EFFECT OF PROTEIN SOURCE ON CALCIUM EXCRETION IN ADULT RATS FED HIGH PROTEIN DIETS

by

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B.S., Virginia Polytechnic Institute and State University
Blacksburg, Virginia, 1976

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1978

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INTRODUCTION

Advances in medical technology and improvements in socio-economic conditions have contributed to increased life expectancy of men and women in the United States and throughout the world. The increase in longevity in man is accompanied by a rise in the number of health problems of the elderly. One of the major problems that has received considerable attention in recent years is osteoporosis. Lutwak (1) has described osteoporosis as a condition of the skeleton in which the absolute amount of bone is diminished, but the remaining bone is normal in chemical composition. It has been estimated that a minimum of 10 percent of the population over fifty years of age suffers from senile osteoporosis severe enough to cause vertebral, hip or long-limb fractures (1). Bone-loss occurs in both sexes and in all races, but its incidence appears to be four times greater in women than in men, and white women of Anglo-Saxon origin are particularly susceptible to the condition (2).

Explanations for osteoporosis have been numerous and varied. Nordin (3) has attributed the disease to prolonged suboptimal intake or inadequate absorption of calcium. Dietary surveys of patients with osteoporosis have indicated that they consume diets lower in calcium content than age-matched populations without bone demineralization (4). Adaptation to lower calcium intake occurs eventually, but this may not be

true for all individuals (3). It has been argued that adaptation cannot occur without jeopardy of bone loss resulting in fractures, especially for the elderly (2). The Food and Nutrition Board of the National Academy of Sciences-National Research Council has established the recommended dietary allowance for calcium at 800 mg per day for adults (5). Lutwak (4) has suggested that because of the variability of calcium absorption in human beings, this figure may represent a minimum for prevention of osteoporosis. It has been proposed that 1,000 mg of calcium daily would result in maximum absorption in adults (5) and maintain normal, if not optimal, bone density (2).

While a number of authorities (1-4) believe that a diet adequate in calcium is important in the prevention and treatment of osteoporosis, Garn et al. (6) found that, within very broad limits, calcium intake did not relate to bone loss, either on a population basis, or within individuals in a single population sample. Intakes of calcium above 1,500 mg per day did not appear to be "protective", and levels of calcium below 300 mg per day were not associated, demonstrably, with bone loss. They suggested that all people ultimately lose bone, but those who have achieved a larger bone mass by age 30 are slower to show clinical symptoms of osteoporosis.

Further evidence that bone loss is not caused totally by calcium deficiency are observations of bone atrophy in the paralyzed side of hemiplegic patients (2, 7). Bone loss will occur in both sexes at any age if physical activity is reduced,

markedly. Mack and LaChance (8) reported that healthy young male astronauts lost significant amounts of calcium in the os calcis and phalanges 5-2 during a seventy-two-hour flight. Smith (9) observed a significant increase in bone mineral in human subjects with increased activity.

The high incidence of osteoporosis in postmenopausal women has suggested that bone loss could be attributed to the cessation of estrogen stimulus for bone formation. Davis et al. (10) reported that estrogen administration resulted in the retardation or prevention of bone loss in menopausal women.

The effect of different dietary patterns on calcium metabolism has been a concern of numerous investigators. Krook et al. (11) suggested that periodontal disease resulting from resorption of alveolar bone, an early sign of osteoporosis, might be related to inadequate calcium intake in combination with excess dietary phosphorus. North Alaskan Eskimos with a history of high meat consumption, resulting in high protein, low calcium and high phosphorus intakes, have been reported to have a high incidence of osteoporosis (12). Ellis et al. (13) reported a higher prevalence of the disease in omnivores than in ovo-lacto vegetarians.

Many years ago, researchers observed that consumption of high meat diets caused increased calcium excretion (14, 15). More recently, investigators (16, 17) have suggested that excess dietary protein may have an adverse effect on skeletal tissue, and that it may be an etiologic factor in osteoporosis. Information regarding the effect of protein source on calcium

excretion is limited. This study was designed to investigate the effect of protein source on calcium excretion in adult rats fed high protein diets.

REVIEW OF LITERATURE

Effects of Dietary Protein Level on Calcium Excretion

As early as 1920, Sherman (14) observed that the addition of meat to the diets of healthy, male subjects resulted in increased urinary calcium excretion. Based on a comparison of dietary records and actual food supplies with laboratory evidence of nutritive requirements, he suggested that many more American diets were deficient in calcium than in protein. In 1930, McClellan et al. (15) found that two men were in negative calcium balance throughout experimental periods during which they consumed diets consisting of meat only. Urinary calcium excretion and urine and fecal calcium output were greater when the meat diets were fed than when the men consumed mixed diets. Several years later, Hegsted (18) observed that a high meat diet not only increased urinary excretion of calcium in adult males, but resulted in a considerable shift in the distribution of calcium excretion from the feces to the urine.

In 1939, Pittman and Kunerth (19) reported that consumption of a medium protein diet resulted in greater calcium retention in young adult women than consumption of a low protein diet (20). Calcium intakes were not controlled in the two experiments, and the subjects were not in positive calcium balance when either diet was fed.

McCance et al. (21) observed that increased protein intake

resulted in increased calcium absorption and urinary calcium excretion in five healthy adults during metabolic studies. Hall and Lehmann (22) reported the same results from a study of patients fed amino acid supplements to increase dietary protein. They considered calcium absorption to be the amount of calcium in the diet minus the amount of calcium in the feces and assumed that calcium in intestinal secretions was constant.

In 1947, Knapp (23) reviewed the factors including dietary protein, that influence urinary excretion of calcium. Based on the results of several studies, she concluded that increase in dietary protein without notable concurrent increase in acidity of ash of the diet or change in dietary calcium to phosphorus ratio caused a small but consistent increase in urinary calcium. However, the magnitude of increase was not sufficient to raise urine calcium outside normal limits in any of the studies.

The effect of various nutrients, including protein, on the urinary excretion of divalent cations by healthy male volunteers, ages 19 to 63 years, was investigated by Lindeman et al. (24). Ingestion of glucose, galactose, fructose, protein or ethanol resulted in increased urinary calcium and magnesium, and a decrease in urinary potassium. They observed increased glucose uptake and glycolysis in renal tubular cells with each of those nutrients, and proposed that calcium and magnesium reabsorption and potassium secretion was in some way inhibited in the distal tubules or collecting ducts by the

enhanced glucose uptake and glycolysis.

