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Effects of varying protein and energy value of diets on nitrogen utilization and body composition of adult female rats

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NOTE TO USERS

EFFECTS OF VARYING PROTEIN AND ENERGY VALUE OF DIETS ON NITROGEN UTILIZATION AND BODY COMPOSITION OF ADULT FEMALE RATS

by

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INTRODUCTION

Although the fundamental importance of food energy to protein nutrition has been established, little is known concerning the ratio of protein to food energy at which optimum protein utilization can be achieved when intakes of food energy are low. Such information would be of great value during times of food shortages, in weight reduction programs and in devising military combat rations. Knowledge along this line would be of prime importance during an illness or following an injury and in post-surgical conditions when patients are temporarily unable to ingest an adequate diet. In all these instances, it is desirable to know when the need for calories takes priority over that for protein.

Food shortages prevailed in many parts of the world as a direct result of World War II. Masses of people in war-stricken areas subsisted mainly on cereal grains, potatoes and garden vegetables which provided limited amounts of protein. Severe undernutrition became increasingly prevalent as the war continued. Need for extensive relief feeding and rehabilitation programs was, therefore, inevitable. Since very little information

was available concerning effective relief programs, a group of workers led by Keys (1946, 1950) investigated the physiological and psychological changes occurring during voluntary semi-starvation and rehabilitation. Thirty-two men were subjected to semi-starvation for a period of 6 months and subsequently rehabilitated on different relief diets which provided varying levels of calories, proteins and vitamins. The following conclusions were drawn: (a) calories are of greatest importance in relief feeding of people starved on the European type of famine diet which had been employed; (b) protein and/or vitamin supplementation had little or no effect on the rate of recovery from starvation; (c) from a practical standpoint, any increase in caloric intake from cheap sources such as cereal grains and potatoes, but not refined sugar, is accompanied by a corresponding increase in the protein and vitamin content of the diet; (d) in relief feeding, "quality" of the diet is apt to be secondary to "quantity".

These conclusions are not in agreement with those recorded by Benditt, Woolridge and Stepto (1948). They maintained that although caloric intake is a major factor in the rehabilitation of depleted individuals, it is not the only factor. As a result of studying experimental data compiled from reports of other workers, Benditt and

his co-workers concluded that a high protein intake was merited in the rehabilitation of protein-depleted individuals.

In more fortunate areas of the world like the United States where food shortage is less likely to occur, the most serious nutritional problem is that of "obesity". Current interest in the problem is a direct result of insurance statistics as well as experimental and clinical studies which have indicated that obesity is associated with an increased incidence of degenerative diseases. Since excess overweight essentially results from a caloric imbalance (i.e. caloric intake exceeding energy output), several attempts have been made to devise weight reduction diets which are adequate in all known essential nutrients, and which permit loss of fat without loss of other body reserves.

Survival in certain military situations involving subsistence on a severely reduced food consumption has concerned the Quartermaster Corps for a number of years. Efforts have been directed toward designing a nutritionally adequate "survival" ration which is compact because there are space and weight limitations in military transportation. The problem involves the incorporation of an adequate supply of protein to a ration which provides limited

sources of food energy (Specter, 1952). A study carried out on 10 enlisted men has shown that 43 gms. of protein added to a non-protein ration supplying approximately 900 calories did not improve nitrogen balance but was merely utilized for energy purposes (Quinn et al., 1953). Additional research investigations of a similar nature are needed.

Exposure to trauma, illness or post-operative conditions is associated with significant nitrogen losses (Mulholland et al., 1943; Howard and Parson et al., 1944; Howard and Winternitz et al., 1944; Elman, 1944). The problem of feeding an adequate diet to patients who are temporarily unable to tolerate food has confronted clinicians almost daily. Some authors maintain that a high protein intake is of primary importance for rapid recovery (Elman, 1944; Cannon, 1947), while others believe that the energy demands must first be satisfied if optimum protein utilization is to be accomplished (Van Itallie, 1953). The question of feeding such patients becomes more complex when parenteral alimentation is indicated. According to Van Itallie (1953), parenteral feeding will be effective only if the caloric needs of the patient are met either parenterally or from body stores of fat. With parenteral preparations now available, the demands for energy by

patients cannot be satisfied. Attempts still are being made to develop a suitable concentrated source of calories for parenteral use which could be given to insure maximum utilization of dietary protein for tissue synthesis.

That the total energy intake plays an important role in protein utilization of healthy people is evident from the observations reported by Ohlson and her co-workers (1952) in their study of the nutritional status of 136 women over 30 years of age. Although these women on self-selected diets appeared to be maintaining their weights, the majority of them were in negative nitrogen balance. Average daily nitrogen intakes ranged from 8 to 10 gms. With a consumption of 1200 to 1500 calories, negative retentions occurred despite the seemingly adequate protein intake. Retention of nitrogen improved with progressive increments in the intake of food energy. These observations appear to indicate that nitrogen retention may be dependent on the total energy consumption of an individual.

In view of the findings by Ohlson and her co-workers (1952), it seemed worthwhile to undertake the present investigation using adult female albino rats in an attempt to relate varying levels of dietary protein and calories to nitrogen utilization. An exploratory study had been carried out earlier by Samvik (1953) on adult male albino

rats. Nitrogen balance studies were conducted to provide an overall picture of nitrogen metabolism. Although the information obtained from such studies has proven invaluable for measuring nitrogen utilization, a knowledge of body composition for studying metabolic changes also seemed desirable. The present study provides information about body fat, carcass and liver nitrogen and the activities of certain enzymes in the liver. The enzymes systems investigated were xanthine oxidase, succinic dehydrogenase and cytochrome oxidase.

REVIEW OF LITERATURE

The profound influence exerted by total energy intake on protein metabolism has long been recognized. However, the underlying biochemical mechanism still remains to be clarified. At an extremely low intake of food energy, dietary protein which ordinarily would be utilized for tissue synthesis and repair may be used to meet the energy demands of the organism. Under conditions of caloric inadequacy, the energy and protein intakes must be integrated properly to insure maximal efficiency of dietary protein utilization.

The present review will be limited to studies reported in the literature which provide information about nitrogen utilization at varying intakes of protein and food energy. Experimental evidence, derived from both human and animal studies, has been based on one or more of the following criteria for evaluating protein utilization:

1. growth as a measure of tissue synthesis,
2. maintenance of nitrogen equilibrium,
3. changes in body composition during protein depletion and repletion as reflected by loss or gain in (a) body weight, (b) total plasma protein level,

(c) hemoglobin concentration in the blood, (d) body fat and (e) carcass and liver nitrogen.

Human Studies

The single criterion most often employed for studying nitrogen utilization in human subjects under varying experimental conditions has been the maintenance of nitrogen equilibrium. Relatively few human studies have been carried out to investigate the effects of varying the intake of dietary protein and food energy on nitrogen utilization.

Chittenden (1904) made one of the earliest observations showing that nitrogen equilibrium was possible at a low food energy intake. He restricted his diet to approximately 1600 cal. and 38 gm. of protein daily. On this dietary regime, which he claimed was beneficial to his health, he was able to maintain nitrogen equilibrium for a period of 9 months.

Neumann's (1902) self-experiment which extended over a period of almost 2 years probably represents one of the first attempts to conduct a carefully controlled study with maintenance diets low in proteins and calories. His average body weight and height were 66.5 kg. and 165 cm.

respectively. Data from his experiment have been recalculated for a body weight of 70 kg. by Keys and his co-workers (1950).

The recalculated data showed that Neumann maintained nitrogen equilibrium for a period of 305 days on an average diet containing 2427 cal. and 69.1 gm. of protein. Muscular activity was assumed to be light. During the subsequent experimental period of 66 days, the dietary intakes of protein and calories were increased progressively at 6 different time intervals from 51 gm. of protein and 1535 cal. to 79.5 gm. of protein and 2777 cal. Negative nitrogen balances continued as the protein intake was increased from 51 to 69 gm. and the caloric intake, from 1535 to 1937 cal. At these levels of intake, nitrogen loss was not related to caloric intake. With intakes of 2659 cal. and 76 gm. of protein, nitrogen equilibrium was attained and body weight increased by 1.3 kg. over the initial weight. During the subsequent period of 240 days, Neumann was able to maintain his body weight on a diet supplying 2000 cal. and 74 gm. of protein. According to Keys and his associates, a possible explanation for the apparent difference between this diet and the first maintenance diet may have been a difference in the composition of the diets. The second maintenance diet which supplied 2000

cal. had a higher protein and fat content with lower amounts of carbohydrates and alcohol than the first maintenance diet of 2427 cal. It is also possible that there was a decrease in his energy expenditure although no such change was indicated.

