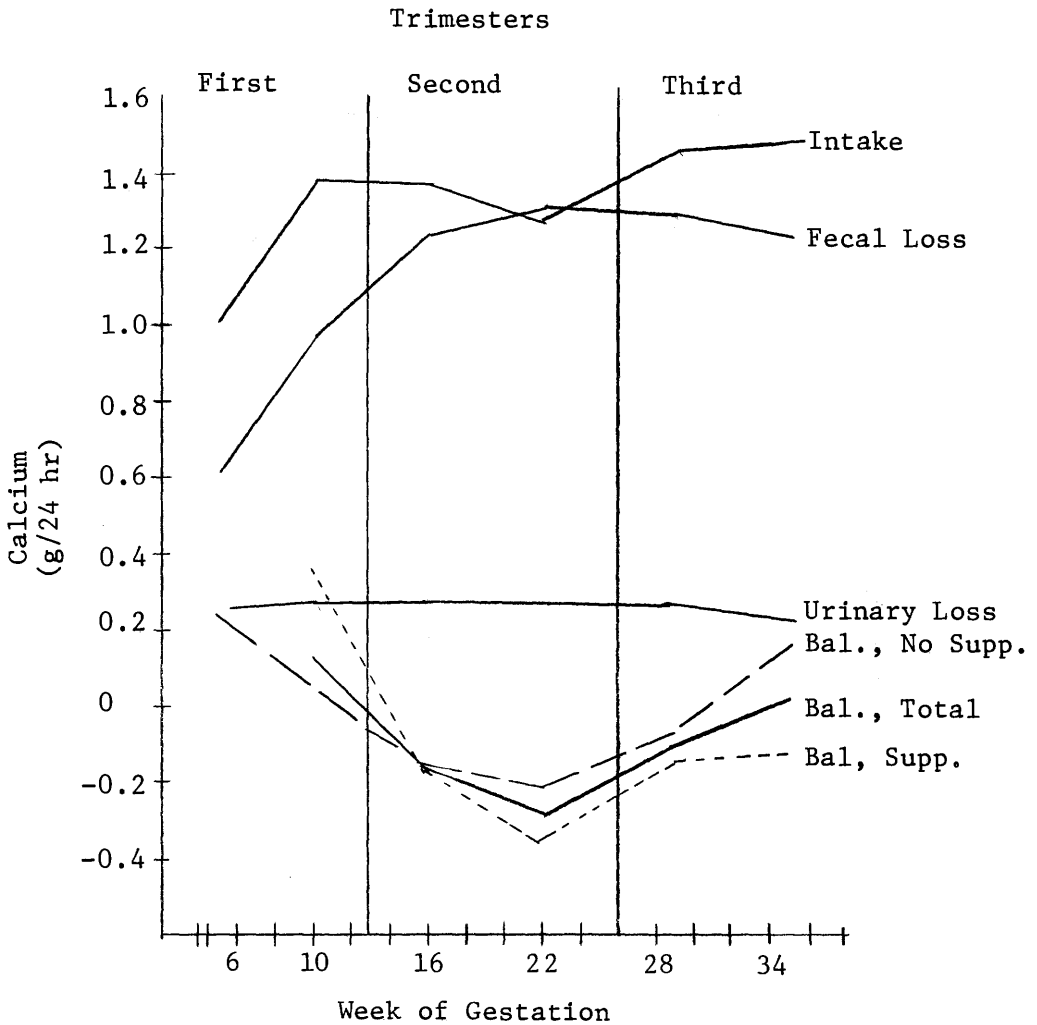

FIGURE 1

MEAN CALCIUM UTILIZATION BY WEEK OF GESTATION

and 24 seven-day experimental periods in which a supplement containing calcium carbonate provided approximately 20% of the calcium intake. The Student t test (128) verified that the calcium balance values of these two groups were not significantly different (Table 16, Appendix B).

Post Partum

The post partum calcium data for Subjects 1, 2, 4, 6, 8, 9, and 10 are presented in Table 7, page 63. Only Subject 8 continued to receive a multiple vitamin-mineral supplement containing 0.250 g of calcium as calcium carbonate (Tables 2 and 3, pages 39 and 40). The mean calcium intake of the five subjects not lactating during the post partum experimental period was 0.860 $\pm$ 0.295 g daily. However, Subjects 2 and 6 had calcium intakes less than 0.800 g and were in negative calcium balance. Subject 9 had an intake of 0.819 g, but was also in negative calcium balance. Subject 4 was in equilibrium ($\pm$ 5% of the intake); only Subject 10 indicated calcium retention. Generally, the retention of calcium by these subjects during the post partum experimental period was lower than the calcium retention during pregnancy. The fecal loss of calcium was large, and there was a mean negative balance of -0.187 $\pm$ 0.298 g daily for the five subjects.

Subjects 1 and 8 had calcium intakes less than the recommended daily allowance of 1.3 g for lactating women (4). Nevertheless, Subject 8 appeared to be in equilibrium ($\pm$ 5% of the intake), while Subject 1 showed a retention of calcium. However, the loss of calcium due to lactation was not determined.

IV. PHOSPHORUS

Intake

No supplement received by any subject contained phosphorus. However, an analysis of variance (128) indicated a significant difference ($P<0.005$, Table 17, Appendix B) in the phosphorus intakes (Table 9). Duncan's new multiple-range test (128) specified that with the exception of Subjects 7 and 9 the phosphorus intake of Subject 6, who had a large milk consumption, was significantly greater ($P<0.05$, Table 18, Appendix B) than that of the other subjects. The phosphorus intakes of Subjects 7 and 9 were significantly greater ($P<0.05$, Table 18, Appendix B) than the intakes of Subjects 2, 4, and 5. The intakes of Subjects 1, 2, 3, 4, 5, 8, and 10 were not significantly different (Table 18, Appendix B) from one another.

The mean phosphorus intake of the 10 subjects was 1.335 ± 0.278 g daily. Seven of the subjects met the recommended daily allowance of 1.2 g for pregnant women (4). The mean phosphorus intakes of Subjects 2, 4, and 5 were approaching this level.

Phosphorus Balance

The mean fecal loss of phosphorus for the 10 subjects (Table 9) was 0.619 ± 0.211 g daily. The apparent absorption of phosphorus (the intake minus the fecal loss with no consideration for endogenous losses) was found to be significantly dependent ($P<0.001$, Table 13, Appendix B) on the phosphorus intake using the test for simple linear regression (128). The apparent absorption of phosphorus increased as the intake increased.

TABLE 9

PHOSPHORUS UTILIZATION BY INDIVIDUAL SUBJECTS

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		g/24 hr		
		Subject 1		
20	1.242	0.478	0.702	0.062
24	1.411	0.563	0.683	0.165
29	1.305	0.460	0.954	-0.109
36	1.523	0.674	0.785	0.064
Mean	1.370	0.544	0.781	0.046
SD	±0.123	±0.097	±0.123	±0.113
Post Partum*	1.511	0.683	0.940	-0.112
		Subject 2		
8	1.320	0.748	0.915	-0.343
13	1.246	0.579	0.776	-0.109
19	0.932	0.384	0.680	-0.132
25	1.406	0.645	0.768	-0.007
30	1.024	0.533	0.858	-0.367
36	0.853	0.427	0.678	-0.252
Mean	1.130	0.553	0.779	-0.202
SD	±0.225	±0.135	±0.094	±0.142
Post Partum	0.997	0.370	0.814	-0.187
		Subject 3		
16	1.432	0.993	0.667	-0.228
22	0.843	1.398	0.282	-0.837
29	1.318	0.682	0.565	0.071
35	1.544	0.919	0.542	0.083
Mean	1.284	0.998	0.514	-0.228
SD	±0.308	±0.298	±0.164	±0.431

TABLE 9 (continued)

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		g/24 hr		
		Subject 4		
10	1.535	0.649	0.809	0.077
16	1.150	0.630	0.626	-0.106
22	0.917	0.617	0.524	-0.224
29	0.932	0.776	0.579	-0.423
35	1.032	0.488	0.594	-0.050
Mean	1.113	0.632	0.626	-0.145
SD	±0.253	±0.102	±0.108	±0.189
Post Partum	1.390	0.613	0.580	0.197
		Subject 5		
16	1.022	0.594	0.601	-0.173
22	1.166	0.698	0.633	-0.165
29	1.037	0.530	0.442	0.065
35	0.960	0.626	0.471	-0.137
Mean	1.046	0.612	0.537	-0.102
SD	±0.086	±0.069	±0.094	±0.112
		Subject 6		
9	1.675	0.988	0.696	-0.009
22	1.564	0.562	0.738	0.264
29	1.676	0.412	0.643	0.621
35	2.002	0.313	0.934	0.755
Mean	1.729	0.569	0.753	0.408
SD	±0.189	±0.297	±0.126	±0.346
Post Partum	0.814	0.873	0.453	-0.512
		Subject 7		
5	1.535	0.330	0.843	0.362
9	1.364	0.366	0.929	0.069
16	1.332	0.392	0.781	0.159
21	1.686	0.511	1.057	0.118
Mean	1.479	0.400	0.902	0.177
SD	±0.164	±0.078	±0.119	±0.128