Linkswiler and coworkers (16, 25-27) conducted a series of three studies to determine the urinary calcium, calcium absorption and calcium retention of 33 young adult males fed 47, 95 and 142 g protein daily at calcium intakes of 500, 800 and 1,400 mg. In each study, protein intakes were varied while calcium intakes were held constant. When the calcium intake was maintained at 1,400 mg daily (a Ca:P ratio of 1.0), increasing the protein intake from 48 to 141 g daily caused an increment of 163 mg daily in urinary calcium but an increment of only 69 mg in apparent absorption (25). The level of protein had no significant effect on fecal calcium. When the calcium intake was held at 800 mg daily (a Ca:P ratio of 0.8), calcium balance of subjects was positive with intakes of 47 and 95 g protein daily but negative with 142 g protein (26). The lowest protein diet resulted in a significant increase in fecal calcium. All subjects were in negative calcium balance when they were fed diets containing 500 mg calcium (a Ca:P ratio of 0.6) and either 95 or 142 g of protein (27). Fecal calcium was not affected significantly by protein intake. From those studies, Linkswiler et al. (16) suggested that the calcium requirement of the adult human male is influenced by the level of dietary protein.

Schwartz et al. (28) studied the effect of protein level on calcium balance in adolescent boys (ages 13 to 14 years) during two 30-day experimental periods. Dietary protein was held constant during each period at either 43 or 93 g for

each subject per day. Urinary calcium and apparent absorption of calcium were increased by the increased dietary protein, but a reduction in overall calcium balance was not observed. In general, apparent calcium absorption and retention were highest at the beginning of each 30-day period and decreased with time.

Margen et al. (17) found a positive correlation between the level of protein ingestion and calciuria in several studies with healthy male volunteers, 20 to 32 years of age. Varying protein intake from 0 g to 90 g nitrogen per day resulted in approximately an 800% increase in calcium excretion, irrespective of calcium intake. When purified crystalline amino acids were used as the nitrogen source in the diet, they induced a calciuretic effect similar to whole protein.

In a study with six healthy men fed diets containing 0.1 g calcium/day at three levels of protein intake: 0.9, 12 and 24 g nitrogen/day, Chu et al. (29) observed that fecal calcium decreased significantly as protein intake increased from 0.9 to 12 g nitrogen/day, but on the average there was no further decrease as protein increased to 24 g nitrogen/day. The decrease in fecal calcium was equal to or greater than the increase in urinary calcium. The medium protein diet resulted in the least negative calcium balance for five of the subjects. Using creatinine clearance rate as glomerular filtration rate (GFR), calcium excretion relative to 100 ml of glomerular filtrate increased as protein intake increased. An additional intake of 900 mg calcium resulted in an average increase in

calcium excretion of slightly more than double the amount as occurred on the same protein intake without calcium supplement. They postulated an inhibition of tubular calcium reabsorption, possibly coupled with increased glomerular filtration, as the principal mechanism involved in the calciuretic effect of high-protein intake.

Oddoye[1] reported a significant increase in urinary calcium excretion with an increase in nitrogen intake from 12 to 36 g daily in a long-term calcium balance study with six young men. The higher protein diet resulted in a more negative calcium balance in all cases.

In studies with adult male rats fed diets containing adequate amounts of calcium and phosphorus (0.6 and 0.3%, respectively) and low, moderate or high concentrations of protein (10, 20 or 40%), Bell et al. (30) observed enhanced absorption of dietary calcium and increased urinary calcium excretion with increasing levels of protein. However, fecal calcium excretion decreased with increments in dietary protein and consequently there was no increase in total calcium excretion attributable to the high protein intake. They concluded that in adult rats, the high protein intake led to increased excretion of calcium in the urine because of increased absorption of dietary calcium and a shift in the route of excretion

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[1]Oddoye, E.A. (1976) The effect of high and low nitrogen intake on calcium balance. Federation Proc. 35, 499. (Abstr.)

of endogenous calcium from the feces to the urine. No bone resorption attributable to the high protein intake was detected.

Denis et al. (31) studied the effects of three dietary protein levels (3.3, 11 and 33%) on bone resorption in young male rats fed diets inadequate in calcium and phosphorus. Urinary calcium excretion and bone resorption increased with increasing levels of dietary protein. The researchers concluded from the study that the amount of dietary protein determines the magnitude of the physiological and histological changes observed in growing rats fed diets inadequate in calcium and phosphorus.

Adaptation to the calciuretic effect of dietary protein in growing rats over time was observed by Allen and Hall.[2] Groups of 56-day old male rats were fed control (18%) or high (36%) casein diets adequate in calcium, phosphorus and vitamin D. Urinary calcium excretion was increased significantly to 1.27 mg/day in the group of rats fed the high protein diet for 2 days and to 1.07 mg/day in the group fed that diet for 14 days. After 28 days, excretion of calcium in the urine of rats fed the high protein diet did not differ significantly (0.75 mg/day) from that of the group consuming the control diet (0.77 mg/day). No significant change was detected in any other parameters of calcium metabolism, including absorption or changes in bone.

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[2]Allen, L.H. & Hall, T.E. (1976) The rat as an experimental model for protein-induced calcinuria. Federation Proc. 35, 499. (Abstr.)

Effects of Dietary Protein Source on Calcium Excretion

It has been suggested that all proteins may not have the same effect on urinary calcium and calcium retention (16). Lee et al. (32) fed groups of weanling rats diets containing 10% protein as bean, bean-cornmeal mixture and skim milk, 0.4 or 0.8% calcium, and phosphorus was held constant at 0.67%. Calcium utilization was determined by calcium balance studies and analysis of bone mineral content. Rats fed the skim milk diet utilized calcium significantly better than rats fed the bean-cornmeal mixture, and the bean diet resulted in the least calcium utilization. They concluded that dietary protein quality was a more important factor in calcium utilization than the levels of dietary calcium fed in their study.

Dull[3] studied the effect of dietary gelatin on calcium metabolism in human subjects. He observed that feeding normal diets plus 50 g of gelatin per day to three subjects resulted in increased urinary and fecal calcium. During 30 days of gelatin administration, a negative calcium balance was maintained in two of the subjects, with one subject having a negative calcium balance that ranged from 100-215 mg/day.