Studies of rehabilitation of undernourished populations have provided valuable information on the problem of protein metabolism under conditions of caloric deficit. Beattie and his co-workers (1947) have studied 6 undernourished Dutch subjects and 11 undernourished German subjects in order to determine the levels of caloric and protein intakes that would secure optimum nitrogen retention and promote rapid weight recovery. Previous dietary history indicated that the German subjects lived on diets which were low in protein and energy value for a period of 1 year. They were in slight negative nitrogen balance when the first observations were made.

The experimental periods were from 10 to 15 days duration. The caloric intake of the Dutch patients ranged from 2860 to 3180 cal. and the nitrogen intake varied from 23.8 to 50.2 gms. The lowest intake the German subjects had was 1700 cal. and 9 gm. of nitrogen and the highest was 2500 cal. and 19 gm. of nitrogen. The average weight changes during each balance period did not exceed 1.5 kgs; in most cases, they were less than 1 kg. Nitrogen

retention was found to be proportional to energy intake. Nitrogen intakes above 0.17 gm. of nitrogen per kg. of body weight per day were associated with positive nitrogen balance provided the daily caloric intakes exceeded 35 cal. per kg. of body weight. With food energy intakes varying from 67.0 to 76.5 cal. per kg. of body weight per day and daily nitrogen intakes ranging from 0.6 to 1.2 gm. of nitrogen per kg. of body weight, nitrogen retention remained constant at 0.23 and 0.24 gm. of nitrogen per kg. of body weight per day. According to the authors, this suggests that a major portion of the dietary protein was being utilized for energy purposes. The most efficient nitrogen retentions were obtained when the proportion of non-protein to protein calories exceeded a 5:1 ratio.

Metabolic studies performed on obese individuals during weight reduction likewise provide some information about the protein-calorie interrelationship when caloric restrictions are imposed. Evans and Strang (1931) reported a study on 5 obese patients with an average initial body weight of 157.5 kgs. At the beginning of the experiment, they received a diet containing 2413 cal. and 69 gms. of protein which maintained nitrogen balance and body weight. During the period of weight reduction, a diet supplying 335 cal. and 59 gms. of protein daily produced a small

nitrogen loss of 2 gms. of nitrogen in 5.5 weeks. By increasing the total energy intake to 445 cal. per day while keeping the protein intake constant, these patients were able to attain nitrogen equilibrium. One of the subjects maintained nitrogen equilibrium for a period of 260 days while losing 67.1 kgs.

Urinary excretion of nitrogen by obese patients on low-calorie diets containing different amounts of protein was reported by Keeton and Dickson (1933). The subjects were either ambulatory or "bed patients". Repeated observations had shown that the basal rates of these subjects were within the normal limits of individuals of the same surface area, sex and age. The authors, therefore chose to express the energy value of the diets in terms of percentages below the normal basal requirement.

The majority of the obese subjects maintained positive nitrogen balance on a diet which supplied 90 gm. of protein per day and on 40 to 50 per cent less calories than their calculated basal requirement. The authors suggested that patients in negative nitrogen balance were less obese than those who were in positive balance. When 13 to 14 gms. of protein were given to these obese patients on a caloric intake 30 per cent below their basal requirements, only a slight negative nitrogen balance was noted. The

findings in this study demonstrate that body fat has a powerful nitrogen-sparing action. In general, the results obtained with obese patients on a very restricted diet are strikingly different from observations made on normal lean subjects during semi-starvation. According to Keys and his associates (1950), normal men appear to require from 2500 to 3000 calories per day in order to maintain nitrogen equilibrium on 60 to 70 grams of protein.

One of several studies on weight reduction in overweight college women was that reported by Brewer and her co-workers (1952) and Cederquist et al. (1952). Two groups of college women were studied in 2 experiments. On self-selected diets, the first group of 4 women had an average daily caloric intake of 2278 cal. and the second group of 10 women received an average of 2710 cal. All subjects retained nitrogen on their protein intakes of 76 to 84 gms. of protein per day. During the weight reduction period of 16 weeks, the first group of women was fed a low-fat diet which supplied an average daily intake of 1200 cal. and 10.5 gm. of nitrogen. The other group received a low-carbohydrate diet which provided 1500 cal. and 16.2 gms. of nitrogen daily. At the end of the experimental period, nitrogen retentions were noted in 3 of the 4 subjects fed the low-fat reducing diet while weight losses

were 0.1 to 7.9 kgs. According to the authors, the proportion of protein to calories in this low-fat diet may be a critical ratio for maintenance of nitrogen balance during weight reduction. The low-carbohydrate, high protein diet which supplied 1500 cal. per day promoted nitrogen retentions in all 10 subjects, while permitting weight losses of 8.8 to 16.8 kgs.

Young (1952a, 1952b) had studied the weight losses and nitrogen response to diets which supplied 1400 cal. and 90 gms. protein during a weight reduction period of eight and one-half weeks. The average weight loss was 2 pounds per week, but two-thirds of the subjects were in negative nitrogen balance during the 7th and 8th week of weight reduction.

Weight reduction on diets supplying approximately 1200 cal. and either 60 or 105 gms. of protein daily was reported by Leverton and Rhodes (1949). The average weekly weight losses on the moderate protein diet and high protein diet were 1.5 and 1.7 pounds respectively indicating no significant difference. The average daily nitrogen retention on the high protein diet and moderate protein diet were 2.44 gms. and 0.31 gms. of nitrogen respectively. In a later report by Leverton and Gram (1951), an average daily nitrogen retention of 0.36 gm. was observed on a

reducing diet which supplied 1200 cal. and 63 gms. of protein daily.

More recently, the effects of protein addition to subcaloric diets were reported by Quinn and his co-workers (1954a). Two groups of healthy adult men were given 43 gms. of protein and 0.17 gm. of protein respectively, 900 cal. and 800 ml. of water daily for a period of 9 days. Metabolic balance studies of nitrogen, sodium, chlorine, potassium and water were made during the experimental period. Body fat was estimated by the skin-fold technique.

The mean body weight loss for the protein and non-protein group was 5.5 and 4.1 kg. respectively and was accompanied by mean body fat losses of 2.7 kg. and 2.0 kg. Both groups were in negative nitrogen balance during the entire period. The average daily negative balance was 6.3 gms. of nitrogen for the protein group and 6.7 gms. for the non-protein group. This difference in nitrogen balance was not considered significant. During the latter part of the experiment, the non-protein group appeared to be losing less nitrogen than the group which received protein in the diet. Added protein, therefore, did not decrease the nitrogen loss during caloric restriction.

A second study was conducted on the same group of subjects provided with liberal water intakes (Quinn et al.,

1954b). It was found that increasing the amounts of water did not decrease the nitrogen loss observed during water restriction.

These observations made by Quinn and his associates indicating that added protein had no beneficial effect on nitrogen balance during caloric restriction are not in conformity with those reported earlier by Schwimmer and McGavack (1948). The latter group of workers were able to decrease the negative nitrogen balance by increasing the daily intake of protein from 18.7 to 45.0 gms. on energy and fluid intakes of 900 cal. and 800 ml. respectively.

Animal Studies

Basic information relating to the metabolic interaction of proteins and calories has been obtained from animal experiments designed to ascertain the extent of tissue synthesis as reflected by growth during calorie insufficiency. Bosshardt and his co-workers (1946) have presented evidence which showed that the efficiency of utilization of dietary protein for growth in rats and mice remained constant with successive reductions in the energy content of the diet until a critical caloric level was

reached. Restriction in the intake of food energy beyond this point resulted in a marked decrease in the efficiency of protein utilization.

