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Frequency of eating and utilization of protein and calcium for bone growth

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GUJRAL, Sunder M., 1925-
FREQUENCY OF EATING AND UTILIZATION OF PROTEIN
AND CALCIUM FOR BONE GROWTH.

Iowa State University, Ph.D., 1973
Health Sciences, nutrition

Frequency of eating and utilization of protein

and calcium for bone growth

by

Sunder M. Gujral

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food and Nutrition
Major: Nutrition

Approved:

In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University
Ames, Iowa

1973

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INTRODUCTION

Experimental findings with animals have suggested that normal metabolic pathways change in response to eating frequency. These changes have led to alterations in body composition (Cohn and Joseph, 1959), glucose absorption (Leveille and Chakrabarty, 1968), and nitrogen excretion (Cohn et al., 1963).

Cohn and Joseph (1959) reported that meal eating resulted in 35% more body fat than nibbling although food intakes were similar. Leveille and Chakrabarty (1968) demonstrated that both the intestinal weight relative to body weight and the glucose absorption coefficient were increased about 40% in meal-fed rats compared with nibbling rats. The increased absorption was associated with an increase in surface area available for absorption. According to Cohn et al. (1963), the mean amount of urea nitrogen excreted during a 7-day period was 43% larger for force-fed rats than those fed ad libitum although their intakes of protein were equal (914 mg vs 639 mg urea nitrogen excreted).

When the influx of amino acids into the livers of dogs was increased as a consequence of eating a meal providing 43% calories from meat, urea formation was increased (Elwyn et al., 1968). Of the amino acids absorbed, 57% were converted to urea. Elwyn et al. concluded that the liver prevented abrupt changes in free amino acids in the blood by removing their amino groups before they reached the systemic circulation.

Earlier Cohn et al. (1964) had reported that when dietary protein intakes were equal, the activities of hepatic arginine synthetase and arginase were greater for meal-fed rats than for rats fed ad libitum. Thus

a change from nibbling to meal feeding increased the catabolism of dietary amino acids.

Information on the effect of meal feeding on mineral metabolism is incomplete. A metabolic study conducted by Irwin and Feeley (1967) showed no significant differences in the retention of calcium, magnesium, and phosphorus when the same amounts of these nutrients were consumed in three or six equal meals, or two small and one large meal. However, recently Kales and Phang (1971) reported that adult volunteers benefited from divided calcium intakes. Milk, supplying 700 mg calcium of the daily 1000 mg intake, was served at one meal or divided into six equal portions. Based on calcium balance studies and tracer doses of radiocalcium, the authors calculated that calcium absorption increased 20% while bone resorption decreased 12% when milk was given six times daily instead of once. The discrepancy between the results reported by Irwin and Feeley (1967) and Kales and Phang (1971) could be attributed to the frequency of calcium intakes.

El-Maraghi et al. (1965) demonstrated that the simultaneous presence of adequate dietary protein and calcium was essential for optimal bone growth and ossification. Rats developed osteoporosis when they were fed diets providing 10.2 net dietary protein calories percent (NDpCal%) with 0.11% calcium or 4.5 NDpCal% with 0.44% calcium. The ash content of bone expressed as mg per cm^3 decreased when dietary protein was raised from 4.5 to 10.2 NDpCal% if both diets contained 0.11% dietary calcium. Yet increasing the calcium intake from 0.11 to 0.44% without a concomitant increase in protein had no effect on ash per cm^3 values. Bone growth was greatest when the diet provided 10.2 NDpCal% and 0.44% calcium, the maximum amounts of both nutrients used in the experiment by El-Maraghi and his co-workers.

The present investigator (Gujral, 1968) supplemented the home diets of two-year-old Indian children for eight months with an additional source of food energy equivalent to 297 kilocalories (Kcal) and 9.0 g cereal protein. The home diets provided 8.7 g protein per day and food energy equivalent to 452 Kcal. Based on the appearance of ossification centers in the children's hands (radiographic observations), she reported greater mineralization in the supplemented group. Since the calcium intakes were similar for the two groups (287 and 265 mg calcium per day), improved mineralization of the bone might have been due to increased protein intake.

In the study of two-year-old children (Gujral, 1968), diet surveys were conducted initially and repeated during the last week of supplementation. No records of the number of times supplemented and unsupplemented groups ate each day were obtained. However, the supplement was a powder which was made into a gruel and ingested separately from the home prepared foods. Thus the number of meals per day also could have increased. The observed improvement in mineralization of bone in these children might have been due to an increase in feeding frequency as well as to the increase in amount of food eaten.

The investigator's interest in child feeding programs in India and in the possible effect of feeding frequency on protein and calcium utilization for bone growth suggested the present experiments.

This study investigated: (a) the utilization of dietary protein and calcium for bone growth when rats were fed for 2 hours ad libitum twice daily compared with utilization when rats were fed for 24 hours ad libitum and (b) the interaction of protein and calcium on bone growth.

The diets contained two different concentrations of protein and two (Experiment I) or three (Experiment II) different concentrations of calcium.

Protein and calcium utilizations were evaluated on the basis of 5-day balance studies initiated on the 25th day of Experiment I. In Experiment II, the concentrations of nitrogen and fat in the livers and carcasses and the activity of hepatic arginase were measured to evaluate protein utilization and meal feeding effects.

The right femur was chosen to represent the skeletal system. Calcium utilization was assessed using the following data for femurs: fresh weight, length, volume, widths of middiaphysis and distal epiphysis, dry weight, and ash weight.

REVIEW OF LITERATURE

As early as 1937 Werthessen noted that rats fed once a day had significantly higher respiratory quotients (RQ) than those fed ad libitum. Later Brobeck and his co-workers (1943) pair-fed two groups of rats, one of which had been subjected to hypothalamic lesions; the animals with lesions consumed 1½ times as much oxygen as those with no lesions. The two groups also differed in their rates of food ingestion. Rats with lesions finished their allotted food within a short period of time whereas rats without lesions nibbled it for 24 hours.

In 1943 Tepperman et al. reported that obese rats with hypothalamic lesions exhibited a significantly higher RQ than intact controls although the amount of food eaten was equal. For both groups, the diet, laboratory chow, contained 18% protein, 5% fat, and 68% carbohydrate. The animals with hypothalamic lesions ate their day's ration within 3 to 4 hours while intact controls ate throughout the day. To investigate whether elevated RQ was caused by the rate of food ingestion or by hypothalamic lesions, Tepperman et al. (1943), in a second experiment, trained one set of intact rats to eat their food allowance for the whole day within 3 hours while other intact rats were allowed to eat all day long. After 3 weeks on these feeding patterns, the RQ's for both groups of rats were measured as in Experiment I. The RQ values of the trained group of intact rats in the second experiment were similar to those observed for the hypothalamic rats in the first experiment and were higher than the values of the untrained intact rats. Thus alternate periods of fasting and food consumption led to an increased RQ.

In their third experiment, Tepperman et al. (1943) reduced the feeding period for a trained group from 3 hours to 1 hour. At the same time, a synthetic diet, similar in percentage of fat and carbohydrate to the laboratory chow used previously and containing sucrose as the main carbohydrate source, was given to the rats. With the reduced time period and the synthetic diet, the mean RQ value was much higher for the trained group (1.22) than for the untrained group (1.05). Thus Tepperman et al. (1943) found the rate of ingestion of food influenced the amount of carbohydrate converted to fat.

Frequency of Eating and Protein Utilization

Cohn et al. (1963) determined the effect of tube feeding and the amount of protein on fat accumulation by rats fed diets containing 0 to 67% protein. In comparing the effects of tube-feeding with ad libitum feeding, rats fed by tube had significantly more body fat than those fed ad libitum except when the protein-free diet was fed. Protein affected fat accumulation differently depending on the feeding pattern. In rats fed ad libitum, fat accumulation decreased with increasing protein intake, whereas no consistent trend in decreasing fat deposition with increasing protein levels was observed in tube-fed rats. Since the energy intakes of all the rats were comparable, except those fed a diet containing 67% protein, the difference between the two groups was the pattern of feeding and the amount of protein intake. The authors believed that when tube-fed animals were provided with large amounts of protein in a short period of time, they catabolized greater amounts of ingested amino acids than ad libitum fed rats, thereby providing precursors for fat synthesis.

To test whether tube-feeding increased catabolism of amino acids, Cohn et al. (1963) measured urinary urea nitrogen excretion. Rats were fed a diet containing 18.5% protein either ad libitum or twice daily by stomach tube. After 7 days of adaptation to the diet and feeding pattern, rats were transferred to metabolic cages, and the tube-fed rats were pair-fed amounts equivalent to those of rats fed ad libitum. Urine collections were analyzed daily for nitrogen. Total excretion of urinary nitrogen for 7 days by tube-fed rats was 43% higher than that of controls fed ad libitum (914 vs 639 mg N). The feeding patterns were reversed on the 8th day of the urine collection period. Nitrogen excretion started to increase on the first day that the feeding pattern was changed. Seven days later, tube-fed rats excreted 29% more urinary nitrogen than those fed ad libitum. The total excretions of urinary nitrogen by tube-fed rats and by their controls were 907 and 701 mg N, respectively.

Thus rats subjected to a large amount of protein in a short period of time (tube-fed) appeared to catabolize a greater proportion of their ingested amino acids than the controls. The results also indicated that with tube-feeding, less dietary protein was available to participate in protein anabolic reactions, and more was probably degraded to the carbon moiety for conversion to fat.

Cohn et al. (1964) confirmed their previous findings (1963) that the proportion of ingested amino acids utilized for anabolic purposes was limited when large amounts of protein were ingested in a short period of time. Rats were fed a diet containing 20% protein either ad libitum or by stomach tube twice daily. After one week of adaptation to the diet and feeding pattern, three rats from each group were transferred to metabolic

cages for a 3-day nitrogen balance study. On the first day only of the

3-day balance period, ^{15}N enriched yeast protein was added to the diet.

The tube-fed rats were pair-fed with those fed ad libitum. Tube-fed rats

excreted more total urea nitrogen (473 vs 317 mg N per 3 days) and more

urea ^{15}N (48.8 vs 30.0 mg N per 3 days) than their ad libitum fed controls.

The authors postulated that as a consequence of tube-feeding, a large por-

tion of the ingested amino acids were channeled into the urea cycle. The

deaminated carbon skeleton then was oxidized either to CO_2 or used as a

precursor for fat synthesis.

The effect of meal feeding on lipogenesis in response to low protein

intakes compared with adequate protein intakes was reported by Beaton

et al. (1964). They investigated the incorporation of ^{14}C-acetate into

fatty acids of adipose tissue of rats fed isocaloric diets containing 5%

or 20% protein. Both diets were provided either for 2 hours each day or

for 24 hours. The meal-fed rats (restricted to eating 2 hours per day)

incorporated significantly more radioacetate into the fatty acids of their

adipose tissue at both protein intakes than those fed ad libitum. These

results agreed with those of Cohn et al. (1963) that meal-fed rats accumu-

lated more body fat than those fed ad libitum.

The effect of dietary protein on incorporation of radioacetate into

fatty acids of adipose tissue was also reported by Beaton et al. (1964).

Lipogenesis was not significantly different when rats were given 5% or 20%

protein in the diets. The authors concluded that the ability to synthesize

fat was not appreciably impaired when rats were given a 5% protein diet,

either ad libitum or meal-fed.

Utilization of protein by human beings, when the number of meals per day has been varied, has been examined by only a few investigators. In 1950, Wu and Wu observed that nitrogen utilization was more efficient when 4 meals were fed than when 2 meals were fed, if 1.14 g protein per kg body weight was provided per day. However, subjects receiving 0.73 g protein per kg body weight per day retained nitrogen to the same extent whether they ate 2 or 4 meals a day. These authors suggested that with the lower protein intakes (0.73 g protein per kg body weight), the amino acid concentration in the metabolic pool would be smaller than when 1.14 g protein per kg body weight was fed. Consequently the excretion of urea nitrogen would be smaller with the lower protein intake.

Barja et al. (1972) examined nitrogen balances in growing children when animal protein was distributed unequally among meals. The diet had an energy value of 2050 Kcal and contained 63 g protein, 60% of which was animal in origin. In one period of 10 days, the animal protein was served in equal amounts at breakfast and lunch whereas in another period of 10 days, the animal protein was distributed evenly among 4 meals. The children were adjusted to the diet and the feeding patterns for 7 days before nitrogen balance was measured over 3 days. When animal protein was fed 4 times daily, 3.5 times as much nitrogen was retained as when the protein was fed twice a day (1472.9 vs 426.8 mg N per day). However, the children lost on the average 0.10 kg weight per day when the animal protein was given only for breakfast and lunch; they gained 0.34 kg weight per day when the animal protein was distributed evenly among 4 meals. Barja et al. (1972) believed that protein was better utilized when all amino acids, especially the sulfur containing ones, were supplied at the same time. Conversely, when ani-

mal protein was given only in the first half of the day, a relative deficiency of sulfur containing amino acids in the second half of the day perhaps developed. The authors postulated that this relative deficiency caused an impairment of protein synthesis, and, hence, more of amino nitrogen was excreted as suggested by Wu and Wu (1950).

Shortridge and Linkswiler (1963) measured nitrogen balances when human subjects were fed either 3 or 6 times a day. Of the 5.3 g nitrogen supplied daily, 4.8 g were obtained from intact egg protein or the equivalent of purified amino acids. More nitrogen was retained when egg protein was included in the diet than when purified amino acids were fed. However, nitrogen retention was similar whether 3 meals or 6 meals were fed daily.

The studies cited above suggested that when daily food intake was equal, 2 meals per day compared with nibbling resulted in increased excretion of urea nitrogen (Cohn et al., 1963, 1964) and increased accumulation of fat in rats (Cohn and Joseph, 1959; Cohn et al., 1963; Beaton et al., 1964). However, increased nitrogen excretion in humans was not observed when diets containing a lower level of protein were fed 4 vs 2 times a day (Wu and Wu, 1950).

Hepatic arginase

In 1950, Miller reported the loss and regeneration of hepatic arginase in response to change in dietary protein. After adaptation for a minimum of 10 days to a diet containing 25% protein, adult rats were transferred to diets containing 6 or 0% protein. After 21 days on 6% protein or 14 days on 0% protein, the arginase activity per g hepatic protein had not changed significantly. However, hepatic protein had decreased from 745 mg per

100 g initial body weight to 573 and 437 mg per 100 g initial body weight on the diets containing 6 or 0% protein, respectively. Therefore, the total arginase activity actually had decreased when dietary protein was decreased.

To examine regeneration of hepatic arginase, rats were fed a diet containing 6% protein for 23 days (Miller, 1950). For the following 9 days, they were fed a diet containing 0% protein; then for the next 7 days, a diet containing 25% protein. Increasing the dietary protein from 0 to 25% resulted in an increase in total arginase activity. The authors concluded that arginase, a cellular protein, was lost when protein was lost from the body, i.e., when rats were fed a diet containing 6 or 0% protein. Conversely, arginase was regenerated when dietary protein was increased.

Mandelstam and Yudkin (1952) evaluated the activity of hepatic arginase after rats weighing 60 to 70 g had eaten diets containing 17, 33, or 67% protein for 22 to 24 weeks. The arginase activity increased with increases in dietary protein. According to the investigators, with increasing protein intakes, larger amounts of proteins were metabolized, hence increasing amounts of arginase were formed to produce urea. Furthermore, the authors postulated that arginase activity not only could have been responding to increasing intakes of protein but also to an increasing supply of arginine released from the dietary protein.

Ashida and Harper (1961) observed that the activity of arginase fluctuated with the amount of protein consumed. They adapted rats weighing 60 to 65 g to a diet containing 25% protein, then transferred them to diets containing 25, 45, or 70% protein. Arginase activity was determined at intervals for the following 10 days. When protein was increased from 25 to

45%, a peak value of 1.6 times the original value of arginase activity was observed after 4 days. When the dietary protein was increased from 25 to 70%, the arginase activity peaked at 2.5 times the original value after 7 days.

In another experiment (Ashida and Harper, 1961) to determine whether reduced protein intake would reduce enzyme activity as quickly as increased protein intake had increased enzyme activity, rats were fed a diet containing 25% protein. Within one day, the enzyme activity was reduced by nearly 1/3, a drop which was as great as the increase had been when protein intake had been changed in the other direction. Therefore, when dietary protein was increased or decreased, arginase activity changed within 24 hours, in the same direction as the protein intake.

In yet another experiment, Ashida and Harper (1961) demonstrated a nearly linear relationship between activity of hepatic arginase and excretion of urinary nitrogen. The rats were adapted to a diet containing 25% protein for 4 days, then divided into two groups. One group was fed a diet containing 25% protein in amounts equal to that eaten by rats fed a diet containing 70% protein. Throughout 10 days, the activity of hepatic arginase increased with the increase in urea nitrogen excretion after changing the protein content of the diet from 25% to 70%.

Schimke (1962) also has reported that activity of arginase increased with increasing protein intakes. Rats weighing 50 to 60 g were fed diets containing 15, 30, or 60% protein for 14 days. When rats were fed diets having 60% protein, the activity of their hepatic arginase was 84,500 μmoles of urea per g wet liver compared with 38,300 μmoles for rats fed 15% protein. However, when dietary protein was raised from 15 to 30%, no

appreciable changes in arginase activity occurred, 38,300 to 40,000 μmoles urea per g wet liver.

Schimke (1962) has suggested that addition of dietary protein at the expense of carbohydrates might reduce the carbohydrate to amounts below that required to fulfill the energy needs of rats. For example, when diets containing protein above 30% were fed, rats needed to catabolize amino acids for energy, whereas below 30%, carbohydrate alone could meet the need. Since unutilized nitrogen would be excreted as urea, the activity of arginase would need to be increased more when protein was raised from 30 to 60% than when it was raised from 15 to 30%.

To determine the effect of sudden reduction in protein intake on the activity of arginase, Schimke (1962) transferred a group of rats which has been fed a diet containing 60% protein to a diet containing 15% protein. The activity of arginase per g liver decreased progressively with time reaching 58% of its initial value 18 days later (from 50,000 to 29,000 units).

The effect of frequency of eating on hepatic arginase was investigated by Cohn et al. (1964). Rats were fed diets containing 18.5% protein either ad libitum or by stomach tube twice daily for 9 days. They observed that tube-fed rats excreted more urea nitrogen than ad libitum fed controls. They postulated that tube-fed rats had greater arginase activity than their controls, an adaptation similar to that observed with high protein intakes. Hepatic arginase was estimated from the amount of urea excreted. Although the activity of arginase for tube-fed rats appeared to be 118% of that in those fed ad libitum, the increase was not statistically significant.

Frequency of Eating and Calcium Utilization

Phang et al. (1968) investigated calcium excretion of three subjects who were accustomed to consuming a diet providing 1000 mg calcium daily. Metabolic balances were determined while they were eating a diet containing 300 mg calcium from foods other than milk and 700 mg calcium from 400 ml reconstituted milk. For one balance period of 3 days, all of the reconstituted milk was given with breakfast. For a second balance period, the milk was divided into six equal portions and served at intervals throughout the day. The mean daily urinary excretion of calcium in 3 subjects was 0.213 g and 0.153 g when milk was given six times and once a day, respectively. The authors postulated that the larger urinary excretion of calcium, when it was provided throughout the day, reflected absorption of a larger percent of calcium when it was consumed frequently in smaller amounts than when it was ingested all at once.

Serum calcium levels were also measured by Phang et al. (1968). Concentration of serum calcium rose to 10.4 mg/100 ml when subjects consumed milk six times a day compared with 10.2 mg when they consumed milk once a day. The authors suggested that when the subjects received calcium from milk six times a day, concentration of plasma calcium rose slightly during a 24-hour period; such small changes, however, could have suppressed parathyroid secretion which in turn increased renal calcium excretion and reduced renal calcium reabsorption.

Recently, Kales and Phang (1971) extended studies of divided intakes of calcium on its metabolism. The feeding plan was similar to the earlier study reported by Phang et al. (1968). Again urinary excretion of calcium was significantly higher after calcium from milk was provided six times a

day than after it was provided at a single meal. To determine if the time at which the milk was consumed had an influence on calcium absorption, milk was given either once in the morning or once in the evening, or six times a day. The divided servings led to higher urinary calcium excretion than the single serving, whether it was given in the morning or in the evening.

Kales and Phang (1971) also studied the rate of bone accretion and bone resorption using tracer amounts of radiocalcium and metabolic balances. At the beginning of each balance period, 10 μc of $^{47}CaCl_2$ was injected intravenously. Urine and feces were collected for 18 days. Based on the specific activity of calcium in the serum and urine and the cumulative radioactivity in the urine and feces, bone accretion rate was calculated from the amount of calcium transferred from labile pool into the bone per day. Rate of bone resorption also was calculated from the amount of calcium transferred from the bone to the labile pool each day. The gastro-intestinal absorption (g per day) was 20% higher when calcium from milk was provided six times per day than when it was provided once. The rate of bone accretion was not different, but the rate of bone resorption was 12% less when calcium was given six times than when it was given once each day. Kales and Phang (1971) concluded that the single large dose of calcium saturated the mechanism for absorption and resulted in less efficient absorption than when the same amount of calcium was administered in smaller amounts more frequently.

Based on a study using radiocalcium, Birge et al. (1969) reported that the percentage of calcium absorption decreased as the gut wall was sub-jected to increasing calcium loads. Human subjects accustomed to a diet providing an intake of 800 mg calcium daily were given a meal which

contained only 12 mg calcium but which was supplemented with varying amounts of calcium lactate dissolved in lemonade. The added calcium varied from 0 to 400 mg. Two and a half hours after a meal, [47]calcium was given orally. The percentage of [47]calcium absorbed was 60% when there was no calcium added to the meal but decreased to approximately 42% when the added calcium equalled 100 mg. A further increase of added calcium to 300 mg reduced the percent absorbed to approximately 33%. Raising the added calcium from 300 to 400 mg had no further effect on absorption. Birge et al. (1969) postulated that calcium absorption was suppressed because circulating plasma calcium affected the transport mechanism in the intestinal mucosa.

With everted gut sacs, Kimberg et al. (1961) demonstrated that intestinal mucosa from weanling rats fed a diet containing 0.2% calcium transported greater amounts of calcium than intestinal mucosa from rats fed a diet containing 1.2% calcium. Everted gut sacs from proximal duodenum, distal ileum, and two intermediate parts of the intestines were prepared after rats had been fed for 15 to 23 days and were incubated in a medium containing [45]calcium. The transfer of [45]calcium from the mucosal to the serosal side of the duodenal sac was twice as large for rats fed 0.2% calcium as for rats fed 1.2% calcium. Although sacs prepared from the distal ileum and two intermediate parts of the small intestine transported less [45]calcium than sacs prepared from proximal duodenum, they also exhibited the same relative difference in transfer of [45]calcium due to the diets consumed by the weanling rats.

To test the response of the active calcium transport mechanism to intakes of 0.2% calcium, everted gut sacs were filled with a solution con-

taining $CaCl_2$ and ^{45}calcium and incubated for 3 hours. The initial ratio of ^{45}calcium on the serosal side to that of the mucosal side was 1.0.

Three hours later, the ratio was greater than 1.0. The higher ratio was due to an increase in active transport of ^{45}calcium from the mucosal to serosal side of gut sacs of rats fed a diet containing 0.2% calcium. Thus the authors suggested that the observed increase in transport of calcium from mucosal to serosal side might have been due to increase in active transport mechanism.

Factors Influencing Bone Growth

Hormones produced by the pituitary, the thyroid glands, the parathyroid glands, the adrenal glands, and the gonads have been reported to influence skeletal growth and development. Because the present study was planned to evaluate bone growth as a function of frequency and amount of calcium intake, the discussion on endocrinological influence has been limited to parathyroid hormone and thyrocalcitonin which have been associated, mainly, with calcium and phosphorus metabolism.

Calcium homeostasis appears to be maintained by the direct effects of parathyroid hormone on bone resorption and gastrointestinal absorption of calcium (Rasmussen et al., 1970) whereas the extracellular concentration of phosphorus is regulated by thyrocalcitonin which decreases renal reabsorption of phosphorus (Nielsen et al., 1971).

Parathyroid hormone

Jahan and Pitts (1948) measured the concentrations of calcium and phosphorus in the serum and urinary excretion of calcium and phosphorus for the same dog before and after administration of parathyroid extract. Urine

was collected by an indwelling catheter for periods of 10 minutes at a

time. Blood was drawn from the jugular vein at the mid-point of each urine

collection period. The administration of 600 units of parathyroid extract

did not decrease appreciably the concentration of serum phosphorus. Excre-

tion of urinary phosphorus also was not changed. At the same time, the

concentration of serum calcium increased from 9.6 to 14.1 mg per 100 ml and

the rate of urinary excretion of calcium from 0.007 to 0.348 mg per minute.

The authors suggested that the concentration of serum calcium and excretion

of urinary calcium rose because parathyroid extract enhanced mobilization

of calcium from body stores.

Sherwood et al. (1966) reported a significant negative correlation

($r = -0.6$) between the concentration of plasma calcium and plasma parathy-

roid hormone. The concentration of calcium was varied by infusing calcium

chloride or disodium EDTA into the blood stream of cows. When the concen-

tration of plasma calcium had been raised to 14 mg per 100 ml, parathyroid

hormone disappeared completely from the plasma. On the other hand, admin-

istration of EDTA reduced plasma calcium and increased the concentration of

plasma parathyroid hormone by as much as 3 to 4 times.

Sammon et al. (1970) investigated the effect of parathyroid hormone on

calcium homeostasis in parathyroidectomized (PTX) rats. Rats were fed

diets containing 24% casein with 0.05% calcium, 0.5% calcium, or 1.4% cal-

cium. Between 6 to 72 hours after injecting ^{45}calcium intravenously, total

calcium and ^{45}calcium were measured in the urine, feces, and serum. Data

obtained by labeling and balance techniques indicated that the net absorp-

tion of calcium increased as calcium intakes increased in both PTX and

intact rats. However, the effect of parathyroidectomy on increases in cal-

cium absorption varied with calcium intakes. On intakes providing 1.4%
calcium, the net absorption of calcium was 64.8 mg per day in intact and
50.9 mg per day in PTX rats. When the calcium intake was 0.5%, the net
absorption was 39.3 mg per day in intact and 38.4 mg per day in PTX rats.
When calcium intake was 0.05%, the calcium absorption was 6.3 and 3.4 mg
per day in intact and PTX rats, respectively. At the two higher calcium
intakes, the differences in percent calcium absorption between intact and
PTX rats were of a smaller magnitude than the difference at the lowest cal-
cium intake. Thus, the authors suggested that the absorption mechanism
which comes into play at higher calcium intakes might be regulated by para-
thyroid hormone. Absorption at lower calcium intakes might be independent
of parathyroid hormone regulation.

At each calcium intake, i.e., 0.05%, 0.5%, and 1.4%, PTX rats
deposited less and released less calcium from the bone than did intact
ones. The fact that at each calcium intake the plasma calcium was lower in
the PTX rat than in the intact one supported the idea that parathyroid hor-
mone enhanced calcium absorption and bone resorption.

The question of how parathyroid hormone secretion is regulated was
examined by Care et al. (1966). The left parathyroid gland of a year-old
goat was perfused with blood containing known concentrations of calcium.
Variations in calcium concentrations in the blood were made by adding
either disodium EDTA or calcium gluconate in 0.9% saline in the perfusion
fluid. Perfusion of the gland with hypercalcemic blood containing 14 mg
per 100 ml suppressed parathyroid hormone secretion to 20% of its initial
value. On the other hand, perfusion of the gland with hypocalcemic blood
increased parathyroid hormone secretion within 20 minutes. These findings

indicated that parathyroid secretion was regulated by the concentration of plasma calcium.

Thyrocalcitonin

Contrary to the action of parathyroid hormone on bone mineral, thyrocalcitonin inhibits bone resorption. Johnston and Deiss (1966) administered radiocalcium (^{45}calcium) subcutaneously to male rats after injecting them intraperitoneally with thyrocalcitonin. The concentration of the stable isotope in the serum, ^{40}calcium, was reduced significantly in thyrocalcitonin treated rats but ^{45}calcium in their serum was not different from that of control rats. These investigators attributed the decreased ^{40}calcium in the serum of thyrocalcitonin treated animals to the inhibitory effect of thyrocalcitonin on release of calcium from bones. They also reported that serum ^{40}calcium was reduced significantly when thyrocalcitonin only was given but was not affected when parathyroid hormone was coadministered with thyrocalcitonin. These data supported the hypothesis that thyrocalcitonin inhibited release of calcium from the bone.

Foster et al. (1966) observed osteoclasts by histochemical methods in proximal and distal metaphysis of vertebrae of thyrocalcitonin-injected, parathyroidectomized rats and non-thyrocalcitonin treated controls. Fewer osteoclasts were reported for the former than for the latter. The fact that the osteoclasts were decreased was consistent with other evidences that thyrocalcitonin inhibited bone resorption.

Bell and Stern (1970) demonstrated that parathyroid extract-induced hypocalcemia could be reduced by administration of thyrocalcitonin. Parathyroid extract and thyrocalcitonin were administered subcutaneously to

thyroparathyroidectomized (TPTX) and to intact control rats. Serum was

obtained at 0, 1, 3, and 5 hours after administration of thyrocalcitonin.

Serum calcium in TPTX rats decreased from the initial value of 6.6 to 5.4

mg per 100 ml within 3 hours. Thereafter no further decrease was observed.

The serum calcium level remained at a value lower than that of the intact

thyrocalcitonin treated rats. Conversely, in control rats serum calcium

was reduced from 9.7 to 8.2 mg per 100 ml one hour after thyrocalcitonin

injection, but five hours after thyrocalcitonin injection, the concentra-

tion of serum calcium had returned to 9.3 mg per 100 ml. In TPTX rats,

the prolonged decrease in serum calcium might have been due to the absence

of parathyroid gland.

When calcium deficient diets were fed to intact and TPTX rats, admin-

istration of thyrocalcitonin had no effect on values for serum calcium for

either group, although the values differed widely, i.e., 8.8 mg % and 4.6

mg % in intact and TPTX rats, respectively (Bell and Stern, 1970). Thus,

the serum calcium lowering effect of thyrocalcitonin depended upon serum

calcium values at the time thyrocalcitonin was administered. When the

serum calcium was low due to low calcium intakes, the thyrocalcitonin

effect was generally less than when the serum calcium was normal.