TABLE 9 (continued)

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		g/24 hr		
		Subject 8		
6	0.960	0.315	0.625	0.020
12	1.257	0.465	0.664	0.128
16	1.354	0.597	0.624	0.133
22	1.399	0.714	0.633	0.052
27	1.705	0.737	0.853	0.115
35	1.417	0.598	0.623	0.196
Mean	1.349	0.571	0.670	0.107
SD	±0.242	±0.159	±0.090	±0.062
Post Partum*	1.413	1.072	0.743	-0.402
		Subject 9		
11	1.440	0.544	0.629	0.267
16	1.267	0.647	0.602	0.018
22	1.408	0.707	0.652	0.049
29	1.670	0.924	0.711	0.035
35	2.029	1.081	0.679	0.269
Mean	1.563	0.781	0.655	0.128
SD	±0.298	±0.218	±0.041	±0.128
Post Partum	0.912	0.594	0.604	-0.286
		Subject 10		
11	1.392	0.546	0.697	0.149
16	1.479	0.587	0.688	0.204
22	1.307	0.474	0.612	0.221
29	1.427	0.662	0.728	0.037
34	1.269	0.527	0.581	0.161
Mean	1.375	0.559	0.661	0.154
SD	±0.085	±0.070	±0.061	±0.071
Post Partum	0.981	0.333	0.587	0.061

*Subject was lactating during the post partum experimental period.

(The data for Subject 7, the fifth week of gestation, and Subject 8, the sixth week of gestation, were determined by the test described by Steel and Torrie (128) as being significantly different ($P<0.05$) from the population group tested and were omitted from the calculation of simple linear regression).

The mean urinary loss of phosphorus of the 10 subjects (Table 9) was 0.688 ± 0.142 g daily; the mean phosphorus balance for the 10 subjects (Table 9) was 0.028 ± 0.256 g daily. Subjects 6 through 10 had a positive mean phosphorus balance, while Subject 1 was in equilibrium ($\pm$ 5% of the intake). Subjects 2 through 5 showed a negative mean phosphorus balance.

Using the test for simple linear regression (128) it was determined that the phosphorus balance was directly related to the phosphorus intake ($P<0.02$, Table 13, Appendix B). As the intake increased, the phosphorus balance increased. (The data for Subject 7, the fifth week of gestation, and Subject 8, the sixth week of gestation, were determined by the test described by Steel and Torrie (128) as being significantly different ($P<0.05$) from the population group tested and were omitted from the calculations of simple linear regression).

Phosphorus Utilization by Week of Gestation

In order to determine if the gestational period was a factor affecting utilization of phosphorus, phosphorus data were grouped into six gestational periods in the same manner as the calcium balances (Table 8, page 67). Except for a rise after the first experimental period, the fecal loss of phosphorus was relatively constant. The

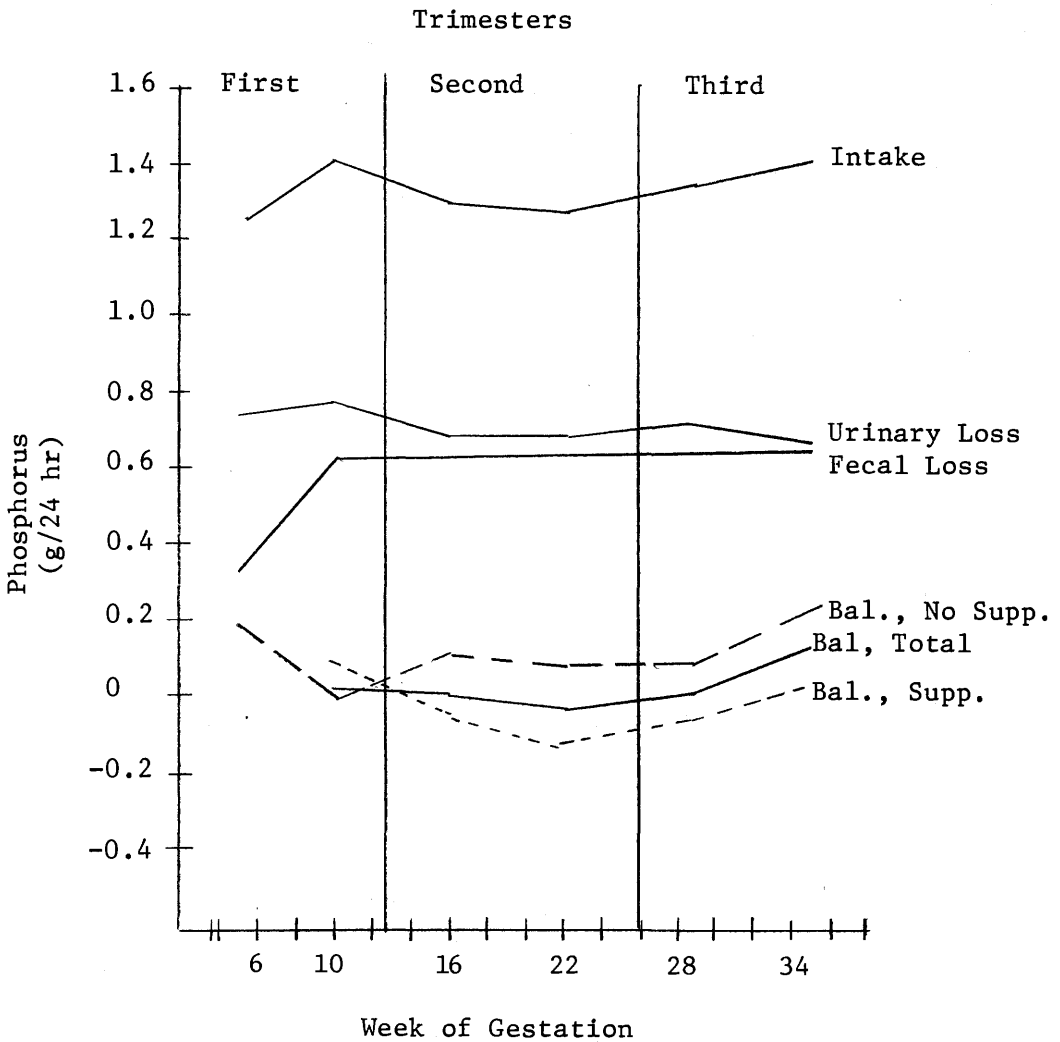

FIGURE 2

MEAN PHOSPHORUS UTILIZATION BY WEEK OF GESTATION

The phosphorus intakes of Subjects 1 and 8 exceeded the recommended daily allowance of 1.3 g for lactating women (4). Nevertheless, both subjects were in negative phosphorus balance. If the phosphorus losses in the breast milk had been determined, the negative phosphorus balance would have been greater.

V. MAGNESIUM

Intake

Only Subjects 1 and 8 received any supplemental magnesium. Their multiple vitamin-mineral supplement provided only a small quantity of magnesium, 0.15 mg as the oxide (Tables 2 and 3, pages 39 and 40). However, an analysis of variance (128) indicated a significant difference ($P<0.025$, Table 20, Appendix B) in the magnesium intakes. Duncan's new multiple-range test (128) established that the magnesium intake of Subject 5 was significantly lower ($P<0.05$, Table 18, Appendix B) than the magnesium intake of the other subjects. With the exception of Subject 5, the magnesium intakes of the subjects were not significantly different (Table 18, Appendix B).

The magnesium intakes were surprisingly low (Table 10). The mean intake of the 10 subjects was 269 $\pm$ 55 mg daily. With the exception of Subject 5, the mean magnesium intakes were within 255 to 310 mg daily. This is below the recommended daily allowance of 450 mg for pregnant women (4).

Magnesium Balance

The mean fecal loss of magnesium for the 10 subjects (Table 10) was 215 $\pm$ 69 mg daily. The urinary loss of magnesium was relatively

TABLE 10

MAGNESIUM UTILIZATION BY INDIVIDUAL SUBJECTS

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		mg/24 hr		
		Subject 1		
20	266	165	142	-41
24	303	196	167	-60
29	277	209	177	-109
36	322	203	166	-47
Mean	292	193	163	-64
SD	±24	±17	±10	±30
Post Partum*	352	201	147	4
		Subject 2		
8	271	208	109	-46
13	227	160	96	-29
19	245	135	128	-18
25	266	157	122	-13
30	311	197	132	-18
36	250	197	71	-18
Mean	262	176	110	-24
SD	±28	±28	±20	±10
Post Partum	219	113	85	21
		Subject 3		
16	298	316	121	-139
22	202	370	22	-190
29	226	202	62	-38
35	297	303	80	-86
Mean	256	298	71	-113
SD	±49	±70	±40	±65

TABLE 10 (continued)