These findings were further substantiated by the same group of workers (Bosshardt et al., 1948) in a later study on growing mice receiving low levels of energy intake with varying amounts of protein intake levels. When the caloric intake was reduced while maintaining the protein intake constant, the growth rate decreased as did the efficiency of protein and calorie utilization for growth. Increments in dietary protein intake produced a corresponding increase in growth of animals receiving either an adequate supply of calories or calories restricted to about 50 per cent of the ad libitum intake. Equicaloric replacement of dietary fat or carbohydrates by protein resulted in an increased growth response. The authors concluded that the efficiency of protein utilization of dietary protein at a reduced energy intake was dependent upon the extent of caloric restriction imposed and that within certain limits, increased utilization of dietary protein for growth could be effected by increasing the protein intake without necessarily changing the caloric consumption. If the protein intake was held constant, any increase in the energy intake improved nitrogen utilization. On the other hand, if additional sources of calories were supplied in form

of protein rather than as fat and carbohydrates, the amount of protein utilized for growth purposes correspondingly increased. According to the authors, their findings provide additional evidence of the importance of protein nutrients in conditions of semi-starvation.

Cox and his associates (1953) studied the extent of protein utilization during caloric restriction using growing rats with different nutritional reserves. Animals weighing 100 gms. were treated in 4 different ways for a period of 2 weeks prior to the experimental feeding. One group of rats was depleted by feeding a protein-free diet and a second group was partially starved on a diet which supplied 20 to 22 cal. daily so that body weight was maintained. A third group was fed a stock ration and half of these animals were scalded. Equicaloric quantities of dextrose and of dextrose-protein hydrolysate diets were fed to these groups of animals for 5 weeks. The diets supplied 11, 16, 21, 31 or 51 cal. daily. The intake of the diet supplying 51 cal. was considered ad libitum. When dextrose diets were offered, only those supplying 11 and 16 cal. were completely consumed so that only the 2 lowest levels of caloric intakes were used for comparisons of growth performance.

All the animals fed the dextrose diets lost weight. On the protein-containing diet, there was slight growth

at the 16 cal. intake and weight maintenance at 11 cal. daily in the protein-depleted rats. The partially starved animals showed slight growth at an intake of 21 cal. and lost weight on the 2 lowest levels of caloric intake. Both the stock and the scalded animals lost weight at the 3 lowest levels of caloric intake. At the end of the experiment, the protein-fed rats weighed more than the animals fed dextrose alone regardless of the caloric intake or the initial nutritional state of the animals. Carcass analyses showed that the protein-fed rats had larger stores of body protein than those fed the dextrose diet. At the 3 lowest levels of caloric intake, the amount of carcass fat was not significantly different in all the groups of animals. With a daily intake of 21 cal. or more, the rats fed the protein diet contained more fat in the carcasses than the animals fed dextrose alone. From these findings, the authors inferred that the greater need for protein by the depleted animals as compared to the other 3 groups of animals resulted in increased utilization of dietary protein for growth on a restricted caloric intake of 11 cal. daily (equivalent to 25 per cent of the ad libitum intake).

In the same experiment, when the protein-depleted rats were fed 20 cal. daily as diets containing dextrose or protein hydrolysate alone or various mixtures of

dextrose and protein hydrolysate, the largest weight gains were obtained when 20 per cent of the total calories was fed as protein.

Stevenson and her co-workers (1946) observed that the addition of egg protein to a diet fed to protein-depleted rats caused a marked decrease in the urinary excretion of nitrogen. When the energy value of the diet was successively reduced, the sparing action of egg proteins was lost when the calories were cut to less than 50 per cent of the normal intake.

Further support for the concept that dietary protein can be utilized for tissue synthesis or for maintenance of nitrogen equilibrium even if energy requirements are not fully satisfied has been given by Benditt and his colleagues (Benditt and Humphreys et al., 1948). Groups of protein-depleted adult rats with an average weight of 154 gms. were fed various combinations of protein and calories for a period of 14 days. A half and half mixture of bovine lactalbumin and vitamin-free casein was used as the source of protein. Successive increments in the caloric consumption while maintaining a constant protein level of 1.50 gms. per kg. of body weight per day produced corresponding increases in protein utilization until a peak was reached at an intake of 1240 cal. per square meter of body surface per day. Protein utilization

at the level of 560 cal. per square meter per day was poor.

Varying the protein intake from 2.8 to 14.8 gms. per kg. of body weight per day at a constant energy intake of 1630 cal. per square meter per day increased the utilization of dietary protein. The rate of tissue synthesis, however, gradually diminished beyond an intake of 6 gms. of protein per kg. of body weight per day. Variations in the protein and energy intakes while maintaining the ratio of proteins to calories in the diet constant, resulted in an increased rate of protein utilization with increasing consumption of the diets.

Fat deposition at a constant protein intake but with progressive increases in caloric intake were found to be proportional to the number of calories consumed. On the other hand, when the energy intake was maintained at a constant value, body fat gains increased with increments in the protein intake up to a level of 8 gms. of protein per kg. of body weight per day. Further increments beyond 8 gms. of protein per kg. of body weight per day were associated with a moderate decrease in the amount of fat deposited.

In another study by Benditt, Woolridge and Stepto (1948), the protein levels fed to protein-depleted adult rats were approximately 0, 1, 2, 3 or 4 gms. of protein

per kg. of body weight per day at a daily intake of 48 calories. When these amounts of protein were consumed, the rate of protein utilization for tissue synthesis was proportional to the intake of dietary protein.

The metabolic response to varying levels of protein and energy intake is also partly dependent on the amount of tissue reserves in a normal adult animal. Allison and his associates (1946) have demonstrated that the nitrogen balance index of the dietary protein fed to well-nourished dogs was altered only when the energy content of the diet was reduced to 50 per cent of the normal requirement. In a later report from the same laboratory (Rosenthal and Allison, 1951), normal adult dogs were fed diets containing casein which supplied a constant nitrogen intake of 3.82 gms. per day per square meter of body surface on varying intakes of food energy. Successive reductions in caloric intake from 3,190 to 95 cal. per square meter of body surface per day were associated with corresponding decreases in nitrogen retention. The lowest level of caloric intake that maintained nitrogen equilibrium in dogs was approximately 2,290 cal. per square meter of body surface per day. The response to caloric restriction varied with the amount of tissue reserves in the body of the animals. One dog with an ample supply of body stores of nitrogen was in acute nitrogen balance when caloric restriction was imposed,

then approached nitrogen equilibrium. Another dog whose initial body protein reserve was low, retained nitrogen at the beginning of caloric restriction, but this was succeeded by gradually increasing loss of nitrogen.

Evidence was also presented by Elman and his co-workers (1945) that healthy dogs on restricted caloric intakes of either 25 or 50 cal. per day per kg. body weight were able to maintain nitrogen equilibrium when the proportion of the protein to carbohydrate content of the diet was 4 to 1 but not when it was 1 to 4.

The influence of caloric restriction on nitrogen metabolism was studied by Calloway and Spector (1953) using adult rats that had been standardized on one of 4 different diets, namely: (a) commercial stock diet, (b) commercial stock diet plus sucrose, (c) purified diet containing 28 per cent casein, and (d) purified diet containing 18 per cent casein. All animals maintained their body weight at 300 gms. when 46 cal. was fed to them. At this level of energy intake, the commercial stock diet and the purified diet containing 28 per cent casein each supplied 450 mgs. of nitrogen daily. The commercial stock diet with added sucrose and the purified diet containing 18 per cent casein each supplied 270 mgs. of nitrogen daily. All the standardized animals were in positive

nitrogen balance and their nitrogen stores in the liver, plasma and carcass were comparable. During the subsequent period of 12 days, the energy intake was reduced to 50 per cent of the maintenance level. A high fat-egg albumin diet supplying 160 mgs. of nitrogen daily was fed to the animals. The animals which were pre-fed commercial rations showed greater body weight and nitrogen losses during the early phase of caloric restriction than those given either casein diets. However, the differences in response between these groups of animals diminished toward the latter part of the experimental period.