Nguyen and Jowsey (1970) infused either parathyroid extract or thyro-

calcitonin into an artery in the leg of an anesthetized dog. Serum calcium

was measured in samples taken at 30-minute intervals by means of a catheter

inserted into an artery and vein. The difference in serum calcium values

between arterial and venous blood was expressed as a percentage of the

value in arterial blood. With the administration of parathyroid extract,

hypercalcemia correlated significantly (r = 0.86) with the increased number

of osteoclasts observed histochemically in the shaft of the right meta-

tarsal. On the other hand, administration of thyrocalcitonin increased

bone mineralization significantly as indicated by an increased number of

osteoblasts and a decreased serum calcium value. These investigators con-

cluded that parathyroid extract enhanced bone resorption whereas thyrocal-

citonin inhibited it.

Dietary calcium and phosphorus

Bell et al. (1941) fed weanling rats diets varying in calcium from

0.075 to 1.390%. After 8 weeks, the dry weight and calcium content of

their femurs had increased as dietary calcium increased from 0.075 to

0.365%. Additional amounts of dietary calcium, however, did not produce

additional increases in dry weight or calcium content of the femurs. Bell

et al. (1941) postulated that calcium uptake by the femur had an upper

limit beyond which increased calcium intake did not increase femur mineral-

ization.

Toothill and Hosking (1968) demonstrated that the fresh weight and ash

content of long bones (humerus, radius, ulna, femur, tibia, and fibula ana-

lyzed collectively) were significantly greater in rats fed a diet contain-

ing 0.744% calcium than in rats fed a diet containing 0.131% calcium; how-

ever, they did not investigate intermediate intakes of calcium.

Increased calcium content of the bone with increased calcium intakes

has been reported also in other species. Schryver et al. (1970) fed ponies

a diet containing 1.5%, 0.8%, or 0.15% calcium. A balance study was con-

ducted before and within 24 hours as well as 10 days after an intravenous

injection of radiocalcium ([47]calcium). The negative calcium balance of

ponies was -0.75 g calcium per 100 kg body weight per day when they were

fed a diet containing 0.15% calcium. However, their calcium balance was

+2.4 g and +5.6 g calcium per 100 kg body weight per day when dietary cal-

cium was 0.8% and 1.5%, respectively. On diets containing 0.8 and 1.5%

calcium, ponies had excreted 25% of the injected [47]calcium dose in the

urine within 24 hours and 35% of the dose after 10 days. On the other

hand, the same ponies excreted 2.5% and 3.8% within 24 hours and 10 days

after the injected dose, respectively, when they had been fed diets con-

taining 0.15% calcium. In the same experiment, calcium kinetics based on

the data obtained indicated that calcium deposition in the bone did not

vary with intakes of calcium (13.9, 9.1, and 13.5 g calcium per 100 kg body

weight per day deposited), but calcium withdrawal from the bone was signif-

icantly decreased from 14.7, 5.0, and 4.2 g calcium per 100 kg body weight

per day as dietary calcium increased from 0.15, 0.8, to 1.5%. These inves-

tigators postulated that higher calcium intakes maintained calcium homeo-

stasis by suppressing resorption. Their data could be interpreted to sup-

port an earlier hypothesis that higher calcium intakes suppressed parathy-

roid activity and, consequently, bone resorption (Sherwood et al,, 1966).

Protein Interaction with Calcium and Bone Composition

Shenolikar and Rao (1968) studied the effect of protein restruction on

calcium balance and on composition of the femur. One group of weanling

rats was fed ad libitum a diet containing 5% protein and 0.5% calcium

(group 1). A second group of rats consumed a diet containing 20% protein

and 0.5% calcium in amounts equal to that eaten by rats in group 1. A

third group of rats was fed the same diet as the second group but

restricted to amounts that permitted the rats to grow at the same rate as those in group 1.

Rats in group 1 excreted (fecal plus urine calcium) more calcium (16.8 vs 15.0 mg calcium per day) and retained less (11.3 vs 13.1 mg calcium per day) than their pair-fed controls although both groups had the same intake of calcium. The third group fed on a weight-gain basis with group 1 consumed slightly less calcium (24.0 vs 28.1 mg per day), excreted less calcium (9.7 vs 15.0 mg per day), but retained more calcium (14.3 vs 13.1 mg per day) than group 1. The femurs of rats in group 1 were lighter (353 mg) than the femurs of pair-fed controls (478 mg, group 2) and of rats in group 3 (381 mg). The percentage ash in femurs of group 1 was less than that in femurs of group 2 (45.4 vs 47.4%). Femurs of rats in group 3, however, contained no more ash than the femurs of rats in group 1 (44.8 vs 45.4%).

Shenolikar and Rao (1968) also examined the effect of restriction in the energy value of food consumed on the same parameters discussed in the preceding paragraph. Restriction was achieved by feeding a diet containing 40% protein and 1.0% calcium in exactly half the amounts eaten by rats receiving the 20% protein diet. Calcium retention, femur weight, and ash content were lower in restricted rats than in the pair-fed controls. The data from Shenolikar and Rao's experiments indicated that either a decrease in protein intake or energy value of the food intake lowered calcium absorption (calcium intake minus fecal calcium) and decreased femur weight and ash content.

The effect of protein on calcium absorption was also studied by McCance et al. (1942) with human subjects consuming either 55 or 165 gm

protein daily. The calcium intake was relatively constant, varying between 600 to 700 mg per day. Increasing the protein intake from 55 to 165 g per day increased calcium absorption (calcium intake minus fecal calcium) from 32 to 94 mg and urinary calcium excretion from 72 to 107 mg per day. In another experiment, calcium absorption increased from 43 to 149 mg daily when subjects consumed 1 to 1.5 lb bread which was fortified with 0.1 g calcium per 100 g bread. Based on these two experiments, these investigators suggested that the diet must provide both protein and calcium to increase calcium absorption.

Frandsen et al. (1954) studied skeletal growth in rats fed diets containing 24%, 6%, 3%, or 0% protein. Additional groups received 24% protein in amounts equal to those ingested by the rats fed 6%, 3%, or 0% protein. The length of tibias was measured from roentgenographs. The length of the tibia was shorter by 12.8 mm (37.1 vs 24.3 mm) in rats fed 0% protein than in those fed 24%. However, the length was shorter by 8.1 mm (37.1 vs 29.0 mm) when protein was reduced from 24% to 6%. Whereas the length was only 2.5 mm or 2.2 mm shorter (29.0 vs 26.5 mm or 26.5 vs 24.3 mm) when dietary protein was reduced from 6% to 3% or 3% to 0%, respectively. Thus, smaller differences in length were observed between rats fed the lower protein intakes. The length of tibia of rats fed a diet containing 24% protein in amounts equal to the amounts eaten by 6, 3, or 0% protein were longer than the tibias of rats eating 6, 3, or 0% protein diets and shorter than the tibia of rats fed 24% protein diets ad libitum. The data indicated that the restriction on food intake also influenced length of tibia but not as much as did restriction in protein.

Since protein and calcium are both involved in bone growth and ossification, El-Maraghi and his co-workers (1964, 1965) have done a series of experiments to investigate the interaction of protein and calcium on bone growth. El-Maraghi and Stewart (1964) fed adult rats a diet containing either 6.0 or 1.6 NDpCal% and 0.44 or 0.11% calcium in different combinations. After 20 weeks, the bones of the animals fed the higher protein diets were denser (based on X-ray photographs) than those fed lower protein diets irrespective of their calcium content. Increasing calcium in the diet from 0.11 to 0.44% when the diet contained 1.6 NDpCal% increased the ash by 62 mg per cm^3, i.e., from 472 to 534 mg per cm^3. The same increase in calcium when the protein content of the diet was 6.0 NDpCal% increased the ash by only 18 mg per cm^3, i.e., from 615 to 633 mg per cm^3. On the other hand, with 0.44% calcium diets, reducing protein from 6.0 to 1.6 NDpCal% decreased the femur ash content by 99 mg per cm^3, i.e., 633 to 534 mg per cm^3, while a similar decrease in protein when dietary calcium was 0.11% reduced the ash content by 143 mg per cm^3, i.e., from 615 to 472 mg per cm^3. This group of investigators suggested that protein and calcium were both required for maintenance of bone tissue in adult rats.

El-Maraghi et al. (1965) then designed an experiment for growing animals and fed them diets containing 10.2, 6,0, 5.1, and 4.5 NDpCal% and 0.44, 0.22, or 0.11% calcium in all possible combinations. After 11 weeks, femurs of rats fed 4.5 NDpCal% contained approximately the same amount of ash 93, 91, or 87 mg whether the calcium content of the diet was 0.44, 0.22, or 0.11%. The amount of femur ash was 93 mg for rats fed 4.5 NDpCal% compared with 220 mg when they were fed 10.2 NDpCal% when dietary calcium was 0.44%. A similar increase in protein with the lower dietary calcium

of 0.11% also resulted in a larger amount of femur ash, i.e., 87 vs 108 mg. The change in femur ash was less in response to an increase in dietary protein when the dietary calcium was low (0.11%) than when the dietary calcium was high (0.44%). Nevertheless the findings indicated that changes in both protein and calcium intake had influenced femur mineralization.

When femur ash was expressed as mg per cm^3 at the two higher dietary calcium concentrations (0.44 and 0.22%), reducing dietary protein from 10.2 to 4.5 NDpCal% reduced ash values from 516 to 336 mg per cm^3 and from 419 to 360 mg per cm^3, respectively. On the other hand, when dietary calcium was 0.11%, a similar reduction in protein increased ash values from 282 to 328 mg per cm^3. Thus, according to El-Maraghi et al. (1965), the higher protein intakes with lower calcium levels or lower protein intakes with higher calcium levels resulted in matrix or mineral osteoporosis.

In summary, a greater load of amino acids in the liver caused by large ingestion of food within a short period of time has increased urinary urea nitrogen excretion (Cohn et al., 1963). With equal intakes of calcium, ingestion of calcium in a single large dose was absorbed less efficiently compared with the same amount given in small portions (Kales and Phang, 1971). Diets containing adequate protein with inadequate calcium or adequate calcium with inadequate protein led to osteoporosis (El-Maraghi et al., 1965).

EXPERIMENTAL PROCEDURE

This study investigated the utilization of protein and calcium for bone growth in weanling rats fed for 2 hours twice daily or for 24 hours ad libitum. The rats were fed diets containing two levels of prótein and two or three levels of calcium in different combinations.

The study was conducted in two parts. The first experiment was designed to determine (a) food intake of meal-fed rats relative to that of rats fed ad libitum, (b) the weight gain of rats fed 8 or 4% lactalbumin protein when 0.4 and 0.1% calcium were included in diets, (c) food consumption of meal-fed rats when one meal was offered in the dark (laboratory lights off) and when both meals were consumed in the light (laboratory lights on), (d) percent of ingested nitrogen and calcium retained as estimated by nitrogen and calcium balances for 5 days, (e) the effect of meal or ad libitum feeding on the fresh weight, length, volume, and dry and ash weight of femurs when diets containing 8 and 4% lactalbumin were fed with 0.4 and 0.1% calcium.

The second experiment investigated the effect of eating frequency and variations in dietary protein and calcium on the utilization of protein and calcium for bone growth when intakes of protein and calcium were equal in meal-fed and ad libitum fed rats. Protein utilization was estimated by measuring the retention of nitrogen in the liver and in the carcass. The liver and carcass were also analyzed for total fat content because meal feeding had increased the accumulation of liver and body fat according to Cohn and Joseph (1959). The activity of hepatic arginase was determined because an increase in excretion of urea nitrogen had been reported in

force-fed rats (Cohn et al., 1963), and the force-feeding regimen resembled a meal eating pattern. The change from nitrogen balance in Experiment I to analysis of liver and carcass nitrogen in Experiment II was made to achieve an estimate of nitrogen utilization throughout the experiment rather than for a short period.

Selection of Animals and Feeding Schedules

Experiment I

Wistar strain male weanling rats weighing 45-55 g each were shipped by air from Simonsen-Thorpe Laboratories.[1] On their arrival at the nutrition laboratory in the evenings (after 6:00 p.m.), they were offered stock diet (Table 24) and distilled water ad libitum until the following morning. Rats were received within 2 weeks in 3 shipments, 4 litters of 5 male rats per shipment. A restricted randomization was used to distribute rats into 8 groups (Table 25). One litter mate was autopsied on day 1 of the experiment and provided zero-time contral values for bone composition. To minimize weight loss and to train rats to eat food immediately after it was offered, food was offered to the first 2 shipments of animals four times daily (8:00 to 10:00 a.m., 2:00 to 3:00 p.m., 4:00 to 6:00 p.m., and 8:00 to 10:00 p.m.) for 2 days. During the following 3 days, the fourth meal was eliminated. From the 6th day onwards, a two-hour, twice-daily (8:00 to 10:00 p.m. and 4:00 to 6:00 p.m.) pattern was adopted. Since the average food intake from 2:00 to 3:00 p.m. and from 4:00 to 6:00 p.m. was always less than 1 gm, the 3rd shipment of rats was fed three times a day (8:00 to

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[1]Simonsen-Thorpe Laboratories, Inc., 5228 Centerville Rd., St. Paul, Minnesota.

10:00 a.m., 2:00 to 3:00 p.m., and 8:00 to 10:00 p.m.) for the first 5

days. Beginning on the 6th day, the rats in the third shipment were fed

twice daily (8:00 to 10:00 a.m. and 6:00 to 8:00 p.m.).

To determine the effect of darkness on food intake and to shorten the

overnight fasting period, the evening meal for all rats was given from

6:00 to 8:00 p.m. instead of 4:00 to 6:00 p.m. This change occurred on the

14th day of the experiment for the first shipment of rats and on the 7th

day for the second shipment. The data given below indicated a trend

towards a greater daily food intake when rats were fed from 6:00 to 8:00

p.m. than when they were fed from 4:00 to 6:00 p.m.

Mean daily food intake, 4 days before and 4 days
after changing time for the evening meal

Food intake

Dietary treatment	When evening meal was given from 4:00 to 6:00 p.m.		When evening meal was given from 6:00 to 8:00 p.m.	
	g/day	g/100 g body wt.	g/day	g/100 g body wt.
8% protein, 0.4% calcium	6.4 (2)[1]	10.0	7.0	9.4
8% protein, 0.1% calcium	8.4 (2)	13.1	9.6	12.9
4% protein, 0.4% calcium	5.7 (2)	10.1	6.8	10.3
4% protein, 0.1% calcium	4.6 (2)	9.8	5.5	11.1

Rats were weighed twice a day at 8:00 a.m. and 1:00 p.m. to determine

the effect of the long overnight fast and the morning meal on the body

weights of the meal-fed rats. Meal-fed rats weighed 3 to 5 g more at

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[1]Number of rats.

1:00 p.m. than at 8:00 a.m. No difference in weights was observed in rats fed ad libitum.

Experiment II

Male weanling rats were obtained as described in Experiment I except that 23 litters of 6 each were ordered. The rats were obtained in 4 shipments, 3 shipments of 6 litters and 1 shipment of 5 litters. They arrived in the morning (about 8:00 a.m.) and were offered stock diet and distilled water ad libitum for 24 hours. A restricted randomization was used to distribute rats (Table 26) into 10 groups according to the design below.

	Protein %			
Calcium %	8	12	4	6
	A[1]	M[2]	A	M
0.2	12[3]	-	11	-
0.3	-	11	-	11
0.1	12	-	11	-
0.15	-	11	-	12
0.05			12	-
0.075			-	12

One male from each litter was autopsied on the first day of the experiment to obtain zero-time control values for liver, carcass, and bone composition. Food was offered three times a day for the first 3 days (7:30 to 9:30 a.m., 1:30 to 2:30 p.m., and 7:30 to 8:30 p.m.) and then twice daily for the next 25 days until the end of the experiment (7:30 to 9:30 a.m. and

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[1]Ad libitum.

[2]Meal-fed.

[3]Number of rats.

5:30 to 7:30 p.m.). Food intake for all rats was recorded every other day. Rats were weighed every evening between 4:30 and 5:30 p.m. before the second meal was offered to the meal-fed rats.

General Care of the Animals

Animals were housed individually in wire mesh cages. The laboratory was maintained at $24\pm1^\circ C$ and at approximately 40% relative humidity. The lighting in the laboratory was regulated automatically. Laboratory lights were off from 6:00 p.m. to 7:30 a.m. (Experiment I) and 5:30 p.m. to 6:30 a.m. (Experiment II).

For the first 5 days, food was provided in ceramic cups; later glass jars were used. A ceramic cup or jar containing food and a paper towel were weighed together. Paper towels were placed under each cage to hold spilled food and fecal material. The paper towel and food jars were changed every other day. Fecal material was carefully separated from the food particles and discarded. Paper towels were placed in appropriate food jars and air dried overnight. Food jars containing leftover food and dried paper towels were weighed together. The food intake was the difference between the two weights.

Experimental Diets

Table 1 presents the composition of each diet for Experiments I and II. In Experiment I, whether the rats were fed ad libitum or twice a day, they gained less weight when their diet contained 4% protein and 0.4% calcium than when it contained 4% protein and 0.1% calcium. When diets contained 0.4 or 0.1% calcium, rats fed ad libitum gained 30.3 g and 40.5 g while those fed twice daily gained 14.5 and 24.5 g. Because the diets con-

Table 1. Composition of experimental diets

Diet	Lactalbumin[a] (g)	Non-nutritive fiber[b] (g)	Corn oil[c] (g)	Mineral mix[d] (g)	Calcium carbonate[e] (g)	Booster salt mix[d] (g)
Experiment I						
8% protein, 0.4% calcium	10.2	2.0	5.0	1.2	0.10	4.4
8% protein, 0.1% calcium	10.2	2.0	5.0	1.2	0.10	0.7
4% protein, 0.4% calcium	5.1	2.0	5.0	1.2	0.10	4.4
4% protein, 0.1% calcium	5.1	2.0	5.0	1.2	0.10	0.7
Experiment II						
Ad libitum						
8% protein, 0.2% calcium	9.8	2.0	5.0	1.2	0.10	2.0
8% protein, 0.1% calcium	9.8	2.0	5.0	1.2	0.10	0.7
4% protein, 0.2% calcium	4.9	2.0	5.0	1.2	0.10	2.0
4% protein, 0.1% calcium	4.9	2.0	5.0	1.2	0.10	0.7
4% protein, 0.05% calcium	4.9	2.0	5.0	1.2	0.10	0.1
Meal-fed						
12% protein, 0.3% calcium	14.7	1.5	5.5	1.9	0.15	2.9
12% protein, 0.15% calcium	14.7	1.5	5.5	1.9	0.15	1.1
6% protein, 0.3% calcium	7.4	1.5	5.5	1.9	0.15	2.9
6% protein, 0.15% calcium	7.4	1.5	5.5	1.9	0.15	1.1
6% protein, 0.075% calcium	7.4	2.5	5.5	1.9	0.15	0.2

[a] Nutritional Biochemical Corporation, Cleveland, Ohio.

[b] General Biochemicals, Inc., Chargin Falls, Ohio.

[c] Mazola, Best Foods Division, Corn Products Company, New York, New York.

[d] See Table 3.

[e] J. T. Baker Chemical Company, Phillipsburg, New Jersey

Zinc carbonate[e] mg	Corn starch g	Calculated mg N/g diet	Analyzed mg N/g diet	Calcium calculated %	Calcium analyzed %	Phosphorus %	Protein:calcium ratio g pro./g Ca	Ca:p g Ca/g p	Energy value k cal/g diet
Experiment I									
4.1	77.0	13.02	13.27	0.40	0.39	1.20	20	0.34	3.9
-	80.7	13.02	13.23	0.10	0.13	0.31	80	0.32	4.1
4.1	82.1	6.51	6.73	0.40	0.39	1.20	10	0.34	3.9
-	85.8	6.51	6.71	0.10	0.09	0.31	40	0.32	4.1
1.4	79.9	12.51	12.53	0.20	0.19	0.60	40	0.33	4.0
-	81.1	12.51	12.50	0.10	0.10	0.31	80	0.32	4.1
1.4	84.8	6.25	6.59	0.20	0.17	0.60	20	0.33	4.0
-	86.0	6.25	6.58	0.10	0.09	0.31	40	0.32	4.1
-	86.6	6.25	6.75	0.05	0.05	0.16	80	0.31	4.1
Experiment II									
2.0	73.3	18.77	19.09	0.30	0.29	0.90	40	0.33	4.0
-	75.2	18.77	19.11	0.15	0.16	0.46	80	0.32	4.1
2.0	80.7	9.45	9.66	0.30	0.29	0.90	20	0.33	4.0
-	82.5	9.45	9.85	0.15	0.14	0.46	40	0.32	4.1
-	82.4	9.45	9.70	0.75	0.80	0.24	80	0.31	4.1

taining 0.4% calcium had had an adverse effect on weight gain, dietary cal-cium was reduced to 0.2% in the second experiment.

In Experiment II, the diets were to be formulated so that ad libitum and meal-fed rats would have similar intakes of protein and calcium. Hence the daily food intake of each group in Experiment I was calculated for sev-eral different periods of time (Table 2). These periods were:

1. The full experimental period, 29 days.

2. A period of 24 days. The 5-day metabolic period from the 25th through the 29th day was omitted from the full experimental period. This period, therefore, included the first 24 days.

3. A period of 15 days. The first 9 days were omitted because the rats were being adapted to meal feeding, i.e., 2 hours twice daily by reducing the numbers of meals gradually from 4 to 2.

4. A period of 10 days. In order to get comparable data from differ-ent shipments of rats, the days 9 through 14 were omitted.

   The time for the evening meal was changed from 4:00 to 6:00 p.m. to 6:00 to 8:00 p.m. on the 14th day for the rats in the first shipment; therefore, the data for all shipments were excluded for 14 days.

On examining the food intake in the different periods of time when diets contained 8 or 4% protein and 0.1% calcium, the meal-fed rats con-sumed a mean 68 or 70% as much food as ad libitum fed rats. To formulate diets for meal-fed rats, factors of 1.46 and 1.43 were obtained by defining the food intake of ad libitum fed rats as 100% and dividing by 68 and 70%, respectively. To facilitate weighing, a factor of 1.5 was used. Thus, the amounts of lactalbumin, mineral mix, calcium carbonate, and booster salt

Table 2. Food intake of rats in Experiment I

Periods (days)	8% protein, 0.4% calcium			8% protein 0.1% calcium			4% protein, 0.4% calcium			4% protein, 0.1% calcium		
	Ad lib-itum	Meal-fed	% intake of ad lib-itum	Ad lib-itum	Meal-fed	% intake of ad lib-itum	Ad lib-itum	Meal-fed	% intake of ad lib-itum	Ad lib-itum	Meal-fed	% intake of ad lib-itum
29 Full experiment	12.0 (10.9- 13.0)	7.8 (5.9- 10.2)	65	11.7 (10.2- 12.9)	8.2 (7.1- 9.9)	70	9.2 (8.5- 9.9)	6.4 (5.6- 9.5)	69	9.6 (8.2- 11.4)	7.0 (5.6- 9.5)	73
24 First through 24th	11.1 (10.3- 11.7)	6.8 (5.6- 7.9)	61	11.4 (10.4- 12.6)	7.7 (6.8- 9.3)	68	8.7 (7.6- 9.9)	5.6 (4.7- 6.2)	64	9.3 (8.2- 10.8)	6.5 (4.9- 8.4)	70
15 10th through 24th	12.6 (11.7- 13.9)	7.5 (5.9- 9.1)	60	12.5 (11.5- 13.6)	8.6 (7.4- 10.6)	69	9.5 (8.7- 10.6)	6.2 (5.3- 9.2)	65	10.1 (8.6- 11.7)	7.0 (5.1- 9.2)	70
10 15th through 24th	14.6 (12.2- 15.4)	8.4 (7.2- 10.4)	58	14.2 (12.2- 15.7)	9.5 (8.1- 12.1)	67	10.2 (8.1- 11.7)	6.5 (5.6- 9.3)	64	10.8 (9.0- 12.5)	7.5 (5.1- 9.5)	69

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mix were increased by a factor of 1.5 in those diets which were to be fed as meals. The increase in lactalbumin and minerals was made mainly at the expense of corn starch.

The lactalbumin was not analyzed for nitrogen before it was used to prepare diets for Experiment I. However, the lot number of the batch was the same as the batch of lactalbumin used by a co-worker in this laboratory; so the value of 79 g protein per 100 g lactalbumin (N x 6.25 analyzed by a co-worker) was the basis on which diets were formulated. Later the lactalbumin was analyzed for nitrogen and the amounts incorporated into diets for Experiment II were calculated from the value of 127.7 mg N per g lactalbumin. Protein equalled nitrogen times 6.38.

All diets were planned to be isocaloric. As a result, corn oil was increased from 5% in diets for rats fed ad libitum to 5.5% in the diets to be fed as meals. All diets were prepared in amounts sufficient for an entire experiment and stored in plastic containers at $4^{\circ}C$.

Jones-Foster salt mix (Jones and Foster, 1942) was modified (Table 3) to supply all the minerals required for growth (National Academy of Sciences-National Research Council, 1962) except calcium. A booster salt was made and added in amounts that maintained the calcium:phosphorus ratio of all the diets between 0.31 to 0.33, a ratio that has been observed commonly in Indian diets.

Table 3. Composition of mineral mix and booster salt mix

Mineral Mix		gm
Potassium phosphate monobasic[a]	KH_2PO_4	15.0000
Sodium chloride[a]	$NaCl$	6.0000
Ferrous sulfate[a]	$FeSO_4 \cdot 7H_2O$	2.6960
Magnesium sulfate[a]	$MgSO_4$	5.7302
Potassium iodide[b]	KI	0.0790
Zinc chloride[a]	$ZnCl_2$	0.0259
Zinc carbonate[a]	$ZnCO_3$	0.0140
Manganous sulfate monohydrate[c]	$MnSO_4 \cdot H_2O$	0.4453
Cupric sulfate[d]	$CuSO_4 \cdot 5H_2O$	0.0475
Cobalt chloride[a]	$CoCl_2 \cdot 6H_2O$	0.0002
Corn starch		0.9619
	Total	31.0000

Booster Salt Mix		gm
Calcium phosphate monobasic[a]	$Ca(H_2PO_4)_2 \cdot H_2O$	21.0
Potassium phosphate monobasic[a]	KH_2PO_4	10.0
Sodium phosphate monobasic[a]	$NaH_2PO_4 \cdot H_2O$	10.0
	Total	41.0

elements g/g booster salt mix

Ca	0.0814
P	0.2365
K	0.0700
Na	0.0407

[a] J. T. Baker Chemical Co., Phillipsburg, New Jersey.

[b] Allied Chemical, General Chemical Division, Morristown, New Jersey.

[c] General Chemical Division, Allied Chemicals and Dye Corporation, New York, New York.

[d] Mallinckrodt Chemical Works, St. Louis, Missouri.

All vitamins (Table 4) except cod liver oil[1] and DL-alpha-tocopherol[2] were thoroughly mixed with sufficient dextrin so that 500 mg of the mixture furnished the daily recommended allowances for growth (National Academy of Sciences-National Research Council, 1962). The vitamin mixture was stored in a dark bottle at 4°C. One gram of DL-alpha-tocopherol was diluted with 99 grams of oil of undetermined origin.[3] Two drops (50 mg) of DL-alpha-tocopherol in the oil mixture provided 0.75 mcg of DL-alpha-tocopherol. Vitamin A and D were provided by adding 50 mg (two drops) of cod liver oil to the vitamin mixture. The cod liver oil supplied 42.5 units of vitamin A and 4.25 units of vitamin D.

Analysis of Diets

Nitrogen

Diets were analyzed for nitrogen by a modification of the macro-Kjeldahl method (Bradstreet, 1965; Fleck and Munro, 1965). Approximately 1 to 2 g samples of diets containing 8% protein or 2 to 3 g samples of diets containing 4% protein were digested with 25 ml concentrated sulfuric acid (c.p., low N value), 15 g potassium sulfate, and 0.7 g mercuric oxide. After digestion, 75 milliliters of a saturated solution of sodium hydroxide were used to release ammonia. Excess mercuric oxide was reduced by adding 0.2 g of powdered zinc during distillation to prevent the formation of mercury-ammonium complexes. The ammonia was collected in 75 ml of a 4% boric

[1] Squibb and Sons, New York, New York, 850 USP units vitamin A, 84 USP units vitamin D per gm.

[2] General Biochemicals, Inc., Chagrin Falls, Ohio.

[3] Wesson Sales Co., Fullerton, California.

Table 4. Composition of water soluble vitamin mixture

Vitamin[a]	Allowance per rat per day	Per 1000 doses
Thiamine HCL	40 mcg	40 mg
Riboflavin	60 mcg	60 mg
Pyridoxine HCL	40 mcg	40 mg
Calcium-pantothenate	100 mcg	100 mg
Nicotinic acid	500 mcg	500 mg
Folic acid	8 mcg	8 mg[b]
Biotin	1 mcg	100 mg[c]
Vitamin B_{12}	0.75 mcg	750 mg
Ascorbic acid	1 mg	1 gm
Choline chloride	5 mg	5 gm
Inositol	10 mg	10 gm
P-aminobenzoic acid	10 mg	10 gm
Dextrin		to make 500 gm

[a]General Biochemicals, Inc., Chagrin Falls, Ohio.

[b]Biotin dextrin mixture (100 mg mixture contained 1 mg biotin).

[c]Vitamin B_{12} in mannitol furnished 0.1 mg of vitamin B_{12} per 100 mg of mixture.

acid solution containing mixed indicator (methyl red-methylene blue) and was titrated with standardized 0.1N HCl (Sobel et al., 1937).