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		mg/24 hr		
		Subject 4		
10	346	286	118	-58
16	266	199	92	-25
22	226	154	75	-3
29	282	225	78	-21
35	246	164	91	-9
Mean	273	206	91	-23
SD	±45	±52	±14	±20
Post Partum	223	171	93	-41
		Subject 5		
16	166	134	84	-52
22	219	184	92	-57
29	161	97	69	-5
35	152	116	77	-41
Mean	174	133	80	-39
SD	±28	±36	±0	±22
		Subject 6		
9	293	254	101	-62
22	300	387	118	-205
29	289	252	102	-65
35	343	213	132	-2
Mean	306	276	113	-84
SD	±24	±75	±14	±86
Post Partum	218	195	86	-63
		Subject 7		
5	340	188	72	80
9	238	114	76	48
16	281	215	85	-19
21	275	195	92	-12
Mean	284	178	81	24
SD	±41	±44	±0	±47

TABLE 10 (continued)

Week of Gestation	Intake	Fecal Loss	Urinary Loss	Balance
		mg/24 hr		
		Subject 8		
6	151	133	71	-53
12	229	164	78	-13
16	280	202	68	10
22	271	232	76	-37
27	310	281	105	-76
35	287	196	101	-10
Mean	255	201	83	-30
SD	±56	±51	±14	±30
Post Partum*	214	147	50	17
		Subject 9		
11	250	153	80	17
16	247	188	85	-26
22	246	194	76	-24
29	343	249	104	-10
35	457	412	104	-59
Mean	309	239	90	-20
SD	±92	±102	±10	±26
Post Partum	226	232	98	-104
		Subject 10		
11	312	266	71	-25
16	307	278	63	-34
22	263	300	72	-109
29	257	226	63	-32
34	269	283	73	-87
Mean	282	271	68	-57
SD	±24	±26	±0	±37
Post Partum	214	209	61	-56

*Subject was lactating during the post partum experimental period.

constant for each subject throughout the pregnancy (Table 10) having a mean of 94 ± 28 mg daily for the 10 subjects.

With the exception of Subject 7, the mean magnesium balance of all subjects was negative (Table 10). Balance periods for Subject 7 were completed only through the twenty-first week of gestation. The positive magnesium balances of Subject 7 occurred during the first trimester of pregnancy, while one balance completed during the second trimester was negative and the other was in equilibrium (± 5% of the intake). Seven subjects were not in positive magnesium balance during any seven-day experimental period. The mean balance of the 10 subjects was -41 ± 50 mg daily.

Magnesium Utilization by Week of Gestation

The grouping of the magnesium data into six gestational periods as was done for calcium balance (Table 8, page 67) revealed that there was little change in magnesium balance throughout the pregnancy. An analysis of variance (128) established that the change in magnesium balance throughout the weeks of gestation was not significant (Table 21, Appendix B).

Post Partum

The post partum magnesium balance data for Subjects 1, 2, 4, 6, 8, 9, and 10 are presented in Table 10. The mean magnesium intake for the five subjects not lactating during the post partum experimental period was 220 ± 0 mg daily. All magnesium intakes were below the recommended daily allowance of 300 to 350 mg daily for nonpregnant women in these

age groups (4), and four of these subjects were in negative magnesium balance. One subject showed a small retention. The mean magnesium balance for the five subjects was -48 ± 45 mg daily.

Neither Subject 1 nor 8 met the recommended daily allowance of 450 mg of magnesium for lactating women (4). Nevertheless, Subject 1 appeared to be in magnesium equilibrium (± 5% of the intake), and Subject 8 showed a small retention. However, magnesium losses via breast milk were not determined.

VI. IRON

Intake

Subjects 3, 4, 5, 7, and 8 received a multiple vitamin-mineral supplement containing iron as ferrous fumerate or ferrous sulfate (Tables 2 and 3, pages 39 and 40). Subject 1 received ferrous fumerate from both a multiple vitamin-mineral supplement and an iron supplement. Subjects 2 and 6 received ferrous sulfate supplementation only, while Subjects 9 and 10 received no supplemental iron. The mean daily iron supplement actually ingested by the subjects for each seven-day experimental period is indicated in Table 11. Subjects 4, 6, 7, and 8 began iron supplementation following the first seven-day balance period. Subject 2 began supplementation before beginning the third experimental period, but the supplementation continued for only two and one-half weeks.

Because Subject 2 received iron supplementation during only the experimental period conducted during the nineteenth week of gestation and because this supplementation was discontinued approximately a month

TABLE 11

IRON UTILIZATION BY INDIVIDUAL SUBJECTS

Week of Gestation	Supp. of Fe	Intake*	Fecal Loss	Apparent Absorption
			mg/24 hr	
		Subject 1		
20	30	46.8	50.0	-3.2
24	30	41.8	45.6	-3.8
29	80	104.0	105.6	-1.6
36	80	103.4	122.2	-18.8
Mean		74.0	80.8	-6.8
SD		±34.4	±38.8	±8.0
Post Partum**	--	18.1	15.6	2.5
		Subject 2		
8	--	18.2	17.9	0.3
13	--	18.3	20.5	-2.2
19	105***	(122.2)	(57.0)	(65.2)
25	--	11.5	35.5	-24.0
30	--	18.5	7.5	11.0
36	--	18.2	1.6	16.6
Mean		16.9	16.6	0.3
SD		±3.0	±13.0	±15.6
Post Partum	--	14.1	9.0	5.1
		Subject 3		
16	40	59.6	60.4	-0.8
22	40	51.9	76.6	-24.7
29	40	49.9	52.1	-2.2
35	40	50.8	47.6	3.2
Mean		53.0	59.2	-6.1
SD		±4.4	±12.8	±12.6

TABLE 11 (continued)

Week of Gestation	Supp. of Fe	Intake*	Fecal Loss	Apparent Absorption
			mg/24 hr	
		Subject 4		
10	--	11.7	7.7	4.0
16	40	53.2	55.4	-2.2
22	40	49.3	40.0	9.3
29	40	53.6	43.6	10.0
35	40	57.2	50.6	6.6
Mean		45.0	39.5	5.5
SD		±18.8	±18.7	±4.9
Post Partum	--	12.4	8.1	4.3
		Subject 5		
16	34	47.2	36.6	10.6
22	40	55.7	50.3	5.4
29	40	62.0	49.6	12.4
35	40	68.9	59.5	9.4
Mean		58.5	49.0	9.4
SD		±9.2	±9.4	±3.0
		Subject 6		
9	--	12.8	12.6	0.2
22	45***	(68.7)	(135.6)	(-66.9)
29	90***	(111.2)	(122.3)	(-11.1)
35	150***	(203.6)	(187.7)	(15.9)
Mean		12.8	12.6	0.2
SD		--	--	--
Post Partum	--	14.9	9.6	5.3
		Subject 7		
5	--	23.8	9.5	14.3
9	40	85.0	37.4	47.6
16	40	65.7	63.8	1.9
21	40	65.3	48.5	16.8
Mean		60.0	39.8	20.2
SD		±25.8	±22.9	±19.4

TABLE 11 (continued)

Week of Gestation	Supp. of Fe	Intake*	Fecal Loss	Apparent Absorption
			mg/24 hr	
		Subject 8		
6	--	11.5	8.5	3.0
12	40	63.9	41.6	22.3
16	40	69.8	65.8	4.0
22	60	88.8	90.9	-2.1
27	60	99.4	74.5	24.9
35	60	97.5	78.3	19.2
Mean		71.8	59.9	11.9
SD		±32.9	±30.1	±11.6
Post Partum**	60	116.2	64.0	52.2
		Subject 9		
11	--	13.0	9.6	3.4
16	--	17.9	12.4	5.5
22	--	11.1	12.1	-1.0
29	--	20.4	18.5	1.9
35	--	19.4	14.1	5.3
Mean		16.4	13.3	3.0
SD		±4.1	±3.3	±2.7
Post Partum	--	14.8	9.8	5.0
		Subject 10		
11	--	22.7	24.5	-1.8
16	--	24.4	27.3	-2.9
22	--	22.8	19.0	3.8
29	--	34.1	33.3	0.8
34	--	9.5	12.2	-2.7
Mean		22.7	23.3	-0.6
SD	--	±8.7	±8.0	±2.8
Post Partum	--	13.4	8.0	5.4

*Intake includes supplement.

**Subject was lactating during the post partum experimental period.

***Because of irregularity in iron supplementation, data for Subject 2 and Subject 6 appearing in parentheses were not included in iron calculations.

before the next experimental period began, the data collected during the nineteenth week of gestation for Subject 2 were not included in the analysis of the iron data. The data collected during the remaining experimental periods were analyzed as not including an iron supplement.

The ingestion of the iron supplement by Subject 6 was notably irregular (Table 11). This irregularity in the iron intake could obscure physiological adaptation of the subject during pregnancy. For this reason, only the iron data of Subject 6 collected before supplementation began, the ninth week of gestation, were included in the analysis of the iron data.

The iron intakes of the 10 subjects (Table 11) had a mean of 44.9 $\pm$ 28.6 mg daily. An analysis of variance (128) indicated a significant difference ($P<0.005$, Table 22, Appendix B) in the iron intakes of subjects. Using Duncan's new multiple-range test (128) it was determined that the iron intakes of Subjects 1, 3, 5, 7, and 8 were significantly greater ($P<0.05$, Table 18, Appendix B) than the intakes of Subjects 2, 9, and 10. That is, with the exception of Subject 4, the intake of the subjects receiving iron supplementation were significantly higher than the intake of the subjects receiving no supplementation. Nevertheless, with the exception of two unsupplemented subjects with a mean intake of 16.6 mg, all subjects met the recommended daily allowance of 18 mg for pregnant women (4).