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[3]Dull, T. (1963) Effect of dietary gelatin on Ca, Mg, and P metabolism. Clin. Res. 11, 404. (Abstr.)

MATERIALS AND METHODS

Animals

Male, 5-month-old rats of the NLR Strain, Wistar origin, weighing approximately 450 g, were obtained from National Laboratory Animal Company, O'Fallon, Mo. All animals had been fed a commercial stock diet, Wayne Lab Blox, since weaning. Wayne Lab Blox contains 24.5% protein, 4.2% fat, 3.2% fiber, 1.2% calcium, 1.0% phosphorus and other essential minerals and vitamins.

During the experimental period, the rats were housed individually in 24x18x18 cm metabolism cages and provided feed and distilled water ad libitum. Mean daily feed intakes were determined during and between urine and fecal collection periods. Body weights of the rats were obtained at the beginning and end of each collection period. The animals were weighed weekly between collection periods.

Diets

The rats were divided randomly into four groups and assigned to one of four dietary treatments (table 1). Diet A, which provided 20% protein as casein, was fed to one group of of animals for comparison of calcium excretion. Diets B, C and D provided 40% protein as casein, casein and wheat gluten or casein and gelatin, respectively.

The dietary proteins were analyzed for nitrogen, using a

TABLE 1

Percent composition of semisynthetic diets

Component	Dietary treatment			
	A (20% casein)	B (40% casein)	C (40% casein-gluten)	D (40% casein-gelatin)
Casein, vitamin free[1]	22.04	44.08	22.04	22.04
Gluten[1]	-	-	25.97	-
Gelatin[2]	-	-	-	22.00
Dextrose[1]	51.10	29.42	25.36	29.24
Fat (lard)[1]	20.00	20.00	20.00	20.00
Alphacel[1]	2.50	2.50	2.50	2.50
Vitamin mix[3]	1.00	1.00	1.00	1.00
Mineral mix[4]	1.47	1.47	1.47	1.47
$CaCO_3$	1.23	1.44	1.31	1.15
$Ca(H_2PO_4)_2 \cdot H_2O$	0.66	0.09	0.35	0.60

[1]Vitamin-free casein, gluten, dextrose, lard and alphacel (nonutritive bulk), ICN Nutritional Biochemicals, Cleveland, Ohio.
[2]Gelatin, United States Biochemical Corp., Cleveland, Ohio.
[3]Vitamin Diet Fortification Mixture, ICN Nutritional Biochemicals, Cleveland, Ohio. Supplying (in mg/100 g diet): retinyl acetate, 0.265; ergocalciferol, 0.002; D,L-tocopherol acetate, 5.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.25; p-aminobenzoate, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine·HCl, 1.0; thiamine·HCl, 1.0; calcium pantothenate, 3.0; biotin, 0.02; folic acid, 0.09; vitamin B-12, 0.0013.
[4]Containing (% of diet): $ZnCO_3$, 0.0096; $FeSO_4 \cdot 7H_2O$, 0.0124; $CuSO_4 \cdot 5H_2O$, 0.002; $MnSO_4 \cdot H_2O$, 0.015; KI, 0.00013; NaCl, 0.23; Na_2CO_3, 0.16; K_2CO_3, 0.353; $4MgCO_3 \cdot Mg(OH)_2 nH_2O$, 0.69.

Labcono Micro-Kjeldahl Digestion Unit and the AOAC method (33), in the Analytical Services Laboratory, Department of Animal Science and Industry. Based on the nitrogen determinations, the diets were formulated to provide 20 or 40% protein. All diets contained 20% fat to simulate the fat content of human diets that are high in protein (30). The diets were made isocalorie by adjusting the dextrose level.

The calcium and phosphorus intakes of all groups of rats were maintained at 0.6 and 0.3%, respectively, by adjusting the $CaCO_3$ and $Ca(H_2PO_4)_2 \cdot H_2O$ levels in the salt mixtures. Those concentrations have been shown to be required for maximal inhibition of bone resorption in adult rats (34). Diet analyses for calcium and phosphorus in the Analytical Services Laboratory, Department of Animal Science and Industry, confirmed the percentages of those minerals in the diets. A Jarrell Ash model 82500 MV flame spectrophotometer was used to measure dietary calcium concentration by a modified AOAC method (33). Dietary phosphorus concentration was measured using a Gilford model 240 spectrophotometer and the Fiske and SubbaRow method (35). The mineral and vitamin mixes were incorporated in the diets at levels that provided maintenance requirements for adult rats (5).

Urine and Fecal Collections

After the rats had been fed the experimental diets for 35 days, 96-hour collections of urine and feces were obtained from each rat. Three successive urine and fecal collections

were obtained beginning on days 50, 63, and 78 of the study. The number of animals for which complete data were obtained for each collection period is given in table 2.

During collection periods, urine samples were collected every 24 hours in 120 ml, acid-rinsed glass bottles using 0.2 ml of a 10% (w/v) solution of thymol in propanol as a preservative (36). Each day freshly prepared collection bottles were provided and the funnels of the metabolism cages were rinsed with distilled water. At the end of each 24-hour period, the urine in each collection bottle was transferred to an acid-rinsed centrifuge tube and centrifuged[4] for 8 minutes at approximately 1,500 x g to remove feed particles from the sample. Then the urine was decanted into an acid-rinsed polyethylene bottle, covered and refrigerated until the 96-hour collection was completed. At the end of each four-day collection period, the pooled 24-hour urine samples were stored at 0° until analyzed.

Fecal samples were collected every 24 hours during collection periods and stored at room temperature in 120 ml, acid-rinsed, wide-mouth glass jars covered with cheesecloth. At the end of each 96-hour collection period, the pooled fecal samples were air-dried at room temperature for 72 hours and then dried in a 105° oven for 24 hours. Dried samples were weighed, transferred to acid-rinsed, plastic scintillation vials and stored at 0° until analyzed.

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[4]IEC Clinical Centrifuge.