Calcium

Triplicate 4 to 5 g samples of diets were weighed into acid boiled (10% HCl) crucibles and dried in a muffle furnace at 50-75°C overnight. The temperature was then raised by 50°C every 1½ hour until it reached 300°C. Thereafter it was raised by 50°C every hour until it reached 540°C. The samples were held overnight at this temperature. Despite the drying of samples and the initial slow elevation of temperature, all ash samples

klinked. Five drops of 70% nitric acid[1] was added to the klinked ash and stirred with a thin glass rod. After the ash was dissolved, the glass rod was rinsed with ½ ml of deionized water and the crucibles were heated on a hot plate at 100° to 150°C to evaporate the solvent. To re-ash, the temperature of the muffle furnace was held at 150°C for 2 hours then raised to 540°C and held overnight.

The ash was dissolved by adding 8 drops of concentrated hydrochloric acid and about 10 ml of hot deionized water to the crucibles and heating them on a hot plate at 150° to 200°C. The solutions were boiled gently for 8 to 10 minutes then quantitatively transferred to clean volumetric flasks. Appropriate dilutions were made so that 4 ml aliquots contained about 0.4 mg calcium.

Calcium was determined by the method of Kramer and Tisdall (1921) as modified by Tisdall (1923) and Clark and Collip (1925). Four milliliters of each ash solution were measured into a centrifuge tube. The pH was adjusted to 4.8-5.2 with 2% ammonium hydroxide[2] and 0.025 N nitric acid using bromcresol purple as the pH indicator. One milliliter of a saturated solution of ammonium oxalate[2] was added to precipitate the calcium. The tubes were allowed to stand for one hour. Then they were centrifuged at 2000 rpm for 15 minutes. The supernatant was decanted carefully and the tubes inverted on Whatman No. 40 filter paper for 5 minutes. After the

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[1]Bakers Analyzed Reagent. J. T. Baker Chemical Co., Phillipsburg, New Jersey.

[2]J. T. Baker Chemical Co., Phillipsburg, New Jersey.

precipitate was washed with 4 ml of 2% ammonium hydroxide, the process of centrifuging and discarding the supernatant was repeated.

The precipitate was dissolved in 2 ml of warm 1 N sulfuric acid and titrated with 0.01 N potassium permanganate[1] at 70°C.

Autopsy Procedure

Experiment I

On the morning of the 30th day, rats were weighed, their food jars removed, and food intakes recorded. The rats eating ad libitum were offered jars of fresh food, and meal-fed rats were given their morning meal. After weighing the rats at 12:00 noon, they were stunned with a blow on the head. Lengthwise and crosswise incisions were made in the abdominal and thoracic regions. The portal vein was cut to exsanguinate the liver, which was excised quickly, freed from non-liver tissue, blotted on damp filter paper, placed on a watch glass, and weighed. Each liver was wrapped in aluminum foil, labeled, frozen rapidly in liquid nitrogen, and stored at -20°C.

The right femur was removed with the help of a dissecting knife blade,[1] cleared of adhering connective tissue, and weighed immediately to the nearest 0.1 mg using a Mettler type H6 Digital balance. The length of the bone was measured with vernier calipers[2] and the volume determined by water displacement in a 5 ml graduate cylinder. The bones were wrapped in aluminum foil, labeled, and stored at -20°C.

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[1] Fisher Scientific Co., Chicago, Illinois.

[2] Curtin Scientific Co., 2218 University Ave., S. E., Minneapolis, Minnesota.

Experiment II

On the morning of the 29th day of the experiment, the food jars were removed and weighed as in Experiment I. All rats, i.e., meal-fed and those fed ad libitum, were fasted for 3 hours before autopsy. The autopsy board was covered with paper towels and waxed paper (Waxtex). Whatman No. 40 filter paper was used to absorb blood when hemorrhaging occurred. The liver was removed, as in Experiment I, and blotted on the same filter paper which was used to absorb the blood.

To prepare a sample of liver for determination of arginase activity, one gram of liver was sampled (a small portion from each lobe) and homogenized immediately in ice cold distilled water. The homogenate was diluted to 20 ml with distilled water (Schimke, 1962) and transferred to pre-chilled tubes. The homogenate was held in an ice bath for 60 to 80 minutes until analyzed.

The gastrointestinal tract (GI) was removed from the cardiac end of the stomach to the anus and blotted on filter paper. Mesenteric fat was trimmed and added to the carcass. The carcass was weighed and stored, along with the blood-soaked filter paper, in a pre-labeled ½-lb Mason jar at -20°C. The GI tract and its contents were weighed and discarded.

The right femurs were removed within 2 to 3 days following the autopsy, and their weights, lengths, widths, and volumes were measured as in Experiment I.

Analytical Procedures

Experiment I

Collection of samples for determination of nitrogen and calcium balance   On the morning of the 25th day, three rats from each group of 6 were transferred to metabolic cages for 5 days. Meal-fed rats whose food intake for 6 days averaged 60% or above of the intake of rats fed ad libitum were selected for metabolic balance study. The metabolic cages were equipped with stainless steel funnels fitted with a piece of wire mesh to separate fecal material. Urine trickled into a pharmacy bottle containing 4 ml toluene and 4 ml 0.1 N hydrochloric acid.[1] Fecal material was removed every morning at about 7:45 a.m.

Adhering food particles were brushed off, and the fecal matter was stored in acid washed (10% HCl) sample bottles in a refrigerator (4°C). At the end of the metabolic period, the cage, mesh piece, and funnel were washed with distilled water and the volume made to 200 ml. Since food particles were observed in the urine as well as in the cage washings, both were filtered through Whatman No. 40 filter paper and mixed thoroughly. The filter papers were air dried and then oven dried at 80°C for 6-8 hours. The average weight of a filter paper was subtracted from the total weight to estimate the amount of food recovered from urine and cage washings. The amount of food recovered varied from 8.0 to 12.4 g and was subtracted from the food intake recorded during the 5-day metabolic study to correct for spilling. The diluted urine samples were refrigerated until analyzed for

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[1] Bakers Analyzed Reagent. J. T. Baker Chemical Co., Phillipsburg, New Jersey.

nitrogen and calcium. Fecal material was dried at 80°C for 12 hours and ground to a powder in a pyrex mortar and pestle. The powder was analyzed for nitrogen and calcium.

Fecal and urine nitrogen

Nitrogen was estimated in 0.2 to 0.4 g powdered fecal material and in 15 to 20 ml diluted urine by the macro-Kjeldahl method described under Analysis of Diets.

Fecal and urine calcium

Approximately 0.5 g powdered fecal material was ashed in a muffle furnace at 600°C for 12 to 14 hours. The ash was dissolved in 4 to 6 drops of concentrated hydrochloric acid and the volume made to 100 ml with distilled water. Aliquots of 4 ml were used for calcium analysis.

Twenty-five milliliters of diluted urine were measured into test tubes. To decolorize the diluted urine, approximately 0.4 g decolorizing carbon[1] was added to each aliquot, and the mixture was allowed to stand for 15 to 20 minutes. Then it was filtered through Whatman No. 42 filter paper. Four milliliters of the filtrate were used for each calcium analysis.

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[1]Norit A, pharmaceutical grade. Fisher Scientific Co., Pittsburgh, Penna. USA.

Experiment II

Hepatic nitrogen   Each frozen liver was thawed and homogenized with

15 ml distilled water in a Tri-R-Teflon tissue homogenizer[1] tube. The

homogenate was diluted to 50 ml with distilled water when the liver weighed

less than 5 g and to 100 ml when it weighed more than 5 g. Ten milliliters

of diluted homogenates were estimated to contain between 10 and 30 mg

nitrogen.

Duplicate aliquots were analyzed for hepatic nitrogen by the modifica-

tion of the macro-Kjeldahl method described under Analysis of Diets.

Carcass nitrogen

The carcass homogenate was prepared by the method of Michelsen and

Anderson (1959) with some modifications. The thawed carcass was autoclaved

at 15 lb pressure, $120^{\circ}C$ for 45 minutes. The time of autoclaving was

increased from 15 minutes (Michelsen and Anderson, 1959) to 45 minutes to

soften the carcass enough so that it could be homogenized in a Waring

blender. The autoclaved carcass was cooled in a refrigerator ($4^{\circ}C$) over-

night. This treatment reduced the unpleasant odor that was observed when

blending took place without refrigeration. The refrigerated carcass was

transferred from a Mason jar to a pre-weighed Waring blender container, and

distilled water equivalent to the carcass weight was added. The blender

motor was operated first at low speed for 5 minutes and then at high speed

for an additional 5 minutes. The lid and sides of the blender container

were washed with a minimum amount of distilled water; then the container and

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[1] Physicians and Hospital Supply Co., Scientific Laboratory Division,
1400 Harmon Place, Minneapolis 3, Minnesota.

homogenate were weighed together. The homogenate was poured into a Mason jar, sampled for nitrogen and fat determinations immediately, and then stored at $-20^{\circ}C$.

Sampling of homogenate for nitrogen determination

The homogenate was mixed by inverting the jar 15 times between samplings. Triplicate aliquots of 1 to 2 g each were weighed in a tared beaker. Weighed homogenates were transferred quantitatively into Kjeldahl flasks. Nitrogen was determined by a modification of the macro-Kjeldahl method described under Analysis of Diets. Carcass weight was divided by the weight of the homogenate to calculate the weight of carcass per gram homogenate.

Hepatic fat

Fat was extracted by the method of Soderhjelm and Soderhjelm (1949). To 10 ml of homogenate in a Mojonnier flask, 12 ml of 95% ethyl alcohol, 20 ml of anhydrous ether, and 25 ml of hexane were added. The extraction was repeated with 5 ml of 95% ethyl alcohol, 15 ml of anhydrous ether, and 15 ml of hexane. The upper layer containing the extracted fat was decanted into a pre-weighed beaker. The fat solvents were evaporated on a steam bath and the residue dried in an oven for 15½ hours at $80^{\circ}C$. The beakers were cooled in a desiccator for 4 hours and weighed.

Sampling of homogenate for carcass fat determination

Triplicate aliquots of homogenate weighing approximately 4 to 5 g each were transferred quantitatively to Mojonnier flasks using a small amount of distilled water plus 95% ethyl alcohol. The use of alcohol insured the complete transfer of fat. Carcass fat was extracted by the same procedure described for hepatic fat.

Hepatic arginase (L-arginine amidinohydrolase (E.C. 3.5-3.1)

Arginase activity was measured by the procedure of Hagan and Dallam (1968). The enzyme activity in 0.1 ml homogenate was based on the hydrolysis of L-arginine to urea and ornithine. The urea produced was reacted with p-dimethylaminobenzaldehyde[1] (Ehrlich reagent) in dilute sulfuric acid to form N(p-dimethylamine)benzalurea. The concentration of N(p-dimethylamine)benzalurea was determined spectrophotometrically at 450 mμ.

The reagents were prepared in amounts sufficient for all the assays (see Table 27 for description of the reagents).

Assay procedure   Tubes containing 0.9 ml maleate buffer or 0.9 ml L-arginine solution were prepared a day prior to the autopsy and were kept frozen (arginine) or refrigerated (buffer). To measure the activity of hepatic arginase, 0.1 ml of homogenate was pipetted into tubes containing buffer. The tubes were placed in a water bath at 55°C for exactly 5 minutes for enzyme activation (Schimke, 1962) and then transferred to an ice bath. The ice cold solution was centrifuged immediately for 15 minutes at 4°C and the supernatant removed for enzyme assay.

Tubes containing L-arginine solution were incubated for 5 minutes at 37°C, then 0.1 ml supernatant was pipetted into them. They were incubated at 37°C for 10 minutes. Enzyme activity was stopped by adding 2.5 ml Ehrlich reagent which also provided the necessary chemicals for color development. After an hour at room temperature, absorbance was read in a

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[1] J. T. Baker Chemical Co., Phillipsburg, New Jersey.

DU Spectrophotometer[1] at 450 mμ against an Ehrlich reagent blank. Homogenate blanks were carried out with each assay. Asborbence values for the samples were compared with a calibration curve which was a plot of absorbence values for standard solution of urea expressed in μmoles per 0.1 ml.

Volume of femurs

Into a 5.00 ml graduated cylinder containing exactly 4.00 ml water, a femur was dropped with its distal end down. The rise in the meniscus of the water above 4.00 ml was recorded as the femur volume.

Drying and ashing of femurs

Femurs were dried to constant weight at 80°C in pre-dried, pre-weighted aluminum cups. Values were expressed to the nearest 0.1 mg. Dried femurs were ashed twice (18 hrs each time) in a muffle furnace at 600°C. The ashes were cooled in a desiccator and weighed to the nearest 0.1 mg.

Statistical Evaluation

The data were subjected to analysis of variance in which treatment variations were subdivided into those due to feeding patterns, protein intakes, calcium intakes, and those due to the interaction of these three factors. The analysis of variance is outlined below:

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[1] Beckman Instrument Inc., Fullerton, California.

Source	d.f.
Total reduction	31
Mean	1
Calcium	2
Protein	1
Feeding	1
Litters	22
Ca x Pro	1
Ca x F	2
Pro x F	1
Remainder	83

Adjusted means were computed and compared for significant differences using a t-test.

The model used was:

$$Y = U + L_i + T_i + e_{ij}$$

where

Y = true adjusted mean

U = the overall mean

L_i = deviation of particular litter mean from overall mean

T_i = deviation of particular treatment mean from overall mean

e_{ij} = the error term

All tests were reported as significant when $P < .05$ (Snedecor and Cochran, 1967). Data were handled by the Iowa State University Computation Center under the supervision of Dr. D. Hotchkiss of the Statistics Department.

RESULTS AND DISCUSSION

In Experiment I, 8 groups of weanling rats (6 each) were fed diets containing 8 or 4% protein and 0.4 or 0.1% calcium. These diets were given either ad libitum or for two hours twice daily. This study was planned, mainly, to determine food intake of meal-fed rats relative to those fed ad libitum. The effect of feeding frequency and variation in dietary protein and calcium on utilization of protein and calcium for bone growth was evaluated by measuring nitrogen and calcium balances and by determining weight, length, volume, dry weight, and ash weight of the right femur.

Experiments I and II had similar experimental plans. However, diets for Experiment II were formulated so that intakes of protein and calcium would be equal for ad libitum and meal-fed rats if food intake differed as it had in Experiment I. The diets for Experiment II were prepared with calcium to protein ratios of 20, 40, or 80 for diets containing 4% protein and 40 or 80 for diets containing 8% protein.

In Experiment II, rats were assigned randomly to 10 different treatment groups (11 or 12 rats per group). Protein utilization and feeding effects were determined by measuring nitrogen in the liver and carcass, fat in the liver and carcass, and activity of hepatic arginase.

For the sake of brevity and clarity in Experiment II, the total intake of food during 28 days and the feeding regimen were coded as follows: the diets with the lowest concentration of dietary calcium (0.05%) and protein (4%) provided an intake of 1Ca and 1P. Other dietary calcium and protein intakes were multiples of 1Ca and 1P. "A" and "M" denoted ad libitum and meal-feeding, respectively. For example, the ad libitum group fed 8% pro-

tein and 0.2% calcium diet for 28 days had an intake of 2P4Ca. Data for

similar or related variables in both experiments will be discussed together.

Food Intake, Nitrogen Intake, and Weight Gain

Food intake

In Experiment I, meal-fed rats ate 65 to 73% as much food as rats fed
the same diets ad libitum for 29 days; consequently, the energy values of
their food intakes as well as their intakes of nitrogen were less than
those of ad libitum fed rats (Table 5). In Experiment II, the food intake
of meal-fed rats was 66 to 78% of the food intake of rats fed ad libitum
with the exception of 1P1CaM group which ate nearly as much food as their
ad libitum fed controls (Tables 6a, 6b).

The food intakes in the present experiments agreed fairly well with
those reported by Leveille (1972) who fed young growing rats either ad
libitum or only once a day for 2 hours. He reported that food intakes of
meal-fed rats were 75 to 80% of the amounts consumed by nibblers.

When dietary protein was decreased from 2P to 1P, the energy value of
the food eaten by meal-fed rats decreased significantly from 918 to 749
Kcal in 29 days in Experiment I and from 844 to 651 in 28 days in Experi-
ment II. Similarly, the energy value of the food eaten by ad libitum fed
rats was reduced from 1362 to 1082 Kcal in Experiment I and from 1224 to
803 in Experiment II (Tables 5 and 6a) when dietary protein was reduced.

Energy value of food intake (Kcal 29 days)

Dietary groups	"M"	"A"
Experiment I		
8% protein (0.4 and 0.1% calcium)	918	1362
4% protein (0.4 and 0.1% calcium)	749	1082

Table 5. Means for food, nitrogen, and energy needed to produce each gram of weight gained in Experiment I

Dietary treatments	No. of rats	Food consumption g	Energy[a] value of intake Kcal	Weight gain g	FER	Nitrogen[ab] intake mg	NER	Kcal consumed /g wt. gain
8% protein, 0.4% calcium								
Ad libitum	6	339.7	1323 (1189-[c] 1453)	106.3 (92.0-[c] 107.0)	0.313 (0.287-[c] 0.333)	4494 (4068-[c] 4929)	23.7 (21.7-[c] 25.2)	12
Meal-fed	6	219.8	858 (684- 1067)	60.0 (42.0- 74.0)	0.273 (0.239- 0.327)	2908 (2320- 3620)	20.6 (18.1- 24.7)	14
8% protein, 0.1% calcium								
Ad libitum	6	341.7	1401 (1310- 1562)	101.8 (97.0- 111.0)	0.298 (0.289- 0.304)	4534 (4239- 5056)	22.4 (21.2- 22.9)	14
Meal-fed	6	238.5	978 (844- (1173)	72.0 (59.0- 95.0)	0.302 (0.285- 0.332)	3165 (2732- 3795)	22.8 (21.5- 25.0)	14
4% protein, 0.4% calcium								
Ad libitum	6	256.5	1017 (852- 1151)	30.3 (21.0- 41.0)	0.118 (0.087- 0.139)	1721 (1466- 1980)	17.6 (13.0- 20.7)	34
Meal-fed	6	176.0	686 (556- 954)	14.8 (9.0- 24.0)	0.084 (0.063- 0.103)	1181 (956- 1641)	12.5 (9.4- 15.4)	46

[a]See Table 1 for energy and nitrogen value of the diets.

[b]Calculations based on analyzed values.

[c]Range.

Table 5.   (Continued)

Dietary treatments	No. of rats	Food consump-tion g	Energy value of intake Kcal	Weight gain g	FER	Nitrogen intake mg	NER	Kcal consumed /g wt. gain
4% protein, 0.1% calcium								
Ad libitum	6	279.6	1146	40.5	0.145	1884	21.5	28
			(1011-1366)	(31.0-57.0)	(0.119-0.171)	(1659-2059)	(17.7-25.4)	
Meal-fed	6	198.1	812	24.8	0.125	1332	18.6	33
			(581-1028)	(20.0-30.0	(0.099-0.145)	(954-1688)	(14.7-21.6)	

Table 6a. Adjusted means for food, nitrogen, and energy needed to produce each gram of weight gained in Experiment II

Groups	No. of rats	Food consumption g	Energy[a] value of intake Kcal	% intake of ad libitum	Body weight gain g	Kcal/ g gain	FER	Nitrogen[ab] intake mg	NER
2P4CaA	12	290.8 ±10.19[c]	1175 ±42[c]		77.7 ±4.2[c]	15	0.267 ±0.013[c]	3619 ±144[c]	21.7 ±0.96[c]
2P4CaM	11	194.3 ± 0.70	779 ±44	66	73.6 ±4.5	11	0.371 ±0.014	3681 ±151	19.9 ±1.01
2P2CaA	12	311.3 ±10.19	1273 ±42		88.4 ±4.2	14	0.284 ±0.013	3833 ±144	22.6 ±0.96
2P2CaM	11	222.6 ±10.71	910 ±44	71	88.2 ±4.5	10	0.368 ±0.014	4280 ±151	19.2 ±1.01
1P4CaA	10	194.6 ±11.30	786 ±46		16.9 ±4.7	46	0.091 ±0.014	1232 ±159	14.4 ±1.07
1P4CaM	11	144.2 ±10.70	578 ±44	74	18.3 ±4.5	32	0.126 ±0.014	1387 ±151	13.2 ±1.01
1P2CaA	11	212.2 ±10.70	868 ±44		23.1 ±4.5	38	0.110 ±0.014	1371 ±151	17.1 ±1.01
1P2CaM	12	165.3 ±10.20	676 ±42	78	28.6 ±4.3	24	0.164 ±0.013	1623 ±144	16.8 ±0.96
1P1CaA	12	183.4 ±10.20	754 ±42		14.6 ±4.2	51	0.080 ±0.013	1247 ±144	11.3 ±0.96
1P1CaM	12	171.3 ±10.20	700 ±42	93	31.9 ±4.3	22	0.175 ±0.013	1715 ±144	17.7 ±0.96

[a]See Table 1 for energy and nitrogen values of the diets in Experiment II.

[b]Calculations based on analyzed values.

[c]Standard error.

Table 6b. Probability of significance of variables in Table 6a

Comparisons	Food consump-tion	Energy value of intake[a]	Body weight gain[a]	FER[a]	Nitrogen intake[a]	NER[a]
Feeding effect						
Ad libitum vs						
meal-fed						
2P4Ca	<.001	<.001	ns[b]	<.001	ns[b]	ns[b]
2P2Ca	<.001	<.001	ns	<.001	<.05	<.025
1P4Ca	<.001	<.001	ns	ns[b]	ns	ns
1P2Ca	<.005	<.005	ns	<.005	ns	ns
1P1Ca	ns[b]	ns[b]	<.001	<.001	<.025	<.001
Protein effect						
2P4CaA vs 1P4CaA	<.001	<.001	<.001	<.001	<.001	<.001
2P4CaM vs 1P4CaM	<.005	<.005	<.001	<.001	<.001	<.001
2P2CaA vs 1P2CaA	<.001	<.001	<.001	<.001	<.001	<.001
2P2CaM vs 1P2CaM	<.005	<.005	<.001	<.001	<.001	ns
Calcium effect						
2P4CaA vs 2P2CaA	ns	ns	ns	ns	ns	ns
2P4CaM vs 2P2CaM	ns	ns	ns	ns	<.05	ns
1P4CaA vs 1P2CaA	ns	ns	ns	ns	ns	ns
1P4CaM vs 1P2CaM	ns	ns	ns	<.05	ns	<.01
1P2CaA vs 1P1CaA	ns	ns	ns	ns	ns	<.005
1P2CaM vs 1P1CaM	ns	ns	ns	ns	ns	ns
1P4CaA vs 1P1CaA	ns	ns	ns	ns	ns	<.025
1P4CaM vs 1P1CaM	ns	ns	<.05	<.01	ns	<.001

[a]See Table 39 for least squares analysis of variance.

[b]Non-significant.

Experiment II	(Kcal/28 days)	
2P (4Ca and 2Ca)	844	1224
1P (4Ca and 2Ca and 1Ca)	651	803

Crews et al. (1969) also had observed that over a 3-month period the energy value of the food consumed by rats fed 22% protein was 7638 Kcal while that of rats fed 8% protein was only 6037 Kcal. Earlier Meyer (1958) had suggested that rats fed low protein diets consumed less food because they grew

more slowly and because they had limited capacity to store or dissipate energy. He also suggested that the amount of energy needed to retain one gram of nitrogen was so large that it limited the food intake of rats fed low protein diets.

Food intake was not affected by the protein:calcium ratios of the diets (Tables 7a, 7b). Whether the protein:calcium ratio was 40 or 80, the food intake was significantly higher with 2P than with 1P intakes. These data supported the hypothesis that dietary protein concentration rather than the protein:calcium ratio was related to the food intake when the ratio was 40 or 80. With the intakes of 1P, the food intake did not differ whether diets contained protein:calcium ratio 20 or 40. However, in Experiment I, when diets contained 4% protein, rats ate 10% less when dietary protein:calcium ratio was 10 than when the ratio was 40. Therefore, protein:calcium ratio 10 also appeared to limit the food intake.

All rats with 1P intakes, whether ad libitum or meal-fed, grew more slowly than those with intakes of 2P. The slower growth rate, in turn, may have limited their food intakes. Hegsted and Haffenreffer (1949), who had noted a high positive correlation (r = 0.8) between food intake and basal metabolism, suggested that if rats were fed diets containing inadequate protein, they grew more slowly and had a lower basal metabolism.

Weight gain and FER

Meal-fed rats in Experiment II gained as much or more weight than rats fed ad libitum (Table 6a). One meal-fed group, 1P1CaM, gained more weight than its ad libitum control 1P1CaA, i.e., 31.9 vs 14.6 g. When partial correlation was calculated (Table 8), weight gain was positively correlated

Table 7a. Adjusted means for energy value of intake and nitrogen intake per g weight gain for rats fed different ratios of protein and calcium

Diets	No. of rats	Pro:Ca ratio	Energy value of intake Kcal	Nitrogen[a] intake mg	FER	NER
1P4Ca	21	20	682 $\pm 32^b$	1309 $\pm 111^b$	0.109 $\pm 0.010^b$	13.8 $\pm 0.78^b$
1P2Ca	23	40	771 ± 30	1498 ± 104	0.137 ± 0.009	16.9 ± 0.74
1P1Ca	24	80	772 ± 29	1480 ± 101	0.128 ± 0.009	14.5 ± 0.71
2P2Ca	23	40	976 ± 30	3651 ± 104	0.319 ± 0.009	20.7 ± 0.74
2P1Ca	23	80	1092 ± 30	4081 ± 104	0.326 ± 0.010	20.9 ± 0.74

[a] See footnote Table 6a.

[b] Standard error.

with both nitrogen intake (r = 0.95) and energy value of the food intake (r = 0.89); thus, the higher body weight of the 1P1CaM group compared with the 1P1CaA group might have been due to a higher nitrogen intake (1715 vs 1247 mg) together with approximately the same food energy intake (750 vs 700 Kcal).

In Experiment I, meal-fed rats ate less than those fed ad libitum, consequently, their nitrogen and food energy intakes were also less and they gained less weight. Meal-fed rats gained, on the average, 42.9 g while those fed ad libitum gained 69.7 g.

Table 7b. Probability of significance of variables in Table 7a

Comparisons	Energy value of intake	Nitrogen intake	FER	NER
(1P) Pro:Ca ratio 20 vs 40	ns[a]	ns[a]	ns[a]	<.01
20 vs 80	ns	ns	ns	ns[a]
40 vs 80	ns	ns	ns	ns
(2P vs 1P) 40 vs 40	<.001	<.001	<.001	<.001
80 vs 80	<.001	<.001	<.001	<.001

[a]Non-significant.

Weight gain in grams

Dietary treatments	"M"	"A"
8% protein, 0.4% calcium	60.0	106.3
8% protein, 0.1% calcium	72.0	101.8
4% protein, 0.4% calcium	14.8	30.3
4% protein, 0.1% calcium	24.8	40.5
Mean	42.9	69.7

Reducing the dietary protein from 8 to 4% also resulted in reduced food consumption and weight gain, i.e., on the average from 66.0 to 19.8 g for meal-fed and from 104.0 to 35.4 g for ad libitum fed rats as summarized on page 62.

Table 8. Partial correlation coefficients for the variables in Experiment II

	Weight gain	Energy intake	Nitrogen intake	FER	NER
Energy intake	.8935**				
Nitrogen intake	.9513**	.9287**			
FER	.8467**	.6835**	.7229**		
NER	.7471**	.6854**	.5979**	.9057**	
Hepatic nitrogen	-.1892	-.1798	-.1374	-.2905**	-.3893**
Hepatic fat	.2442**	.2510**	.2071*	.2267*	.3048**
Carcass nitrogen	-.0767	-.0633	-.0963	-.1538	-.1928*
Carcass fat	.5627**	.6274**	.4728**	.5097**	.6742**
Femur fresh weight	.7570**	.7501**	.7453**	.6379**	.5898**
Femur length	.7858**	.8026**	.7776**	.7060**	.6543**
Femur dry weight	.7250**	.7219**	.7098**	.6100**	.5427**
Femur ash weight	.7642**	.7592**	.7748**	.6432**	.5385**

**Significant at the 1% level.

*Significant at the 5% level.

| Hepatic nitrogen | Hepatic fat | Carcass nitrogen | Carcass fat | Right femur | | | |
				Fresh weight	Length	Dry weight	Ash weight
-.3199**							
-.3736**	-.1378						
-.3421**	.3239**	-.1811					
-.1431	.1726	.0464	.4557**				
-.0837	.1539	-.0689	.4679**	.8548**			
-.0487	-.0561	.1573	.4470**	.8930**	.8482**		
-.0834	.0664	.1207	.3865**	.8000**	.8208**	.9233**	

Weight gain in grams

Dietary treatments	"M"	"A"
8% protein (0.4 and 0.1% calcium)	66.0	104.0
4% protein (0.4 and 0.1% calcium)	19.8	35.4

In Experiment II, reducing dietary protein significantly decreased weight gain in ad libitum as well as in meal-fed rats (Figure 1); however, reduction in dietary calcium did not affect weight gain except for the group 1P1CaM (Table 6a). This group was only significantly heavier than 1P4CaM group (31.9 vs 18.3 g). Although its nitrogen intake and the energy value of its food intake were higher (700 vs 578 Kcal and 1715 vs 1387 mg), they were not significantly different.

Meal-fed rats consumed significantly less food and weighed approximately the same as those fed ad libitum when the protein intake was 2P. However, meal-fed rats gained 26.3 g while those fed ad libitum gained

Weight gain in grams

Deitary groups	"M"	"A"
1P4Ca	18.3	16.9
1P2Ca	28.6	23.1
1P1Ca	31.9	14.6
Mean	26.3	18.2

18.2 g when protein intake was 1P. These data indicated that meal-fed rats used food more efficiently than those fed ad libitum.