In another experiment of a similar nature, Spector and Calloway (1953) investigated the effects of varying intakes of nitrogen on protein utilization in adult rats standardized on either a commercial stock ration or a purified diet containing 18 per cent casein. Nitrogen levels ranging from 0 to 160 mgs. daily were employed when the caloric intake was reduced to half the maintenance energy requirement. The animals which were standardized on the commercial ration lost 11 per cent of their body weights during 4 days of caloric restriction while those pre-fed the casein diet lost 7 per cent. In both instances, body weight losses were found to be independent of nitrogen intake during restricted feeding. Nitrogen losses decreased with increments in nitrogen intake but none of

the animals attained nitrogen equilibrium. Variations in dietary nitrogen intake during restriction did not influence liver nitrogen losses in the animals standardized on commercial ration. On the other hand, liver nitrogen losses in animals pre-fed a purified casein diet were found to be inversely proportional to the level of nitrogen in the diet during restricted feeding.

That protein metabolism is in a "state of dynamic equilibrium with energy intake" even when the animals are in nitrogen balance was shown by Munro and Naismith (1953). Adult male rats were fed either a protein-containing or a protein-deficient diet in combination with varying levels of energy intake ranging from 850 to 1700 calories per square meter of body surface per day for 4 days following a 7-day adjustment period. Body weight changes in the presence or absence of dietary protein were found to be directly related to the energy intake. However, the caloric intake exerted a greater influence on body weight in the presence of adequate amounts of protein in the diet than in the absence of dietary protein.

A linear relationship between nitrogen balance and energy intake, likewise, was demonstrated when the diet contained protein. The total amount of liver nitrogen also increased with increasing levels of energy intake.

In the absence of dietary protein, neither the nitrogen balance nor the total liver nitrogen was directly related to caloric intake.

According to the authors, their findings appear to indicate that the influence of protein and energy intake on nitrogen metabolism operates through a common biochemical mechanism, namely, their effect on protein synthesis. It was suggested that in the absence of dietary protein, the supply of amino acids from endogenous sources becomes the limiting factor in the rate of protein synthesis when the energy intake is low. This limitation no longer holds when adequate amounts of protein are included in the diet and energy intake then influences the rate of protein synthesis.

The present investigation on the interrelationship between proteins and calories is an extension of the exploratory work conducted by Samvik (1953) at the Iowa State College nutrition laboratory. Adult male albino rats at 4 and 1/2 to 5 months of age were employed to study the effects of variations in dietary protein and calories on the maintenance of nitrogen balance. Three different diets supplying 15, 10 and 5 per cent of the energy value of the diet as lactalbumin were fed <u>ad libitum</u> to groups of animals. As the animals approached nitrogen equilibrium,

caloric restriction was imposed by reducing the energy intake to two-thirds of the ad libitum intake while the nitrogen intakes were maintained. When the nitrogen balance was achieved at two-thirds of the ad libitum intake, the caloric intake was reduced to one-third of the normal voluntary intake, but the nitrogen intake remained unchanged.

Body weight changes appeared to be directly related to protein intake during ad libitum feeding. When caloric restriction was imposed, weight losses seemed to increase with increments in nitrogen consumption. A linear relationship between body weight and nitrogen balance was noted. The degree of negative nitrogen balance following calorie reduction appeared to be of greater magnitude in animals which received higher amounts of protein in the diet during ad libitum feeding than those pre-fed lower levels of protein. Carcass nitrogen was found to be directly related to nitrogen intake, but no significant effect on liver nitrogen was noted. The hemoglobin concentration in the blood was lower than normal at the end of the experiment.

Most of the literature pertaining to liver enzyme systems relate enzyme activity to alterations in dietary protein or to inanition. That liver protein and the

concomitant production of hepatic enzymes are readily affected by variations in dietary protein intake have been repeatedly demonstrated. Early investigations reported by Addis and co-workers (1936a, 1936b) on rats during inanition have shown that the liver loses its protein content more rapidly than any other organs studied.

The depletion of protein "reserves" in the liver is associated with a considerable reduction in metabolic activity as a consequence of the loss in protein which comprises enzyme systems. Miller (1948) has demonstrated that the decrease in liver enzyme activity during inanition may be attributed to a loss in enzyme protein *per se* rather than to a diminution in available prosthetic groups or enzyme activators or to an accumulation of enzyme inhibitors. He measured the activities of various enzyme systems in the liver of adult albino rats following a 7-day period of fasting. A decrease was noted in the unit activites of catalase, alkaline phosphatase, xanthine dehydrogenase and cathepsin accompanied by a simultaneous reduction in the liver protein.

The study was later extended to include rats maintained on either a high or low protein-containing ration for 21 to 23 days (Miller, 1950). In addition to the enzyme systems previously investigated, the unit activity

of arginase was also determined. Loss in enzyme activity was associated with a decrease in liver protein of the animals which received low protein diets. Realimentation using a control diet containing 25 per cent casein immediately restored the concentration of liver protein and the unit activity of enzymes to normal values. The same loss in enzyme activity as noted during low protein or non-protein feeding was observed in animals given a protein-deficient diet with glycine added to bring the total nitrogen content to that of the control ration. This finding underlines the importance of protein quality for enzyme synthesis.

Wainio and his associates (1953) have studied the effects of protein depletion on eight oxidative enzyme systems in liver tissues of adult rats using pair-fed and ad libitum-fed animals as controls. Protein depletion decreased the unit activities (activity per mg. of nitrogen) of succinoxidase, succinic dehydrogenase, D-amino acid oxidase, DPN-cytochrome c reductase and uricase systems in liver tissues. Pyruvate oxidation showed no change and the unit activity of cytochrome oxidase increased during protein depletion. A marked reduction in the unit activity of xanthine oxidase was observed in the livers of both the depleted and pair-fed animals. Food restriction produced no change in the unit activities of the liver enzyme

systems studied except xanthine oxidase.

The total activities (activity per liver) of succin-oxidase, succinic dehydrogenase, D-amino acid oxidase, DPN-cytochrome c reductase and uricase systems also decreased as a result of protein depletion. Since the total activity of xanthine oxidase was markedly reduced by food restriction alone, further omission of dietary protein had no significant effect.

Other workers also have shown that the unit activity of cytochrome oxidase in liver tissue of rats was not affected by protein depletion (Millman, 1951).

Xanthine oxidase is one of the most labile enzymes and responds readily to protein deprivation (Litwack and his co-workers, 1950). A 25 per cent reduction in the unit activity of liver xanthine oxidase occurred after male rats were fed a protein-free diet for 48 hours. Within a period of 5 days on the same diet, the unit activity of this enzyme had decreased very markedly. Enzyme activity was restored immediately by feeding a protein-containing ration to animals which had received a non-protein diet. Although the liver nitrogen showed the same trend, it was much less sensitive to the effects of non-protein feeding than xanthine oxidase. The marked reduction in liver xanthine oxidase activity observed in animals fed a

protein-free diet did not appear to be associated with a loss of flavine adenine dinucleotide in the liver.

Most workers have reported that increases in liver enzymes are a direct result of increases in the dietary protein intake. Mandelstam and Yudkin (1952) observed a linear response of hepatic arginase when rats were fed increasing amounts of protein. However, these authors attempted to explain this phenomenon in terms of their "mass action" theory of enzyme adaptation. It was pointed out that increments in dietary protein intake produce a corresponding increase in the substrate concentration which would, therefore, require additional quantities of arginase for urea production. With a high protein intake, the proportion of arginase in combined forms may be increased because the additional ammonia formed will go through the arginine cycle or because substances derived from protein such as arginine, ornithine and lysine will combine with the enzyme. If the amount of enzyme in a combined for is increased, increasing amounts of arginase will be produced. According to these workers, the linear relationship between dietary protein and the amount of enzyme produced substantiates the "mass action" theory of enzyme adaptation.

METHOD OF PROCEDURE

General Plan of Study

The effects of varying the intake of both dietary protein and calories on nitrogen utilization and on body composition of adult female albino rats were studied in 5 successive experiments. Since several criteria for evaluating protein utilization and body composition were studied, only the general plan of each experiment with its corresponding main objective will be described in this section. Detailed information about the experimental animals, the composition of the experimental diets and the analytical procedures will be presented in subsequent sections.

Daily records of food consumption and of body weight were kept for individual animals during each experiment. Carcasses and livers of all experimental animals were analyzed for total nitrogen. Carcass fat was estimated volumetrically in Experiments II, III, IV and V.

Experiment I

Objective: To obtain nitrogen balances of animals fed three different but constant protein intakes during

both ad libitum feeding and caloric restriction.