TABLE 2

Number of animals for each period-diet combination

Collection period	Dietary treatment			
	20% protein	40% protein		
	Casein	Casein	Casein-gluten	Casein-gelatin
1 (days 35-39)	4[a]	6	5[b]	8
2 (days 50-54)	6	6	7	8
3 (days 63-67)	6	6	7	8
4 (days 78-82)	6[c]	6	6[b]	8

[a]6 aminals in table 5. [b]7 animals in table 5.

[c]5 animals in table 5.

Determination of Calcium

Urine samples from each collection period were thawed at room temperature, transferred to acid-rinsed graduated cylinders, and 96-hour urine volumes were determined. Each urine sample was transferred to an acid-rinsed Coors 120 ml evaporating dish and charred under heat lamps for 36 hours. Charred urine samples were placed in a cold muffle furnace and ashed at 550° for 24 hours. Each ashed sample was dissolved in 10 ml of 6 N HCl and heated to boiling on a hot plate. The

sides of the evaporating dish were washed down with demineralized water and the solution was heated to boiling again. The ash solution was transferred to a 50 ml volumetric flask and diluted to volume with demineralized water.

Fecal samples from each collection period were thawed at room temperature. Each fecal sample was transferred to an acid-rinsed Coors 30 ml crucible, placed in a hot muffle furnace and ashed at 550° for 24 hours. Samples were removed, cooled, moistened with 8-10 drops of concentrated nitric acid and returned to the muffle furnace for 5 hours to digest the organic compounds completely. Each fecal sample was dissolved in 5 ml of 6 N HCl and heated to boiling on a hot plate . The sides of the crucible were washed down with demineralized water and the solution was heated to boiling again. The ash solution was transferred to a 100 ml volumetric flask and diluted to volume.

The ash solutions of urine and fecal samples were analyzed for calcium using a Jarrell Ash model 82500 MV flame spectrophotometer by a modified AOAC method (33) in the Analytical Services Laboratory, Department of Animal Science and Industry.

Statistical Analyses

Two way analysis of variance with diet and period as the main effects were performed on the data for each measurement (37). Least significant difference (LSD) at the 5% level of probability was calculated when the F-value was significant.

RESULTS AND DISCUSSION

Feed Intakes and Weight Gains

The analyses of variance for feed intakes and weight gains is presented in table 3. There were no significant differences in feed intakes attributable to dietary treatments (table 4). Feed intakes during collection periods 2 and 3 were not significantly different, but there was a decrease ($P<0.05$) in feed intakes between period 1 and periods 2 and 3; between periods 2 and 3 and period 4; and between period 1 and period

TABLE 3

Analyses of variance of feed intakes and weight gains

Source of variation	df	Mean squares	
		Feed intake	Weight gain
Diet	3	16.834	40.97
Rat/diet	23	6.375	40.95
Period	3	27.106***	96.45
Diet x period	9	0.792	63.04*
Residual[1]	64	1.395	36.52

[1]68 df for weight gain. ***$P<0.001$. *$P<0.05$.

TABLE 4

Effects of dietary treatments and collection periods on feed intake

	Dietary treatment			
	A (20% casein)	B (40% casein)	C (40% casein-gluten)	D (40% casein-gelatin)
Feed intake, g/day	14.46 ± 0.56[1]	12.50 ± 0.52	12.91 ± 0.53	13.69 ± 0.45
	Collection period			
	1 (days 35-39)	2 (days 50-54)	3 (days 63-67)	4 (days 78-82)
Feed intake, g/day	14.88 ± 0.26	13.42 ± 0.23[a2]	12.98 ± 0.23[a]	12.27 ± 0.24

[1]Values are means ± SE.

[2]Means in a row sharing a common superscript are not significantly different (P>0.05) using LSD test.

4 (table 4).

When the weight gain data were analyzed, there was interaction between dietary treatments and collection periods (table 3). Therefore, the effects of diet and collection period on weight gains were compared. Rats fed diet D, 40% protein as casein and gelatin (table 5) had higher ($P<0.05$) mean weight gain during collection period 1 than rats fed diet A (20% protein as casein), diet B (40% protein as casein) or diet C (40% protein as casein and wheat gluten). During periods 2, 3 and 4 mean weight gains did not differ significantly for rats fed diets A, B, C and D.

The mean weight gains of rats fed diets A, B and C did not differ during any of the collection periods (table 5). Rats fed diet D had higher ($P<0.05$) mean weight gain during the first collection period than during periods 2, 3 and 4. The mean weight gain of those rats did not differ during periods 2 and 3, but it was lower ($P<0.05$) during period 4 than during period 2.

The feed intakes of rats in this study were lower than those reported by Newell and Beauchene (38). They found that four-month old male rats fed semi-synthetic diets, containing 20% casein, 60% carbohydrate, 10% fat and 500 mg of calcium/100 g, had mean feed intakes of 17.9 g/rat/day, during weeks 4, 8 and 16 of a study. Decreases in feed intake with time in this study cannot be attributed to the high protein content of the diets, because similar decreases were observed in rats fed the 20% casein diet.

TABLE 5

Effects of dietary treatments and collection periods on weight gain

Collection period	Dietary treatment			
	A (20% casein)	B (40% casein)	C (40% casein-gluten)	D (40% casein-gelatin)
	gms			
1 (days 35-39)	-0.33 ± 2.27[a] [1,2,3]	1.67 ± 2.27[a]	2.57 ± 2.10[a]	11.00 ± 1.96
2 (days 50-54)	6.00 ± 2.27[a]	1.50 ± 2.27[a]	2.29 ± 2.10[a]	4.87 ± 1.96[a]
3 (days 63-67)	3.83 ± 2.27[a]	1.33 ± 2.27[a]	1.86 ± 2.10[a]	0.75 ± 1.96[ab]
4 (days 78-82)	2.83 ± 2.27[a]	0.83 ± 2.27[a]	0.14 ± 2.10[a]	-0.75 ± 1.96 [b]

[1]Values are means ± SE.

[2]Means in a row sharing a common line are not significantly different (P>0.05) using LSD test.

[3]Means in a column sharing a common superscript are not significantly different (P>0.05) using LSD test.

The reason for the observed decreases in feed intakes was not apparent from the data. Respiratory infection developed in the rats prior to the first collection period. Although treatment with sulfametazine in the drinking water was started immediately and continued throughout the study, the infection may have affected the feed intakes of the animals, adversely.