Food efficiency ratios (FER) were calculated by dividing cumulative weight gain (g) by cumulative food intake (g). With the exception of the 1P4CaM group, all meal-fed rats had significantly higher mean FER values than those fed ad libitum (Table 6b). The FER values are summarized below for Experiment II.
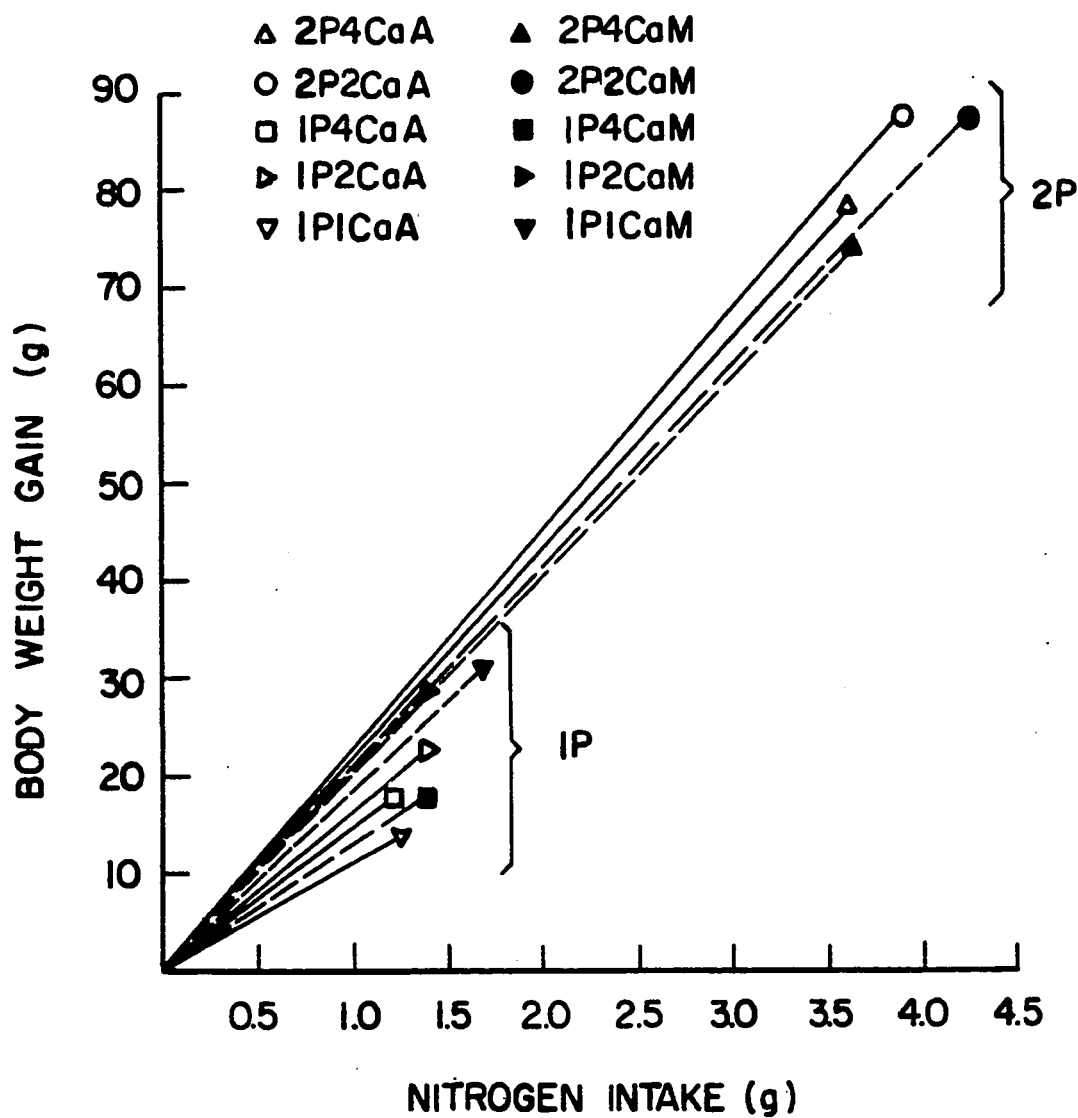
Figure 1. Influence of nitrogen intake on weight gain

FER values

Dietary group	"M"	"A"
2P4Ca	0.371	0.267
2P2Ca	0.368	0.284
1P4Ca	0.126	0.091
1P2Ca	0.164	0.110
1P1Ca	0.175	0.080
Mean (except 1P4Ca)	0.270	0.185

Excluding 1P4Ca, therefore, the mean FER value for ad libitum fed rats was two-thirds that of rats consuming similar amounts of protein but less available food energy (0.270 vs 0.185).

Reducing dietary protein from 2P to 1P resulted in a significantly lower FER for rats fed ad libitum than those fed 2 meals per day. The mean FER value were as follows:

FER values

Dietary groups	"M"	"A"
2P (4Ca and 2 Ca)	0.370	0.276
1P (4Ca and 2Ca and 1Ca)	0.155	0.094

On the other hand, calcium intakes did not affect FER values with the exception of those for meal-fed rats receiving diets providing the lower of the two protein intakes. The mean FER for the 1P4CaM group was 0.126, a value significantly smaller than 0.164 for the 1P2CaM group and 0.175 for 1P1CaM group. The FER for the 1P4CaM group probably was due to the low cumulative food and nitrogen intakes by rats in this group (144.2g food and 1387 mg N) compared with those by rats in the 1P2CaM and 1P1CaM groups (165.3 and 171.3 g food, 1623 and 1715 mg N).

The data from Experiment I, when nitrogen intakes of meal-fed rats were less than the nitrogen intakes of rats fed ad libitum, lend support to the hypothesis that lower nitrogen intakes resulted in lower FER values (Table 5). The meal-fed rats used food less efficiently than those fed diets of the same composition ad libitum with one exception, the rats consuming diets with 8% protein and 0.1% calcium. The FER values for Experiment I are summarized below:

FER values

Dietary treatments	"M"	"A"
8% protein, 0.4% calcium	0.273	0.313
8% protein, 0.1% calcium	0.302	0.298
4% protein, 0.4% calcium	0.084	0.118
4% protein, 0.1% calcium	0.125	0.145
Mean (except 8% protein, 0.1% calcium)	0.161	0.192

The energy value of the food consumed per unit of weight gained indicated that all meal-fed rats required less energy than those fed ad libitum (see Table 6a). The values for the amount of food energy used to add one gram of weight are summarized below by protein intakes:

Kcal needed per g weight gained

Dietary groups	"M"	"A"
2P (4Ca and 2Ca)	10.5	14.5
1P (4Ca and 2Ca and 1Ca)	26.7	45.0

With intakes of 2P or 1P, meal-fed rats required 10.5 or 26.7 Kcal per g weight gain compared with 14.5 or 45.0 Kcal required by rats fed ad libitum. In Experiment I, when intakes of nitrogen were not equal between meal-fed and ad libitum fed rats, meal-fed rats required 14 or 40 Kcal to produce

one gram of weight while ad libitum fed rats used 13 or 31 when dietary

protein was 8 or 4%, respectively.

<u>Kcal needed per g weight gained</u>

Dietary treatment	"M"	"A"
8% protein (0.4 and 0.1% calcium)	14.0	13.0
4% protein (0.4 and 0.1% calcium)	39.5	31.0

Rats with intakes of 1P required 2 to 3 times as much food energy to

produce the same amount of body weight as rats with 2P intakes, whether ad

libitum or meal-fed, therefore, weight gain was related to the amount of

protein consumed. These data do not agree with those of Crews et al.

(1969). They reported that rats utilized 52 Kcal per g weight gain when

they were fed a diet containing 8% protein while 24 Kcal per g body weight

gain were needed when dietary protein was reduced to 22%.

Protein Utilization

Nitrogen intake and NER[1]

In Experiment II, nitrogen intakes of meal-fed rats were similar to

those of rats fed ad libitum except for the group 2P2CaM and 1P1CaM (Tables

6a, 6b). The diets had been formulated so that intakes of nitrogen and

calcium would be the same for rats with 2 meals per day and those fed ad

libitum. Based on food intakes in Experiment I, it was assumed that the

meal-fed rats would eat two-thirds as much food as those fed ad libitum.

However, the actual food intake of the 2P2CaM group was slightly higher

than expected, i.e., 71% instead of 67%, while that of the 1P1CaM group

was 93% of that consumed by its ad libitum control group. The increased

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[1]Weight gained (g) divided by nitrogen intake (g).

nitrogen intake may have promoted increased growth which further stimulated food consumption as suggested by Hegsted and Haffenreffer (1949).

Food energy value of the intake was not affected by protein:calcium ratios of 20, 40, or 80 when 1P was eaten. With the intakes of 2P, however, the food energy value of the diets consumed by rats fed protein:calcium ratio of 80 was higher than those fed a protein:calcium ratio of 40 (1092 vs 976 Kcal, see Table 7a).

Feeding frequency did not influence NER values for groups 1P2Ca, 1P4Ca, and 2P4Ca but did affect the NER values for the groups 2P2Ca and 1P1Ca. For these latter two groups, nitrogen intakes of meal-fed rats were significantly different from those of the corresponding ad libitum fed groups. The NER values were 19.2 and 17.7 for the 2P2CaM and 1P1CaM groups compared with 22.6 and 11.3 for the 2P2CaA and 1P1CaA groups. Differences in NER between meal-fed and ad libitum fed groups might have been due to differences in nitrogen and food intakes. The group 2P2CaM ingested 10% more nitrogen (4280 vs 3833 mg), but the energy value of the food eaten was 28% less than that of the 2P2CaA group (910 vs 1273 Kcal). The 1P1CaM group consumed 38% more nitrogen (1715 vs 1247 mg) while the energy value of its food intake was only 7% less than that of the 1P1CaA group (700 vs 754 Kcal). Thus, the lower NER for 2P2CaM group appeared to be related to the fact that the food energy value of its intake was 28% less than its control; on the other hand, the higher NER for 1P1CaM group appeared to be related to the fact that the nitrogen intake was 38% higher than its control, and the energy value of food intake was similar.

In Experiment I, the nitrogen intakes of meal-fed and ad libitum fed rats were not equal; as a result, meal-fed rats had lower NER values than rats fed the same diets ad libitum except for the group fed a diet contain-

ing 8% protein and 0.1% calcium. The NER values for the treatment groups were as follows:

<u>NER values</u>

Dietary treatments	"M"	"A"
8% protein, 0.4% calcium	20.6	23.7
8% protein, 0.1% calcium	22.8	22.4
4% protein, 0.4% calcium	12.5	17.6
4% protein, 0.1% calcium	18.6	21.5
Mean (without 8% protein, 0.1% calcium)	17.2	20.9

Nitrogen concentrations of the liver and carcass are summarized in Tables 9a, 9b. Although values for hepatic nitrogen tended to be higher for meal-fed rats than for those fed ad libitum, the differences were significantly higher for 2P2CaM, 1P2CaM, and 1P1CaM groups but not for 2P4CaM and 1P4CaM groups. For rats in the 2P2CaM and 1P1CaM groups, nitrogen intakes were significantly greater than for their ad libitum fed controls; therefore, the hepatic nitrogen concentrations may have reflected these differences in intakes. Rats in the 1P2CaM group also consumed more nitrogen than the 1P2CaA group (1623 vs 1371 mg) although these differences were not statistically significant. The higher hepatic nitrogen value for the 1P2CaM group might also be due to more nitrogen intakes.

The livers of meal-fed rats were slightly heavier than those of rats fed ad libitum, consequently, total hepatic nitrogen averaged 116 mg when values for all meal-fed rats were combined compared with 97 mg for all rats fed ad libitum (Table 9a).

With the exception of the 1P1CaM group, all groups of meal-fed rats had significantly higher concentrations of carcass nitrogen than groups fed

Table 9a. Adjusted means for nitrogen content of livers and carcasses in Experiment II

Groups	Hepatic N mg/g wet tissue	Carcass N mg/g	Liver wt. % body wt.[a]	Total N liver + carcass g	% N liver + carcass	Carcass wt. g	Liver wt. g	Total hepatic N mg
2P4CaA	27.2[b] $\pm$0.66[b]	27.5[b] $\pm$0.46[b]	3.99[b] $\pm$0.14[b]	3.29[b] $\pm$0.14[b]	2.75[b] $\pm$0.04[b]	114.3[b] $\pm$4.20[b]	5.14[b] $\pm$0.26[b]	140
2P4CaM	28.2 $\pm$0.70	31.3 $\pm$0.48	4.52 $\pm$0.15	3.55 $\pm$0.14	3.11 $\pm$0.05	108.1 $\pm$4.41	5.74 $\pm$0.27	162
2P2CaA	26.1 $\pm$0.66	27.9 $\pm$0.46	4.48 $\pm$0.14	3.65 $\pm$0.14	2.78 $\pm$0.04	125.1 $\pm$4.20	6.42 $\pm$0.26	168
2P2CaM	28.1 $\pm$0.70	30.6 $\pm$0.48	4.92 $\pm$0.15	3.87 $\pm$0.14	3.04 $\pm$0.05	120.6 $\pm$4.41	7.07 $\pm$0.27	199
1P4CaA	23.8 $\pm$0.74	26.1 $\pm$0.51	3.79 $\pm$0.16	1.57 $\pm$0.15	2.60 $\pm$0.05	58.9 $\pm$4.66	2.46 $\pm$0.29	58
1P4CaM	25.1 $\pm$0.70	28.0 $\pm$0.48	3.54 $\pm$0.15	1.66 $\pm$0.14	2.79 $\pm$0.05	57.6 $\pm$4.41	2.41 $\pm$0.27	60
1P2CaA	23.0 $\pm$0.70	26.6 $\pm$0.48	3.99 $\pm$0.15	1.77 $\pm$0.14	2.65 $\pm$0.05	63.9 $\pm$4.41	2.86 $\pm$0.27	66
1P2CaM	25.6 $\pm$0.66	28.6 $\pm$0.46	3.82 $\pm$0.14	1.99 $\pm$0.14	2.85 $\pm$0.04	67.7 $\pm$4.21	2.98 $\pm$0.26	76
1P1CaA	20.5 $\pm$0.66	27.4 $\pm$0.46	4.34 $\pm$0.14	1.58 $\pm$0.14	2.70 $\pm$0.04	55.3 $\pm$4.20	2.69 $\pm$0.26	55
1P1CaM	24.3 $\pm$0.66	28.6 $\pm$0.46	4.16 $\pm$0.14	2.11 $\pm$0.14	2.84 $\pm$0.04	70.8 $\pm$4.20	3.46 $\pm$0.26	84
Zero time control	31.4 $\pm$0.62	25.6 $\pm$0.35	3.70 $\pm$0.07	1.06 $\pm$0.02	2.58 $\pm$0.33	39.1 $\pm$0.80	1.76 $\pm$0.04	55

[a] Body wt. taken at the time of autopsy.

[b] Standard error.

Table 9b. Probability of significance of variables in Table 9a

Comparisons	Hepatic nitrogen mg/g wet tissue[a]	Carcass nitrogen mg/g carcass	Liver % body weight[a]	Total nitrogen liver + carcass	% nitrogen liver + carcass	Carcass weight	Liver weight
Feeding effect							
ad libitum vs meal-fed							
2P4Ca	ns[b]	<.001	<.01	ns[b]	<.001	ns[b]	ns[b]
2P2Ca	<.05	<.001	<.025	ns	<.001	ns	ns
1P4Ca	ns	<.01	ns[b]	ns	<.01	ns	ns
1P2Ca	<.01	<.005	ns	ns	<.005	ns	ns
1P1Ca	<.001	ns[b]	ns	<.01	<.025	<.001	ns
Protein effect							
2P4CaA vs 1P4CaA	<.001	<.025	ns	<.001	<.025	<.001	<.001
2P4CaM vs 1P4CaM	<.005	<.001	<.001	<.001	<.001	<.001	<.001
2P2CaA vs 1P2CaA	<.005	<.05	<.025	<.001	<.05	<.001	<.001
2P2CaM vs 1P2CaM	<.01	<.005	<.001	<.001	<.005	<.001	<.001
Calcium effect							
2P4CaA vs 2P2CaA	ns	ns	<.025	ns	ns[b]	ns	ns
2P4CaM vs 2P2CaM	ns	ns	<.05	ns	ns	ns	ns
1P4CaA vs 1P2CaA	ns	ns	ns	ns	ns	ns	ns
1P4CaM vs 1P2CaM	ns	ns	ns	ns	ns	ns	ns
1P2CaA vs 1P1CaA	<.01	ns	ns	ns	ns	ns	ns
1P2CaM vs 1P1CaM	ns	ns	ns	ns	ns	ns	ns
1P4CaA vs 1P1CaA	<.001	ns	<.01	ns	ns	ns	ns
1P4CaM vs 1P1CaM	ns	ns	<.005	<.025	ns	ns	ns

[a]See Table 39 for least squares analysis of variance.

[b]Non-significant.

ad libitum. Reducing dietary protein by about 50% significantly reduced carcass nitrogen concentrations regardless of feeding frequency or calcium concentrations of the diets.

With 1P intakes, protein:calcium ratios of 20 and 40 led to significantly higher hepatic nitrogen concentrations (24.4 and 24.3 mg/g liver) than did a protein:calcium ratio of 80 (22.4 mg/g liver) (see Tables 10a, 10b). The protein:calcium ratios of 40 and 80 did not affect concentration of liver nitrogen differently when protein intake was 2P.

Table 9a also includes total nitrogen in liver plus carcass and percent nitrogen in liver plus carcass. Feeding frequency had no effect on total nitrogen in the liver plus the carcass except for the 1P1CaM group. The group 1P1CaM accumulated significantly more nitrogen (2.1 g/liver plus carcass) than did the 1P1CaA group (1.6 g/liver plus carcass).

At each level of dietary protein, liver plus carcass of meal-fed rats contained significantly greater concentrations of nitrogen than liver plus carcass of rats fed ad libitum. The greater accumulation of nitrogen by meal-fed rats compared with rats fed ad libitum differed from the findings reported by other investigators. Cohn and Joseph (1959) demonstrated that with identical food intakes, when diets contained 18.5% protein, the newly formed tissue (body weight of an experimental group minus body weight of a zero-time control group) of tube-fed rats contained lower concentrations of nitrogen than that of rats fed ad libitum (17.7 vs 22.4%). Likewise, Wardlaw et al. (1969) reported that when rats were fed equal amounts of a 26% protein diet ad libitum and by tube, the tube-fed rats had lower concentrations of carcass nitrogen than nibblers (17.8 vs 19.4%).

Table 10a. Adjusted means for nitrogen and fat in liver and carcass of
rats fed different ratios of protein and calcium

Diets	No. of rats	Pro:Ca ratio	Hepatic N mg/g wet tissue	Carcass N mg/g carcass	Hepatic fat conc. %	Carcass fat conc. %
1P4Ca	21	20	24.4 $\pm$0.51[a]	27.0 $\pm$0.35[a]	7.2 $\pm$0.66[a]	12.5 $\pm$0.57[a]
1P2Ca	23	40	24.3 $\pm$0.48	27.6 $\pm$0.33	7.9 $\pm$0.62	13.9 $\pm$0.54
1P1Ca	24	80	22.4 $\pm$0.32	27.9 $\pm$0.32	9.2 $\pm$0.60	12.8 $\pm$0.52
2P4Ca	23	40	27.7 $\pm$0.48	29.4 $\pm$0.33	4.5 $\pm$0.62	11.5 $\pm$0.54
2P2Ca	23	80	27.1 $\pm$0.48	29.2 $\pm$0.33	6.4 $\pm$0.62	11.0 $\pm$0.54

[a]Standard error.

The findings of the present experiment may have differed from those

reported by Cohn and Joseph (1959) and Wardlaw et al. (1969) because

(1) the source of dietary protein was lactalbumin instead of casein and

(2) the energy value of food consumed by meal-fed rats was less than that of

nibblers. Geiger (1951) has reported that lactalbumin disappeared from the

intestinal tract more slowly than casein. He fasted rats for 24 hours,

then fed them diets containing approximately 100 mg nitrogen from either

lactalbumin or casein. The nitrogen content of the gastrointestinal tract

was analyzed after 2, 4, and 6 hours. Lactalbumin was absorbed more slowly

than casein; after 2 hours, 31.8 and 56.6% of lactalbumin and casein had

been absorbed, respectively, after 4 hours, 66.7 and 81.3%, and after 6

hours, 83.8 and 99.0%. If amino acids were released from lactalbumin more

Table 10b. Probability of significance of variables in Table 10a

Comparisons	Hepatic N mg/g wet tissue	Carcass N mg/g carcass	Hepatic fat conc. %	Carcass fat conc. %
Pro:Ca ratio				
(1P)				
20 vs 40	ns[a]	ns[a]	ns[a]	ns[a]
20 vs 80	<.005	ns	<.05	ns
40 vs 80	<.05	ns	ns	ns
(2P vs 1P)				
40 vs 40	<.001	<.001	<.001	<.001
80 vs 80	<.001	<.001	<.001	<.05

[a]Non-significant.

slowly than from casein, then they would be provided in smaller amounts from the gut for protein synthesis. Moreover, because lactalbumin is a protein with higher biological value than casein, protein synthesis would be more efficient. These reasons may account for the fact that carcasses of meal-fed rats contained significantly higher concentration of nitrogen than carcasses of rats fed ad libitum.

The data on nitrogen balance (Experiment I) also supported the idea that when dietary protein was 8%, meal-feeding had no adverse effect on protein utilization (Table 11). When meal-fed rats were given a diet containing 8% protein, regardless of calcium concentrations, they retained as much nitrogen as was retained by rats fed the same diet ad libitum (69%).

Table 11. Nitrogen balance data for 5 days at the end of Experiment I

Dietary treatments	No. of rats	Nitrogen intake mg/5 days	Nitrogen in feces mg/5 days	% absorbed[a] of intake	Urinary excretion mg/5 days	Total excretion mg/5 days	Retained mg/5 days	Nitrogen retained % of intake
8% protein, 0.4% calcium								
Ad libitum	3	913.8 (807.0-[b] 1004.2)	80.3 (48.5-[b] 122.0)	91	228.7 (201.1-[b] 272.5)	309.0	604.8 (464.2-[b] 680.5)	66
Meal-fed	3	749.7 (625.8- 844.1)	75.4 (47.8- 95.3)	90	181.6 (100.0- 300.6)	257.0	492.7 (395.4- 604.6)	66
8% protein, 0.1% calcium								
Ad libitum	3	936.4 (895.7- 959.4)	68.6 (60.5- 77.3)	93	199.7 (125.7- 303.5)	268.3	668.1 (573.3- 765.7)	71
Meal-fed	3	779.0 (683.4- 844.0)	69.8 (51.4- 100.2)	91	157.8 (92.2- 235.2)	227.6	551.4 (348.0- 694.1)	71
4% protein, 0.4% calcium								
Ad libitum	3	335.9 (304.0- 383.1)	43.3 (26.6- 63.2)	87	96.1 (77.8- 124.4)	139.4	196.5 (186.1- 207.9)	58
Meal-fed	3	262.8 (204.7- 367.0)	42.5 (25.2- 57.7)	84	79.6 (41.6- 139.5)	122.1	140.7 (105.4- 182.9)	54

[a] $\dfrac{\text{Nitrogen intake minus fecal nitrogen}}{\text{Nitrogen intake}} \times 100.$

[b] Range.

Table 11. (Continued)

Dietary treatments	No. of rats	Nitrogen intake mg/5 days	Nitrogen in feces mg/5 days	% absorbed[a] of intake	Urinary excretion mg/5 days	Total excretion mg/5 days	Retained mg/5 days	Nitrogen retained % of intake
4% protein, 0.1% calcium								
Ad libitum	3	362.8 (332.5- 383.6)	54.3 (47.9- 60.3)	85	77.2 (52.5- 111.0)	131.5	231.3 (216.4- 259.6)	64
Meal-fed	3	290.1 (214.7- 350.0)	47.1 (40.9- 51.8)	84	83.1 (44.9- 150.0)	130.2	159.9 (119.5- 212.1)	55

When diets contained 4% protein, meal-fed rats retained slightly less nitrogen than did ad libitum fed rats (54 vs 61%).

The results of nitrogen balance differed from those reported by Cohn et al (1963, 1964). In 1963, these investigators fed rats a diet containing 18.5% protein either ad libitum or by tube. Excretion of urea nitrogen was 43% higher for tube-fed rats than for those fed ad libitum. Later Cohn et al. (1964) again demonstrated that rats fed ad libitum excreted less urea nitrogen (317 mg/3 days) than tube-fed rats (473 mg/3 days) when their food intakes were equal.

The discrepancy between the results reported by Cohn et al. and those of the present experiment might have been due (1) to the differences in the amounts of dietary protein provided and (2) to the lower energy value for the food intake of meal-fed rats compared with that of rats fed ad libitum. Cohn et al. fed their rats diets containing either 18.5% (in 1963) or 20% (in 1964) casein whereas the diets of the study reported herein contained 8 or 4% lactalbumin. Wu and Wu (1950) had observed that, with a liberal protein intake (1.14 gm/kg body weight), nitrogen was more efficiently utilized when human subjects ate 4 meals per day than when they ate 2 meals per day. When protein intake was reduced to 0.73 gm per kg body weight so that little or no surplus protein was provided, no differences in efficiency of nitrogen utilization were observed whether subjects ate 4 or 2 meals a day. Similarly, Shortridge and Linkswiler (1963) reported no differences in nitrogen retention by young women with low protein intakes (5.3 g N/subject/day) whether the food was eaten in 6 equal meals or in 3 equal meals.

In the experiments reported here, the dietary nitrogen was limited rather than generous, hence rats retained similar nitrogen under the feeding regimens imposed on them.

Hepatic arginase

In Experiment II, the activity of arginase was measured in the livers of 6 rats from each of 8 experimental groups. These groups were 2P4Ca, 2P2Ca, 1P4Ca, and 1P2Ca, both ad libitum and meal-fed. Since the activity of arginase was 5 to 6 times higher with the intakes of 2P than 1P, the data for the activity of arginase were analyzed for 2P and 1P intakes separately.

Tables 12a and 12b present the results of the enzyme assays as units of arginase per mg hepatic nitrogen and per liver. The activity of hepatic arginase did not differ among meal and ad libitum fed groups whether the protein intake was 2P or 1P. When Cohn et al. (1964) examined the effect of tube-feeding on the activity of hepatic arginase, they fed rats an equal amount of a diet containing 18.5% protein. The arginase activity as units per mg nitrogen was slightly higher (1.18 times) for tube-fed rats than for rats fed ad libitum. The variation in the findings of the study of Cohn et al. from that of the present experiment might be due to differences in the amount of dietary protein eaten and in food intake between meal-fed rats and those fed ad libitum.

The activity of arginase was significantly higher per unit of hepatic nitrogen and per unit of liver with intakes of 2P than with 1P intakes. The mean values of arginase activity, when feeding frequency and calcium concentrations in the diets were ignored, were 250 or 127 units per mg

Table 12a. Hepatic arginase in Experiment II

Groups	No. of animals	Arginase units[a]/ mg hepatic nitrogen	Arginase units[a]/ liver
2P4CaA	6	274 $\pm 36^b$	37300 $\pm 6700^b$
2P4CaM	6	248 ± 36	40500 ± 6700
2P2CaA	6	276 ± 36	44900 ± 6700
2P2CaM	6	212 ± 36	45500 ± 6700
1P4CaA	6	107 ± 25	6090 ± 2130
1P4CaM	6	88 ± 25	5220 ± 2130
1P2CaA	6	154 ± 25	10600 ± 2130
1P2CaM	6	160 ± 25	12200 ± 2130

[a]Units expressed as μmoles area formed in 10 minutes at 37°C.

[b]Standard error.

hepatic nitrogen and 42050 or 8528 units per liver for 2P and 1P intakes, respectively.

Schimke (1962) studied the effect of dietary protein on the activity of hepatic arginase. He fed rats diets containing 15, 30, or 60% protein for 14 days. The activity of arginase per g liver did not increase appreciably when dietary protein increased from 15 to 30% (38,300 vs 40,400 μmoles urea formed/hour), but a further increase in protein from 30 to 60% doubled the activity of hepatic arginase (40,400 to 84,500 μmoles urea formed/hour). Likewise, Mandelstam and Yudkin (1952) noted an increase in the activity of hepatic arginase from 12.0 to 13.9 and 19.0 units when

Table 12b. Probability of significance of variables in Table 12a

Comparisons	Arginase/ mg hepatic nitrogen	Arginase/ liver
Feeding effect		
Ad libitum vs		
meal-fed		
2P4Ca	ns[a]	ns[a]
2P2Ca	ns	ns
1P4Ca	ns	ns
1P2Ca	ns	ns
Protein effect		
2P4CaA vs 1P4CaA	<.001	<.001
2P4CaM vs 1P4CaM	<.001	<.001
2P2CaA vs 1P2CaA	<.001	<.001
2P2CaM vs 1P2CaM	<.001	<.001
Calcium effect		
2P4CaA vs 2P2CaA	ns	ns
2P4CaM vs 2P2CaM	ns	ns
1P4CaA vs 1P2CaA	ns	ns
1P4CaM vs 1P2CaM	<.05	<.05

[a]Non-significant.

dietary protein was increased from 17 to 33 and 67% (enzyme activity was expressed as mg urea produced in 30 minutes).

Decreasing dietary calcium did not affect the activity of arginase except for one group (Tables 12a, 12b). The 1P2CaM group had significantly higher arginase activity than 1P4CaM group (12,200 vs 5220 units). The nitrogen intake of 1P2CaM group was higher although not significantly different from 1P4CaM group (1623 vs 1367). The higher arginase activity for 1P2CaM group than 1P4CaM group may have been due to the higher nitrogen intake.

The variables related to protein utilization, i.e., nitrogen balance, nitrogen retained in the liver and carcass, and the activity of hepatic arginase, indicated that meal-fed rats utilized protein as efficiently as rats fed ad libitum.

Hepatic and carcass fat

Fat concentration in the livers of meal-fed rats consuming 2P4Ca, 1P2Ca, and 1P1Ca was significantly lower than that in the livers of rats consuming the same amounts of calcium and protein ad libitum (Tables 13a, 13b), while hepatic fat for 2P2CaM did not differ from 2P2CaA and 1P4CaM from 1P4CaA. Porta and Hartroft (1970) have reported that fatty livers did not develop if a protein deficiency was associated with a substantial caloric deficiency as in the case of marasmus. In the present experiment, the energy value of the food intakes was significantly less for meal-fed groups (except when the intake was 1P1Ca) than those fed ad libitum; therefore, the relative shortage of available food energy deficiency might have prevented fat deposition in livers of meal-fed rats.