Groups of animals were fed ad libitum one of three diets which supplied 15, 10 or 5 per cent of the calories as protein. The animals consumed approximately 200, 140 or 70 mg. of nitrogen daily. Nitrogen balances were measured for 4 periods of 5 days each following a 7-day adjustment period. During the succeeding 35 days, caloric restriction was imposed by reducing the energy intake to two-thirds of the ad libitum intake. The intake of dietary protein, however, was maintained at the ad libitum amount. Nitrogen balance information was obtained for 7 periods of 5 days each. Hemoglobin concentration in the blood was determined at the end of the experiment.

Experiment II

Objective: To study the effects on nitrogen balance of increasing or decreasing the amount of protein in the diet during caloric restriction following ad libitum feeding.

The feeding plan employed in Experiment I was modified in the subsequent 4 experiments. Two groups of animals were given either 15 per cent of their calories as protein or 5 per cent of their calories as protein during 20 days of unrestricted food intake. At the end of this period,

each group was divided into 3 sub-groups and each animal was fed food energy equivalent to two-thirds of its ad libitum intake. The sub-groups pre-fed 15 per cent of the dietary calories as protein were fed the same, two-thirds or one-third as much protein as they had consumed voluntarily. The sub-groups pre-fed 5 per cent of their dietary calories as protein were fed the same, twice as much or three times as much protein per rat per day as they had consumed voluntarily. Reduction in food energy intake was, therefore, accompanied by variations in nitrogen consumption.

Nitrogen balances were determined for two successive 5-day periods following an adjustment period of 10 days on unrestricted food consumption. Nitrogen balance data were also collected during the seven periods of 5 days each following caloric restriction.

The concentration of hemoglobin in the blood was measured at the end of the experiment. An attempt was made to estimate carcass fat by the specific gravity technique using eviscerated, unclipped carcasses. The results were unsatisfactory because errors were introduced by air bubbles trapped in the fur of the animals.

Experiment III

Objective: To study the effects of varying intakes of dietary proteins and calories on the amount of body fat.

Since the fat stores of an animal may determine the course of nitrogen metabolism by preventing excessive destruction of tissue protein, it appeared worthwhile to investigate the effects of different intakes of protein and calories on the amount of body fat.

Specific gravity measurements were carried out with clipped, eviscerated carcasses of animals which were sacrificed at 3 different intervals during the experiment, namely, (1) at the beginning of the experiment (stock animals which served as controls), (2) at the end of ad libitum feeding (animals fed 15 or 5 per cent of their food energy intake as protein), (3) at the end of restricted feeding (animals fed 3 different levels of protein).

Nitrogen balance information was collected for two periods of 5 days each during ad libitum feeding after 10 days of adjustment to the diets. During 30 days of caloric restriction, nitrogen balance data were obtained only for the last 5 days. Continuous nitrogen balance determinations were made on 2 sub-groups of animals which had been fed 5 per cent of the energy value of the diet as protein during the period of unrestricted feeding. One group

received twice as much protein after restriction of food energy as during ad libitum feeding. The other received three times as much protein after caloric restriction as before. The nitrogen balance response of these two groups of animals were repeated in order to verify the results obtained in Experiment II.

Experiment IV

Objective: To obtain information on the effects of feeding varying protein and calorie levels in the diet on the activities of the following enzyme systems in liver tissue of adult female rats: xanthine oxidase, succinic dehydrogenase, and cytochrome oxidase.

Protein reserves that are readily available for rapid utilization during periods of protein inadequacy are found in the liver. Some enzymes have been shown to respond to variations in protein intake in a manner similar to liver nitrogen.

Since the liver of only one animal could be analyzed for enzyme activities in one day, one animal chosen at random was placed on experiment each day on consecutive days until the groups were completed. Experiment III was divided into 2 parts, namely, Series I and Series II. When the last animal of the group studied in Series I had

been placed on experiment, Series II was started. The animals were of comparable ages at the time they were started on experiment.

Control and experimental animals were sacrificed at the same time intervals in Experiment III. Xanthine oxidase activity expressed as mg. of uric acid produced per gm. of liver (wet weight) per hour was measured colorimetrically using the method of Van Pilsum (1953). Succinic dehydrogenase and cytochrome oxidase activities were determined by manometric measurements of oxygen uptake according to the procedure of Schneider and Potter (1943) as described by Umbreit and his co-workers (1949).

Experiment V

Objective: To obtain additional information on liver enzyme activities and specific gravity of clipped, eviscerated carcasses using the same dietary regimes as in Experiments III and IV.

Technical difficulties had been encountered with the determination of cytochrome oxidase activity in Experiment IV but a satisfactory technique was achieved for this experiment. Experiment V was divided into 2 parts, namely, Series III which was devoted to the determination of the various enzyme activities and Series IV, to specific gravity measurements.

Experimental Animals

Adult female albino rats of Wistar stock, strain A, which had been inbred from 109 to 110 generations were used. These animals were weaned at the age of 28 days and if they weighed over 50 gms., they were housed in pairs in round wire-mesh cages up to the time an experiment was initiated. During this period, they were fed the laboratory stock diet (Steenbock XVII) supplemented with ground raw lean beef (5 gms., 3 times a week), fresh carrots (10 gms., twice a week) and raw cabbage (10 gms., once a week). Three drops of cod liver oil were given to each rat 3 times a week. Weekly records of body weights were kept during this period.

The number of animals used in each of 5 experiments is indicated in Table 1. Average body weights at weaning, and ages and body weights of the rats at the beginning of each experiment are included in the same table.

Animals used in these experiments were carefully selected on the basis of a uniform growth performance. It was also assumed that growth had terminated when the body weights became stationary for 3 or 4 weeks at the age of approximately 4-1/2 months. Rats showing signs of

Table 1. Average weaning weight, age at the beginning of experiment and weight at the beginning of experiment

Experiment number	Number of animals	Average wt. at weaning (gm.)	Average age (days)	Average wt. at beginning of experiment
I	9	56.3 ± 3.71[a]	148.0 ± 3.12	192.3 ± 3.35
II	18	57.0 ± 3.96	129.1 ± 6.31	185.9 ± 7.03
III	36	59.0 ± 3.77	132.3 ± 5.65	187.7 ± 9.21
IV	42	57.4 ± 4.36	168.5 ± 15.87	208.8 ± 11.08
V	39	58.0 ± 4.48	149.4 ± 6.83	200.8 ± 9.28

[a]Standard deviation.

respiratory or eye infections were discarded. For each study, animals were distributed so that each group was comparable with respect to initial weights. Litter mates were not assigned to the same experimental group.

At the conclusion of each experiment, the general appearance of the animals was recorded. The animals were sacrificed under anesthesia using 0.5 ml. of nembutal solution (4.5 gr. of nembutal diluted to 10 ml. with water) injected into the abdomen. As soon as the anesthetic had taken effect, blood was withdrawn from the portal vein with a 5 ml. hypodermic syringe. The blood anti-coagulant used was heparin. The animals were exsanguinated by cutting the portal vein. Livers were carefully removed and weighed. These were coarsely minced and placed in individual 250 ml. Erlenmeyer flasks containing 50 ml. of 20 per cent HCl. The visceral organs were examined for obvious signs of abnormalities and the amount of visible fat in the viscera was recorded. Eviscerated carcasses which had been cut up with a pair of scissors were placed in individual Erlenmeyer flasks (1000 ml.) containing 350 ml. of 20 per cent HCl.

The livers and carcasses were autoclaved in 20 per cent HCl for 1 hour at 15 pounds of pressure. The liver and carcass acid digests were diluted with distilled water

to a final volume of 250 ml. and 1 liter respectively. These were stored in pharmacy bottles until suitable aliquots were analyzed for nitrogen. The acid digests of livers obtained after portions of the organ were removed for measurement of enzyme activities were diluted to a final volume of 200 millliliters.

The animals whose livers were removed for measurement of enzyme activities were stunned with a blow on the head using a rubber mallet, decapitated and exsanguinated. The liver was removed and immediately chilled in cracked ice and blotted free of moisture with filter paper.