Urinary Volume

The analysis of variance for urinary volume is given in table 6. In table 7 it can be observed that urinary volume was lower ($P<0.05$) for rats fed diet A (20% protein) than for rats fed 40% protein diets (B, C and D). The results are

TABLE 6

Analysis of variance for urinary volume

Source of variation	df	Mean squares
Diet	3	6199.91***
Rat/diet	23	301.58
Period	3	726.99***
Diet x period	9	135.56
Residual	64	71.20

***$P<0.001$

TABLE 7

Effects of dietary treatments and collection periods on urinary volume

	Dietary treatment			
	A (20% casein)	B (40% casein)	C (40% casein-gluten)	D (40% casein-gelatin)
Urinary volume, ml	21.64 ± 3.83[1]	50.25 ± 3.54[ab2]	48.96 ± 3.66[a]	59.53 ± 3.07[b]
	Collection period			
	1 (days 35-39)	2 (days 50-54)	3 (days 63-67)	4 (days 78-82)
Urinary volume, ml	53.67 ± 1.88	42.99 ± 1.64[a]	42.77 ± 1.64[a]	40.95 ± 1.68[a]

[1]Values are means ± SE.

[2]Means in a row sharing a common superscript are not significantly different (P>0.05) using LSD test.

similar to those reported by Bell et al. (30). They estimated that rats fed a 40% protein diet excreted at least five times as much urine as rats fed a 10% protein diet, and that rats fed a 20% protein diet excreted about twice as much. They suggested that the enlarged urine volume resulting from an enhanced synthesis of urea may be an important factor in the increased calcium excretion observed in animals fed a high protein diet. The urine volumes of rats fed diets B and C were not significantly different. Rats fed diet D, 40% protein as casein and gelatin, excreted more urine than rats fed any other diet, but the mean urinary volume did not differ significantly from that of rats fed diet B, 40% protein as casein.

Urinary volumes of all rats were higher ($P<0.05$) during collection period 1 than during periods 2, 3, and 4; but the volumes did not differ significantly during periods 2, 3 and 4 (table 7). Decreased urinary volume may have been caused by the decreased solute load that accompanied decreased feed intake, or adjustment to the high protein intake may have occurred.

Calcium Intake, Urinary and Fecal Calcium and Calcium Balance

Table 8 presents the analyses of variance for calcium intake, urinary and fecal calcium and calcium balance. There were no significant differences in calcium intakes attributable to dietary treatments (table 9). Calcium intakes followed the same pattern as feed intakes (table 4). During collection periods 2 and 3, calcium intakes were not significantly different, but there was a decrease ($P<0.05$) in calcium intakes

TABLE 8

Analyses of variance for calcium intake, urinary and fecal calcium and calcium balance

Source of variation	df	Mean squares			
		Calcium intake	Urinary calcium	Fecal calcium	Calcium balance
Diet	3	9696.256	43.88*	6345.14	747.15
Rat/diet	23	3671.872	11.11	2275.07	1327.89
Period	3	15613.184***	86.41***	16674.02***	1504.35
Diet x period	9	456.160	6.09	658.14	1090.57
Residual	64	803.760	6.27	883.41	965.22

*P<0.05 ***P<0.0001

TABLE 9

Effects of dietary treatments and collection periods on calcium intakes, urinary and fecal calcium and calcium balance

	Dietary treatment			
	A (20% casein)	B (40% casein)	C (40% casein-gluten)	D (40% casein-gelatin)
Calcium intake, mg/96 hr	347.04 ± 13.36[1]	300.08 ± 12.36	309.80 ± 12.76	328.56 ± 10.72
Urinary calcium, mg/96 hr	1.69 ± 0.73[a2]	3.45 ± 0.68[ab]	3.69 ± 0.70[b]	4.92 ± 0.59[b]
Fecal calcium, mg/96 hr	298.15 ± 10.52	261.05 ± 9.74	271.22 ± 10.06	287.75 ± 8.43
Calcium balance, mg/96 hr	47.22 + 8.04	35.60 ± 7.44	34.90 ± 7.68	35.91 ± 6.44

	Collection period			
	1 (days 35-39)	2 (days 50-54)	3 (days 63-67)	4 (days 78-82)
Calcium intake, mg/96 hr	357.16 ± 6.32	322.20 ± 5.48[2]	311.64 ± 5.48[a]	294.56 ± 5.64
Urinary calcium, mg/96 hr	6.37 ± 0.56	2.89 ± 0.49[a]	2.49 ± 0.49[ab]	1.98 ± 0.50[b]

TABLE 9

(Continued)

	Collection period			
	1 (days 35-39)	2 (days 50-54)	3 (days 63-67)	4 (days 78-82)
Fecal calcium, mg/96 hr	312.56 ± 6.61[a]	298.95 ± 5.76[a]	261.38 ± 5.76[b]	254.28 ± 5.93[b]
Calcium balance, mg/96 hr	38.23 ± 6.91	29.35 ± 6.02	47.76 ± 6.02	38.29 ± 6.20

[1]Values are means ± SE.

[2]Means in a row sharing a common superscript are not significantly different (P>0.05) using LSD test.

between period 1 and periods 2 and 3; between periods 2 and 3 and period 4; and between period 1 and period 4 (table 9).

Urinary calcium excretion of rats fed diet A was lower ($P<0.05$) than that for rats fed diets C and D, but it did not differ significantly from calcium excretion of rats fed diet B (table 9). Although it was not statistically significant diet D-fed rats tended to excrete more calcium in the urine than rats fed diets A, B or C. Increased urinary calcium excretion in human subjects fed normal diets supplemented with gelatin has been reported by Dull.[5]

Urinary calcium excretions were higher ($P<0.05$) during period 1 than during periods 2, 3 and 4 (table 9). They were higher ($P<0.05$) during the second period than in the fourth period. During periods 3 and 4, excretions of calcium did not differ significantly. Decreasing feed intake during the study, thus decreasing calcium intake, was accompanied by a corresponding drop in calcium excretion in the urine.

There were no significant differences in fecal calcium excretion attributable to the dietary treatments in this study. However, there was a tendency for rats fed diets B, C and D, containing 40% protein, to excrete more calcium in the urine and less in the feces than rats fed the 20% protein diet (table 9). The observation is in agreement with that of Bell et al. (30) who reported a shift in the route of excretion of endogenous calcium from the feces to the urine in rats fed a high

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[5]See footnote 3.

protein diet.