Decreasing the dietary protein significantly increased hepatic fat concentration for the 1P4CaM group compared with 2P4CaM (7.3 vs 3.3%) and for the 1P2CaA group compared with the 2P2CaA group (9.8 vs 6.7%). These results were in harmony with those of Channon and Wilkinson (1935), who had reported a progressive increase in hepatic fat (5.60, 6.12, and 6.50%) with a decrease in dietary protein from 20 to 10 and 5%.

An increase in dietary calcium was associated with a decrease in the concentration of hepatic fat for only 3 out of 8 comparisons made in Experiment II (Table 13a). These comparisons were 2P2CaM versus 2P4CaM (6.1 vs

Table 13a. Adjusted means for fat in the liver and carcass of rats in
Experiment II

Groups	No. of rats	Hepatic fat conc. %	Carcass fat conc. %	Total fat in liver + carcass g	% fat in liver + carcass
2P4CaA	12	5.7 $\pm$0.85[a]	15.2 $\pm$0.74[a]	17.9 $\pm$1.03[a]	14.8 $\pm$0.72[a]
2P4CaM	11	3.3 $\pm$0.89	7.8 $\pm$0.78	8.7 $\pm$1.08	7.6 $\pm$0.76
2P2CaA	12	6.7 +0.85	14.2 $\pm$0.74	18.6 $\pm$1.03	13.8 $\pm$0.72
2P2CaM	11	6.1 (0.89)	7.9 $\pm$0.78	10.6 $\pm$1.08	7.8 $\pm$0.76
1P4CaA	10	7.1 $\pm$0.94	15.5 $\pm$0.82	9.6 $\pm$1.14	15.2 $\pm$0.80
1P4CaM	11	7.3 $\pm$0.89	9.6 +0.78	5.8 +1.08	9.5 $\pm$0.76
1P2CaA	11	9.8 $\pm$0.89	16.1 $\pm$0.78	10.5 $\pm$1.08	15.8 $\pm$0.76
1P2CaM	12	5.9 $\pm$0.85	11.8 +0.74	8.7 $\pm$1.03	11.5 $\pm$0.72
1P1CaA	12	11.9 $\pm$0.85	14.7 +0.74	8.6 $\pm$1.03	14.6 $\pm$0.72
1P1CaM	12	6.5 $\pm$0.85	11.0 $\pm$0.74	8.1 $\pm$1.03	10.8 $\pm$0.72
Zero-time control	23	4.3 $\pm$0.13	5.9 $\pm$0.35	2.4 $\pm$0.16	5.8 $\pm$0.33

[a]Standard error.

3.3%), 1P2CaA versus 1P4CaA (9.8 vs 7.1%), and 1P1CaA versus 1P4CaA (11.9
vs 7.1%).

The values for concentration of carcass fat, total fat per liver plus
carcass, and percentage fat in liver plus carcass were significantly lower
in meal-fed rats than in rats fed ad libitum (Tables 13a, 13b). In con-
trast to the results of the present experiment, more fat was accumulated in

Table 13b. Probability of significance of variables in Table 13a

Comparisons	Hepatic fat[a] %	Carcass fat[a] %	Total fat liver + carcass	% fat liver + carcass
Feeding effect				
Ad libitum vs				
meal-fed				
2P4Ca	<.05	<.001	<.001	<.001
2P2Ca	ns[b]	<.001	<.001	<.001
1P4Ca	ns	<.001	<.025	<.001
1P2Ca	<.005	<.001	ns[b]	<.001
1P1Ca	<.001	<.001	ns	<.001
Protein effect				
2P4CaA vs 1P4CaA	ns	ns[b]	<.001	ns[b]
2P4CaM vs 1P4CaM	<.005	ns	<.05	ns
2P2CaA vs 1P2CaA	<.01	ns	<.001	ns
2P2CaM vs 1P2CaM	ns	<.001	ns	<.001
Calcium effect				
2P4CaA vs 2P2CaA	ns	ns	ns	ns
2P4CaM vs 2P2CaM	<.05	ns	ns	ns
1P4CaA vs 1P2CaA	<.05	ns	ns	ns
1P4CaM vs 1P2CaM	ns	<.05	<.05	<.05
1P2CaA vs 1P1CaA	ns	ns	ns	ns
1P2CaM vs 1P1CaM	ns	ns	ns	ns
1P4CaA vs 1P1CaA	<.001	ns	ns	ns
1P4CaM vs 1P1CaM	ns	ns	ns	ns

[a]See Table 39 for least squares analysis of variance.

[b]Non-significant.

the liver and carcass by meal-fed rats than by rats fed ad libitum according to Cohn and Joseph (1959), Tepperman and Tepperman (1958), and Fabry et al. (1964). The discrepancy in results might be explained by:

(1) differences in feeding schedules;

In the present experiments, rats were fed for 2 hours twice daily, i.e., for a total of 4 hours, whereas Cohn et al. fed rats by stomach tube twice daily. Tepperman and Tepperman fed rats for one hour once a day, and

Fabry et al. allowed the rats to eat twice a day but for one hour each meal. Therefore, food ingestion by meal-fed rats would have taken place over a shorter period of time in the published reports than in the present experiments. With equal amounts of food intake, the faster rate of food ingestion resulted in increased conversion of carbohydrate into fat (Tepperman et al., 1943). Thus, the relatively slower rate of food ingestion by meal-fed rats in the present experiments might be a contributing factor in preventing fat deposition.

(2) source of protein;

Casein has been used as the protein source in most of the studies that investigated the effect of feeding frequency on protein utilization. In the present experiment, lactalbumin was used. The difference in absorption of amino acids between casein and lactalbumin has already been discussed under hepatic nitrogen (Geiger, 1951). Moreover, lactalbumin would have supplied more methionine than casein for lipotropic activity. According to White et al. (1968, p. 502), if choline or methyl groups for synthesis of S-adenosylmethionine were adequate, the liver could synthesize lecithin and fat would be transported from the liver normally.

(3) differences in dietary NDpCal%;

In order to achieve similar nitrogen and calcium intakes for meal-fed and ad libitum fed rats, the concentration of both protein and calcium was increased in diets of meal-fed rats at the expense of carbohydrate (Experiment II). Consequently, diets fed to meal-eaters had higher values for NDpCal% (11.73 and 5.86 NDpCal%) than the diets fed to ad libitum groups (7.82 and 3.91 NDpCal%). Yeh and Leveille (1969) reported an inverse relationship between NDpCal% and incorporation of labeled acetate into fatty

acids by liver slices of rats fed 18 or 12% protein (protein was replaced
by carbohydrate). In the present Experiment II, significantly lower con-
centrations of fat in livers of 3 out of 5 meal-fed groups (2P4Ca, 1P2Ca,
and 1P1Ca) had significantly lower concentrations of carcass fat in all
meal-fed groups may have been due to the higher NDpCal% in the diets fed as
meals. Another explanation for these results might be that meal-fed rats
consumed less food than those fed ad libitum, hence less surplus food
energy was available to be deposited as fat.

(4) physical activity of the meal-fed rats.

Although no record was kept regarding physical activity of the rats,
meal-fed rats were observed to be more aggressive and restless than rats fed
ad libitum. Nejjar and Heggeness (1969) reported that intermittently fed
rats (3 days ad libitum and 3 days restricted feeding) exercised signifi-
cantly more than did continuously fed rats. Thus, in the present Experiment
II, meal-fed rats must have been utilizing more of their available food
energy for physical activity because they were observed to be relatively
more active than rats fed ad libitum. Hence meal-fed rats may have had
less food energy for deposition of fat than their nibbling controls.

Total nitrogen and fat accumulation over the 28-day period was calcu-
lated by subtracting mean values of zero-time control group from the mean
values of experimental groups (Table 14). The percentage of energy
retained was computed assuming 9 Kcal/g fat and 4 Kcal/g protein. The car-
bohydrate content of the rats was disregarded. Energy retention calculated
from gains in fat and protein indicated that all rats fed 2P were equally
efficient in food utilization whether ad libitum or meal-fed. All rats fed
1P were also equally efficient. On the basis of FER (Table 6a), however,

Table 14. Energy and nitrogen retention based on carcass data for rats in Experiment II

Groups	No. of rats	Total N retained[a] g	Cumulative N intake g	% N retained of intake	Kcal retained as fat	Kcal retained as protein[b]	Total Kcal retained	Kcal intake	% Kcal retained
2P4CaA	12	2.23	3.62	62	139.2	55.7	194.9	1175	17
2P4CaM	11	2.49	3.68	68	57.1	62.2	119.3	779	15
2P2CaA	12	2.59	3.83	68	146.3	64.8	211.1	1273	17
2P2CaM	11	2.81	4.28	66	74.3	70.2	144.5	910	16
1P4CaA	10	0.51	1.23	41	65.4	12.8	78.2	786	10
1P4CaM	11	0.60	1.39	43	31.0	15.0	46.0	578	8
1P2CaA	11	0.71	1.37	52	73.4	17.8	91.2	868	10
1P2CaM	12	0.93	1.62	57	56.9	23.2	80.1	676	12
1P1CaA	12	0.52	1.25	42	55.7	13.0	68.7	754	9
1P1CaM	12	1.05	1.72	61	51.8	26.2	78.0	700	11

[a] Total N at the end of experiment minus zero-time control values.

[b] N x 6.25.

meal-fed rats except those in the 1P4Ca group, were more efficient in using food for weight gain than those fed ad libitum. These data indicated the importance of changes in body composition in evaluating the efficiency of food utilization for growth.

Calcium Utilization, Bone Composition, and Interaction of Protein with Calcium

Calcium balance (Experiment I)

Calcium balance data (Table 15) indicated that meal-fed rats retained as much calcium as did those fed ad libitum. These results differed with those reported by Kales and Phang (1971). These authors demonstrated that when adult human subjects consumed milk, providing 700 mg of calcium in 6 equal portions, the rate of calcium absorption increased by 20% from that when milk was given in a single portion. The discrepancy in results might be due to the differences in dietary calcium levels. Kales and Phang worked with adequate calcium intakes whereas in the present experiment, the dietary calcium levels 0.4 and 0.1% were less than that recommended for growth by the National Academy of Sciences-National Research Council (1962). However, on equal calcium intakes, Irwin and Feeley (1967) had reported no differences in calcium retention whether young women were allowed to eat 3 times or 6 times a day. In the present Experiment I, although meal-fed rats consumed 18 to 21% less calcium than their ad libitum fed controls, meal-fed rats retained 58 to 84% of their ingested calcium compared with 68 to 81% retained by those fed ad libitum.

When diets contained 8% protein, irrespective of dietary calcium levels, the calcium was apparently absorbed by meal-fed rats to a slightly greater extent (93%) than by those fed ad libitum (86%). When diets con-

Table 15. Calcium balance data for 5 days at the end of Experiment I

Dietary treatments	No. of rats	Calcium intake mg/5 days	Calcium in feces mg/5 days	% absorbed[a] of intake	Urinary excretion mg/5 days	Total excretion mg/5 days	Retained mg/5 days	Calcium retained % of intake
8% protein, 0.4% calcium								
Ad libitum	3	276.3 (244.0-[b] 303.6)	30.1 (10.8-[b] 65.2)	89	39.5 (11.0-[b] 77.2)	69.6	206.7 (185.8-[b] 218.6)	75
Meal-fed	3	226.7 (189.2- 255.2)	16.4 (2.2- 43.6)	95	23.7 (15.8- 35.9)	40.1	186.6 (167.5- 216.0)	83
8% protein, 0.1% calcium								
Ad libitum	3	70.6 (67.5- 72.3)	12.9 (2.9- 25.9)	82	9.3 (6.9- 12.0)	22.2	48.4 (32.6- 62.1)	68
Meal-fed	3	58.7 (51.5- 63.6)	5.4 (4.0- 6.5)	91	3.8 (0.8- 7.4)	9.2	49.5 (42.5- 56.2)	84
4% protein, 0.4% calcium								
Ad libitum	3	200.3 (181.2- 228.4)	13.9 (5.4- 29.8)	93	29.8 (12.5- 56.3)	43.7	156.6 (129.5- 209.5)	77
Meal-fed	3	156.7 (129.2- 218.8)	9.7 (4.5- 17.2)	94	16.4 (10.9- 21.6)	26.1	130.6 (95.5- 190.7)	82

[a] $\dfrac{\text{Calcium intake minus fecal calcium}}{\text{Calcium intake}} \times 100.$

[b] Range.

Tabl2 15.   (Continued)

Dietary treatments	No. of rats	Calcium intake mg/5 days	Calcium in feces mg/5 days	% absorbed of intake	Urinary excretion mg/5 days	Total excretion mg/5 days	Retained mg/5 days	Calcium retained % of intake
4% protein, 0.1% calcium								
Ad libitum	3	55.0 (49.4-58.5)	2.2 (1.4-3.2	96	8.3 (5.7-11.1)	10.5	44.5 (39.5-48.1)	81
M2al-fed	3	43.6 (31.9-52.0)	5.4 (2.4-11.2)	82	12.8 (5.6-24.1)	18.2	25.4 (5.3-35.9)	58

tained 4% protein, differences in calcium absorption between meal-fed and

ad libitum fed rats were not consistent.

Bone composition

Table 16 presents the composition of rat femurs in Experiment I.

Irrespective of dietary protein and calcium, when the femurs of all meal-

fed rats were averaged together, they were slightly lighter in weight than

those of all rats fed ad libitum (0.323 vs 0.363 g), were slightly shorter

in length (2.33 vs 2.47 cm), and were slightly lower in volume (0.28 vs

0.34 cm^3). The lower values for fresh weight, length, and volume of femurs

of meal-fed rats reflected the fact that they ate only 65 to 73% as much

food as rats fed ad libitum. However, these values for the femur were

reduced less than the food intake, i.e., the weight was only 10% less, the

length only 5%, and volume only 15% than their controls.

Fresh femur weights were influenced by concentration of dietary pro-

tein. Regardless of dietary calcium, reducing the protein content of the

diet from 8 to 4% reduced femur weights by 64% for rats fed ad libitum

(from 0.442 to 0.285 g) and by 70% for meal-fed rats (from 0.384 to 0.262).

Fresh femur weight in grams

Calcium treatment	"M"		"A"	
	8%	4%	8%	4%
0.4% calcium	0.383	0.258	0.460	0.283
0.1% calcium	0.384	0.267	0.423	0.282
Mean	0.384	0.262	0.442	0.285

Table 16. Weight, length, and volume for right femurs of rats in Experiment I

Dietary treatments	No. of rats	Fresh weight g	Length cm	Volume cm^3	Dry weight g	Ash weight g
8% protein, 0.4% calcium						
Ad libitum	6	0.460 [a] (0.365- 0.502)	2.70 [a] (2.65- 2.77)	0.44 [a] (0.40- 0.46)	0.229 [a] (0.202- 0.250)	0.112 [a] (0.090- 0.124)
Meal-fed	6	0.383 (0.357- 0.454)	2.48 (2.42- 2.63)	0.34 (0.31- 0.38)	0.185 (0.167- 0.224)	0.080 (0.068- 0.102)
8% protein, 0.1% calcium						
Ad libitum	6	0.423 (0.382- 0.488)	2.68 (2.62- 2.74)	0.42 (0.39- 0.47)	0.175 (0.162- 0.194)	0.063 (0.050- 0.075)
Meal-fed	6	0.384 (0.370- 0.419)	2.53 (2.46- 2.52)	0.33 (0.25- 0.38)	0.160 (0.146- 0.168)	0.056 (0.051- 0.061)
4% protein, 0.4% calcium						
Ad libitum	6	0.288 (0.272- 0.329)	2.25 (2.18- 2.30)	0.24 (0.20- 0.29)	0.146 (0.126- 0.171)	0.063 (0.052- 0.071)
Meal-fed	6	0.258 (0.235- 0.300)	2.14 (2.05- 2.26)	0.22 (0.19- 0.29)	0.133 (0.123- 0.161)	0.053 (0.046- 0.070)
4% protein, 0.1% calcium						
Ad libitum	6	0.282 (0.256- 0.334)	2.26 (2.18- 2.40)	0.24 (0.20- 0.30)	0.129 (0.110- 0.160)	0.045 (0.041- 0.048)
Meal-fed	6	0.267 (0.234- 0.328)	2.18 (2.04- 2.33)	0.24 (0.19- 0.30)	0.126 (0.104- 0.147)	0.044 (0.034- 0.054)
Mean value for all meal-fed rats	24	0.323	2.33	0.28	0.151	0.058
Mean value for all ad libitum fed rats	24	0.363	2.47	0.34	0.170	0.066
Zero-time control values	11	0.215 (0.183- 0.251)	1.71 (1.59- 1.80)	0.22 (0.15- 0.30)	0.073 (0.057- 0.083)	0.027 (0.022- 0.032)

[a] Range.

According to Table 16 disregarding dietary calcium, reducing dietary protein decreased the femur length from 2.69 to 2.26 cm for ad libitum fed rats and from 2.50 to 2.16 cm for meal-fed rats. At the same time, the

Femur length in centimeters

	"M"		"A"	
Calcium treatment	8%	4%	8%	4%
0.4% calcium	2.48	2.14	2.70	2.25
0.1% calcium	2.53	2.18	2.68	2.26
Mean	2.50	2.16	2.69	2.26

mean femur volume was reduced from 0.43 to 0.24 cm^3 for rats fed ad libitum and from 0.34 to 0.23 cm^3 in meal-fed rats. These data suggested that

Femur volume

	"M"		"A"	
Calcium treatment	8%	4%	8%	4%
0.4% calcium	0.34	0.22	0.44	0.24
0.1% calcium	0.33	0.24	0.42	0.24
Mean	0.34	0.23	0.43	0.24

dietary protein influenced femur weight, length, and volume more than food intake.

Femur weight, length, and volume did not change appreciably when dietary calcium was reduced from 0.4 to 0.1% whether rats were allowed to nibble food all day long or were restricted to two meals per day. Bachmann et al. (1940) demonstrated that reducing calcium content of a diet from 0.67 to 0.40% without changing the protein content did not affect the fresh weight of the femurs for weanling rats fed these diets for 70 days.

The dry weight and ash weight of femurs of meal-fed rats were less than those weights of femurs of rats fed ad libitum (Table 13a). The dry weight and ash weight of the femurs were positively correlated with energy value of food intake (r = 0.72 and 0.75, respectively) in Experiment II, Table 8). Therefore, lower values of dry weight and ash weight of the femurs in meal-fed rats as compared with those fed ad libitum in Experiment I may have been due to their lower food intakes. In addition, meal-fed and ad libitum fed rats ate the same diet, therefore, meal-fed rats also had a lower intake of nitrogen which also may have contributed to the lower dry weight and ash weight of femurs.

Reducing dietary protein from 8 to 4% reduced dry weight of femurs from 0.202 to 0.138 g for rats fed ad libitum and from 0.172 to 0.130 g for

	Dry weight in grams			
	"M"		"A"	
Calcium treatment	8%	4%	8%	4%
0.4% calcium	0.185	0.133	0.229	0.146
0.1% calcium	0.160	0.126	0.175	0.129
Mean	0.172	0.130	0.202	0.138

meal-fed rats (Table 16). Likewise ash weight was decreased from a mean value of 0.088 to 0.045 g for ad libitum fed rats and from 0.068 to 0.048 g for meal-fed rats. The decrease in dry weight was more marked when protein was reduced in the diet containing 0.4% calcium than when it was reduced in the diet containing 0.1% calcium (from 0.229 to 0.146 and from 0.175 to 0.129 g for rats fed 8 and 4% protein ad libitum and from 0.185 to 0.133 and from 0.160 to 0.126 g when dietary protein was reduced from 8 to 4% for meal-fed rats).

Ash weight in grams

	"M"		"A"	
Calcium treatment	8%	4%	8%	4%
0.4% calcium	0.080	0.053	0.112	0.063
0.1% calcium	0.056	0.044	0.063	0.045
Mean	0.068	0.048	0.088	0.054

Similarly the decrease in ash weight was more pronounced when protein was reduced from 8 to 4% in diets containing 0.4% calcium than when diets contained 0.1% calcium. When rats were fed 8% protein ad libitum, the ash weight of the right femur averaged 0.112 g if the diet contained 0.4% calcium but only 0.063 g if it contained 0.1% calcium. With 4% protein in the diets fed ad libitum, the resulting ash weights were 0.063 and 0.045 g when 0.4 and 0.1% calcium were included in the diets. For meal-fed rats, if diets contained 8% protein, ash weights were 0.080 and 0.056 g when 0.4 and 0.1% calcium were provided; if diets contained 4% protein, ash weights were 0.053 and 0.044 g when calcium was included at 0.4 and 0.1%. The decrease in ash weight was more when protein was reduced from 8 to 4% in diets containing 0.4% calcium than 0.1% calcium (0.049 vs 0.018 g in rats fed ad libitum and 0.027 vs 0.012 g in meal-fed rats). Therefore, it appeared that 0.1% calcium in the diet was less adequate for bone mineralization when diets contained 8% protein than when they contained 4% protein.

When rats were fed ad libitum and dietary calcium was reduced from 0.4 to 0.1% in diets containing 8% protein, the dry weight and ash weight were lowered from 0.229 to 0.175 g and from 0.112 to 0.063 g, respectively. When rats were given continuous access to a diet containing 4% protein, the

reduction in dietary calcium from 0.4 to 0.1% decreased dry weight from 0.146 to 0.129 g and ash weight from 0.063 to 0.045 g.

For meal-fed rats, reduction in dietary calcium from 0.4 to 0.1% when dietary protein was 8% reduced dry weight from 0.185 to 0.160 g and ash weight from 0.080 to 0.056 g. A similar reduction in calcium when dietary protein was 4% resulted in little or no change in dry weight, 0.133 to 0.126 g and in ash weight, 0.053 to 0.044 g. Therefore, protein limited mineralization even when the calcium intake was 0.4%.

Meal-feeding did not affect femur ash per unit of length or per unit of volume (Table 17). Regardless of feeding frequency and dietary calcium concentrations, when dietary protein was reduced from 8 to 4%, ash per unit of length reduced. The mean ash values were 32.5 and 24.0 mg for rats fed ad libitum when dietary protein was 8 and 4%, respectively, and 32.2 and 22.5 mg for meal-fed rats.

Ash per cm length in milligrams

	"M"		"A"	
Calcium treatment	8%	4%	8%	4%
0.4% calcium	32.3	24.8	41.5	28.1
0.1% calcium	22.1	20.2	23.5	19.9
Mean	32.2	22.5	32.5	24.0

The decrease in ash per unit of length associated with reduction in dietary protein was more pronounced when diets contained 0.4% calcium (41.5 and 28.1 mg per cm length in rats fed ad libitum; 32.3 and 24.8 mg ash per cm length in meal fed rats) than when diets contained 0.1% calcium (23.5 and 19.9 mg per cm length in rats fed ad libitum; 22.1 and 20.2 mg per cm length in meal-fed rats). Reducing calcium content of diets decreased ash

Table 17. Ash values for right femurs of rats in Experiment I

Dietary treatments	No. of rats	Ash per cm length mg	Ash per cm^3 mg	% femur wt. to body wt.
8% protein, 0.4% calcium				
Ad libitum	6	41.5 (34.0-[a] 45.4)	254 (196-[a] 288)	0.299 (0.245-[a] 0.341)
Meal-fed	6	32.3 (28.0- 38.8)	235 (197- 354)	0.350 (0.318- 0.379)
8% protein, 0.1% calcium				
Ad libitum	6	23.5 (18.4- 27.4)	150 (122- 168)	0.265 (0.209- 0.291)
Meal-fed	6	22.1 (20.7- 23.6)	170 (155- 204)	0.320 (0.286- 0.332)
4% protein, 0.4% calcium				
Ad libitum	6	28.1 (23.8- 30.9)	262 (234- 300)	0.358 (0.333- 0.378)
Meal-fed	6	24.8 (21.3- 31.0)	241 (185- 274)	0.405 (0.384- 0.427)
4% protein, 0.1% calcium				
Ad libitum	6	19.9 (16.0- 20.9)	188 (160- 220)	0.317 (0.296- 0.329)
Meal-fed	6	20.2 (16.7- 23.5)	183 (156- 245)	0.365 (0.341- 0.410)
Zero-time control values	11	15.8 (11.1- 20.0)	123 (88- 180)	0.446 (0.332- 0.562)

[a]Range.

per unit of length in the femur of rats irrespective of the dietary protein and the feeding pattern imposed.

The reduction in ash per unit of length of femurs with decreases in dietary protein or calcium has also been reported by El-Maraghi et al.

(1965). When they reduced dietary protein from 10.2 to 4.5 NDpCal%, the ash decreased from 67.1 to 36.3 mg per cm when rats were fed a diet containing 0.44% calcium. When they decreased calcium in the diet from 0.44 to 0.11% and provided 10.2 NDpCal%, ash decreased from 67.1 to 35.4 mg per cm. The femurs of rats fed 10.2 NDpCal% with 0.11% calcium or 4.5 NDpCal% with either 0.44% calcium or 0.11% calcium contained less ash per unit length than femurs of rats fed 10.2 NDpCal% with 0.44% calcium. These data indicated that the intakes of higher protein with lower calcium and lower protein with higher calcium resulted in lower ash values than the intake of higher protein with higher calcium.

The values of ash per unit of volume were not changed when dietary protein was reduced and calcium held constant at 0.4% whether rats were fed meals or ad libitum (see Table 17). Regardless of the feeding frequency, on the other hand, femurs of rats fed 4% protein and 0.1% calcium contained more ash per unit volume than those of rats fed 8% protein and 0.1% calcium. When diets contained 8% protein instead of 4% protein, perhaps the amount of matrix formed was in excess of that which could be fully mineralized by the 0.1% calcium provided. El-Maraghi et al. (1965) reported that when diets containing 10.2 NDpCal% were fed ad libitum to rats, decreasing dietary calcium from 0.44 to 0.11% decreased the ash per cm^3 from 516 to 282 mg.

When values for the femur weights were expressed as percentage of body weight (Table 17), the values were higher for meal-fed rats than for rats fed ad libitum. These data indicated that growth of tissues other than bone was hindered when protein and calcium intakes of ad libitum and meal-fed rats were not equal.

McMeekan (1940) fed protein deficient diets to pigs and observed that skeletal weight was affected relatively less than the weight of muscle and fat. Later Gunther and Tekin (1968) investigated the effect of dietary calcium and phosphorus levels on mineralization of the entire skeleton in 1- to 28-day-old chicks. Based on the distribution of total ash in different skeletal parts, they observed that legs calcified to a greater extent than the rest of the skeleton when supply of calcium and phosphorus was limited.

Table 18 presents the mean changes in femur values at the end of the experiment from the zero-time control values. Regardless of the amount of dietary calcium and feeding frequency, diets containing 8% protein were associated with greater increases in femur weight, length, volume, and dry weight than diets containing 4% protein.

Reduction in dietary protein decreased ash per unit length less when dietary calcium was 0.1% (7.7 to 4.1 mg in ad libitum and 6.3 to 4.4 mg in meal-fed rats) than when it was 0.4% (25.7 to 12.3 mg in ad libitum and 16.5 to 9.0 mg in meal-fed rats). Increasing the calcium concentration of the diet from 0.1 to 0.4% without a simultaneous increase in dietary protein resulted in lower ash per unit length (12.3 mg per cm) than when calcium and protein both were increased simultaneously (25.7 mg per cm). These values confirmed the need for simultaneous increase of calcium and protein for greatest increase in femur growth and mineralization.

In Experiment II, when intakes of protein and calcium were equal for rats fed ad libitum and twice daily, femur weight did not differ significantly although energy values of the food intake were lower for meal-fed rats (Tables 19a, 19b).

Table 18. Changes[a] in mean values for size and composition of the right femurs of rats in Experiment I

Dietary treatments	No. of rats	Δ in weight g	Δ in length cm	Δ in volume cm^3	Δ in dry weight g	Δ in ash weight g	Δ in mg ash/ cm length mg	Δ in mg ash/ cm^3 mg
8% protein, 0.4% calcium								
Ad libitum	6	0.245	0.99	0.217	0.156	0.085	25.7	131
Meal-fed	6	0.168	0.77	0.122	0.112	0.053	16.5	112
8% protein, 0.1% calcium								
Ad libitum	6	0.208	0.97	0.195	0.102	0.036	7.7	27
Meal-fed	6	0.169	0.82	0.108	0.087	0.029	6.3	47
4% protein, 0.4% calcium								
Ad libitum	6	0.073	0.54	0.015	0.073	0.036	12.3	139
Meal-fed	6	0.043	0.43	0.002	0.060	0.026	9.0	116
4% protein, 0.1% calcium								
Ad libitum	6	0.067	0.55	0.022	0.056	0.018	4.1	65
Meal-fed		0.052	0.47	0.017	0.053	0.017	4.4	61

[a]Final values minus the values of zero-time control group.