Experimental Diets

The experimental diets used in all the experiments were constructed by Samvik (1953). They differed from each other only with respect to their protein content. Lactalbumin was used as the source of protein. A half and half mixture of lard and butterfat supplied the fat. Nitrogen-free dextrin was the source of carbohydrate. Samvik (1953) has presented the derivation of each diet in detail. The composition of the diets is indicated in Table 2.

In diets I-A, I-B and I-C, the per cent of the calories supplied by protein were 15, 10 and 5 respectively. The

Table 2. Composition of the experimental diets

Diet	Ingredients per 100 grams of diet (gm.)							Calculated calories/gm. diet
	Lard[a]	Butter fat[b]	Dextrin[c]	Lactal-bumin[d]	Osborne and Mendel salts[1]	Ruffex[e]	NaCl	
I-A	9.3	9.3	53.4	23.3	4.0	2.0	1.0	4.55
I-B	9.3	9.3	60.5	15.5	4.0	2.0	1.0	4.58
I-C	9.3	9.3	67.6	8.0	4.0	2.0	1.0	4.61
	Ingredients per 67 grams of diet (gm.)							
II-A	6.2	6.2	28.6	23.3	2.7	1.3	0.3	4.52
II-B	6.2	6.2	35.7	15.5	2.7	1.3	0.3	4.56
II-C	6.2	6.2	42.4	8.0	2.7	1.3	0.3	4.61

[1]Osborne, T. B. and Mendel, L. B., J. Biol. Chem. 37, 557-601 (1919).

[a]Pure leaf lard purchased from local market.

[b]Purchased as butter from local market and prepared in the nutrition laboratory.

[c]Fisher Scientific Co., St. Louis, Mo.

[d]Nutritional Biochemicals Corp., Cleveland, Ohio.

[e]Fisher Scientific Co., Pittsburgh, Pa.

fat content comprised approximately 18.6 per cent of the diet. Similar caloric densities were maintained in all of the diets by adjusting the carbohydrate component when protein was increased or decreased. Diets I-A, I-B or I-C were used during the period of ad libitum feeding.

When diets II-A, II-B or II-C were fed in quantities equivalent to two-thirds of the ad libitum food intake, the energy intake was correspondingly reduced by the same proportion but the absolute amounts of protein were maintained, increased or decreased. The protein in diets II-A, II-B and II-C supplied 25, 17 and 8 per cent respectively of the food energy.

The diets were prepared in the following manner: The butter was melted and filtered through several thicknesses of cheesecloth to remove the milk solids. The aqueous layer which separated during the process of melting was discarded. Butterfat and lard were weighed together in a tared beaker. The melted fats were combined with the sifted dry ingredients and thoroughly blended by hand. Diets stored in suitable containers were immediately refrigerated after preparation. Sufficient quantities of diets were prepared to last for the duration of each experiment.

Five hundred milligrams of vitamin mixture were given daily. This supplement contained all the known crystalline

vitamins with the exception of vitamin K. Vitamin B_{12}, alpha tocopherol and the fat-soluble vitamins contained in cod liver oil were given separately. Alpha tocopherol in Wesson oil, vitamin B_{12} (Betalin 12) and cod liver oil were measured with individual droppers calibrated to deliver daily doses of 50 mg. of each liquid vitamin mixture. Fifty milligrams of alpha tocopherol in Wesson oil and of vitamin B_{12} as Betalin 12 provided 0.75 mg. of alpha tocopherol and 1.5 mg. of vitamin B_{12} respectively. The composition of the vitamin preparation is given in Table 3. The experimental diets and vitamin mixtures prepared for each experiment were analyzed for nitrogen by the Kjeldahl procedure (Table 4).

Analytical Procedures

Collection of samples for nitrogen determination

During the preliminary adjustment period, experimental diets were fed ad libitum to all the animals. Food was removed from the cage 6 hours preceding the beginning of each balance period. At the beginning of each period, the animals were transferred to individual metabolism cages. Daily records of food consumption and of body weight were kept. During caloric restriction, diets were offered in

Table 3. Composition of the vitamin mixture

Vitamin	Gm. per 500-gram mixture
Thiamine	0.040
Riboflavin	0.060
Niacin	0.500
Pyridoxine	0.040
Calcium pantothenate	0.100
Ascorbic acid	1.000
Inositol	10.000
Para-aminobenzoic acid	10.000
Biotin	0.001
Folic acid	0.008
Choline chloride	5.000
Dextrin was added to make 500 grams	

Table 4. Nitrogen contents in milligrams per gram of experimental diet and of vitamin mixture as determined by the Kjeldahl method

Experiment No.	I	II	III	IV	V
Diets:					
I-A	28.1705 28.8811	28.4408	26.8718 26.6675	27.3489	27.7868
I-B	18.7336 19.1094	--	--	--	--
I-C	9.9577 10.0255	9.3702	9.6301 9.5614	9.9527	9.7983
II-A	41.9217	40.8131	39.9323	40.8196	40.8958
II-B	28.6192	27.6661	27.2990	27.8561	27.8561
II-C	15.1537	14.4386	14.4987	14.6593	14.5913
Vitamin mixture	3.5294	3.5068	3.5418	3.6476	3.6476

weighed amounts equivalent to two-thirds of the ad libitum food intake of each animal. The vitamin supplements were fed in separate small Pyrex cups before food was placed in the cages.

Composite collections of urine and feces were made during each 5-day balance period. The urine was collected on acid-treated filter paper[1]. For each test interval, seven of these papers were placed on the Pyrex plates which supported each metabolism cage. One filter paper was removed daily and placed in 1-liter Erlenmeyer flasks containing 200 ml. of 20 per cent HCl. The feces were brushed free from spilled food and hair and collected in Erlenmeyer flasks (250 ml.) containing 50 ml. of 20 per cent HCl. The fecal marker employed was ferric oxide. Red diets[2] were fed on the first day of each balance period and on the day following the completion of each period. The appearance of red feces marked the beginning and the end of each collection period.

At the end of each balance period, metabolism cages and Pyrex plates were washed quantitatively with hot

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[1]Five hundred 9-inch filter papers were soaked overnight in a solution of 900 ml. of 95 per cent alcohol and 100 ml. of glacial acetic acid, then dried.

[2]One hundred mg. of ferric oxide per 100 gm. of diet.

distilled water. The washings were added to the flasks containing the urine samples.

Urine and fecal collections were autoclaved under 15 pounds of pressure for 1 hour. The contents of the flask containing urine collections were transferred quantitatively to a volumetric flask (1 liter) and the volume was adjusted with distilled water. The fecal acid digest was forced through a sieve into a 250 ml. volumetric flask and distilled water was added. Urine and fecal samples were stored in pharmacy bottles for nitrogen determination.

Food, urine and feces were analyzed for nitrogen using the Kjeldahl-Gunning-Arnold procedure. Weighed samples of diets containing approximately 20 to 40 mg. of nitrogen were used in the analyses for nitrogen. The nitrogen content of one gram samples of vitamin mixtures was determined. Twenty-five ml. aliquots of urine and fecal samples were used in the nitrogen determinations. The amount of urine catalyst used per sample contained 10 gm. of K_2SO_4 and 0.7 gm. of mercuric oxide. The fecal catalyst contained 15 gm. of K_2SO_4 and 0.7 gm. of mercuric oxide. For the nitrogen determinations on food samples, the fecal catalyst was employed.

Carcass and liver samples (see section on experimental animals for preparation of these samples) were analyzed for

total nitrogen in a manner similar to fecal samples. Five ml. aliquots of the carcass acid digest and 50 ml. aliquots of the liver acid digest were used in the nitrogen determinations.

The distillation procedure following digestion with concentrated H_2SO_4 and catalyst was carried out according to a modification proposed by Hiller and his co-workers (1948). Zinc dust was employed to reduce the mercuric oxide catalyst and an excess of saturated NaOH solution to neutralize H_2SO_4. The liberated ammonia was collected in approximately 0.1N HCl using methylene blue-methyl red indicator. Standard NaOH (0.1N) was used to titrate the excess acid.