Fecal calcium excretions for all rats were higher ($P<0.05$) during collection periods 1 and 2 than during periods 3 and 4 (table 9). Fecal excretions of calcium did not differ significantly during the first and second periods nor during the third and fourth periods. Decreasing levels of calcium excretion in the feces may have been caused by the decreased calcium intake.

The diets fed to rats in this study produced no significant differences in calcium balance in the animals (table 9). All groups of rats were in positive calcium balance regardless of dietary treatment. Rats fed the 40% protein diets (B, C and D) tended to be in less positive calcium balance than rats fed the 20% protein diet (A). The trend toward less positive calcium balance in rats fed high protein diets is similar to the findings of Linkswiler et al. (16) and Oddoye[6], who observed negative calcium balances in adult human males fed high protein diets.

In the present study, varying the protein source in the diets (B, C and D) had no significant effect on calcium balance in adult rats (table 9). Lee et al. (32) reported better calcium utilization and more positive calcium balance in weanling rats fed a diet in which protein was supplied by skim milk than in diets in which protein was supplied by bean or a bean-cornmeal mixture. Protein source may be a less critical factor in calcium utilization in adult rats than in growing animals.

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[6]See footnote 1.

SUMMARY

Numerous studies have shown that dietary protein level influences retention and excretion of calcium in the body, and recently, it has been suggested that high protein diets may be a factor in osteoporosis in human adults. It is not known if all proteins have a similar effect on calcium metabolism, therefore a study was designed to determine the effect of protein source on calcium excretion and calcium balance.

Groups of adult male rats were fed diets containing 20% protein as casein or 40% protein diets as casein, casein and wheat gluten or casein and gelatin. All diets contained 20% fat to simulate the fat content of human diets high in protein. The calcium and phosphorus intake of all groups were maintained at 0.6 and 0.3%, respectively. Four 96-hour collections of urine and feces were obtained from each rat, beginning on days 35, 50, 63 and 78 of the study. Urine volumes and the calcium contents of urine and fecal samples were determined. Feed intakes and animal weights were recorded during and between collection periods.

In this study, there were no significant differences in feed intake or weight gain attributable to dietary treatments. Urinary volume was lower ($P<0.05$) for rats fed the 20% protein diet than for rats fed diets containing 40% protein. Urinary calcium excretion of rats fed the 20% casein diet was lower ($P<0.05$) than that for rats fed the 40% casein and wheat gluten

diet or the 40% casein and gelatin diet, but it did not differ significantly from that of rats fed the 40% casein diet.

Feed and consequently calcium intakes decreased with each successive collection period, although the decrease between periods 2 and 3 was not significant. Urinary volumes were highest ($P<0.05$) during collection period 1, and they tended to decrease with each period thereafter, but the differences were not significant. Urinary calcium excretions were higher ($P<0.05$) during period 1 than during periods 2, 3 and 4. They tended to decrease with each successive period, but they were not significantly different during periods 3 and 4. Fecal calcium excretions were higher ($P<0.05$) during the first two collection periods than during the last two periods.

Regardless of dietary treatment, all groups of rats maintained positive calcium balance throughout the study, but rats fed the 40% protein diets tended to be in less positive balance than rats fed the 20% protein diet. Dietary protein source had no significant effect on calcium balance in adult male rats in this study.

ACKNOWLEDGMENT

The author wishes to express appreciation to Dr. Kathleen Newell for her inspiration and patient guidance throughout the research experiment, and for her assistance in the preparation of this thesis. An expression of thanks is extended to the committee members Dr. Beth Fryer and Dr. Gerald Reeck.

Special thanks are also due to Dr. Arthur Dayton for his counsel and direction in the experimental design and the statistical analyses.

Deep gratitude is expressed to the author's husband, Joseph H. Klotz, Jr., for his constant support and patient confidence, and to family and special friends for their interest and encouragement.

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APPENDIX

TABLE 10

Average daily feed intakes during collection periods

Dietary treatment	Rat no.	Collection period 1 (days 35-39)	2 (days 50-54)	3 (days 63-67)	4 (days 78-82)
		g/day			
A (20% casein)	1	14.5	16.5	16.0	14.1
	2	16.5	14.0	12.8	11.9
	3	(13.9)[1]	12.8	16.5	12.0
	4	(5.3)	16.1	14.0	14.6
	5	16.1	15.3	15.0	13.1
	6	14.1	13.8	13.3	12.9
B (40% casein)	7	11.3	11.5	11.1	9.6
	8	15.3	11.5	12.9	11.9
	9	12.4	12.3	12.3	12.3
	10	16.3	13.1	12.1	12.4
	11	14.9	14.0	13.3	12.7
	14	13.6	12.4	11.1	9.8
C (40% casein-gluten)	15	(----)[2]	11.4	10.5	10.3
	17	15.0	12.9	12.9	12.8
	18	18.6	14.8	13.8	13.4
	19	12.1	11.9	11.9	11.9
	20	10.9	12.4	11.4	10.9
	21	17.5	13.8	12.4	12.4
	22	(----)	12.1	13.5	(11.0)
D (40% casein-gelatin)	23	16.3	14.8	13.1	13.0
	24	14.4	13.1	11.1	9.8
	25	18.9	13.6	10.8	11.3
	26	18.4	14.5	16.6	15.8
	27	13.4	12.5	11.6	12.0
	28	13.4	12.5	11.8	11.8
	29	12.9	12.9	13.5	12.9
	30	17.3	15.9	14.4	13.8

[1]Data in parentheses were not included in the analyses of variance computations.

[2]Dash indicates that values were not obtained.

TABLE 11

Weight gains or losses during collection periods

Dietary treatment	Rat no.	Days of collection			
		35-39	50-54	63-67	78-82
		g/96 hrs			
20% casein	1	5	10	4	3
	2	7	7	10	(--)[1]
	3	5	2	-2	-2
	4	-29	10	7	9
	5	7	4	4	-1
	6	3	3	0	2
40% casein	7	-6	4	3	-1
	8	6	2	7	1
	9	-4	2	2	2
	10	8	2	-5	3
	11	7	0	5	2
	14	-1	-1	-4	-2
40% casein-gluten	15	-3	7	-2	0
	17	0	-5	2	-1
	18	6	0	4	2
	19	-1	0	-1	1
	20	-3	3	2	-2
	21	11	4	8	5
	22	8	7	0	-4
40% casein-gelatin	23	18	8	-1	1
	24	12	-1	-5	-1
	25	17	9	6	-2
	26	6	0	-2	1
	27	6	1	6	0
	28	4	6	-6	-3
	29	1	-4	2	1
	30	24	20	6	-3

[1]Dash indicates that value was not obtained.