Apparent Absorption

The mean fecal loss of iron (Table 11) was 40.7 $\pm$ 28.0 mg daily. Using simple linear regression (128) the fecal loss was determined to be

significantly dependent ($P<0.001$, Table 13, Appendix B) on the iron intake. As the intake increased, the loss of iron in the feces increased.

The apparent absorption of iron (the intake minus the fecal loss) did not consider the endogenous losses of the mineral (Table 11, page 84). Subjects 1 and 3 had negative mean apparent absorptions of iron; Subjects 2 and 10 were in equilibrium ($\pm$ 5% of the intake); Subjects 4, 5, 7, 8, and 9 had positive apparent absorptions. The mean apparent absorption of the 10 subjects was 4.2 mg with a standard deviation of 12.2.

Iron Utilization by Week of Gestation

To determine if iron absorption was dependent on the period of gestation, iron data were divided into six groups as to week of gestation in the same manner as is shown for apparent absorption (Table 12). Iron intakes generally increased throughout pregnancy except for a decreased intake at approximately the twenty-second week of gestation. The fecal excretion increased rapidly as the intake increased until approximately the sixteenth week of gestation and then remained relatively constant at that level, continuing to increase only slightly. There was a decrease in the mean apparent absorption of iron during the second trimester of pregnancy followed by an increasing absorption after the twenty-second week of gestation (Figure 3). However, as determined by analysis of variance (128), the apparent absorption of iron was not significantly different (Table 23, Appendix B) throughout the pregnancy.

Utilization of Supplemental Iron

A greater mean apparent absorption of iron by the iron supplemented subjects was evident during every gestational period except the latter

TABLE 12

APPARENT ABSORPTION OF IRON BY WEEK OF GESTATION

Subject	Week of Gestation	Apparent Absorption Fe Unsupp. mg/24 hr	Apparent Absorption Fe Supp. mg/24 hr	Subject	Week of Gestation	Apparent Absorption Fe Unsupp. mg/24 hr	Apparent Absorption Fe Supp. mg/24 hr
			First Trimester				
				2	8	0.3	
				7	9		47.6
				6	9	0.2	
				4	10	4.0	
				9	11	3.4	
				10	11	-1.8	
7	5	14.3		8	12		22.3
8	6	3.0		2	13	-2.2	
Mean		8.6	--	Mean		0.6	35.0
Total Mean		8.6		Total Mean		9.2	
SD		±8.0		SD		±17.4	
			Second Trimester				
				1	20		-3.2
				7	21		16.8
				8	22		-2.1
9	16	5.5		5	22		5.4
10	16	-2.9		4	22		9.3
3	16		-0.8	3	22		-24.7
4	16		-2.2	10	22	3.8	
5	16		10.6	9	22	-1.0	
7	16		1.9	1	24		-3.8
8	16		4.0	2	25	-24.0	
Mean		1.3	2.7	Mean		-7.0	-0.3
Total Mean		2.3		Total Mean		-2.3	
SD		±4.8		SD		±13.2	

TABLE 12 (continued)

Subject	Week of Gestation	Apparent Absorption Fe Unsupp. mg/24 hr	Apparent Absorption Fe Supp. mg/24 hr	Subject	Week of Gestation	Apparent Absorption Fe Unsupp. mg/24 hr	Apparent Absorption Fe Supp. mg/24 hr
			Third Trimester				
8	27		24.9	10	34	-2.7	
9	29	1.9		9	35	5.3	
10	29	0.8		3	35		3.2
1	29		-1.6	4	35		6.6
3	29		-2.2	5	35		9.4
4	29		10.0	8	35		19.2
5	29		12.4	1	36		-18.8
2	30	11.0		2	36	16.6	
Mean		4.6	8.7	Mean		6.4	4.0
Total Mean		7.1		Total Mean		4.9	
SD		±9.2		SD		±11.9	

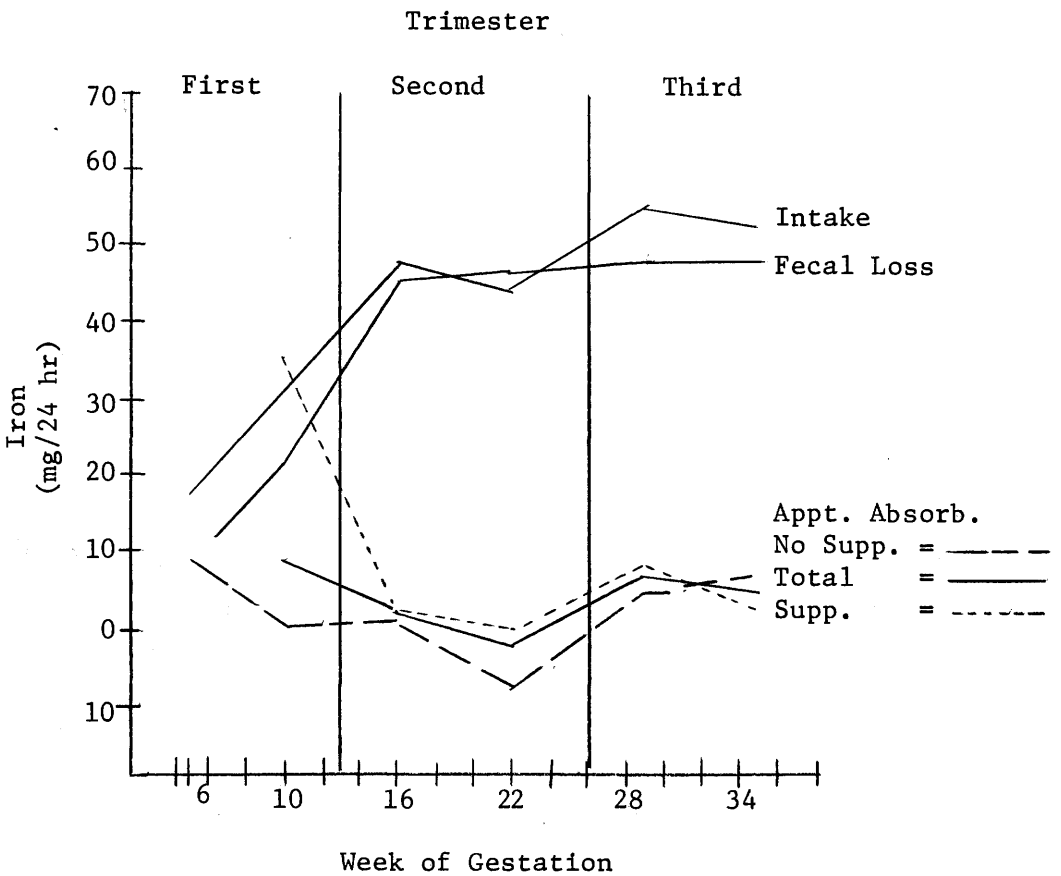

FIGURE 3

MEAN IRON UTILIZATION BY WEEK OF GESTATION

part of the third trimester (Table 12). The Student t test (128) was used to determine if the greater apparent absorption of iron when a supplement was included was significant. The experimental periods were grouped as 24 seven-day experimental periods in which iron supplementation had been ingested and 19 seven-day experimental periods in which no iron supplement had been included. The test revealed that the total amount of iron absorbed when an iron supplement was present was significantly higher ($P<0.01$, Table 16, Appendix B) than the amount absorbed when no supplement was included in the intake.

Post Partum

The post partum iron data of Subjects 1, 2, 4, 6, 8, 9, and 10 are presented in Table 11, page 84. Only Subject 8 continued to receive a multiple vitamin-mineral supplement containing 60 mg of iron as ferrous sulfate (Tables 2 and 3, pages 39 and 40). Since the recommended daily allowance of iron is not increased during lactation, the two lactating subjects were not considered separately in the post partum iron data. The mean iron intake of the six unsupplemented subjects was 14.6 ± 2.0 mg with an apparent absorption of 4.6 ± 1.0 mg daily, a 31.3% absorption. The mean iron intake of all seven subjects was 29.1 ± 38.4 mg daily or 39.2% absorption. This value far exceeds the mean apparent absorption of approximately 9.1% during pregnancy.

CHAPTER V

DISCUSSION

In both animal and human studies, the elevated nutrient requirements of pregnancy have been reported to be accompanied by an increased efficiency in the utilization of various nutrients (26,59-61,64,105-107). Despite physiological adaptations, additional daily allowances of nutrients are recommended during pregnancy (4).

In recent years a trend has developed toward including a prenatal supplement providing various vitamins and minerals with a decreasing emphasis on the composition of the prenatal diet. Incidences of leg cramps, reported by some investigators to be related to low serum calcium levels resulting from high phosphorus intakes (84,85), have led to an emphasis on the use of phosphorus-free supplements and, in many instances, to a decreased milk intake. As prenatal supplements are increasingly depended upon to meet the additional requirements of pregnancy, the need for mineral supplementation as well as the utilization of minerals provided by a supplement become of concern.