Determination of hemoglobin concentration in the blood

The hemoglobin concentration was determined colorimetrically as oxyhemoglobin. Twenty microliters of blood measured with a blood pipette were mixed with 10 ml. of 0.5 per cent ammonium hydroxide solution. The extinction of the resulting mixture was measured at a wave length of 540 millimicrons and a slit width of 0.03 mm. in a Beckman spectrophotometer. The hemoglobin concentration was calculated according to the following formula:

$$\text{Hemoglobin in gram per cent} = \frac{\text{Total volume x reading}}{\text{9.18 x volume of blood used}} .$$

Body fat measurements

Specific gravity was determined on clipped, eviscerated rats by the water displacement method of Rathbun and Pace (1945). The hair of anesthetized rats was clipped with a pair of hair clippers. After exsanguination, the viscera were removed leaving the perirenal fat and the genital fat depots. The kidneys and the genital organs with the surrounding fat were left intact in the method described by Rathbun and Pace. However, in the present investigation, examination of the visceral organs for signs of gross abnormalities was made during autopsy and this necessitated the removal of these 2 organs.

The carcass was weighed in air to the nearest tenth of a gram by suspending it on a hook tied to a piece of nylon thread which in turn was attached to the beam of a Toledo platform balance by another hook. The weight in water was obtained by using another piece of nylon thread long enough to insure complete immersion of the carcass in the water contained in a bell jar. The temperature of the water was recorded immediately. The weight in air and in water thus obtained were corrected for the weight

of the hooks and nylon thread used for suspending the carcass.

The difference between the weight in air and the weight in water represented the volume of water displaced. The specific gravity was calculated according to the following formula and corrected for density changes of water with temperature:

$$\text{Specific gravity} = \frac{\text{weight in air}}{\text{(weight in air - weight in water) x density of water at temperature recorded}}.$$

The percentage of body fat was calculated according to the formula of Rathbun and Pace for eviscerated animals:

Per cent body fat = (5.362/specific gravity - 4.880).

Samvik (1953) has described a method for obtaining a rough estimation of carcass fat by means of volumetric measurements. The hot acid digest of the carcass was transferred quantitatively into a graduated cylinder (1 liter). After cooling, the fat layer was measured directly.

A slight modification of this procedure was adopted in Experiments IV and V. The carcass digest contained in an Erlenmeyer flask was placed in the deep freeze and the solidified fat layer was removed. This layer of solidified fat was melted in a 50-ml. beaker on a water bath and

transferred quantitatively to a 50-ml. graduated centrifuge tube. After centrifugation for about 5 minutes at a speed of 1500 rpm, the volume of fat was measured at room temperature.

Calibration of Warburg flasks and manometers[1]

Manometric measurements of oxygen uptake were employed in the determination of succinic dehydrogenase and cytochrome oxidase activities in the liver. Prior to the study of liver enzyme activity, flasks and manometers were calibrated with mercury according to the method proposed by Grisolia (Umbreit et al., 1949).

The flasks to be calibrated were cleaned thoroughly after removing the grease with xylene on a piece of cotton. After rinsing with tap water, they were placed in alcoholic sodium hydroxide solution (120 gm. of NaOH dissolved in 120 ml. of distilled water and diluted to 1 liter with 95 per cent alcohol) for about 30 minutes. The flasks were washed with a hot detergent solution and rinsed about 6 times with distilled water. They were dried in an air oven overnight. This same cleaning procedure was used after each assay.

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[1]Machlett, New York, N. Y.

The manometers were cleaned with HNO_3 diluted 1:2 with water and rinsed thoroughly with distilled water using suction for drawing fluids through the column. Alcohol (95 per cent) and ether (anhydrous) were used in succession for drying the manometers.

The mercury used for calibration was filtered through filter paper with a tiny pin point at the tip. This method proved to be satisfactory for the present purpose since the mercury employed was relatively clean and did not appear to require a more laborious cleaning procedure.

The manometer mounted to a manometer support was placed in an upright position. With a diamond point, a reference mark was scratched about 1 cm. above the ground glass joint. The flask was filled with mercury and the side arm stopper was inserted. A shallow tray made of wrapping paper was used to catch spilled mercury. Trapped air bubbles were removed with the aid of a piece of fine copper wire. The flask was seated firmly on the ground glass joint and the mercury level was adjusted to coincide with the scratched mark. With a medicine dropper, small amounts of mercury were removed or added while adjustments were being made. The mercury contained in the flask was poured quantitatively into a tared weighing bottle after its temperature was recorded. A torsion balance was used for weighing the mercury to the nearest tenth of a gram.

The method for calibrating manometers was slightly modified to permit manipulation by one person. The mounted manometer was held at about a 30° angle in an inverted position. A short piece of rubber tubing fitted with a screw clamp was attached to the tip of the stopcock. The stopcock was turned so that the tail vent was connected to the manometer. Mercury was introduced into the manometer through the rubber tube with the aid of a 5 ml. glass syringe. The manometer was tilted in such a way to allow the mercury to flow into the graduated arm of the manometer. The amount of mercury being introduced was controlled by means of the screw clamp. When the mercury level coincided exactly with the scratched mark and the reference point (150 mm.), the stopcock was closed, screw clamp tightened and the rubber tube together with the syringe were removed carefully. The mercury from the manometer was transferred quantitatively through the straight top of the manometer column into a tared weighing bottle and weighed on an analytical balance. This procedure for calibrating manometers proved to be efficient and minimized the possibility of introducing air bubbles into the manometer column.

Brodie's solution was introduced into the calibrated manometers through the rubber tube at the base of the

reservoir by means of a hypodermic syringe. Brodie's solution was made according to the following formula (Sumner and Somers, 1944):

Sodium chloride	23 gms.
Sodium taurocholate	5 gms.
Water, to make	500 ml.
Colored with Evans blue .	

Determination of liver enzyme activities

Preparation of homogenates. After weighing the whole liver, a representative sample was obtained by taking small portions from each lobe. The sample was rapidly and accurately weighed on an analytical balance. A 1:10 liver homogenate was prepared for determination of succinic dehydrogenase and cytochrome oxidase activities. One gram of liver coarsely minced with a pair of scissors was homogenized for 1 minute with 2 ml. of cold distilled water contained in a glass tube homogenizer provided with a fitted glass pestle. The homogenizer was immersed in a beaker of cracked ice during homogenization. Seven milliliters of cold distilled water was added to the homogenized sample. A 5 per cent homogenate was prepared by adding 2 ml. of cold distilled water to 2 ml. of the 1:10 homogenate.

For xanthine oxidase determinations, a 1:10 liver homogenate was prepared using 4.5 ml. cold phosphate

buffer (0.0666M, pH 7.4) as the diluent and 0.5 gm. of liver. The homogenate was kept in a beaker of cracked ice for about 1 hour until the manometric assays for succinic dehydrogenase and cytochrome oxidase were completed.

Two ml. of the 10 per cent liver homogenate were pipetted in duplicate into tared aluminum foil boats and dried to constant weight in an air oven (105° C). The difference between the weights of the empty aluminum boat and the boat with the dried liver sample represented the dry weight of 200 mg. of fresh liver since a 2 ml. aliquot contained this amount of wet tissue.

Succinic dehydrogenase and cytochrome oxidase. The activities of these 2 liver enzyme systems were measured according to the method of Schneider and Potter (1943) as described by Umbreit and co-workers (1949). Both enzymes can be assayed simultaneously with one sample of liver tissue. Six flasks and manometers were set up as indicated in Table 5. Reagents and solutions prepared for these determinations are presented in the latter part of this section. Graduate 1 ml. pipettes were employed for measuring the different materials comprising the reaction mixture. The reagents and solutions which were stored under refrigeration were brought to room temperature prior to the assay. Except for the liver homogenate to be tested,

Table 5[1]. Reaction mixtures in the succinic dehydrogenase-cytochrome oxidase assay

Flask no.	1	2	3	4	5	6
	Succinic dehydrogenase			Cytochrome oxidase		
	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)
H_2O (to make 3.0 ml.)	0.6	0.5	0.9	0.3	0.25	0.20
0.1 M PO_4 pH 24 with NaOH	1.0	1.0	1.0	1.0	1.0	1.0
0.5 M Na-succinate pH 7.4	0.3	0.3	0.3	--	--	--
1×10^{-4} M cytochrome c	0.4	0.4	--	--	--	--
2.4×2^{-4} M cytochrome c	--	--	--	1.0	1.0	1.0
4×10^{-3} M $CaCl_2$	0.3	0.3	0.3	--	--	--
4×10^{-3} M $AlCl_3$	0.3	0.3	0.3	0.3	0.3	0.3
0.228 M Na-ascorbate[a] pH 7.0	--	--	--	0.3	0.3	0.3
5 per cent rat liver homogenate in water	0.1	0.2	0.2	0.10	0.15	0.20

[1]Parts of Table 5 reproduced from manometric techniques and tissue metabolism by W. W. Umbreit, R. H. Burris and J. F. Stauffer, 1949, p. 139.