Table 19a. Adjusted means for size and composition of the right femurs of rats in Experiment II

Groups	No. of rats	Weight g	Length cm	Volume cm³	Width of middia- physis cm	Width of distal epiphysis cm	Dry weight g	Ash weight g
2P4CaA	12	0.426[a] $\pm$0.013[a]	2.59[a] $\pm$0.03[a]	0.38[a] $\pm$0.01[a]	0.30[a] $\pm$0.01[a]	0.60[a] $\pm$0.01[a]	0.199[a] $\pm$0.006[a]	0.092[a] $\pm$0.003[a]
2P4CaM	11	0.450 $\pm$0.014	2.58 $\pm$0.03	0.38 $\pm$0.01	0.30 $\pm$0.01	0.61 $\pm$0.01	0.208 $\pm$0.006	0.099 $\pm$0.003
2P2CaA	11	0.437 $\pm$0.013	2.62 $\pm$0.03	0.40 $\pm$0.01	0.30 $\pm$0.01	0.61 $\pm$0.01	0.170 $\pm$0.006	0.065 $\pm$0.003
2P2CaM	11	0.475 $\pm$0.014	2.65 $\pm$0.03	0.41 $\pm$0.01	0.31 $\pm$0.01	0.63 $\pm$0.01	0.207 $\pm$0.006	0.092 $\pm$0.003
1P4CaA	10	0.282 $\pm$0.014	2.17 $\pm$0.03	0.26 $\pm$0.01	0.25 $\pm$0.01	0.56 $\pm$0.01	0.140 $\pm$0.007	0.060 $\pm$0.003
1P4CaM	12	0.294 $\pm$0.014	2.23 $\pm$0.03	0.27 $\pm$0.01	0.26 $\pm$0.01	0.56 $\pm$0.01	0.145 $\pm$0.006	0.061 $\pm$0.003
1P2CaA	11	0.280 $\pm$0.136	2.23 $\pm$0.03	0.27 $\pm$0.01	0.26 $\pm$0.01	0.55 $\pm$0.01	0.124 $\pm$0.006	0.046 $\pm$0.003
1P2CaM	12	0.309 $\pm$0.013	2.27 $\pm$0.03	0.27 $\pm$0.01	0.26 $\pm$0.01	0.56 $\pm$0.01	0.140 $\pm$0.006	0.056 $\pm$0.003
1P1CaA	12	0.265 $\pm$0.013	2.14 $\pm$0.03	0.26 $\pm$0.01	0.24 $\pm$0.01	0.54 $\pm$0.01	0.114 $\pm$0.006	0.038 $\pm$0.003
1P1CaM	12	0.302 $\pm$0.013	2.30 $\pm$0.03	0.28 $\pm$0.01	0.26 $\pm$0.01	0.56 $\pm$0.01	0.130 $\pm$0.006	0.045 $\pm$0.003
Zero-time control values	22	0.230 $\pm$0.004	1.73 $\pm$0.01	0.22 $\pm$0.04	0.24 $\pm$0.01	0.54 $\pm$0.003	0.078 $\pm$0.002	0.028 $\pm$0.001

[a]Standard error.

Table 19b. Probability of significance of variables in Table 19a

Comparisons	Weight[a]	Length[a]	Volume	Width middia-physis	Width distal epi-physis	Dry weight[a]	Ash weight[a]
Feeding effect							
Ad libitum vs meal-fed							
2P4Ca	ns[b]	ns[b]	ns[b]	ns[b]	ns[b]	ns[b]	ns[b]
2P2Ca	<.05	ns	ns	ns	ns	<.001	<.001
1P4Ca	ns	ns	ns	ns	ns	ns	ns
1P2Ca	ns	ns	ns	ns	ns	ns	<.025
1P1Ca	<.05	<.05	ns	ns	ns	ns	ns
Protein effect							
2P4CaA vs 1P4CaA	<.001	<.001	<.001	<.001	<.001	<.001	<.001
2P4CaM vs 1P4CaM	<.001	<.001	<.001	<.001	<.001	<.001	<.001
2P2CaA vs 1P2CaA	<.001	<.001	<.001	<.001	<.001	<.001	<.001
2P2CaM vs 1P2CaM	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Calcium effect							
2P4CaA vs 2P2CaA	ns	ns	ns	ns	ns	<.001	<.001
2P4CaM vs 2P2CaM	ns	ns	ns	ns	ns	ns	ns
1P4CaA vs 1P2CaA	ns	ns	ns	ns	ns	ns	<.005
1P4CaM vs 1P2CaM	ns	ns	ns	ns	ns	ns	ns
1P2CaA vs 1P1CaA	ns	<.05	ns	ns	ns	ns	<.05
1P2CaM vs 1P1CaM	ns	ns	ns	ns	ns	ns	<.05
1P4CaA vs 1P1CaA	ns	ns	ns	ns	ns	<.005	<.001
1P4CaM vs 1P1CaM	ns	ns	ns	ns	ns	ns	<.001

[a]See Table 39 for least squares analysis of variance.

[b]Non-significant.

Femurs of the rats fed 2P2Ca and 1P1Ca ad libitum were significantly lighter in weight than the femurs of those presumably fed the same amounts of calcium and protein twice daily. Examination of the nitrogen intakes of these two meal-fed groups indicated that they had eaten significantly more food than anticipated and, therefore, consumed more protein and calcium than ad libitum fed controls. Therefore, significantly higher femur

weights might have been due to increased nitrogen and calcium intakes rather than to feeding frequency. Nitrogen intake rather than calcium intake as the primary factor accounting for the differences in femur weight was suggested by the fact that reducing the nitrogen intake from 2P to 1P significantly reduced fresh weight of femurs from a mean value of 0.447 to 0.289 g irrespective of feeding frequency and calcium intakes.

Femur weight in grams

Calcium intake	2P	1P
4Ca (A and M)	0.438	0.288
2Ca (A and M)	0.456	0.294
1Ca (A and M)		0.284
Mean	0.447	0.289

Shenolikar and Rao (1968) have reported that protein restriction retarded bone growth more than general undernutrition or restriction of the energy value of food intake. They had fed one group of rats ad libitum with a diet containing 5% protein and 0.5% calcium while a second group was pair-fed with a diet containing 20% protein and 0.5% calcium. The food energy value of a third group of rats was restricted by feeding them a diet which contained 40% protein and 1.0% calcium but in amounts equal to one-half that consumed by the first and second groups. After five weeks, the femurs of rats fed the diet containing 5% protein were lighter (0.324 g) than the femurs of pair-fed rats (0.480 g) and rats restricted severely in food intake (0.388 g).

The reduction in femur weight in response to reduced protein intake in Experiment I was confirmed in Experiment II. Gontzea et al. (1962) have reported that femurs were smaller by 60% for rats fed 4.2% protein compared

with those for rats fed 17.7% protein in diets. However, Gontzea et al.
compared the effect of adequate and inadequate protein diets on femur
growth while in the present experiments both protein diets (8 and 4%) were
inadequate to support maximum growth (National Academy of Sciences-National
Research Council, 1962). Nevertheless, the femurs of rats whose protein
intake was 2P in Experiment II were heavier by 35% than those of rats whose
protein intake was one-half as much, i.e., 1P. Because femur weight cor-
related positively with nitrogen intake, (r = 0.74, Table 8), the heavier
femurs of 2P2CaM and 1P1CaM than 2P2CaA and 1P1CaA, respectively, might
have been due to the higher nitrogen intake of 2P2CaM and 1P1CaM groups.

Reducing dietary calcium when nitrogen intake was constant (2P or 1P)
had no effect on femur weight. Likewise, varying the ratios of protein to
calcium in the diet from 20 to 40 or 80 when the protein intake was 1P did
not change femur weights. Femurs weighed 0.288, 0.294, and 0.283 g (Tables
20a, 20b). When protein intakes were 2P, femurs were heavier than when
intakes were 1P whether the protein:calcium ratio was 40 (0.438 vs 0.294 g)
or 80 (0.456 vs 0.283 g). Thus, dietary protein intake rather than the
protein:calcium ratio had influenced the weight of the femurs under the
conditions of the present experiment.

Meal-feeding did not influence femur length except for the 1P1CaM
group (Table 19a) which had significantly longer femurs than their ad libi-
tum fed controls. However, rats in the 1P1CaM group had significantly
higher intakes of protein than rats in 1P1CaA. Hence the difference in
femur length may have been due to the difference in protein intake.

Femur length was correlated positively with nitrogen intake (r = 0.78,
Table 8). Other investigators also have observed an association between

Table 20a. Adjusted means for size and composition of right femurs of rats fed different ratios of protein and calcium

Diets	Pro:Ca ratio	Weight g	Length cm	Dry weight g	Ash weight g	Femur weight % body weight	Ash/ cm length mg	Ash/ cm^3 mg
1P4Ca	20	0.288 $\pm$.010[a]	2.20 $\pm$.022[a]	0.142 $\pm$.005[a]	0.060 $\pm$.002[a]	0.427 $\pm$.009[a]	27.4 $\pm$.75[a]	229 $\pm$5.55[a]
1P2Ca	40	0.294 $\pm$.009	2.25 $\pm$.021	0.133 $\pm$.004	0.051 $\pm$.002	0.393 $\pm$.009	22.6 $\pm$.70	192 $\pm$5.21
1P1Ca	80	0.283 $\pm$.009	2.22 $\pm$0.20	0.122 $\pm$.004	0.042 $\pm$.002	0.395 $\pm$.008	18.7 $\pm$.68	153 $\pm$5.06
2P2Ca	40	0.438 $\pm$.009	2.58 $\pm$.021	0.204 $\pm$.004	0.096 $\pm$.002	0.342 $\pm$.009	36.9 $\pm$.70	252 $\pm$5.21
2P1Ca	80	0.456 $\pm$.009	2.64 $\pm$.021	0.188 $\pm$.004	0.078 $\pm$.002	0.326 $\pm$.009	29.3 $\pm$.70	191 $\pm$5.21

[a]Standard error.

Table 20b. Probability of significance of variables in Table 20a

	Weight	Length	Dry weight	Ash weight	Femur weight % body weight	Ash/ cm length	Ash/ cm^3
Pro:Ca ratio (1P)							
20 vs 40	ns[a]	ns[a]	ns[a]	ns[a]	ns[a]	<.001	<.001
20 vs 80	ns	ns	ns	<.001	<.001	<.005	<.001
40 vs 80	ns	ns	ns	ns	ns	<.001	<.001
(2P vs 1P)							
40 vs 40	<.001	<.001	<.001	<.001	ns	<.001	<.001
80 vs 80	<.001	<.001	<.001	<.001	<.001	<.001	<.001
(2P)							
40 vs 80	ns	ns	<.005	<.001	ns	<.001	<.001

[a]Non-significant.

protein intake and bone length. For example, Frandsen et al. (1954) demonstrated that when dietary protein was increased from 3 to 6%, the tibia of weanling rats had increased in length from 26.5 to 29.0 mm after six weeks. Gontzea et al. (1962) reported that after 30 days, the bones of weanling rats fed 17.7% protein were 25 to 30% longer than the bones of rats fed a diet containing 4.2% protein.

Femur valume, width of middiaphysis, and width of distal epiphysis were not significantly affected by either meal-feeding or calcium intakes (see Tables 19a, 19b). On the other hand, reducing the intakes of dietary protein did reduce significantly the volume, width of middiaphysis, and of distal epiphysis. Frandsen et al. (1954) also had observed that the width

of tibial middiaphysis decreased from 2.9 to 2.0, to 1.8, and to 1.6 mm when weanling rats accustomed to a diet containing 24% protein were fed diets which contained 6, 3, and 0% protein. At the same time, these investigators reported no change in width of tibial distal epiphysis.

Meal-feeding did not affect dry weight and ash weight of the femurs except when the intakes were 2P2Ca (Tables 19a, 19b). The femurs of rats in the 2P2CaM group were significantly greater in dry weight and ash weight than femurs of rats in the 2P2CaA group (0.207 vs 0.170 g and 0.092 vs 0.065 g). These significantly different values for dry weight and ash weight were likely due to higher nitrogen and calcium intakes of the meal-fed group compared with the group fed ad libitum rather than to feeding frequency.

When protein was reduced from 2P to 1P and calcium concentrations of the diets and feeding frequency disregarded, dry weight and ash weight of femurs were significantly reduced (Table 19a). Lower nitrogen intake also had limited bone growth as indicated by shorter femur lengths. When calcium was reduced from 4Ca to 2Ca and protein held constant at 2P, dry weight and ash weight were reduced significantly for rats fed ad libitum but not for meal-fed rats. With the intake of 1P, however, no differences were observed in dry weight of femurs when calcium was reduced from 4Ca to 1Ca. The diet containing 0.1% calcium (2Ca), therefore, was inadequate for maximal mineralization of the matrix formed by rats with 2P intakes. These findings confirmed those of Experiment I, i.e., that intakes of 0.1% calcium with 8% protein resulted in lower dry weight and ash weight of the femurs than intakes of 0.4% calcium with 8% protein.

The dietary protein:calcium ratios of 40 and 80 did not affect dry weight or ash weight of the femurs whether intakes were 1P or 2P.

Figure 2 illustrates that when data for meal-fed and ad libitum fed groups were combined, changing calcium intakes from 4Ca to 2Ca decreased ash weight whether protein intake was 2P or 1P; however, ash weight decreased more when changes in calcium intakes were made with intakes of 2P (0.096 to 0.078 g) than with intakes of 1P (0.060 to 0.051 g).

Changes in femur composition after 28 days were calculated by subtracting zero-time control values from the values obtained at the end of the experiment (Table 21). When all meal-fed rats were compared with all those fed ad libitum, they exhibited greater increases in femur weight, length, volume, width of middiaphysis and of distal epiphysis than the rats fed ad libitum. The increases in weight, volume, width of middiaphysis, and width of distal epiphysis were 3 to 5 times as large with intakes of 2P as with intakes of 1P.

Reducing calcium intakes from 4Ca to 2Ca decreased dry weight and ash weight of femurs in rats fed ad libitum but not in the rats fed twice daily. When the calcium intake was reduced further to 1Ca for rats on the lower protein intake only (1P), the dry weight and ash weight of their femurs were reduced whether they were fed ad libitum or twice daily. Thus, the lowest intake of calcium (1Ca) with 1P was less adequate for mineralization of femurs than the higher intakes of 2Ca and 4Ca with 1P.

Feeding frequency did not affect ash concentration in femurs per unit length and ash per unit volume for 2P4Ca, 1P4Ca, and 1P1Ca groups (Tables 22a, 22b) but did differ for 2P2CaM and 1P2CaM groups. The group 2P2CaM ate significantly more than the expected amount of food, i.e., more than

FEMUR ASH WEIGHT
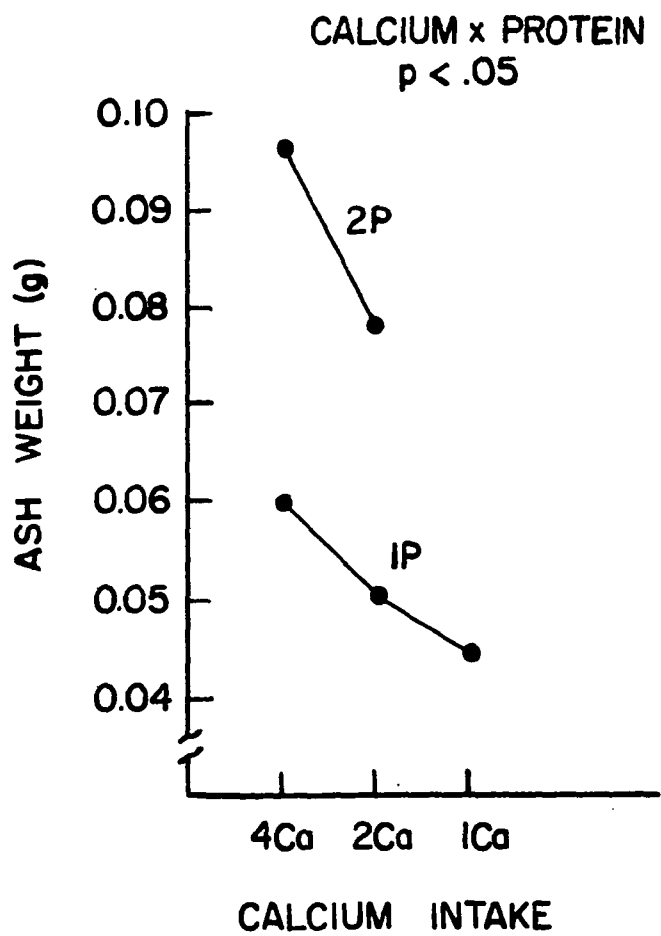
Figure 2. Influence of interaction between calcium and protein on ash weight

Table 21. Changes[a] in mean values for size and composition of the right femurs of rats in Experiment II.

Groups	Δ in weight g	Δ in length cm	Δ in volume cm³	Δ in width of middia- physis cm	Δ in width of distal epi- physis- cm	Δ in dry weight g	Δ in ash weight g
2P4CaA	0.196	0.86	0.157	0.051	0.059	0.121	0.064
2P4CaM	0.220	0.85	0.165	0.057	0.064	0.130	0.071
2P2CaA	0.207	0.89	0.179	0.060	0.071	0.092	0.037
2P2CaM	0.245	0.92	0.196	0.062	0.091	0.129	0.064
1P4CaA	0.052	0.44	0.043	0.008	0.016	0.062	0.032
1P4CaM	0.064	0.50	0.052	0.015	0.016	0.067	0.033
1P2CaA	0.050	0.50	0.048	0.013	0.004	0.046	0.018
1P2CaM	0.079	0.54	0.050	0.017	0.022	0.062	0.028
1P1CaA	0.035	0.41	0.039	0.002	0.001	0.036	0.010
1P1CaM	0.072	0.57	0.067	0.011	0.020	0.052	0.017

[a]Final values minus the values of zero-time control group.

two-thirds as much as that eaten by rats fed ad libitum; therefore, the nitrogen intake of 2P2CaM group was significantly higher than 2P2CaA group. The greater intake of protein by meal-fed rats was associated with significantly higher concentrations of ash wehther expressed as ash per unit length (34.2 vs 24.5 mg per cm) or ash per unit volume (221 vs 162 mg per cm³). Although the nitrogen intake of 1P2CaM group was not significantly different from that of 1P2CaA group, 1P2CaM group did consume more nitrogen, 1623 mg, than 1P2CaA group, 1371 mg. Therefore, the significantly higher ash concentration in femurs per unit length (24.6 vs 20.5 mg per cm) and ash per unit volume (209 vs 173 mg per cm³) of 1P2CaM group might have been due to its higher nitrogen intake.

Table 22a. Adjusted means for ash values of the right femurs of rats in
Experiment II

Groups	Ash/cm length mg	Ash/cm^3 mg	% femur wt. of body wt.[a]
2P4CaA	35.6	247	0.330
	$\pm$0.90[b]	$\pm$6.6[b]	$\pm$0.012[b]
2P4CaM	38.2	257	0.354
	$\pm$0.95	$\pm$6.9	$\pm$0.013
2P2CaA	24.5	162	0.309
	$\pm$0.90	$\pm$6.6	$\pm$0.012
2P2CaM	34.2	221	0.344
	$\pm$0.95	$\pm$6.9	$\pm$0.013
1P4CaA	27.5	232	0.426
	$\pm$1.00	$\pm$7.3	$\pm$0.014
1P4CaM	27.4	226	0.428
	$\pm$0.95	$\pm$6.9	$\pm$0.013
1P2CaA	20.5	173	0.386
	$\pm$0.95	+6.9	$\pm$0.013
1P2CaM	24.6	209	0.398
	$\pm$0.90	$\pm$6.6	$\pm$0.012
1P1CaA	17.8	148	0.424
	$\pm$0.90	$\pm$6.6	$\pm$0.012
1P1CaM	19.6	158	0.366
	$\pm$0.90	$\pm$6.6	$\pm$0.012
Zero-time control values	15.8	130	0.484
	$\pm$0.86	$\pm$7.1	$\pm$0.008

[a]Body weight taken at the time of autopsy.

[b]Standard error.

In Experiment II, ash concentration per unit volume decreased from 232
to 173 and to 148 mg per cm^3 as the calcium intake of animals fed ad libi-
tum was reduced from 4Ca to 2Ca and to 1Ca with 1P intakes (Figure 3). Fig-
ure 3 also includes the ash concentration per unit volume obtained in
Experiment I for femurs of rats fed a diet containing 4% protein and 0.4%
calcium (twice as large as the highest dietary calcium in Experiment II)

Table 22b. Probability of significance of variable in Table 22a

Comparisons	Ash/ cm length[a]	Ash/ cm^3 volume[a]	% femur wt. of body wt.[a]
Feeding effect			
Ad libitum vs			
meal-fed			
2P4Ca	ns[b]	ns[b]	ns[b]
2P2Ca	<.001	<.001	<.05
1P4Ca	ns	ns	ns
1P2Ca	<.005	<.001	ns
1P1Ca	ns	ns	<.001
Protein effect			
2P4CaA vs 1P4CaA	<.001	ns	<.001
2P4CaM vs 1P4CaM	<.001	<.005	<.001
2P2CaA vs 1P2CaA	<.005	ns	<.001
2P2CaM vs 1P2CaM	<.001	ns	<.005
Calcium effect			
2P4CaA vs 2P2CaA	<.001	<.001	ns
2P4CaM vs 2P2CaM	<.005	<.001	ns
1P4CaA vs 1P2CaA	<.001	<.001	<.05
1P4CaM vs 1P2CaM	<.05	ns	ns
1P2CaA vs 1P1CaA	<.05	<.05	<.05
1P2CaM vs 1P1CaM	<.001	<.001	ns
1P4CaA vs 1P1CaA	<.001	<.001	ns
1P4CaM vs 1P1CaM	<.001	<.001	<.001

[a]See Table 39 for least squares analysis of variance.

[b]Non-significant.

(Table 5). The ash per unit volume decreased from 280 mg (Experiment I) to 232 mg (Experiment II) when calcium was reduced from 0.4% to 0.2%. Examining the results of both the experiments, the decrease in ash per unit volume with decrease in dietary calcium from 0.4% to 0.05% when diets contained 4% protein did not confirm the findings reported by El-Maraghi et al. (1965). These authors had reported no decrease in the values of ash per unit volume when dietary calcium was reduced from 0.44 to 0.11% of diets

FEMUR ASH WEIGHT
PER UNIT VOLUME
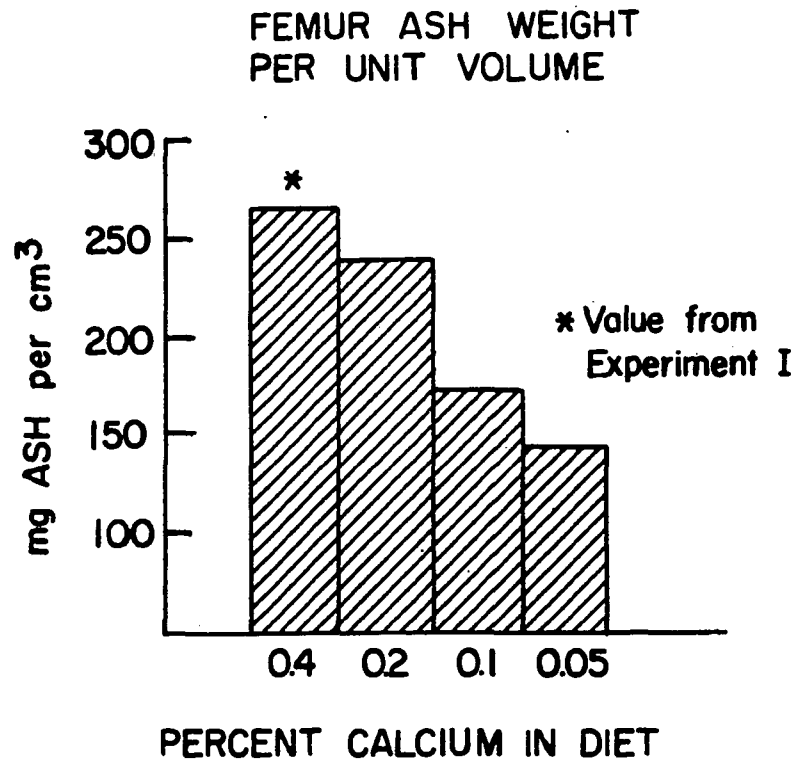
Figure 3.   Influence of varying dietary calcium on ash per unit volume

containing 4.5 NDpCal%. The discrepancy between the present results and those reported by El-Maraghi et al. (1965) might be accounted for in part by the fact that they fed rats for 11 weeks whereas the data here were based on 4-week experiments.

Toothill and Hosking (1968) demonstrated that when weanling rats were fed diets containing 0.131 or 0.744% calcium, the difference in values between treatments became smaller as the experiment was continued for 21, 48, and 60 weeks. At the end of 60 weeks, no significant differences were observed in ash weight, ash concentration, or in fresh weight for the long bones, whether rats were fed diets containing 0.131 or 0.744% calcium.

Figure 4 illustrates the effect of interaction between calcium and protein on the amount of ash per unit length. Decreasing calcium intakes from 4Ca to 2Ca decreased ash per unit length with intakes of 2P (from 36.9 to 29.3 mg per cm, when data for meal-fed and ad libitum fed animals were combined) or with 1P (from 27.4 to 22.6 mg per cm, when data were combined without regard to feeding frequency). When calcium intake was held constant at 4 or 2 Ca, the decrease in protein from 2P to 1P resulted in greater decrease in ash per unit length with intakes of 4Ca than with intakes of 2Ca, i.e., 36.9 to 27.4 vs 29.3 to 22.6 mg.

Figure 5 illustrates the interaction between calcium and protein on ash per unit volume of femurs. Decreasing calcium intakes from 4Ca to 2Ca decreased ash per unit volume irrespective of protein intakes, although the decrease was greater with 2P intakes (252 to 192 mg per cm^3) than with 1P intakes (229 to 191 mg per cm^3). The effect of protein and calcium interaction on ash per unit length and ash per unit volume lends support to the

FEMUR ASH WEIGHT
PER UNIT LENGTH
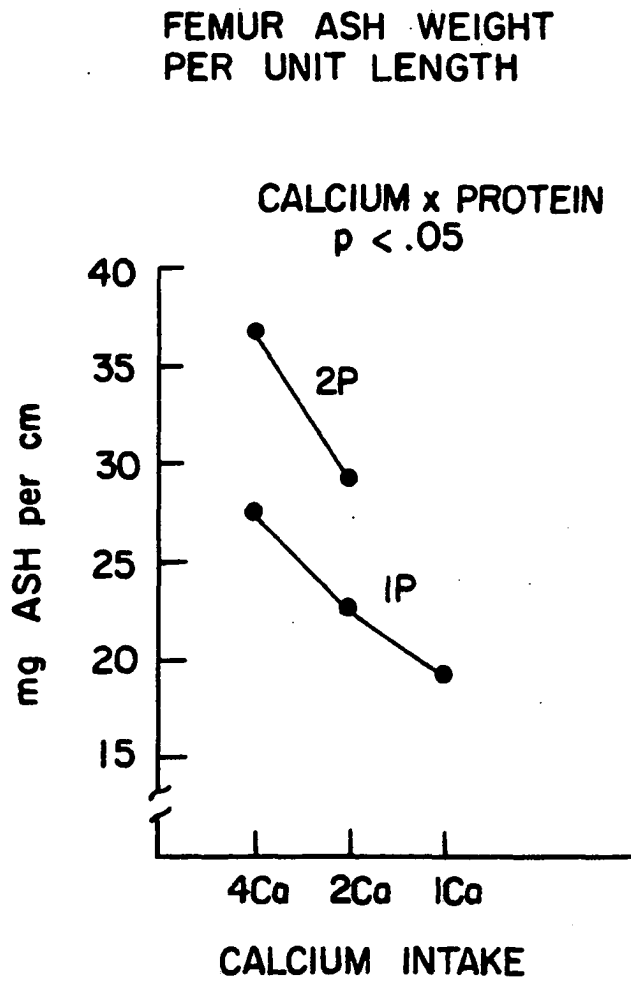
Figure 4. Influence of interaction between calcium and protein on ash per unit length

FEMUR ASH WEIGHT
PER UNIT VOLUME

CALCIUM x PROTEIN
p < .05
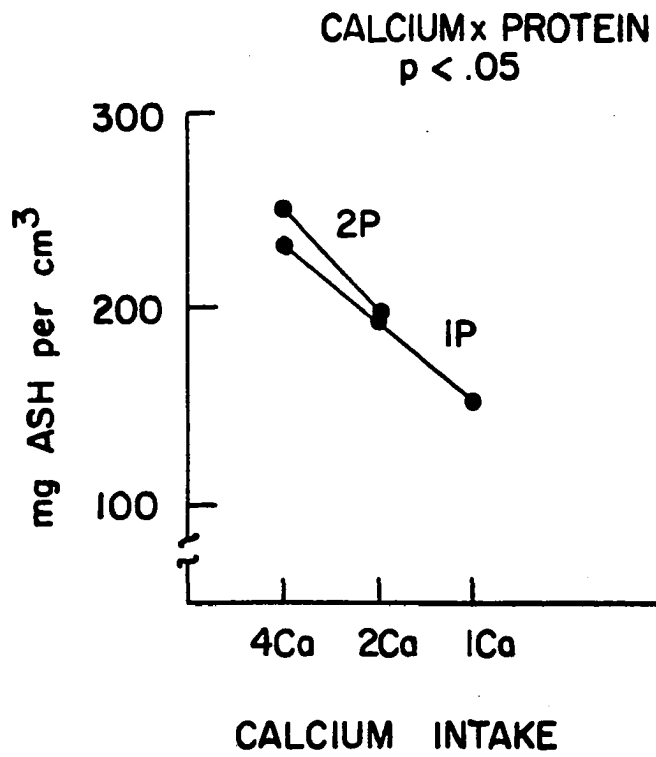
Figure 5.   Influence of interaction between calcium and protein on ash per
unit volume

Ash per unit volume in milligram

Protein intake	2Ca	4Ca
2P (A and M)	192	252
1P (A and M)	191	229

fact that dietary protein and calcium must increase together for greatest bone growth.

Regardless of feeding frequency and calcium intakes, femur weights expressed as percentage body weight were significantly higher when rats were fed diets containing 1P (0.402) than 2P (0.334).

% femur weight of body weight

Calcium intake	1P	2P
4Ca and 2Ca	0.410	0.334
1Ca	0.395	
Mean	0.402	0.334

The higher femur weights expressed as percentage body weight with 1P intakes than with 2P indicated that growth of tissues other than femur was impaired.

Table 23 presents changes in ash values at the end of Experiment II from zero-time control values. The values of ash per unit length and ash per unit volume of femurs for ad libitum or meal-fed rats were lower than other groups with the intakes of 1P1Ca. There could be two possible explanations. The intake of 1Ca could have lowered plasma calcium concentration resulting in increasing parathyroid activity. Therefore, calcium could have been withdrawn from the femurs. Phang et al. (1968) reported that a small decrease in plasma calcium level (from 10.37 to 10.22 mg calcium per 100 ml) significantly increased the secretion of parathyroid hor-

Table 23. Changes[a] in ash values of right femurs of rats in Experiment II

Group	Δ in mg ash/ cm length	Δ in mg ash/ cm^3	Δ in femur wt. % body wt.[b]
2P4CaA	19.8	117	-0.154
2P4CaM	22.4	127	-0.130
2P2CaA	9.7	32	-0.175
2P2CaM	18.4	91	-0.140
1P4CaA	11.7	102	-0.058
1P4CaM	11.5	96	-0.056
1P2CaA	4.7	43	-0.098
1P2CaM	8.8	79	-0.086
1P1CaA	2.0	18	-0.060
1P1CaM	3.8	28	-0.118

[a]Final values minus the values of zero-time control group.