I. NITROGEN

Intake

The apparent absorption of nitrogen significantly increased as the intake increased, and negative balances occurred only when nitrogen intakes were inadequate. Nitrogen intakes were adequate for most subjects; however, the negative balances of Subjects 3 and 5 indicated that their

nitrogen intakes were not sufficient to meet the nitrogen requirements of pregnancy or that the quality of the protein was poor.

Nitrogen Balance

The nitrogen, calcium, phosphorus, magnesium, and iron balances of the women on this study when consuming self-selected diets were markedly irregular from woman to woman and for the same woman at different times of the gestational period. Such variation has often been seen in human studies (59-62, 105, 106). Nitrogen balance generally increased in the latter part of pregnancy, but intake also increased so that any effect of the gestational period on nitrogen utilization could not be discerned. These results were similar to those of Coons and Blunt (60) who found nitrogen retention was most frequently related to intake in pregnant women on self-selected diets, and reported that nitrogen retention did not seem related to the period of gestation.

The lower nitrogen retentions by these subjects as compared to the nitrogen retentions of pregnant women reported in earlier studies (67-69) may partially have resulted from smaller nitrogen intakes. However, caloric intakes may also have been influential in nitrogen retention. The calculated caloric intakes are presented in Table 24, Appendix B. The mean caloric intake of the ten subjects was 1758 $\pm$ 269 kcal daily. Subject 3, the only subject in negative nitrogen balance, was also the subject having the lowest mean caloric intake, 1333 $\pm$ 182 kcal daily. However, Subject 3 was also the smallest woman on the experiment.

II. CALCIUM

Intake

Calcium supplementation was included if it had been prescribed by the obstetrician initially, or if it seemed needed as the pregnancy progressed. Six of the 10 subjects received a supplement of calcium, and with the exception of one subject all women consumed at least the recommended calcium allowance. The calcium intake of the subjects with diets providing a good consumption of dairy products but no supplemental calcium were comparable to the calcium intake of the subjects receiving 0.230 to 0.250 g of calcium daily from a prenatal supplement. This illustrates that for the pregnant woman with an adequate intake of dairy products, a supplement of calcium is not necessary to meet the requirements of pregnancy. Furthermore, four of the six subjects receiving a calcium supplement had a mean daily intake of 1.427 g of calcium. Assuming that 0.250 g of calcium were provided by a prenatal supplement, 1.177 g were provided by the subject's diet. That is, of the 10 subjects, all but two essentially met the recommended daily allowance of 1.2 g of calcium for pregnant women through their diet alone (4).

Calcium Balance

Calcium balances fluctuated from period to period, and in spite of supposedly adequate intakes negative calcium balances occurred in various experimental periods for all subjects. Periods of calcium losses were reported by Macy et al. (61) in pregnant women with calcium intakes of 1.5 to 2.7 g daily, and by Hummel et al. (62) in a pregnant woman with a mean daily intake of 3.1 g.

Fecal losses of calcium were large, often exceeding the calcium intake. Similar findings were reported by Hunscher (63) in lactating women, by Leichsenring et al. (44) in college women, and by Steggerda and Mitchell (130) in men. However, all fecal calcium was not unabsorbed dietary calcium. Stearns (131) estimated that from 0.3 to 0.8 g of calcium daily, with a mean of approximately 0.65 g, was secreted into the intestine. Bronner and Harris (9) and Spencer et al. (10) estimated that 5 to 15% of the fecal calcium output was endogenous.

The large fecal losses of Subject 6 resulting in negative calcium balances may have exemplified adaptation to a large calcium intake. Subject 6 had a dietary history of large milk intakes, consuming at least a quart of milk daily, and continued these high milk intakes during pregnancy. Although negative balances persisted throughout all experimental balance periods, the calcium balances became less negative as the pregnancy progressed. This suggested the possible influence of pregnancy on the calcium balance of a woman whose body had adapted to high calcium intakes.

Although the total calcium of the diet affects the utilization of the mineral, Scoular et al. (132) pointed out that the day-to-day variation in other nutrients which occurs in self-selected diets may also be a deciding factor. Although it cannot be determined from this study, the large negative calcium balances of Subjects 3 and 5 may have represented a relationship between poor calcium utilization and inadequate protein intakes.

Calcium Utilization by Week of Gestation

In periodic balance experiments conducted on pregnant women between the twelfth and thirty-ninth weeks of gestation with a mean calcium intake of 1.049 g daily, Coons and Blunt (60) reported a tendency for calcium retention to rise toward the end of pregnancy. More recently, Shenolikar (59) reported that in balance experiments conducted on pregnant Indian women at different gestational periods the amount of calcium retained during the twenty-eighth to thirty-second weeks of gestation was significantly higher than that retained during the twelfth to sixteenth weeks of gestation. This increased calcium retention continued throughout the thirty-sixth to thirty-ninth weeks of gestation.

The calcium balance of the subjects in this study showed no significant change over the weeks of gestation. However, there was a tendency for the mean calcium balance to decrease until approximately the twenty-second week of gestation followed by an increase which continued throughout the duration of the experimental periods. Since urinary excretion of calcium was relatively constant, this change in calcium balance resulted from changes in calcium intake and fecal loss. Although changes in both of these parameters were insignificant, the intake generally tended to increase throughout pregnancy, except for a slight decrease at approximately the twenty-second week of gestation. Fecal loss increased rapidly until the sixteenth week of gestation, increased more slowly until the twenty-second week of gestation, then gradually decreased throughout the remainder of the experimental periods. The behavior of the fecal loss of calcium suggested an adaptive mechanism

related to the progressive demands of pregnancy. In early pregnancy, an increasing calcium intake was accompanied by a more rapidly increasing fecal loss with the result of a decreasing calcium balance. However, the fecal loss did not further increase after approximately the twenty-second week of gestation although calcium intake continued to rise; the overall effect was an elevation of the calcium balance. The poor utilization of calcium during approximately the twenty-second week of gestation partially resulted from a fall in calcium intake while the fecal excretion remained high. The fall in intake could not be related to experimental error since the collections and analyses for this gestational period for each subject were performed over a long period of time.

For calcium, phosphorus, and iron the retentions appeared highest during the very early weeks of gestation. However, it must be remembered that only two experimental periods were completed before the sixth week of gestation. Nevertheless, retentions of calcium and iron continued at approximately the tenth week of gestation, with the retentions continuing to decrease as fecal losses increased more rapidly than the intakes. Early pregnancy undoubtably represents a period of physiological change; however, the limited data collected during this period make interpretation of these changes unjustified.

Although a relationship between calcium balance and gestational period was suggested, the relationship was not significant. The mean calcium intake of the women studied by Shenolikar (59) was very low, less than 0.50 g daily, but represented their customary intakes. The mean intake of the subjects on this study was generous, 1.37 g daily. Goss (64) reported that the renal conservation of calcium during the

latter weeks of pregnancy was less effective when calcium intakes exceeded the requirement, and suggested that high intakes may obscure the adaptive mechanism. It is possible that the adaptive mechanism of pregnancy indicated in this investigation was partially obscured by the high levels of calcium ingested.

Utilization of Supplemental Calcium

Investigations to compare the utilization of supplemental calcium to calcium from food sources have indicated the influence of the calcium salt used as well as the nutritional needs of the subject. The utilization of dicalcium phosphate by young women was less than the utilization of equivalent amounts of the mineral from food (45). However, the utilization of dicalcium phosphate by children was comparable to the utilization of calcium supplied by milk (76,80-83). On the other hand, the utilization of calcium carbonate by young women was similar to that of milk calcium (79). When supplemental calcium carbonate provided 20% of the calcium intake of six of the pregnant women in this study, the utilization of the calcium by the supplemented group was generally less efficient than the utilization of the calcium by four pregnant women ingesting only food sources of the mineral; however, the difference was not significant.

III. PHOSPHORUS

Intake

Although no supplemental phosphorus was received by any subject, the phosphorus intakes of seven of the subjects met the recommended daily

allowance (4), and the intakes of the remaining three subjects were approaching this level. The phosphorus intakes of the subjects receiving a calcium supplement and those not receiving a supplement were similar. Only Subject 6 with a very high milk consumption had a significantly larger phosphorus intake.

Phosphorus Balance

In both animal and human studies phosphorus utilization has been reported to be directly related to the phosphorus intake (41-43, 46). In this investigation also the apparent absorption of phosphorus and phosphorus balance increased significantly as the intake increased. The subjects having negative phosphorus balances were also the subjects having the lowest phosphorus intakes. However, the phosphorus intakes of these subjects were near the recommended daily allowance (4). The negative phosphorus balances of Subjects 3 and 5 accompanied negative nitrogen and calcium balances. The negative phosphorus balances of Subjects 2 and 4 accompanied positive nitrogen and calcium balances and cannot be explained. The only two positive balances of Subject 4 occurred when there was no calcium supplementation; the possible influence of variation in other nutrients in the self-selected diets cannot be ignored (132). The calcium-phosphorus ratios ranged from 1:0.5 to 1:1.4, but 40 out of 47 fell within the range of 1:0.8 to 1:1.2.