TABLE 12

Urinary volumes during collection periods

Dietary treatment	Rat no.	Days of collection			
		35-39	50-54	63-67	78-82
		ml/96 hrs			
20% casein	1	27.5	26.5	28.0	34.0
	2	19.0	15.0	15.0	14.5
	3	(24.0)[1]	19.5	19.0	20.5
	4	(13.5)	19.0	19.5	18.5
	5	21.5	22.5	25.5	23.0
	6	24.5	19.5	22.5	25.0
40% casein	7	50.5	41.5	43.5	38.5
	8	71.0	40.0	42.0	40.5
	9	72.5	42.0	43.0	39.5
	10	72.0	51.0	49.5	42.5
	11	84.5	59.0	65.5	63.0
	14	47.0	41.5	37.0	29.0
40% casein-gluten	15	(76.5)	54.5	51.0	47.5
	17	50.0	42.5	41.0	41.5
	18	95.5	61.0	59.5	55.0
	19	42.0	52.0	47.5	42.5
	20	38.0	45.0	41.5	44.0
	21	51.0	60.5	52.0	51.5
	22	(41.0)	37.5	35.5	(36.0)
40% casein-gelatin	23	59.0	58.0	58.5	59.0
	24	83.5	62.0	62.5	51.5
	25	79.5	60.5	54.0	60.0
	26	73.5	53.0	79.0	68.5
	27	43.0	50.0	44.5	39.0
	28	50.0	53.0	47.5	53.0
	29	76.0	49.5	45.0	48.5
	30	118.0	57.0	56.0	53.0

[1]Data in parentheses were not included in the analyses of variance computations.

TABLE 13

Fecal dry weights during collection periods

Dietary treatment	Rat no.	Days of collection			
		35-39	50-54	63-67	78-82
		g/96 hrs.			
20% casein	1	4.8786	5.5985	5.4287	4.6792
	2	4.5953	4.0093	3.4292	3.1743
	3	(4.2556)[1]	3.6135	3.7116	3.2717
	4	(1.7705)	4.9693	4.3778	4.4844
	5	5.5631	5.7133	5.8143	4.1838
	6	4.6540	4.1765	4.3031	3.9652
40% casein	7	3.7036	3.4670	2.7837	2.4248
	8	4.7092	3.8852	3.0638	3.8739
	9	3.5529	3.5882	3.5813	3.4014
	10	4.7637	3.6851	3.4663	3.5712
	11	3.4055	4.7060	3.7540	4.1225
	14	4.2990	4.2473	3.2444	3.2350
40% casein-gluten	15	(3.9960)	3.0415	2.9485	2.4665
	17	4.5305	4.1200	3.6877	3.8297
	18	5.3492	4.8871	4.4353	4.1076
	19	3.7131	4.1967	3.3863	3.8268
	20	3.5301	3.8953	3.3731	3.4731
	21	5.0385	4.5517	4.5976	3.9341
	22	(4.1363)	3.6068	4.1473	(3.3974)
40% casein-gelatin	23	5.0207	4.4157	4.1163	4.2828
	24	4.0706	3.9817	3.3324	2.7830
	25	5.7341	3.6170	3.1501	2.9590
	26	5.1817	4.5168	4.9405	4.2550
	27	3.7869	3.8957	3.4405	3.4057
	28	4.1056	3.7880	3.4499	3.6825
	29	3.7796	3.4246	4.0178	3.8592
	30	5.2617	4.6088	3.8796	3.9745

[1]Data in parentheses were not included in the analyses of variance computations.

TABLE 14

Calcium intakes during collection periods

Dietary treatment	Rat no.	Days of collection			
		35-39	50-54	63-67	78-82
		mg/96 hrs			
20% casein	1	348.0	396.0	384.0	338.4
	2	396.0	336.0	307.2	285.6
	3	(333.6)[1]	307.2	396.0	288.0
	4	(127.2)	386.0	336.0	350.4
	5	386.0	367.2	360.0	314.4
	6	338.4	331.2	319.2	309.6
40% casein	7	271.2	276.0	266.4	230.4
	8	367.2	276.0	309.2	285.6
	9	297.6	295.2	295.2	295.2
	10	391.2	314.4	290.4	297.6
	11	357.6	336.0	319.2	304.8
	14	326.4	297.6	266.4	235.2
40% casein-gluten	15	(----)[2]	273.6	252.0	247.2
	17	360.0	309.6	309.6	307.2
	18	446.4	355.2	331.2	321.6
	19	290.4	285.6	285.6	285.6
	20	261.6	297.6	273.6	261.6
	21	420.0	331.2	297.6	297.6
	22	(----)	290.4	324.0	(264.0)
40% casein-gelatin	23	391.2	355.2	314.4	312.0
	24	345.6	314.4	266.4	235.2
	25	453.6	326.4	259.2	271.2
	26	441.6	348.0	398.4	379.2
	27	321.6	300.0	278.4	288.0
	28	321.6	300.0	283.2	283.2
	29	309.6	309.6	324.0	309.6
	30	413.6	381.6	345.6	331.2

[1]Data in parentheses were not included in the analyses of variance computations.

[2]Dash indicates that values were not obtained.