[b]Body weight taken at the time of autopsy.

mone. On the other hand, intake of 1Ca and 1P might not have been suffi-cient for mineralization of femurs. Hence, the limiting factor could be either dietary calcium concentration or mediation of parathyroid activity.

When intakes were 2P2Ca, the increases in ash per unit length and ash per unit volume (9.7 mg per cm and 32 mg per cm^3, respectively) were smal-ler than the increases when intakes were 2P4Ca (19.8 mg per cm and 117 mg per cm^3). Likewise, with intakes of 1P4Ca the increases in ash per unit length and ash per unit volume were smaller (11.5 mg per cm and 96 mg per cm^3, respectively) than these values when the intakes were 2P4Ca. These data indicated the need for simultaneous increase in dietary protein and calcium for maximal femur growth and mineralization.

SUMMARY AND CONCLUSIONS

The present study was planned to explore the effects of feeding frequency on protein and calcium utilization and of interaction of dietary protein and calcium on bone growth. The study consisted of two experiments. In Experiment I, diets contained 8 or 4% protein with 0.4 or 0.1% calcium and were fed to weanling rats either ad libitum or for 2 hours twice daily. The main purpose of Experiment I was to determine food intake of meal-fed rats relative to those fed ad libitum.

Meal-fed rats ate 27 to 35% less food than the rats fed ad libitum. On the basis of these findings, diets were planned for Experiment II so that intakes of protein and calcium would be equal for ad libitum and meal-fed rats although food intake was less for the latter.

Rats fed a diet containing 4% protein and 0.4% calcium gained approximately 40% less weight than rats fed a diet containing 0.1% calcium. Therefore, to avoid the adverse effects of 0.4% calcium in low protein diets, the highest dietary calcium level used in Experiment II was 0.2%. The diets for ad libitum fed groups in Experiment II contained 8 or 4% protein and 0.2, 0.1, and 0.05% calcium. The diets for meal-fed groups contained 1.5 times as much protein and calcium as the diets for ad libitum feeding.

In Experiment I, when nitrogen, calcium, and food intakes of meal-fed were less than those of ad libitum fed rats, meal-fed rats weighed 36% less than those fed ad libitum. On the other hand, in Experiment II when the intakes of nitrogen and calcium of meal-fed and ad libitum fed rats were equal, as in the case of the 2P4Ca, 1P4Ca, and 1P2Ca groups and only food

intakes were lower, meal-fed rats gained as much weight as rats fed ad libitum.

When food, nitrogen, and calcium intakes were not equal (Experiment I), meat-fed rats utilized their food less efficiently and consumed more Kcal per g weight gain than did the rats having access to food all the time. The intakes of nitrogen and calcium, however, for 2P2CaM and 1P1CaM groups were significantly higher than the intake of nitrogen and calcium for 2P2CaA and 1P1CaA groups. Although the intakes of food energy of 2P4CaM, 2P2CaM, 1P4CaM, 1P2CaM groups were significantly lower than those fed ad libitum, meal-fed rats were more efficient in food utilization, and they consumed less Kcal per g weight gain than did those fed ad libitum.

Nitrogen balance data from Experiment I indicated that meal-fed rats utilized nitrogen as efficiently as did rats having access to food all the time. In Experiment II, meal-feeding had no effect on concentration of hepatic nitrogen. Meal-fed rats accumulated significantly higher concentration of nitrogen (except for 1P1Ca group) and lower concentration of hepatic and carcass fat than did those fed ad libitum. Reducing nitrogen intake, however, significantly reduced hepatic and carcass nitrogen concentration and increased concentration of hepatic fat in both ad libitum and meal-fed groups.

There was no difference in activity of hepatic arginase between ad libitum and meal-fed groups. Decreasing nitrogen intake by about 50% significantly reduced the activity of hepatic arginase by 5 to 6 times. Thus both these parameters (concentration of hepatic and carcass nitrogen and activity of hepatic arginase) indicated that meal-feeding had no effect on protein utilization under the experimental conditions imposed. Nitrogen intake influenced weight gain, efficiency of food utilization, and hepatic

and carcass nitrogen and fat concentrations while feeding frequency had no effect on these parameters when nitrogen intake was constant.

Dietary protein:calcium ratio had no effect on concentration of hepatic or carcass nitrogen. Regardless of the protein:calcium ratios, the values for hepatic and carcass nitrogen were significantly higher with 2P intakes than with the intakes of 1P.

Based on calcium balance data, meal-fed rats were as efficient in calcium utilization as rats fed ad libitum. In Experiment I, when meal-fed rats had lower intakes of nitrogen and food than the rats fed ad libitum, the fresh weight, length, volume, dry weight, and ash weight of the femurs were lower in meal-fed rats than those fed ad libitum. With similar nitrogen and calcium intakes, i.e., groups 2P4CaM, 1P4CaM, 1P2CaM, and their respective nibbling controls, the size and composition of femurs did not differ between meal-fed and ad libitum fed rats. Reducing nitrogen intake from 2P to 1P, however, reduced fresh weight, length, volume, dry weight, and ash weight of the femurs in both ad libitum and meal-fed rats.

The values for ash per unit length and ash per unit volume did not vary between ad libitum and meal-fed groups even though nitrogen and calcium intakes of meal-fed rats were only 65 to 71% of ad libitum fed groups (Experiment I). These data demonstrated that perhaps with lower nitrogen and calcium intakes, the femurs grew slowly but that there was no impairment in mineralization of the femurs.

When dietary calcium was reduced from 4Ca to 2Ca, irrespective of protein intakes (2P or 1P), the values for ash per unit length and per unit volume decreased significantly. Reduction in calcium from 2Ca to 1Ca when protein intake was 1P further reduced these values. Therefore, the 1Ca

intake with 1P and 2Ca with 2P were considered inadequate to fully mineral-
ize the growing femurs. With the protein and calcium levels used in this
study, maximal bone growth was observed when rats were fed a diet contain-
ing 8% protein and 0.4% calcium in Experiment I and when rats had intakes
of 2P4Ca in Experiment II.

Meal-feeding did not affect protein and calcium utilization for bone
growth when the intakes of protein and calcium were equal or greater than
ad libitum fed controls, although the energy value of food intake was
higher for the latter. Meal-feeding did affect protein and calcium utili-
zation adversely when these two nutrients as well as the energy value of
the food were less than the amounts consumed by rats fed ad libitum.

The groups fed either 8% protein and 0.1% calcium or 4% protein and
0.05% calcium had lower values for the variables related to femur composi-
tion than all other groups. The interaction of protein with calcium on
bone growth indicated that feeding higher protein with lower calcium or
lower protein with lower calcium did not permit as much growth and miner-
alization as was observed when rats were fed a diet containing higher pro-
tein with higher calcium (8% protein and 0.4% calcium in Experiment I and
2P4Ca in Experiment II).

Under the present experimental conditions and diets used, feeding
frequency had no adverse effect on protein utilization as judged by
nitrogen concentrations in liver, carcass, and liver plus carcass, by weight
gain and by femur length. Likewise, meal-feeding did not impair calcium
utilization as evaluated by fresh weight, dry weight, ash weight, ash per
unit length, and ash per unit volume.

Based on bone composition, protein and calcium both were required to be increased simultaneously for maximal bone growth.

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ACKNOWLEDGMENTS

Sincere appreciation is extended to her friends, Mrs. Beth Yetley and Miss Uma Sriramlu, for their constant encouragement throughout her program of study at Iowa State University.

Immense appreciation is expressed to her parents for their help and moral support given during the entire period of her graduate studies.

APPENDIX

Table 24. Stock ration for male rats, Steenbock XVII

Dietary components	Percent
Corn meal[a]	48.3
Linseed meal[b]	13.8
Dry skim milk[c]	10.2
Wheat germ[d]	8.6
Yeast, Brewer's usp[l]	8.2
Casein, crude B3F[e]	4.3
Mazola corn oil[f]	3.6
Alfalfa meal[g]	1.7
NaCl[h]	0.4
CaCO$_3$[i]	0.4
Yeast (irradiated)[j]	0.4

[a]General Biochemicals, Inc., Chagrin Falls, Ohio.

[b]Froning and Deppe Elevator, Ames, Iowa.

[c]Mid American Dairyman Inc., Des Moines, Iowa.

[d]General Mills, Inc., Minneapolis, Minnesota.

[e]The Borden Co., Chemical Division, New York, New York.

[f]Best Foods, A Div. of CPC International Inc., Englewood Cliffs, New Jersey.

[g]National Alfalfa, Lexington, Nebraska.

[h]Analytical Reagents, Mallinckrodt Chemical Works, St. Louis, Missouri.

[i]J. T. Baker Co., Phillipsburg, New Jersey.

[j]Brewer's Yeast irradiated in this laboratory.

Table 25. Random distribution of 12 litters, each consisting of 5 litter-mates distributed among 8 experimental groups and one zero-time control group in Experiment I

Litters	8% protein 0.4% calcium A[1]	M[2]	8% protein 0.1% calcium A[1]	M[2]	4% protein 0.4% calcium A[1]	M[2]	4% protein 0.1% calcium A[1]	M[2]	Zero-time control group
1			a	b			c	d	e
2	a	b			c	d			e
3	a		b		c		d		e
4		a		b		c		d	e
5					a	b	c	d	e
6	a	b			c	d			e
7			a	b			c	d	e
8	a	b	c	d					e
9	a		b		c		d		e
10		a		b		c		d	e
11					a	b	c	d	e
12	a	b	c	d					e

[1]Ad libitum feeding.

[2]Meal-feeding.

Table 26. Random distribution of 23 litters, each consisting of 6 litter-mates distributed among 10 experimental groups and one zero-time control group in Experiment II

Litters	2P4Ca		2P2Ca		1P4Ca		1P2Ca		1P1Ca		Zero-time control group
	A[1]	M[2]	A[1]	M[2]	A[1]	M[2]	A[1]	M[2]	A[1]	M[2]	
1	a				b	c	d			e	f
2			a	b	c	d			e		f
3	a		b	c				d		e	f
4		a		b			c	d		e	f
5		a			b		c	d	e		f
6	a	b		c		d		e			f
7	a	b	c		d				e		f
8	a		b		c				d	e	f
9			a		b	c	d	e			f
10	a		b	c			d		e		f
11		a		b		c		d		e	f
12		a		b			c	d		e	f
13			a	b	c	d	e				f
14			a			b		c	d	e	f
15	a	b	c	d						e	f
16	a	b			c		d		e		f
17					a	b		c	d	e	f
18			a	b	c				d	e	f
19	a	b			c	d		e			f
20	a	b	c			d	e				f
21	a			b			c	d	e		f
22		a		b		c			d	e	f
23	a						b	c	d	e	f

[1] Ad libitum feeding.

[2] Meal-feeding.

Table 27. Preparation of reagents for hepatic arginase

1. L-arginine 0.11 M solution: 9.668 g L-arginine was dissolved in approximately 450 ml distilled water. The pH was adjusted to 9.5 with 0.1 N HCl using Beckman pH meter.[a] Volume was made to 500 ml and solution was stored at -20 C.

2. Urea stock solution 0.2 M: 1.2 g urea[b] was dissolved in 100 ml of distilled water, 5 ml of stock solution was diluted to 10 ml. The diluted solution contained 1 μmole urea/0.1 ml. The solutions were stored at -20 C.

3. Ehrlich reagent 0.4 M: 29.8 g p-dimethylaminobenzaldehyde was dissolved in 50 ml concentrated sulfuric acid and diluted to 500 ml with distilled water. The solution was stored in a brown bottle at room temperature.

4. Maleate buffer pH 7.2: 116 g maleic acid[b] was dissolved in approximately 900 ml distilled water. The pH was adjusted to 7.2 with 1 N NaOH, 0.1979 g Manganous chloride[c] was added and the volume made to 1 L. The buffer solution was stored in a refrigerator at 4 C.

[a]Beckman expandomatic SS-2. Beckman Instrument Inc., Scientific Instrument Division, Fullerton, California.

[b]J. T. Baker Chemical Co., Phillipsburg, New Jersey.

[c]Matheson Coleman and Bell Manufacturing Chemists, Norwood, Ohio.

Table 28. Individual data for rats fed diets containing 8% protein, 0.4% calcium in Experiment I

Rat no.	Litter no.	Initial wt. g	Cumu- lative wt. gain g	Food consump- tion g	Liver wt. g	Nitrogen intake mg/5 day	Nitrogen in feces mg/5 day	Nitrogen in urine mg/5 day
				(Ad libitum fed group)				
25	2	48	124	372.6	5.97			
26	3	43	106	330.1	6.64	1004.2	122.0	212.6
27	6	51	107	334.2	6.55			
28	8	47	97	337.9	6.76	930.1	48.5	201.1
29	9	52	92	307.5	5.24	807.0	70.3	272.5
30	12	53	112	356.0	6.69			
Mean		49.0	106.3	339.7	6.31	913.8	80.3	228.7
				(Meal-fed group)				
31	2	52	42	175.4	3.69			
32	4	54	74	263.6	5.38	844.1	95.3	144.2
33	6	46	59	214.7	4.02	625.8	47.8	100.0
34	8	46	62	189.7	4.72			
35	10	48	70	273.6	5.06	779.2	83.2	300.6
36	12	52	53	201.5	4.65			
Mean		49.8	60.0	219.8	4.59	749.7	75.4	181.6

Calcium intake mg/5 day	Calcium in feces mg/5 day	Calcium in urine mg/5 day	Right Femurs				
			Fresh wt. g	Length cm	Volume cm³	Dry wt. g	Ash wt. g
(Ad libitum fed group)							
			0.489	2.73	0.46	0.250	0.124
303.6	10.8	77.2	0.365	2.65	0.46	0.202	0.090
			0.502	2.77	0.45	0.237	0.114
281.2	65.2	30.2	0.491	2.65	0.40	0.232	0.110
244.0	14.0	11.0	0.441	2.72	0.43	0.217	0.110
			0.472	2.70	0.42	0.237	0.121
276.3	30.1	39.5	0.460	2.70	0.44	0.229	0.112
(Meal-fed group)							
			0.357	2.43	0.30	0.172	0.068
255.2	3.3	35.9	0.454	2.63	0.38	0.224	0.102
189.2	2.2	19.5	0.374	2.43	0.35	0.167	0.069
			0.377	2.42	0.36	0.181	0.077
235.6	43.6	15.8	0.375	2.54	0.31	0.184	0.090
			0.362	2.44	0.35	0.182	0.070
226.7	16.4	23.7	0.383	2.48	0.34	0.185	0.080

Table 29.   Individual data for rats fed diets containing 8% protein, 0.1% calcium in Experiment I

Rat no.	Litter no.	Initial wt. g	Cumu- lative wt. gain g	Food consump- tion g	Liver wt. g	Nitrogen intake mg/5 day	Nitrogen in feces mg/5 day	Nitrogen in urine mg/5 day
				(Ad libitum fed group)				
1	1	46	110	373.2	7.65			
2	3	44	98	324.8	7.62	959.4	68.0	125.7
3	7	49	97	319.5	7.42			
4	8	47	98	329.2	6.67	895.7	60.5	169.9
5	9	56	97	322.6	6.78	954.1	77.3	303.5
6	12	52	111	381.0	6.76			
Mean		49.0	101.8	341.7	7.13	936.4	68.6	199.7
				(Meal-fed group)				
7	1	46	59	205.9	3.96			
8	4	48	74	243.3	5.58	809.5	51.4	145.9
9	7	43	95	286.0	6.34	844.0	57.7	92.2
10	8	49	78	255.0	5.73			
11	10	56	61	214.0	4.63			
12	12	49	65	226.8	5.36	683.4	100.2	235.2
Mean		48.5	72.0	238.5	5.27	779.0	69.8	157.8

Calcium intake mg/5 day	Calcium in feces mg/5 day	Calcium in urine mg/5 day	Right Femurs				
			Fresh wt. g	Length cm	Volume cm^3	Dry wt. g	Ash wt. g
			(Ad libitum fed group)				
			0.382	2.65	0.41	0.176	0.067
72.3	10.0	12.0	0.398	2.70	0.41	0.162	0.063
			0.402	2.62	0.39	0.164	0.054
67.5	25.9	9.0	0.422	2.65	0.40	0.174	0.067
71.9	2.9	6.9	0.444	2.71	0.41	0.181	0.050
			0.488	2.74	0.47	0.194	0.075
70.6	12.9	9.3	0.422	2.68	0.42	0.175	0.063
			(Meal-fed group)				
			0.349	2.46	0.25	0.146	0.051
61.0	4.0	0.8	0.396	2.55	0.30	0.158	0.054
63.6	6.5	7.4	0.397	2.62	0.38	0.162	0.061
			0.419	2.57	0.34	0.173	0.060
			0.370	2.47	0.32	0.153	0.053
51.5	5.7	3.3	0.376	2.50	0.38	0.168	0.059
58.7	5.4	3.8	0.384	2.53	0.33	0.160	0.056

Table 30. Individual data for rats fed diets containing 4% protein, 0.4% calcium in Experiment I

Rat no.	Litter no.	Initial wt. g	Cumulative wt. gain g	Food consumption g	Liver wt. g	Nitrogen intake mg/5 day	Nitrogen in feces mg/5 day	Nitrogen in urine mg/5 day
(Ad libitum fed group)								
37	2	55	32	283.8	2.94			
38	3	47	23	218.5	2.00	320.7	26.6	86.2
39	5	45	32	237.9	3.59	304.0	40.1	77.8
40	6	43	33	263.8	2.84			
41	9	57	21	240.1	2.09	383.1	63.2	124.4
42	11	54	41	295.1	3.55			
Mean		50.0	30.3	256.5	2.84	335.9	43.3	96.1
(Meal-fed group)								
43	2	47	16	154.7	2.51			
44	4	48	11	168.6	2.74	216.7	25.2	57.7
45	5	45	14	161.6	2.62	204.7	57.7	41.6
46	6	50	9	142.5	2.30			
47	10	50	15	184.1	1.39			
48	11	54	24	244.6	2.90	367.0	44.6	139.5
Mean		49.0	14.5	176.0	2.41	262.8	42.5	79.6

Calcium intake mg/5 day	Calcium in feces mg/5 day	Calcium in urine mg/5 day	Right Femurs				
			Fresh wt. g	Length cm	Volume cm^3	Dry wt. g	Ash wt. g
			(Ad libitum fed group)				
			0.329	2.30	0.29	0.171	0.068
191.2	5.4	56.3	0.233	2.25	0.20	0.135	0.060
181.2	29.8	20.7	0.285	2.19	0.20	0.141	0.064
			0.272	2.18	0.21	0.126	0.052
228.4	6.4	12.5	0.291	2.27	0.25	0.144	0.062
			0.317	2.30	0.26	0.161	0.071
200.3	13.9	29.8	0.288	2.25	0.24	0.146	0.063
			(Meal-fed group)				
			0.254	2.05	0.19	0.126	0.047
129.2	7.3	16.8	0.247	2.08	0.20	0.127	0.053
122.0	4.5	21.6	0.235	2.06	0.19	0.124	0.052
			0.252	2.14	0.21	0.317	0.046
			0.259	2.25	0.26	0.123	0.048
218.8	17.2	10.9	0.300	2.26	0.29	0.161	0.070
156.7	9.7	16.4	0.258	2.14	0.22	0.133	0.053

Table 31. Individual data for rats fed diets containing 4% protein, 0.1% calcium in Experiment I

Rat no.	Litter no.	Initial wt. g	Cumu-lative wt. gain g	Food consump-tion g	Liver wt. g	Nitrogen intake mg/5 day	Nitrogen in feces mg/5 day	Nitrogen in urine mg/5 day
				(Ad libitum fed group)				
13	1	45	41	274.0	4.00			
14	3	47	37	246.5	3.03	332.5	47.9	68.2
15	5	47	44	304.8	3.53			
16	7	44	34	259.3	3.09	372.4	60.3	52.5
17	9	54	31	259.7	2.59	383.6	54.7	111.0
18	11	54	57	333.2	4.13			
Mean		48.5	40.5	279.6	3.39	362.8	54.3	77.2
				(Meal-fed group)				
19	1	43	20	141.8	3.32			
20	4	48	26	182.2	3.00	214.7	40.9	54.3
21	5	48	20	201.3	2.69	305.6	48.6	44.9
22	7	45	25	172.1	2.85			
23	10	54	28	239.9	3.49	350.0	51.8	150.0
24	11	53	30	250.8	3.56			
Mean		48.5	24.8	198.1	3.15	290.1	47.1	83.1

Calcium intake mg/5 day	Calcium in feces mg/5 day	Calcium in urine mg/5 day	Fresh wt. g	Length cm	Volume cm^3	Dry wt. g	Ash wt. g
			(Ad libitum fed group)				
			0.283	2.19	0.20	0.123	0.044
49.4	1.9	8.0	0.269	2.20	0.20	0.130	0.043
			0.269	2.25	0.25	0.125	0.047
58.5	1.4	11.1	0.256	2.18	0.20	0.110	0.041
57.0	3.2	5.7	0.279	2.31	0.28	0.123	0.048
			0.336	2.40	0.30	0.160	0.048
55.0	2.2	8.3	0.282	2.26	0.24	0.129	0.045
			(Meal-fed group)				
			0.239	2.04	0.19	0.104	0.034
31.9	2.5	24.1	0.276	2.23	0.20	0.141	0.049
46.9	2.4	8.6	0.234	2.12	0.20	0.110	0.038
			0.244	2.07	0.25	0.121	0.039
52.0	11.2	5.6	0.329	2.33	0.30	0.147	0.054
			0.283	2.30	0.28	0.133	0.054
43.6	5.4	12.8	0.267	2.18	0.24	0.126	0.044

Table 32. Individual data for rats in the zero-time control group in Experiment I

<u>Right Femurs</u>

Rat no.	Litter no.	Initial wt.	Liver wt. g	Fresh wt. g	Length cm	Volume cm^3	Dry wt. g	Ash wt. g
49	1	42	1.85	0.211	1.60	0.20	0.064	0.020
50	2	45	1.97			Broken		
51	3	45	1.86	0.190	1.65	0.20	0.068	0.024
52	4	45	1.69	0.183	1.59	0.15	0.059	0.021
53	5	49	1.65	0.225	1.69	0.22	0.077	0.024
54	6	53	2.05	0.251	1.71	0.30	0.078	0.030
55	7	44	1.62	0.240	1.66	0.19	0.057	0.030
56	8	56	2.52	0.253	1.78	0.25	0.083	0.032
57	9	49	1.80	0.187	1.75	0.22	0.071	0.027
58	10	50	1.58	0.230	1.78	0.25	0.082	0.029
59	11	53	1.86	0.199	1.79	0.23	0.070	0.025
60	12	54	2.06	0.201	1.80	0.22	0.080	0.032
Mean		48.8	1.88	0.215	1.71	0.22	0.073	0.027

Table 33a. Individual data for rats with intakes of 2P4Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumu-lative wt. gain g	Food consump-tion g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %	Argi-nase[a]/ mg hepatic N units	Argi-nase[a]/ liver units
					(Ad libitum fed group)							
1	1	48	71	255.7	4.19	28.98	6.85	107	28.45	14.69		
2	3	48	69	281.8	5.49	29.06	4.06	·103	29.02	15.30		
3	6	46	86	296.8	4.66	26.73	5.82	118	26.93·	18.24	442	55008
4	7	43	76	299.7	6.08	23.70	7.39	108	27.81	12.77	190	27429
5	8	50	96	323.3	6.55	24.89	5.78	130	29.54	16.26		
6	10	47	80	286.6	4.75	28.17	6.76	116	28.79	15.79		
7	15	49	100	356.6	5.71	27.72	4.54	138	27.07	20.68	324	51259
8	16	49	90	318.8	5.57	26.85	6.72	125	27.80	16.67	292	43665
9	19	46	67	265.8	4.11	28.68	4.67	105	28.34	11.19	217	25592
10	20	51	78	283.9	4.32	26.88	6.69	117	26.40	16.02	180	20894
11	21	53	68	275.5	4.97	25.78	4.78	111	29.12	12.37		
12	23	53	67	269.2	6.12	26.28	5.12	108	24.47	11.08		
Mean		48.6	79.0	292.8	5.21	26.98	5.76	115.5	27.81	15.09	274	35641

[a]Units expressed as μmoles urea formed in 10 minutes at 37°C.

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Table 33a. (Continued)

Right Femurs

Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm³	Width of middia- physis cm	Width of distal epiphysis cm	Dry wt. g	Ash wt. g
1	1	0.430	2.67	0.40	0.30	0.60	0.193	0.086
2	3	0.374	2.50	0.31	0.26	0.60	0.180	0.087
3	6	0.436	2.59	0.40	0.30	0.54	0.206	0.093
4	7	0.388	2.54	0.32	0.30	0.60	0.181	0.089
5	8	0.487	2.74	0.40	0.32	0.64	0.233	0.113
6	10	0.449	2.60	0.40	0.32	0.60	0.218	0.087
7	15	0.476	2.64	0.40	0.32	0.61	0.224	0.104
8	16	0.479	2.64	0.40	0.31	0.64	0.216	0.100
9	19	0.427	2.58	0.38	0.28	0.60	0.196	0.089
10	20	0.402	2.52	0.35	0.29	0.60	0.187	0.090
11	21	0.444	2.60	0.40	0.29	0.64	0.207	0.096
12	23	0.430	2.60	0.39	0.29	0.59	0.188	0.085
Mean		0.435	2.60	0.38	0.30	0.60	0.202	0.093

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Table 33b. Individual data for rats with intakes of 2P4Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumu- lative wt. gain g	Food consump- tion g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %	Argi- nase[a]/ mg hepatic N units	Argi- nase[a]/ liver units
						(Meal-fed group)						
13	4	49	64	175.9	5.26	30.40	3.35	102	29.61	9.93		
14	5	45	86	210.8	6.30	26.73	3.56	117	29.28	7.18		
15	6	50	93	233.6	6.42	28.09	3.89	128	31.76	8.97	198	35665
16	7	48	80	227.5	5.26	29.14	2.91	112	30.04	6.73	233	35703
17	11	48	70	186.3	5.02	28.02	4.31	106	31.00	5.62		
18	12	53	61	181.7	6.20	24.99	3.42	102	30.94	7.70		
19	15	39	65	171.7	4.39	29.12	3.79	91	31.45	5.80	169	21646
20	16	43	77	197.2	5.47	29.22	3.22	105	30.33	9.19	371	50302
21	19	56	76	198.3	6.17	28.50	2.58	119	39.30	10.96	360	63312
22	20	52	83	221.4	6.55	26.33	3.00	121	28.69	8.14	158	27342
23	22	51	59	163.0	5.29	26.57	4.44	100	31.20	7.92		
Mear.		48.5	74.0	197.0	5.67	27.92	3.50	109.4	31.24	8.01	248	38950

[a]Units expressed as μmoles urea formed in 10 minutes at 37°C.

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Table 33b. (Continued)

Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm³	Width of middia-physis cm	Width of distal epiphysis cm	Dry wt. g	Ash wt. g
13	4	0.453	2.60	0.39	0.28	0.60	0.214	0.104
14	5	0.462	2.61	0.40	0.35	0.62	0.210	0.101
15	6	0.515	2.74	0.45	0.32	0.65	0.244	0.125
16	7	0.451	2.60	0.38	0.30	0.60	0.200	0.095
17	11	0.413	2.53	0.32	0.30	0.60	0.188	0.093
18	12	0.446	2.60	0.35	0.30	0.60	0.216	0.102
19	15	0.384	2.47	0.35	0.29	0.60	0.171	0.080
20	16	0.429	2.57	0.40	0.28	0.58	0.205	0.100
21	19	0.519	2.68	0.42	0.31	0.61	0.246	0.108
22	20	0.511	2.60	0.40	0.34	0.64	0.233	0.113
23	22	0.429	2.58	0.38	0.30	0.60	0.197	0.091
Mean		0.456	2.60	0.38	0.31	0.61	0.211	0.101

Right Femurs

Table 34a. Individual data for rats with intakes of 2P2Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumulative wt. gain g	Food consumption g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %	Arginase[a]/mg hepatic N units	Arginase[a]/liver units
						(Ad libitum fed group)						
24	2	50	93	313.5	7.04	25.85	10.74	128	25.64	15.69	216	39361
25	3	44	67	245.0	5.89	20.81	3.88	100	25.47	9.65	99	12129
26	4	48	102	331.4	8.03	26.71	10.90	135	27.40	18.50		
27	7	46	104	358.6	6.93	24.90	6.48	134	27.60	17.24		
28	8	44	83	292.6	6.37	25.30	4.16	116	27.88	11.22		
29	9	46	61	253.3	4.84	26.00	6.26	93	31.53	9.82		
30	10	55	104	334.6	6.81	24.70	6.02	145	25.73	18.08	278	46762
31	13	42	102	339.0	5.95	27.60	5.45	133	27.93	16.48	346	56870
32	14	47	92	316.3	6.96	29.08	3.96	127	26.84	17.51		
33	15	43	75	280.4	4.23	29.74	5.34	108	30.12	13.73	339	42600
34	18	55	92	331.9	6.42	29.95	6.06	132	28.58	12.95	376	72251
35	20	59	99	363.0	7.69	26.24	6.76	145	29.91	13.95		
Mean		48.2	89.5	313.3	6.43	26.41	6.33	124.7	27.88	14.57	276	44996

[a] Units expressed as μmoles urea formed in 10 minutes at 37°C.