Phosphorus Utilization by Week of Gestation

The phosphorus balance was significantly dependent on the intake, and with the exception of early pregnancy, increased and decreased with

the intake. Following an elevation after the earliest gestational period, the fecal loss was relatively constant. Although the renal excretion decreased slightly in the last months of pregnancy, the increasing phosphorus balance during the third trimester of pregnancy was largely dependent on an increased phosphorus intake. No effect of the gestational period on phosphorus utilization was indicated.

Influence of Calcium Supplementation on Phosphorus Utilization

The utilization of phosphorus by the subjects consuming no calcium supplement was generally more efficient than the phosphorus utilization of the calcium supplemented subjects although the difference was not significant. If the calcium supplement had represented a larger percent of the total intake, the effect of the calcium supplement on phosphorus utilization might have been adverse.

IV. MAGNESIUM

Intake

The mean magnesium intake of all subjects was below the recently established recommended daily allowance of 450 mg for pregnant women (4). Only two of the prenatal supplements contained magnesium, and the amount provided in these supplements was negligible, 0.15 mg. The mean magnesium intake of the 10 subjects was 269 mg daily. Neither whole-grain cereals and flour nor coffee, all good sources of magnesium, were consumed regularly in significant amounts. Nuts, also rich in magnesium, were probably excluded because of the caloric value. The low magnesium intakes of these pregnant women indicated that emphasis should be placed on the importance of including whole-grain cereals and flour in the prenatal diet.

Magnesium Balance

Of 47 seven-day balance periods, three magnesium balances were positive, nine were in equilibrium, and 35 were negative. Leverton and Linkswiler (5) found magnesium intakes of 280 to 320 mg were necessary to insure equilibrium in nonpregnant women receiving 11 g of nitrogen, 0.750 g of calcium, and 0.950 g of phosphorus. Coons and Blunt (60) reported that in pregnant women with a mean magnesium intake of 418 mg, nitrogen intakes of 11 g, and calcium and phosphorus intakes of approximately recommended allowances, retention of the mineral was irregular, inconsistent, and balances were often negative. Hummel et al. (62) found that on a continuous study of a pregnant woman during the last half of pregnancy, a mean daily intake of 600 mg of magnesium resulted in a mean balance of 110 mg. Intakes of nitrogen, calcium, and phosphorus were approximately twice the recommended allowances. The negative balances of the subjects in this investigation suggested that the magnesium intakes were not sufficient to produce equilibrium.

Hunt and Schofield (55) reported that young nonpregnant women could remain in magnesium equilibrium at magnesium intakes of 178 to 196 mg if the protein intake was limited to 34 to 48 g daily. Leichsenring et al. (52) reported that college women with intakes of 260 mg of magnesium, 0.30 g of calcium, and 0.80 g of phosphorus exhibited an increased urinary excretion of magnesium when calcium and phosphorus were elevated to 1.50 g and 1.40 g respectively. The intakes of protein, calcium, and phosphorus by the pregnant women in this investigation could have been influential in the severity of the negative magnesium balances.

V. IRON

Intake

The iron intake of all subjects met the recommended allowance or was approaching it (4). However, the subjects receiving an iron supplement had intakes approximately three times higher than the subjects not receiving an iron supplement.

Apparent Absorption

The apparent absorption of iron varied greatly from subject to subject and for a given subject at various periods. The fecal loss of iron was found to be significantly related to the intake, increasing as the intake increased. Subjects 1 and 3 had fecal losses exceeding the intakes. Even after the iron supplement of Subject 1 was increased from 30 to 80 mg daily, the fecal loss continued to be greater than the intake. The large fecal iron losses of Subject 3 accompanied negative nitrogen, calcium, and phosphorus balances. Endogenous iron losses were reported by Boender and Verloop (133) and Dubach et al. (134). These fecal losses were thought to result from desquamation of epithelial cells in the lumen, losses of macrophages that enter the lumen, and iron lost in bile from the catabolism of hemoglobin. This indicated that all iron lost in the feces was not unabsorbed iron. Boender and Verloop (133) found that within two weeks, 23 to 64% of a tracer dose of iron given orally to adult men had been returned to the intestine as endogenous iron.

Nevertheless, the mean apparent absorption of iron was relatively high, 4.2 mg, for the ten subjects. Coons (105) reported that pregnant

women on home diets providing a mean iron intake of 14.7 g daily retained approximately 3 mg of iron daily. Since urinary losses of iron are small (105, 127), the iron retention of these subjects may be assumed to be similar to the retentions found by Coons. The Council on Foods and Nutrition of the American Medical Association reported that although prophylactic iron administration was justified in pregnancy, no more than 30 mg of supplemental iron per day during the latter half of pregnancy was required (115). The iron retentions of these subjects when ingesting approximately 40 mg of supplemental iron per day was high suggesting that larger iron supplements would have been superfluous.

Iron Utilization by Week of Gestation

An enhancement in the absorption of dietary iron in Indian women during the latter half of pregnancy was reported by Apte and Iyengar (106). Hahn et al. (107) found that the absorption of tracer doses of iron increased as the pregnancy progressed beyond approximately the twelfth week of gestation.

The retention of iron by the subjects in this study showed no significant change over the weeks of gestation. However, the retention tended to decrease until approximately the twenty-second week of gestation followed by an increase which continued through most of the experimental periods. Changes in the fecal loss of iron over the duration of pregnancy were not significant, but decreasing fecal losses suggested an adaptive mechanism similar to that described for calcium.

The fecal loss increased more rapidly than the iron intake until approximately the sixteenth week of gestation resulting in a fall of the

iron retention of early pregnancy. Subsequent fecal losses were relatively constant with only a small further increase over the weeks of gestation. Iron intake continued to increase in the third trimester resulting in an increasing retention during that period. The elevated retention of iron may have resulted from an increased absorption and/or a decreased endogenous loss of iron. In iron-deficient subjects there was not only an increase in the absorption of iron from the intestine, but also a decrease in the iron returned to the intestine (133).

Like calcium, the poor utilization of iron during the twenty-second week of gestation was partially the consequence of a fall in the iron intake while the fecal loss remained high. Nevertheless, the change in the iron retention over the weeks of gestation was not significant.

Perhaps, as was suggested for calcium, the relationship between iron utilization and gestational period was partially obscured by the high intake of iron. Large variations in the iron dose administered by Hahn et al. (107) revealed that in addition to a decrease in the percent uptake of iron, there was also less change in the percent uptake of the iron with the progression of pregnancy as the dosage was increased above 9 mg.

Utilization of Supplemental Iron

Although the absorption of iron is inefficient, utilization of the mineral is influenced by the body's needs. Iron-deficient subjects showed an elevated absorption of food iron (106, 111) and an even greater elevated absorption of iron salts (111, 114). In this investigation, during experimental periods in which 30 to 80 mg of iron from either

ferrous fumerate or ferrous sulfate supplemented the iron intake, the retention of iron was significantly higher than in those periods in which no supplement was included. However, the intake of iron during the periods of supplementation was also significantly higher. The utilization of iron whether provided entirely from food sources or partially from ferrous fumerate or ferrous sulfate appeared to be more dependent on the magnitude of the iron intake than on the source of the iron.

VI. POST PARTUM

The post partum experimental periods were originally planned to provide a nonpregnant experimental period with which to compare the experimental periods completed during the pregnancy. However, several subjects were concerned with weight loss following the pregnancy, and their dietary intakes may have been atypical. Subjects 1 and 8 were lactating during this period; mineral losses through the milk were not determined. Only Subject 8 continued receiving a multiple vitamin-mineral supplement providing calcium and iron.

Generally, the calcium and phosphorus retention of the subjects was less than the retention of the minerals during pregnancy. However, for the two lactating subjects, the calcium retention was similar to the retention during pregnancy.

Magnesium balances remained negative during the post partum experimental period except for Subjects 1 and 8. The two lactating subjects were in equilibrium ($\pm$ 5% of the intake) and positive balance respectively in spite of continued low magnesium intakes.

The mean absorption of iron during the post partum period was higher than the absorption of iron during pregnancy. This elevated utilization of iron may have reflected a depletion of iron following the birth of the child.

During lactation negative balances of calcium, magnesium, and phosphorus were reported by Hummel et al. (62) and Hunscher (63). However, the mineral losses in breast milk were analyzed. In this investigation, mineral losses in milk were not included. Based on the mean mineral losses through milk reported by Hummel et al. (62), the lactating subjects of this study would have shown mean negative daily balances of -0.58 g, -0.54 g, and -0.11 g for calcium, phosphorus, and magnesium respectively. These balances were compared to the mean balances reported by Hummel et al. (62) of -0.48 g, -0.37 g, and -0.03 g for calcium, phosphorus, and magnesium respectively when mineral intakes were approximately twice as high as those in this study.

VII. CONCLUSIONS

Many physiological changes and interrelationships were suggested by the data, but the following conclusions were the most pertinent to the well-being of the pregnant woman and the health and development of the fetus:

1. The 0.230 to 0.250 g of supplemental calcium provided by the prenatal supplement did not seem to show any benefit, and may have been detrimental both to calcium and phosphorus retentions.