TABLE 15

Urinary calcium during collection periods

Dietary treatment	Rat no.	Days of collection			
		35-39	50-54	63-67	78-82
		mg/96 hrs			
20% casein	1	1.389	1.400	0.856	1.242
	2	2.007	1.572	1.094	0.956
	3	(4.036)[1]	1.152	0.861	0.589
	4	(2.328)	1.115	0.890	1.367
	5	2.290	1.502	2.373	1.660
	6	4.976	2.405	2.302	2.417
40% casein	7	4.871	4.842	2.634	2.140
	8	5.023	1.628	0.965	1.145
	9	3.166	2.601	1.643	1.333
	10	6.909	2.111	1.638	1.718
	11	16.480	3.037	2.574	2.017
	14	5.467	3.271	3.379	2.098
40% casein-gluten	15	(5.755)	4.986	2.779	2.258
	17	5.416	2.840	1.764	2.004
	19	13.516	4.717	3.440	2.545
	19	3.685	2.638	1.221	2.041
	20	3.044	2.484	2.961	1.380
	21	7.737	4.388	4.723	2.385
	22	(1.932)	1.456	2.858	(1.523)
40% casein-gelatin	23	7.903	4.229	2.588	2.693
	24	3.385	6.301	4.179	3.418
	25	15.780	7.240	5.809	3.985
	26	6.596	1.397	2.158	2.590
	27	3.777	1.813	3.498	0.986
	28	3.642	1.702	1.548	1.602
	29	10.630	4.692	6.252	5.184
	30	23.759	2.780	2.894	2.321

[1]Data in parentheses were not included in the analysis of variance computations.

TABLE 16

Fecal calcium during collection periods

Dietary treatment	Rat no.	Days of collection			
		35-39	50-54	63-67	78-82
		mg/96 hrs			
20% casein	1	321.578	319.904	300.697	320.724
	2	322.677	270.888	237.785	222.457
	3	(-------)[1]	338.953	249.400	251.171
	4	(-------)	255.203	342.543	325.740
	5	354.981	342.564	292.462	282.956
	6	324.058	282.336	273.141	268.087
40% casein	7	295.658	264.459	214.818	182.551
	8	311.062	255.207	202.107	257.852
	9	250.170	254.572	248.004	245.547
	10	349.341	253.270	235.705	229.148
	11	270.560	330.054	291.899	282.552
	14	303.247	283.650	237.331	216.562
40% casein-gluten	15	(294.529)[2]	234.332	242.590	176.389
	17	316.587	310.367	291.599	263.328
	18	344.499	296.087	302.233	266.162
	19	280.027	295.028	229.170	222.853
	20	254.252	259.275	235.460	218.320
	21	343.404	317.501	302.601	231.266
	22	(299.034)	292.162	272.200	(-------)
40% casein-gelatin	23	339.354	330.756	281.106	344.398
	24	317.136	317.889	241.081	222.790
	25	399.885	267.751	216.563	227.541
	26	255.495	309.693	320.256	298.983
	27	303.605	283.720	219.356	243.166
	28	304.081	273.109	240.023	270.830
	29	301.272	259.622	264.386	269.640
	30	369.855	343.418	269.713	301.578

[1]Dash indicates that values were not obtained.

[2]Data in parentheses were not included in the analyses of variance computations.

TABLE 17

Calcium balance during collection periods

Dietary treatment	Rat. no.	Days of collection			
		1	2	3	4
		mg/96 hrs			
20% casein	1	25.033	74.696	82.447	16.434
	2	71.316	63.540	68.321	62.187
	3	(------)[1]	-32.905	145.739	36.240
	4	(------)	130.082	42.648	23.293
	5	29.129	23.134	15.084	29.784
	6	9.366	46.459	43.757	39.096
40% casein	7	-29.329	6.699	48.948	45.709
	8	51.115	19.165	106.528	26.603
	9	44.264	38.027	45.553	48.320
	10	34.950	59.019	53.057	66.734
	11	70.560	2.909	24.737	20.231
	14	17.686	10.679	25.690	16.540
40% casein-gluten	15	(------)	34.282	6.631	68.553
	17	37.997	-3.607	16.237	41.868
	18	88.385	54.396	25.527	52.893
	19	6.688	-12.066	55.209	60.706
	20	4.304	35.841	35.179	41.900
	21	68.859	9.311	-9.724	63.949
	22	(------)	-3.218	48.942	(------)
40% casein-gelatin	23	43.943	20.215	30.706	-35.091
	24	25.079	-9.790	21.140	8.992
	25	37.935	51.409	36.828	39.674
	26	179.509	36.910	75.986	77.627
	27	14.218	14.467	55.546	43.848
	28	13.877	25.189	41.629	10.768
	29	-2.302	45.286	53.362	34.776
	30	21.586	35.402	72.993	27.301

[1]Dash indicates that values were not obtained.

EFFECT OF PROTEIN SOURCE ON CALCIUM EXCRETION IN ADULT RATS FED HIGH PROTEIN DIETS

by

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Blacksburg, Virginia, 1976

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1978

The effect of protein source on calcium excretion and calcium balance in adult rats fed high protein diets was investigated. Groups of adult male rats were fed diets containing 20% protein as casein or 40% protein diets as casein, casein and wheat gluten or casein and gelatin. All diets contained 20% fat to simulate the fat content of human diets high in protein. The calcium and phosphorus intakes of all groups were maintained at 0.6 and 0.3%, respectively. Four 96-hour collections of urine and feces were obtained from each rat, beginning on days 35, 50, 63, and 78 of the study. Urine volumes and the calcium contents of urine and fecal samples were determined. Feed intakes and animal weights were recorded during and between collection periods.

In this study, there were no significant differences in feed intake or weight gain attributable to dietary treatments. Urinary volume was lower ($P<0.05$) for rats fed the 20% protein diet than for rats fed diets containing 40% protein. Urinary calcium excretion of rats fed the 20% casein diet was lower ($P<0.05$) than that for rats fed the 40% casein and wheat gluten diet or the 40% casein and gelatin diet, but it did not differ significantly from that of rats fed the 40% casein diet.

Feed and consequently calcium intakes decreased with each successive collection period, although the decrease between periods 2 and 3 was not significant. Urinary volumes were highest ($P<0.05$) during collection period 1, and they tended to decrease with each period thereafter, but the differences

were not significant. Urinary calcium excretions were higher ($P<0.05$) during period 1 than during periods 2, 3 and 4. They tended to decrease with each successive period, but they were not significantly different during periods 3 and 4. Fecal calcium excretions were higher ($P<0.05$) during the first two collection periods than during the last two periods.

All groups of rats, regardless of dietary treatment, maintained positive calcium balance throughout the study, but rats fed the 40% protein diets tended to be in less positive balance than rats fed the 20% protein diet. Dietary protein source had no significant effect on calcium balance in adult male rats in this study.