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Table 34a. (Continued)

Right Femurs

Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm^3	Width of middia-physis cm	Width of distal epiphysis cm	Dry wt. g	Ash wt. g
24	2	0.449	2.54	0.40	0.32	0.64	0.186	0.073
25	3	0.395	2.50	0.38	0.30	0.54	0.150	0.056
26	4	0.431	2.65	0.38	0.29	0.62	0.174	0.071
27	7	0.456	2.70	0.40	0.32	0.65	0.174	0.067
28	8	0.442	2.60	0.36	0.29	0.58	0.131	0.046
29	9	0.379	2.47	0.38	0.28	0.59	0.157	0.058
30	10	0.382	2.55	0.37	0.31	0.60	0.141	0.051
31	13	0.442	2.65	0.40	0.29	0.60	0.168	0.065
32	14	0.432	2.56	0.40	0.30	0.62	0.176	0.060
33	15	0.383	2.53	0.39	0.29	0.60	0.150	0.056
34	18	0.462	2.74	0.41	0.30	0.64	0.190	0.077
35	20	0.509	2.76	0.44	0.34	0.66	0.217	0.084
Mean		0.430	2.60	0.39	0.30	0.61	0.168	0.064

Table 34b. Individual data for rats with intakes of 2P2Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumu-lative wt. gain g	Food consump-tion g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %	Argi-nase[a]/ mg hepatic N units	Argi-nase[a]/ liver units
						(Meal-fed group)						
36	2	50	125	298.6	9.15	28.09	7.68	156	30.39	10.01	237	61000
37	3	44	128	296.1	8.99	26.02	10.70	155	31.12	9.15	249	58186
38	6	51	98	239.0	7.92	26.84	2.28	135	28.93	9.07		
39	10	45	116	265.5	7.44	29.08	4.07	139	30.74	12.78	229	49597
40	11	48	78	193.3	5.79	27.93	3.23	114	29.75	7.32		
41	12	47	87	231.2	6.99	27.80	3.84	122	30.63	6.19		
42	13	43	114	261.7	8.91	26.84	12.80	138	30.45	9.25	162	38764
43	15	44	83	199.8	6.07	29.03	7.03	110	31.90	8.29	227	40040
44	18	50	-3	78.7	2.34	33.12	3.15	40	30.70	3.66		
45	21	50	85	227.1	7.51	27.32	7.09	121	30.03	6.90		
46	22	47	54	163.1	5.53	26.97	6.90	92	29.93	6.51	171	25438
Mean		46.9	87.7	223.1	7.43	28.09	6.25	120.2	30.42	8.10	212	45504

[a] Units expressed as μmoles urea formed in 10 minutes at 37°C.

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Table 34b. (Continued)

					Width of middia-physis cm	Width of distal epiphysis cm		
Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm^3			Dry wt. g	Ash wt. g
36	2	0.516	2.78	0.41	0.34	0.64	0.224	0.103
37	3	0.632	2.83	0.56	0.34	0.68	0.258	0.111
38	6	0.584	2.73	0.51	0.34	0.67	0.248	0.103
39	10	0.506	2.76	0.45	0.31	0.65	0.225	0.103
40	11	0.459	2.64	0.41	0.29	0.61	0.205	0.093
41	12	0.462	2.64	0.40	0.30	0.63	0.200	0.092
42	13	0.475	2.73	0.42	0.32	0.64	0.204	0.091
43	15	0.466	2.67	0.40	0.30	0.64	0.205	0.088
44	18	0.260	2.18	0.22	0.24	0.55	0.115	0.045
45	21	0.471	2.67	0.40	0.31	0.62	0.213	0.098
46	22	0.404	2.50	0.39	0.31	0.60	0.172	0.079
Mean		0.476	2.65	0.42	0.31	0.63	0.206	0.091

Right Femurs

Table 35a. Individual data for rats with intakes of 1P4Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumu-lative wt. gain g	Food consump-tion g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %	Argi-nase[a]/ mg hepatic N units	Argi-nase[a]/ liver units
						(Ad libitum fed group)						
47	1	51	18	196.8	2.43	23.38	7.58	62	26.89	14.07	165	9372
48	2	50	18	203.2	2.22	26.23	5.68	61	26.16	13.97	131	7655
49	5	45	20	192.2	2.72	21.13	8.49	58	25.40	16.14		
50	7	44	18	187.1	2.67	24.02	7.60	53	28.17	14.13		
51	8	47	22	211.8	2.55	22.47	8.52	62	27.03	15.72		
52	9	45	21	181.8	2.72	22.58	6.02	59	26.33	14.48	46	2821
53	13	44	21	200.9	2.52	23.28	6.17	59	25.30	18.52	106	6245
54	16	55	27	240.0	3.37	20.89	8.86	74	24.14	21.85		
55	17	44	15	172.3	1.98	26.00	6.38	53	26.30	13.10	110	5657
56						discard						
57	19	53	19	205.7	2.52	23.40	11.54	66	26.67	13.83	82	4824
Mean		47.8	19.9	199.2	2.57	23.34	7.68	60.7	26.24	15.58	107	6096

[a]Units expressed as μmoles urea formed in 10 minutes at 37°C.

Table 35a. (Continued)

<u>Right Femurs</u>

Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm^3	Width of middiaphysis cm	Width of distal epiphysis cm	Dry wt. g	Ash wt. g
47	1	0.291	2.20	0.26	0.25	0.58	0.148	0.065
48	2	0.271	2.17	0.29	0.26	0.58	0.133	0.059
49	5	0.283	2.16	0.28	0.24	0.55	0.126	0.054
50	7	0.265	2.20	0.28	0.25	0.56	0.140	0.063
51	8	0.284	2.18	0.26	0.26	0.57	0.147	0.061
52	9	0.264	2.22	0.25	0.24	0.54	0.130	0.056
53	13	0.314	2.14	0.28	0.25	0.55	0.128	0.052
54	16	0.300	2.18	0.21	0.26	0.57	0.154	0.066
55	17	0.254	2.10	0.25	0.24	0.54	0.122	0.052
56				discard				
57	19	0.309	2.30	0.28	0.28	0.56	0.161	0.070
Mean		0.284	2.18	0.26	0.25	0.56	0.139	0.060

Table 35b. Individual data for rats with intakes of 1P4Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumu- lative wt. gain g	Food consump- tion g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %	Argi- nase[a]/ mg hepatic N units	Argi- nase[a]/ liver units
						(Meal-fed group)						
58	1	45	24	153.7	2.33	23.82	7.54	60	27.35	12.53	94	5231
59	2	48	26	167.2	2.84	25.16	7.44	65	28.26	11.66	99	7101
60	6	47	25	160.8	2.82	22.36	11.04	64	26.88	11.55		
61	9	40	20	140.4	2.26	25.52	7.00	52	28.31	9.15	160	9199
62	11	50	19	146.1	2.83	26.70	4.48	61	28.16	8.52		
63	13	46	17	133.3	2.22	25.36	8.00	59	27.88	8.16	75	4229
64	14	42	18	132.8	2.35	25.69	4.84	53	26.24	10.29		
65	17	48	27	185.3	2.52	23.40	13.48	69	27.66	11.60	72	4242
66	19	50	14	135.5	2.22	23.18	8.54	59	28.30	9.70	26	1332
67	20	47	18	136.2	2.43	24.50	4.98	58	28.87	9.10		
68	22	45	17	137.9	2.30	25.21	5.92	56	28.82	8.07		
Mean		46.2	20.4	148.1	2.46	24.77	7.57	59.6	27.88	10.03	88	5222

[a]Units expressed as μmoles urea formed in 10 minutes at 37°C.

Table 35b. (Continued)

					Right Femurs			
Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm^3	Width of middia- physis cm	Width of distal epiphysis cm	Dry wt. g	Ash wt. g
58	1	0.310	2.25	0.29	0.25	0.57	0.153	0.065
59	2	0.344	2.30	0.30	0.28	0.57	0.166	0.071
60	6	0.344	2.33	0.29	0.27	0.58	0.162	0.065
61	9	0.190	2.11	0.24	0.24	0.53	0.125	0.055
62	11	0.331	2.28	0.28	0.28	0.57	0.165	0.070
63	13	0.302	2.25	0.28	0.26	0.55	0.137	0.058
64	14	0.276	2.08	0.24	0.26	0.55	0.140	0.055
65	17	0.309	2.24	0.30	0.25	0.57	0.139	0.061
66	19	0.298	2.25	0.30	0.27	0.55	0.144	0.059
67	20	0.287	2.25	0.26	0.25	0.57	0.145	0.062
68	22	0.289	2.22	0.26	0.26	0.55	0.141	0.059
Mean		0.298	2.23	0.28	0.26	0.56	0.147	0.062

Table 36a. Individual data for rats with intakes of 1P2Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumu-lative wt. gain g	Food consump-tion g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %	Argi-nase[a]/ mg hepatic N units	Argi-nase[a]/ liver units
					(Ad libitum group)							
69	1	47	23	208.8	2.68	22.36	9.92	63	27.64	14.03		
70	4	44	28	215.3	2.99	24.48	7.24	64	24.97	14.43	207	15158
71	5	54	19	213.9	2.42	24.70	8.85	68	27.66	13.30	206	12298
72	9	43	24	187.2	2.28	25.64	9.53	60	25.36	16.80	131	7672
73	10	49	24	227.3	2.26	25.30	8.96	66	26.32	17.60		
74	12	47	18	193.2	1.99	28.12	10.62	60	26.39	14.73	175	9801
75	13	43	22	194.0	3.60	15.75	8.21	61	26.15	18.35		
76	16	47	23	200.1	3.55	19.06	16.70	64	25.49	17.48		
77	20	48	23	213.4	2.82	22.98	10.54	65	28.60	16.88		
78	21	46	26	218.1	3.02	22.28	10.38	66	28.30	13.67	34	2259
79	23	55	26	232.7	4.28	22.70	7.52	72	26.69	16.69	169	16462
Mean		47.5	23.3	209.4	2.90	23.03	9.86	64.4	26.69	15.81	154	10608

[a]Units expressed as μmoles urea formed in 10 minutes at 37°C.

Table 36a. (Continued)

Right Femurs

Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm^3	Width of middia-physis cm	Width of distal epiphysis cm	Dry wt. g	Ash wt. g
69	1	0.326	2.30	0.29	0.26	0.57	0.138	0.047
70	4	0.264	2.20	0.27	0.25	0.56	0.116	0.046
71	5	0.282	2.30	0.27	0.26	0.57	0.125	0.050
72	9	0.234	2.12	0.24	0.24	0.50	0.109	0.040
73	10	0.276	2.23	0.28	0.25	0.55	0.120	0.045
74	12	0.256	2.20	0.24	0.28	0.51	0.126	0.045
75	13	0.249	2.14	0.26	0.23	0.50	0.102	0.038
76	16	0.283	2.23	0.25	0.23	0.55	0.116	0.041
77	20	0.284	2.24	0.26	0.25	0.56	0.130	0.050
78	21	0.294	2.26	0.27	0.28	0.57	0.136	0.051
79	23	0.323	2.34	0.29	0.28	0.56	0.153	0.057
Mean		0.279	2.23	0.26	0.26	0.54	0.125	0.046

Table 36b. Individual data for rats with intakes of 1P2Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumulative wt. gain g	Food consumption g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %	Arginase[a]/ mg hepatic N units	Arginase[a]/ liver units
					(Meal-fed group)							
80	3	44	31	164.3	3.54	25.79	5.57	66	28.51	13.30		
81	4	47	30	145.0	3.20	27.02	4.08	69	28.58	12.30	325	28101
82	5	52	30	160.0	2.64	24.79	9.22	73	28.04	14.03	138	9066
83	6	45	42	202.3	3.54	23.13	7.74	79	26.29	16.41		
84	9	46	30	172.3	2.56	24.92	5.30	69	29.48	8.73	141	9017
85	11	45	31	178.6	2.67	24.16	4.28	69	28.78	11.18		
86	12	46	38	194.6	3.22	24.79	5.48	75	28.25	13.26	129	10287
87	14	45	34	187.1	3.90	25.06	4.00	71	28.38	12.20		
88	17	51	40	201.2	3.68	25.80	5.56	82	28.70	14.03		
89	19	50	23	149.0	3.00	21.75	8.53	67	27.98	11.81	59	3829
90	21	46	19	130.9	2.87	27.10	7.90	58	28.65	8.29	171	13287
91	23	51	-7	76.2	2.12	31.80	2.91	39	31.82	2.60		
Mean		47.0	28.4	163.4	3.16	25.51	5.88	68.1	28.62	11.51	160	12264

[a] Units expressed as μmoles urea formed in 10 minutes at 37°C.

Table 36b. (Continued)

					Right Femurs			
Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm^3	Width middia- physis cm	Width of distal epiphysis cm	Dry wt. g	Ash wt. g
80	3	0.330	2.28	0.29	0.26	0.57	0.140	0.051
81	4	0.310	2.32	0.26	0.25	0.57	0.146	0.058
82	5	0.349	2.30	0.28	0.28	0.57	0.152	0.055
83	6	0.341	2.37	0.28	0.27	0.59	0.156	0.062
84	9	0.303	2.25	0.29	0.25	0.55	0.135	0.057
85	11	0.300	2.27	0.28	0.27	0.55	0.140	0.063
86	12	0.340	2.38	0.29	0.28	0.58	0.166	0.068
87	14	0.304	2.25	0.28	0.25	0.58	0.140	0.058
88	17	0.335	2.37	0.29	0.28	0.58	0.158	0.065
89	19	0.303	2.30	0.25	0.27	0.57	0.141	0.057
90	21	0.294	2.20	0.28	0.24	0.55	0.127	0.054
91	23	0.242	2.06	0.20	0.25	0.50	0.114	0.038
Mean		0.312	2.28	0.27	0.26	0.56	0.143	0.057

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Table 37a. Individual data for rats with intakes of 1P1Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumu- lative wt. gain g	Food consump- tion g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %
				(Ad libitum fed group)						
92	2	39	15	158.5	2.74	17.78	15.36	48	24.31	15.05
93	5	52	10	177.5	2.32	24.26	7.23	55	29.36	10.80
94	7	50	13	191.0	2.84	21.32	9.96	54	27.49	12.83
95	8	44	11	165.6	2.24	21.74	5.76	49	27.78	13.55
96	10	47	16	211.2	2.12	24.34	10.26	56	28.82	13.01
97	14	46	21	212.6	3.24	19.84	10.18	60	25.35	21.09
98	16	52	8	164.8	2.24	22.24	10.26	54	26.21	15.34
99	17	42	23	201.5	3.55	18.08	22.08	59	25.85	16.80
100	18	59	22	237.6	3.60	19.97	10.76	73	26.89	19.11
101	21	47	17	195.9	2.97	17.44	24.63	57	28.10	15.68
102	22	50	12	198.1	2.77	19.86	12.06	55	26.84	15.35
103	23	44	-5	85.5	2.04	23.12	3.69	34	29.57	5.62
Mean		47.7	13.6	183.3	2.78	20.83	11.85	54.5	27.22	14.52

Table 37a.   (Continued)

				Right Femurs				
Rat no.	Litter no.	Fresh wt. g	Length cm	Volume cm^3	Width middia-physis cm	Width of distal epiphysis cm	Dry wt. g	Ash wt. g
92	2	0.243	2.00	0.26	0.23	0.55	0.103	0.035
93	5	0.269	2.24	0.27	0.23	0.55	0.120	0.040
94	7	0.261	2.20	0.26	0.27	0.54	0.120	0.041
95	8	0.267	2.18	0.26	0.25	0.55	0.110	0.032
96	10	0.248	2.10	0.24	0.24	0.53	0.116	0.046
97	14	0.256	2.07	0.25	0.25	0.55	0.122	0.034
98	16	0.247	2.14	0.26	0.25	0.56	0.113	0.035
99	17	0.248	2.15	0.25	0.24	0.55	0.106	0.038
100	18	0.267	2.19	0.27	0.26	0.54	0.119	0.043
101	21	0.314	2.15	0.28	0.25	0.56	0.102	0.033
102	22	0.315	2.20	0.25	0.25	0.57	0.120	0.039
103	23	0.190	1.93	0.19	0.20	0.48	0.086	0.028
Mean		0.260	2.13	0.25	0.24	0.54	0.111	0.037

Table 37b. Individual data for rats with intakes of 1P1Ca in Experiment II

Rat no.	Litter no.	Initial wt. g	Cumu-lative wt. gain g	Food consump-tion g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %
				(Meal-fed group)						
104	1	44	27	149.3	3.75	21.74	4.41	62	29.51	10.49
105	3	52	35	179.7	4.45	23.66	6.50	79	28.21	13.64
106	4	52	21	151.8	3.36	24.06	5.41	65	28.44	10.23
107	8	45	36	194.3	2.90	28.27	7.93	70	30.83	9.39
108	11	49	40	217.4	3.12	26.00	5.69	81	28.06	9.77
109	12	47	25	153.7	3.02	22.08	9.57	66	30.79	11.04
110	14	42	35	169.4	3.33	26.44	5.28	68	28.34	16.54
111	15	41	25	153.5	2.74	22.72	6.62	59	28.80	10.63
112	17	47	37	198.2	4.18	23.81	4.10	76	27.19	11.23
113	18	40	6	82.6	2.55	28.87	3.42	40	28.06	5.20
114	22	47	29	167.6	3.04	23.17	7.48	71	26.79	10.49
115	23	56	25	157.8	3.74	25.69	4.20	73	27.53	9.58
Mean		46.8	28.4	164.6	3.42	24.71	5.88	67.5	28.55	10.68

Table 37b. (Continued)

<table>
<tr><td colspan="9" align="center">Right Femurs</td></tr>
<tr>
<th>Rat
no.</th>
<th>Litter
no.</th>
<th>Fresh
wt.
g</th>
<th>Length
cm</th>
<th>Volume
cm^3</th>
<th>Width
middia-
physis
cm</th>
<th>Width of
distal
epiphysis
cm</th>
<th>Dry
wt.
g</th>
<th>Ash
wt.
g</th>
</tr>
<tr><td>104</td><td>1</td><td>0.266</td><td>2.19</td><td>0.26</td><td>0.20</td><td>0.56</td><td>0.115</td><td>0.037</td></tr>
<tr><td>105</td><td>3</td><td>0.356</td><td>2.39</td><td>0.30</td><td>0.20</td><td>0.59</td><td>0.152</td><td>0.047</td></tr>
<tr><td>106</td><td>4</td><td>0.299</td><td>2.35</td><td>0.26</td><td>0.24</td><td>0.58</td><td>0.134</td><td>0.052</td></tr>
<tr><td>107</td><td>8</td><td>0.283</td><td>2.29</td><td>0.25</td><td>0.23</td><td>0.57</td><td>0.116</td><td>0.043</td></tr>
<tr><td>108</td><td>11</td><td>0.322</td><td>2.37</td><td>0.30</td><td>0.28</td><td>0.56</td><td>0.133</td><td>0.050</td></tr>
<tr><td>109</td><td>12</td><td>0.287</td><td>2.30</td><td>0.29</td><td>0.25</td><td>0.54</td><td>0.126</td><td>0.043</td></tr>
<tr><td>110</td><td>14</td><td>0.278</td><td>2.18</td><td>0.30</td><td>0.23</td><td>0.56</td><td>0.123</td><td>0.036</td></tr>
<tr><td>111</td><td>15</td><td>0.265</td><td>2.15</td><td>0.25</td><td>0.27</td><td>0.52</td><td>0.108</td><td>0.037</td></tr>
<tr><td>112</td><td>17</td><td>0.292</td><td>2.30</td><td>0.29</td><td>0.28</td><td>0.58</td><td>0.130</td><td>0.043</td></tr>
<tr><td>113</td><td>18</td><td>0.235</td><td>2.09</td><td>0.25</td><td>0.23</td><td>0.50</td><td>0.112</td><td>0.039</td></tr>
<tr><td>114</td><td>22</td><td>0.321</td><td>2.38</td><td>0.30</td><td>0.27</td><td>0.57</td><td>0.134</td><td>0.046</td></tr>
<tr><td>115</td><td>23</td><td>0.337</td><td>2.40</td><td>0.28</td><td>0.28</td><td>0.57</td><td>0.146</td><td>0.052</td></tr>
<tr><td>Mean.</td><td></td><td>0.295</td><td>2.28</td><td>0.28</td><td>0.25</td><td>0.56</td><td>0.127</td><td>0.044</td></tr>
</table>

Table 38. Individual data for rats, zero-time control group in Experiment II

Rat no.	Litter no.	Initial wt. g	Liver wt. g	Hepatic N conc. mg/g	Hepatic fat conc. %	Carcass wt. g	Carcass N conc. mg/g	Carcass fat conc. %
116	1	42	1.92	27.36	4.84	34	26.28	3.65
117	2	49	1.64	29.31	4.28	41	25.46	10.07
118	3	53	2.00	32.66	4.40	44	23.24	6.51
119	4	51	1.71	31.15	5.78	44	23.96	8.19
120	5	48	1.82	31.64	4.84	40	25.87	5.21
121	6	43	1.55	30.78	5.37	36	25.04	4.51
122	7	45	1.62	31.50	4.38	36	25.36	5.16
123	8	54	2.15	33.27	4.04	43	23.25	5.50
124	9	44	1.58	31.90	3.99	37	24.80	4.06
125	10	47	1.48	36.72	4.92	39	25.00	5.22
126	11	46	1.57	33.99	3.50	36	26.45	3.37
127	12	49	1.88	27.60	4.24	40	27.45	4.25
128	13	45	1.70	28.92	3.38	37	23.57	7.47
129	14	46	1.73	29.81	3.25	37	24.81	6.21
130	15	41	1.63	27.50	4.05	33	23.90	4.67
131	16	44	1.70	25.92	3.29	36	25.77	6.98
132	17	47	1.66	30.42	4.09	38	25.74	8.84
133	18	48	2.10	32.64	3.82	38	31.18	7.05
134	19	49	1.82	32.65	4.83	41	25.70	5.42
135	20	56	1.90	38.42	4.48	48	25.81	5.62
136	21	43	1.42	33.30	3.80	35	26.83	4.85
137	22	52	2.00	30.84	4.52	43	26.05	4.95
138	23	53	1.91	33.66	4.53	44	26.72	6.72
Mean		48.0	1.76	31.39	4.29	39.1	25.58	5.85

			Right Femurs			
Fresh wt. g	Length cm	Volume cm^3	Width middia-physis cm	Width distal epiphysis cm	Dry wt. g	Ash wt. g
0.192	1.70	0.21	0.25	0.52	0.067	0.023
0.225	1.73	0.21	0.28	0.55	0.079	0.028
0.244	1.82	0.24	0.27	0.56	0.080	0.030
0.246	1.76	0.21	0.28	0.56	0.087	0.037
0.219	1.64	0.20	0.25	0.55	0.077	0.028
0.214	1.73	0.22	0.24	0.54	0.071	0.024
0.199	1.72	0.21	0.21	0.52	0.068	0.024
0.207	1.73	0.21	0.26	0.54	0.076	0.031
0.226	1.77	0.23	0.23	0.54	0.076	0.026
0.248	1.77	0.22	0.23	0.54	0.080	0.027
0.221	1.70	0.20	0.24	0.53	0.076	0.027
0.261	1.82	0.21	0.23	0.55	0.088	0.032
0.222	1.65	0.20	0.26	0.54	0.071	0.023
			Broken			
0.209	1.65	0.21	0.22	0.53	0.077	0.027
0.225	1.68	0.22	0.24	0.54	0.073	0.023
0.228	1.69	0.20	0.23	0.54	0.066	0.022
0.209	1.66	0.20	0.23	0.53	0.103	0.034
0.250	1.77	0.21	0.25	0.54	0.082	0.030
0.263	1.78	0.25	0.28	0.59	0.086	0.036
0.226	1.68	0.21	0.20	0.52	0.071	0.028
0.268	1.77	0.24	0.27	0.56	0.085	0.030
0.268	1.83	0.28	0.26	0.54	0.087	0.033
0.230	1.73	0.22	0.24	0.54	0.078	0.028

Table 39. Least squares analysis of variance

Source of variation	d.f.	M.S.	F	d.f.	M.S.	F
		Energy intake			Nitrogen intake	
Total reduction	31	186026.14	10.06**	31	5661053.70	25.53**
Mean	1	60935.55	3.29*	1	5189577.66	23.40**
Calcium (Ca)	2	102538.39	5.54**	2	939667.71	4.24*
Protein (Pro)	1	1797134.86	97.14**	1	115290334.90	519.96**
Feeding (F)	1	1142657.25	61.76**	1	1814331.85	8.18**
Litters	22	22614.99	1.22*	22	297911.75	1.34*
Ca x Pro	1	2981.83	0.16	1	285842.78	1.29
Ca x F	2	40681.65	2.20	2	251591.14	1.14
Pro x F	1	166099.91	8.98**	1	4059.35	0.02
Remainder	83	18500.60		83	221729.95	
		FER			NER	
Total reduction	31	0.0426	23.59**	31	59.9135	5.98**
Mean	1	0.0328	18.16**	1	8.7612	0.87
Calcium (Ca)	2	0.0034	1.88	2	34.5377	3.44
Protein (Pro)	1	0.7589	420.59**	1	558.9602	55.76**
Feeding (F)	1	0.1458	80.80**	1	11.7359	1.17
Litters	22	0.0039	2.18**	22	22.7596	2.27**
Ca x Pro	1	0.0022	1.20	1	42.4022	4.23*
Ca x F	2	0.0046	2.53	2	94.8294	9.46**
Pro x F	1	0.0124	6.86*	1	16.3582	1.63
Remainder	83	0.0018		83	10.0246	

**Significant at 1% level.

*Significant at 5% level.

Table 39. (Continued)

Source of variation	d.f.	M.S.	F	d.f.	M.S.	F
		Liver nitrogen (conc.)			**Liver fat (conc.)**	
Total reduction	31	21.6786	4.60**	31	24.1962	2.96**
Mean	1	0.5683	0.12	1	0.0336	0.00
Calcium (Ca)	2	26.0915	5.54**	2	29.4500	3.60*
Protein (Pro)	1	175.9401	37.35**	1	83.8615	10.24**
Feeding (F)	1	107.7147	22.87**	1	165.1440	20.18**
Litters	22	5.4964	1.17	22	10.2816	1.26
Ca x Pro	1	1.0995	0.23	1	7.4425	0.91
Ca x F	2	9.8043	2.08	2	25.3953	3.10*
Pro x F	1	0.7317	0.16	1	0.8530	0.10
Remainder	83	4.7105		83	8.1852	
		Carcass nitrogen (conc.)			**Carcass fat (conc.)**	
Total reduction	31	9.5346	4.22**	31	40.5661	6.87**
Mean	1	6.8762	3.04	1	7.0720	1.20
Calcium (Ca)	2	3.1186	1.38	2	3.4592	0.59
Protein (Pro)	1	75.0218	33.22**	1	72.5908	12.30**
Feeding (F)	1	107.3984	47.56**	1	595.5947	100.91**
Litters	22	2.7137	1.20	22	14.1016	2.39**
Ca x Pro	1	2.6203	1.16	1	15.7364	2.67
Ca x F	2	1.7573	0.78	2	8.6925	1.47
Pro x F	1	7.6273	3.38*	1	15.2274	2.58
Remainder	83	2.2581		83	5.9025	

Table 39. (Continued)

Source of variation	d.f.	M.S.	F	d.f.	M.S.	F
		Femur weight			Femur length	
Total reduction	31	0.0256	14.14**	31	0.1474	16.87**
Mean	1	0.0167	9.25**	1	0.1061	12.14**
Calcium (Ca)	2	0.0021	1.16	2	0.0284	3.25
Protein (Pro)	1	0.4625	255.75**	1	2.7923	319.62**
Feeding (F)	1	0.0193	10.67**	1	0.0847	9.70**
Litters	22	0.0025	1.37	22	0.0098	1.13
Ca x Pro	1	0.0007	0.36	1	0.0002	0.02
Ca x F	2	0.0012	0.64	2	0.0217	2.49
Pro x F	1	0.0006	0.31	1	0.0079	0.91
Remainder	83	0.0018		83	0.0087	
		Femur dry weight			Femur ash weight	
Total reduction	31	0.0047	12.24**	31	0.0017	19.39**
Mean	1	0.0013	3.39	1	0.0002	1.97
Calcium (Ca)	2	0.0029	7.53**	2	0.0030	33.56
Protein (Pro)	1	0.0655	169.03**	1	0.0184	208.01
Feeding (F)	1	0.0068	17.41**	1	0.0027	30.70
Litters	22	0.0004	1.02	22	0.0001	0.96
Ca x Pro	1	0.0001	0.33	1	0.0003	3.82
Ca x F	2	0.0010	2.69	2	0.0006	6.40
Pro x F	1	0.0008	1.97	1	0.0007	7.63
Remainder	83	0.0004		83	0.0001	

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Table 39. (Continued)

Source of variation	d.f.	M.S.	F	d.f.	M.S.	F
		Ash per unit length			**Ash per unit volume**	
Total reduction	31	219.2310	24.86**	31	5849.6101	12.55**
Mean	1	842.3363	95.53**	1	885.7943	1.90
Calcium (Ca)	2	610.1542	69.20**	2	43017.8505	92.25**
Protein (Pro)	1	1257.8618	142.66**	1	2530.8542	5.43
Feeding (F)	1	319.8565	36.28**	1	10532.5801	22.59**
Litters	22	9.6699	1.10	22	512.2148	1.10
Ca x Pro	1	33.3556	3.78*	1	2398.7964	5.14*
Ca x F	2	84.9225	9.63**	2	5440.4874	11.67**
Pro x F	1	92.8325	10.53**	1	2009.9599	4.31*
Remainder	83	8.8174		83	466.3023	
		Liver % body weight			**Femur % body weight**	
Total reduction	31	2.4777	11.45**	31	0.0075	4.72**
Mean	1	56.4595	260.95**	1	0.0058	3.67*
Calcium (Ca)	2	2.4777	11.45**	2	0.0073	4.61*
Protein (Pro)	1	9.2285	42.65**	1	0.1085	68.26**
Feeding (F)	1	0.4462	2.06	1	0.0002	0.14
Litters	22	0.5175	2.39**	22	0.0026	1.67
Ca x Pro	1	0.1970	0.91	1	0.0018	1.16
Ca x F	2	0.0018	0.01	2	0.0079	4.98**
Pro x F	1	2.5637	11.85**	1	0.0027	1.70
Remainder	83	0.2164		83	0.0016	