2. Iron supplementation increased the iron intake beyond that feasible in unsupplemented diets and resulted in larger retentions of the

mineral. Supplementation of the pregnant woman's diet with 30 to 40 mg of iron appeared to be desirable; larger iron supplements may have been superfluous.

3. The magnesium intakes were far below the recommended intakes for pregnant women. Emphasis should be placed on the importance of including whole-grain cereals and flours and certain dark green leafy vegetables in the prenatal diet as good sources of magnesium.

The adult pregnant women of this investigation were private obstetric patients of a middle socio-economic group. They were concerned about the well-being and development of the fetus and received regular prenatal care. The conclusions presented here apply to this select group, but may not be applicable to groups of different socio-economic backgrounds, ages, or circumstances.

CHAPTER VI

SUMMARY

The utilization of calcium, phosphorus, magnesium, iron, and nitrogen in 10 healthy pregnant women consuming self-selected diets was investigated. Seven-day balance experiments conducted under ordinary home conditions were spaced periodically throughout the duration of the pregnancy. A multiple vitamin-mineral supplement provided part of the calcium and iron intake of some subjects, whereas others received their total mineral intake from food sources. In addition to the balance periods conducted during pregnancy, seven of the 10 subjects completed a seven-day balance period within three to 11 weeks post partum.

The calcium intake of pregnant women with a balanced diet including dairy products was sufficient without supplementation of calcium. Although six of the 10 subjects received a prenatal supplement providing calcium, all but two subjects essentially met the recommended daily allowance for calcium for pregnant women through their diets alone. The magnesium intake of all 10 subjects was below that recommended for pregnant women. Approximately 75% of the 47 seven-day balance periods were negative indicating that the magnesium intake of these subjects was not sufficient to meet the magnesium requirements of pregnancy. Emphasis should be placed on the importance of including good sources of magnesium, such as whole-grain cereals and flour and certain dark green leafy vegetables, in the prenatal diet. The intakes of nitrogen, phosphorus, and iron generally met recommended allowances. The subjects

receiving an iron supplement had intakes approximately three times greater than the iron intakes of the unsupplemented subjects.

There was little change in the utilization of magnesium throughout the pregnancy. Changes in the nitrogen and phosphorus balances with the progression of pregnancy were related to the changes in the intake of the nutrients. Although changes in the utilization of calcium and iron were not significantly related to the gestational period, an adaptation of the fecal loss of these minerals resulting in an increased retention was suggested during the latter half of pregnancy when fetal demands were greatest.

Mineral salts appeared to be well utilized by the pregnant women. The retention of calcium when calcium carbonate supplied 20% of the calcium intake was usually less than the retention of calcium provided entirely by food sources, but the difference was not significant. The retention of phosphorus was also less when a calcium supplement was included, but the effect was not significant. Although these results were not statistically significant, the biological importance to the individual should be considered. In these subjects, there was no indication that the supplemental calcium was beneficial, whereas it may have adversely influenced the retention of phosphorus. The retention of iron whether partially provided by ferrous fumerate or ferrous sulfate or provided entirely by food sources appeared to be more dependent on the magnitude of the iron intake than the source of the mineral.

During the post partum experimental period, the retention of calcium and phosphorus was generally less than during pregnancy.

Magnesium intakes were below the recommended levels, and balances continued to be negative. The retention of iron during the post partum experimental period was comparable to the iron retention during pregnancy, although the iron intake had decreased.

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APPENDIXES

APPENDIX A

QUESTIONNAIRE, INSTRUCTION SHEETS, AND RECORD CHARTS

Questionnaire

1. How do you think your pregnancy will affect or has affected change in what you eat or drink?

2. Before your pregnancy, how many meals a day did you normally eat?____

3. Could you described a typical day's meals and snacks prior to pregnancy? (Include anything you put in your mouth and swallow.)

   Breakfast:

   Snack:

   Lunch:

   Snack:

   Dinner:

   Snack:

4. Has your physician ever prescribed a modified (special) diet for you?

   _____

5. What vegetables did you eat at least once a week before your pregnancy?

6. What types of meat did you eat most often before your pregnancy?

	once per week	twice per week	more
Fish:			
Beef:			
Lamb:			
Pork:			

7. There are many varieties of milk. What type of milk did you use before your pregnancy? What do you use now?

	Before	Now
Dry skim	_____	_____
Whole	_____	_____
Skim	_____	_____
Butter	_____	_____
2%	_____	_____
Others	_____	_____

Before

Vitamin D Added? _____

Brand _____

Aver. no. cups per day _____

8. Have you ever been overweight or underweight? _____

   a. What is the most you have ever weighed before your pregnancy?

      _____

   b. When were you underweight? _____

      Why were you underweight? _____

9. Before your pregnancy, did you take any type of food supplement? _____

   Name:

   Amount:

10. Did you take any type of medication regularly? _____

    Name:

    Amount:

    Reason:

Instructions for Weighing and Collection of Food Samples

Equally as important as the complete collection of excreta is the accurate weighing or measurement of each portion of all foods and beverages which you consume together with the recording of these foods and their measurements.

a. At the time you eat or drink anything, please weigh on the Hanson scale each solid or liquid to be eaten, and record the weights in the chart provided. Weigh out each portion in the weighing dish provided and transfer to your plate. Measure very small quantities of solids (such as sugar) before transferring to your cup or glass. Water and some beverages may be measured also (see sample sheet).

b. At the time each food or beverage is weighed in the weighing pan or measured, collect an additional serving (like your own and of approximately equal size) in the same weighing pan and slip pan containing the sample into a small plastic bag or container. Seal the bag tightly or faster the lid of the container securely. Be sure that the date, name of the food, and your name is on the small plastic bag or container. All small bags containing samples collected at this meal should be stored in your refrigerator or the ice chest by placing in a larger plastic bag and sealing this bag tightly.

c. You may not eat all or part of some foods weighed onto your plate, or there may be some inedible residue (skin, bones, seeds, etc.). After the meal, please weigh or measure the uneaten residue from each serving of food or beverage. Record these quantities under the column entitled "Discard". A sample sheet has been provided to try and anticipate some of your problems. If there are other questions, please notify us.

d. If the source of your drinking water is the city water supply, samples can be collected in our laboratory, and you need not save us a cup daily, If, on the other hand, you drink water out of town, a sample should be saved.

Sample Sheet

FOODS	WEIGHT (g)	MEASURE (tbs, tsp)	DISCARD	BEVERAGES	WEIGHT OR MEASURE (g,cup,oz)	DISCARD
Apple	150 g		10 g	Coffee	1 cup	
Cake and Icing	120 g				(or weight)	
Beef, roasted	85 g					
Gravy		2 tbs		Cola	10 oz	
Chicken, breast	94 g		18 g		(or weight)	
Corn on Cob	130 g		20 g			
Jelly		1 tbs		Milk	240 g	
Macaroni and cheese	220 g					
Margarine	15 g			Milk Shake	300 g	
Peaches, canned	90 g					
Syrup		3 tbs		Tea	2 cups	
Pork Chop	98 g		15 g		(or weight)	
Salad, pineapple						
Pineapple	112 g			Water	1 cup	
Lettuce	5 g				(or weight)	
Cottage cheese	20 g					
Salad, tossed	60 g					
Salad dressing		2 tbs				
Sandwich						
Bread	72 g					
Bacon	16 g					
Tomato	30 g					
Lettuce	5 g					
Mayonnaise		1 tbs				
Sugar		2 tsp				

Instructions for
Collection of Feces

It is not possible to identify the stools corresponding to a particular day in the balance period. For this reason, you were asked to take the capsule containing the blue dye at the beginning and first day after the balance period. The stool for the collection period is identified by the dye. You will be notified at the end of the balance period when the fecal collection has been completed.

a. Collect feces between Brilliant Blue marker for the 7-day experimental periods. Take two capsules containing methyl cellulose and the dye before breakfast at the beginning of each period and the morning following the last period. The first appearance of the dye taken will mark the beginning of the fecal collection for that period.

b. Collect each stool in one of the plastic cartons. Mark the container with your initials, the date, and hour of collection. Fasten lid of carton securely and place carton containing the sample in the ice chest as soon after collection as possible. This process will be continued throughout the period until you are notified that the collection is complete. When defecating, it is always better to collect any urine first. It is not necessary to save toilet paper.

c. When away from home, collect stools in a plastic carton carried with the pitcher and urine bottles in the waterproof bag provided. On arriving home, promptly transfer the carton to the ice chest and fasten the lid securely.

d. If you should vomit, please collect each vomitus in a separate plastic fecal box, label with the date and hour, and store in ice chest with other samples.

Samples collected will be picked up each day at the same time as the food samples and urine bottles.

Instructions for
Collection of Urine

Begin the collection of each 24-hour urine sample with the first voiding after breakfast and end with the last voiding before breakfast the following morning.

a. Collect each sample in the small plastic pitcher provided and transfer to one of your urine bottles. Toluene has already been added to the bottle as a preservative. (Wipe pitcher dry for reuse). Screw lid of bottle tightly.

b. As promptly as possible, store the bottle in the ice chest and fasten the lid securely.

c. When going away from home, carry your collection pitcher and as many urine bottles as you might need in the waterproof bag provided. On arriving home, promptly transfer the bottle to the ice chest and fasten the lid securely.

Repeat these steps each day during the 7-day period beginning each collection with the first voiding after breakfast and ending with the last voiding before breakfast the following morning.

The 24-hour urine samples for the collection period just completed will be picked up each day. Please be sure that all urine bottles used during the 24-hour collection are labeled with your name and the date.

If, at any time, any part of a sample should be accidentally lost, please estimate the approximate loss and record this in your notebook.

Food and Beverage Chart

Name _____

Address _____ Telephone No. _____

Date _____ Day of Week _____

Food	Weight (g)	Measure (tbs. tsp.)	Discarded (wt. or measure)	Beverage	Measure (cup)	Discarded (measure)	Water (cup)	Discarded (measure)
Breakfast								
Between Meal								

Name _____

Date _____

Food	Weight (g)	Measure (tbs. tsp.)	Discarded (wt. or measure)	Beverage	Measure (cup)	Discarded (measure)	Water (cup)	Discarded (measure)
Noon Meal								
Between Meal								

Name _____

Date _____

Food	Weight (g)	Measure (tbs. tsp.)	Discarded (wt. or measure)	Beverage	Measure (cup)	Discarded (measure)	Water (cup)	Discarded (measure)
Evening Meal								
After Evening Meal								

If you have questions, call us: Jessie Russell 974-3491 U.T. 522-1430 Home Frances Schofield 974-3491 U.T. 584-1681 Home

Medication and Dietary Supplements Chart

Any medication or dietary supplement which you take should be recorded below. Also, place each capsule or pill (if liquid, measure the amount) in a separate small container and store in the ice chest with other samples. Label each container with the name of the preparation, the date, and the hour.

Name of Preparation	Date	Amount (quantity or measure)

APPENDIX B

STATISTICAL TABLES AND CALORIC INTAKE TABLE

TABLE 13

SIMPLE LINEAR REGRESSION OF NITROGEN APPARENT ABSORPTION ON NITROGEN INTAKE, PHOSPHORUS APPARENT ABSORPTION ON PHOSPHORUS INTAKE, PHOSPHORUS BALANCE ON PHOSPHORUS INTAKE, AND IRON FECAL LOSS ON IRON INTAKE

Variables	DF	b	t*	
Nitrogen apparent absorption (y) Nitrogen intake (x)	45	1.039	35.686	Significant P<0.001
Phosphorus apparent absorption (y) Phosphorus intake (x)	43	0.940	8.427	Significant P<0.001
Phosphorus balance (y) Phosphorus intake (x)	43	0.350	2.688	Significant P<0.02
Iron fecal loss (y) Iron intake (x)	41	0.888	13.554	Significant P<0.001

*To test the null hypothesis that $\beta = \beta_0$.

TABLE 14

ANALYSIS OF VARIANCE FOR CALCIUM INTAKES OF INDIVIDUAL SUBJECTS

Source of Variation	DF	SS	MS	F
Subjects	9	1.196	0.133	1.900*
Error	37	2.592	0.070	
Total	46	3.788		

*Not significant, P>0.05.

TABLE 15

ANALYSIS OF VARIANCE FOR CALCIUM BALANCE BY WEEK OF GESTATION

Source of Variation	DF	SS	MS	F
Gestational period	5	1.158	0.232	0.853*
Error	41	11.152	0.272	
Total	46	12.310		

*Not significant, P>0.05.

TABLE 16

STUDENT t TEST* FOR COMPARISON OF MEAN CALCIUM AND PHOSPHORUS BALANCE OF CALCIUM SUPPLEMENTED AND UNSUPPLEMENTED GROUPS, AND MEAN APPARENT ABSORPTION OF IRON FOR IRON SUPPLEMENTED AND UNSUPPLEMENTED GROUPS

Groups	DF	t	
Calcium balance of calcium supplemented vs unsupplemented groups	45	0.511	Not significant P>0.05
Phosphorus balance of calcium supplemented vs unsupplemented groups	45	0.807	Not significant P>0.05
Iron apparent absorption of iron supplemented vs unsupplemented groups	41	-3.322	Significant P<0.01

*For unpaired comparisons.

TABLE 17

ANALYSIS OF VARIANCE FOR PHOSPHORUS INTAKES OF INDIVIDUAL SUBJECTS

Source of Variation	DF	SS	MS	F
Subjects	9	1.820	0.202	4.298*
Error	37	1.729	0.047	
Total	46	3.549		

*Significant, P<0.005.

TABLE 18

DUNCAN'S NEW MULTIPLE-RANGE TEST* FOR PHOSPHORUS, MAGNESIUM, AND IRON INTAKES OF INDIVIDUAL SUBJECTS

Mineral	Mean Intake of Individual Subjects**										DF
	Subjects										
	5	4	2	3	8	1	10	7	9	6	
Phosphorus (g/24 hr)	1.046	1.113	1.130	1.284	1.349	1.370	1.375	1.479	1.563	1.729	37
	Subjects										
	5	8	3	2	4	10	7	1	6	9	
Magnesium (mg/24 hr)	174	255	256	262	273	282	284	292	306	309	37
	Subjects										
	6	9	2	10	4	3	5	7	8	1	
Iron (mg/24 hr)	12.8	16.4	16.9	22.7	45.0	53.0	58.5	60.0	71.8	74.0	33

*For groups with unequal replications.

**Mean intakes underlined by the same line are not significantly different, P>0.05.

TABLE 19

ANALYSIS OF VARIANCE FOR PHOSPHORUS BALANCE
BY WEEK OF GESTATION

Source of Variation	DF	SS	MS	F
Gestational period	5	0.190	0.038	0.551*
Error	41	2.833	0.069	
Total	46	3.023		

*Not significant, P>0.05.

TABLE 20

ANALYSIS OF VARIANCE FOR MAGNESIUM
INTAKES OF INDIVIDUAL SUBJECTS

Source of Variation	DF	SS	MS	F
Subjects	9	0.055	0.006	3.000*
Error	37	0.084	0.002	
Total	46	0.139		

*Significant, P<0.025.

TABLE 21

ANALYSIS OF VARIANCE FOR MAGNESIUM BALANCE
BY WEEK OF GESTATION

Source of Variation	DF	SS	MS	F
Gestational period	5	0.015	0.003	1.500*
Error	41	0.101	0.002	
Total	46	0.116		

*Not significant, P>0.05.

TABLE 22

ANALYSIS OF VARIANCE FOR IRON INTAKES
OF INDIVIDUAL SUBJECTS

Source of Variation	DF	SS	MS	F
Subjects	9	21121.595	2346.844	5.911*
Error	33	13101.950	397.028	
Total	42	43223.545		

*Significant, P<0.005.

TABLE 23

ANALYSIS OF VARIANCE FOR APPARENT ABSORPTION OF IRON BY WEEK OF GESTATION

Source of Variation	DF	SS	MS	F
Gestational period	5	764.244	152.849	1.030*
Error	37	5487.552	148.312	
Total	42	6251.796		

*Not significant, P>0.05.

TABLE 24

CALCULATED* MEAN CALORIC INTAKES BY WEEK OF GESTATION

Week of Gestation	Daily Intake kcal	Week of Gestation	Daily Intake kcal	Week of Gestation	Daily Intake kcal	Week of Gestation	Daily Intake kcal	Week of Gestation	Daily Intake kcal
Subject 1		Subject 3		Subject 5		Subject 7		Subject 9	
20	1,779	16	1,539	16	1,373	5	2,012	11	1,789
24	1,834	22	1,155	22	1,973	9	1,691	16	1,733
29	2,297	29	1,206	29	1,341	16	1,667	22	1,480
36	2,200	35	1,431	35	1,584	21	2,039	29	1,880
Mean	2,028	Mean	1,333	Mean	1,568	Mean	1,852	Mean	1,736
SD	±259	SD	±182	SD	±291	SD	±200	SD	±153
Post Partum	2,347							Post Partum	1,723
Subject 2		Subject 4		Subject 6		Subject 8		Subject 10	
8	1,654	10	1,961	9	1,958	6	1,675	11	1,925
13	1,603	16	1,650	22	1,818	12	1,937	16	1,797
19	1,572	22	1,201	29	2,066	16	1,809	22	1,876
25	1,702	29	1,458	35	2,021	22	1,952	29	1,813
30	2,177	35	1,588	Mean	1,966	27	2,131	34	1,542
36	1,902	Mean	1,572	SD	±108	35	2,061	Mean	1,791
Mean	1,768	SD	±278	Post Partum	1,307	Mean	1,928	SD	±148
SD	±232	Post Partum	1,475			SD	±166	Post Partum	1,482
Post Partum	2,020					Post Partum	1,772		

*Watt, B. K., and A. L. Merrill 1963 Composition of Foods. Agriculture Handbook No. 8, United States Department of Agriculture, Washington, D. C.

VITA

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