[a]Prepared by adding 1 ml. of 0.2 N NaOH to 40 mg. of ascorbic acid just before use.

measured amounts of the materials indicated in Table 5 were added to the main compartment of the clean, dry Warburg flasks equipped with a center well and a side arm. Two-tenths of a milliliter of 2N NaOH was pipetted into the center well of each flask. The stopper, greased with lanolin[1], was inserted into the side arm. At this point, the animal was sacrificed and liver homogenates were prepared. Five per cent liver homogenate was added to the main compartment of six flasks using a 0.2 ml. graduated pipette calibrated in hundreths. This pipette had a fairly large bore which allowed complete and easy drainage of the liver homogenate. Rectangular strips of fluted filter paper (2.3 cm. by 3.0 cm.) were inserted into the center well of each flask. The prepared flasks were attached to their respective manometers by means of copper wire coils and placed in a constant temperature circular bath at 37° C. equipped with a thermobarometer. Equilibration, with shaking, was carried out for 10 minutes. The manometer fluid in the closed arm of the manometer was adjusted to 150 mm. with the stopcock open. With the stopcock closed, initial readings were taken. Subsequent readings of oxygen pressure changes were taken at 10 minute

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[1]Adeps Lanae (Anydrous), Fisher Scientific Co., St. Louis, Mo.

intervals for 40 minutes. Each reading was corrected for thermobarometer changes and the results were averaged.

The determination of succinic dehydrogenase activity was carried out at two levels of tissue concentration, namely, 5 mg. and 10 mg. of fresh liver tissues contained in flasks 1 and 2 respectively. According to Umbreit and his associates (1949), this technique had an added advantage of providing further evidence that oxygen uptake is proportional to tissue concentration. A blank (flask 3) containing all the materials except cytochrome c was included with each determination.

The data obtained were reported as Q_{O_2} (microliters of oxygen taken up per mg. of dry tissue per hour). The Q_{O_2} for succinic dehydrogenase was calculated according to the following formula:

$$Q_{O_2} = \frac{\text{Ave. } O_2 \text{ uptake in microliters per 10 min. x 6}}{\text{Dry weight of tissue in mg.}}$$

where

Ave. O_2 uptake in microliters per 10 min. = Ave. change in O_2 pressure x flask constant.

The Q_{O_2} values at two levels of tissue concentration were corrected for the blank and results were averaged.

Technical difficulties were encountered in the assay for cytochrome oxidase. The method described by Umbreit

and co-workers (1949) recommended the use of ascorbic acid as substrate at a level of 0.3 ml. of 0.114M sodium ascorbate added to each flask. The autoxidation rate of ascorbic acid was measured by using a series of three different tissue concentrations and extrapolating to zero tissue concentration. The levels of tissue concentration recommended with 1.0, 1.5 and 2.0 mg. of fresh liver obtained from a 1 per cent liver homogenate. By using the recommended amounts of sodium ascorbate and tissue concentration, no appreciable differences in the oxygen pressure changes between flasks 4, 5 and 6 were detected. However, when the sodium ascorbate concentration was increased to twice the amount recommended by Umbreit and co-workers (1949) and the liver homogenate concentration five times, measurable differences in oxygen uptakes were obtained in flasks 4, 5 and 6 which contained 5.0, 7.5 and 10.0 mg. of liver tissue respectively.

The correction factor for the autoxidation of ascorbate was obtained in the following manner: The difference in the average oxygen uptake per 10 minutes of 5 mg. (flask 4) and 7.5 mg. (flask 5) of tissue concentration was added to the difference in average oxygen uptake per 10 minutes of 7.5 mg. (flask 5) and 10.0 mg. (flask 6). This sum representing the average O_2 uptake per

10 minutes of 5 mg. of tissue was subtracted from the average O_2 uptake per 10 minutes in flask 4 to obtain the average oxygen uptake per 10 minutes at zero tissue concentration. The average oxygen uptake obtained for each flask was corrected for autoxidation and Q_{O_2} was calculated according to the same formula used for succinic dehydrogenase activity.

Umbreit and co-workers (1949) maintain that the Q_{O_2} values should be defined according to the substrate employed. The data obtained were, therefore, reported as succinate Q_{O_2} for succinic dehydrogenase activity and as ascorbate Q_{O_2} for cytochrome oxidase activity.

Schneider and Potter (1943) found that Q_{O_2} values for the cytochrome oxidase system were higher in young rats than in adult animals. The method described by Schneider and Potter (1943) may be applicable only for assaying liver tissues of young rats. The difficulty encountered in the present cytochrome oxidase assay may be due to the fact that adult rats were used.

The cytochrome oxidase assay was complicated further by the fact that the homogenization of liver tissue affects the rate of oxygen uptake to a great extent. Schneider and Potter (1943) have observed that only disrupted cells contribute to the rate of oxygen uptake in the cytochrome oxidase system. In one instance, the present investigator

noted that poor homogenization of liver tissue resulted in a low oxygen uptake and hence, a low enzyme activity. Therefore, the homogenization technique needs to be perfected for this assay.

The succinic dehydrogenase enzyme system does not appear to be affected by the degree of homogenization. It has been indicated that the succinate when used as a substrate, is able to diffuse into the intact cells, whereas the ascorbate is unable to penetrate the cell membrane (Schneider and Potter, 1943).

The succinic dehydrogenase and cytochrome oxidase activities obtained from the present study were also expressed in terms of microliters of O_2 per mg. of liver nitrogen per hour (Q_{O_2} (N)) and in ml. of O_2 per liver per hour (total activity).

Xanthine oxidase. The xanthine oxidase activity in rat liver tissues was measured according to a method developed by Van Pilsum (1953). The amount of uric acid produced by the oxidation of xanthine served as a basis for measurement of enzyme activity. The reagents used and their preparations are given in the latter part of this section.

Two 1 ml. aliquots of 10 per cent liver homogenate (in phosphate buffer) were pipetted into two 20 ml.

beakers each of which contained 6 ml. of aqueous monosodium xanthine solution (4.02×10^{-2} M) and 3 ml. of distilled water. One ml. aliquots were withdrawn from each mixture to serve as sample blanks. The remaining solution was transferred to Warburg flasks and incubated with shaking for 2 hours at 37° C. in a constant temperature bath. At the end of the incubation period, duplicate 1 ml. aliquots were pipetted from each of the incubated mixtures into 50 ml. Erlenmeyer flasks. Folin-Wu filtrates were prepared from these aliquots and from the two sample blanks. The tungstic acid filtrates (1:10) were prepared according to the method by Folin and Wu (1919). Each 1 ml. aliquot was diluted with 7 ml. of distilled water. One ml. of a 10 per cent sodium tungstate solution was added to each sample followed by 1 ml. of 2/3N H_2SO_4. The contents of each flask were shaken and filtered through fluted filter paper.

The amount of uric acid in the tungstic acid filtrates were determined colorimetrically according to the method by Brown (1945). Two ml. aliquots taken from each of the tungstic acid filtrates were pipetted into test tubes. Two ml. each of a 12 per cent sodium cyanide (dispensed from a burette) and a 50 per cent urea solution, followed by 1 ml. of the phosphotungstic acid reagent were added to

each test tube. Each addition was mixed by shaking. The stoppered tubes stood for 50 minutes at room temperature, then each sample was diluted to a final volume of 10 ml. by the addition of 3 ml. of distilled water and mixed. Optical densities were measured with a Beckman spectrophotometer at a wave length of 540 millimicrons and a slit width of 0.03 mm.

Reagent blanks using 2 ml. of distilled water and treated in the same manner as the unknowns were prepared in duplicate. Uric acid standards were run with each set of determinations. From a 0.1 per cent Folin stock solution of uric acid, 3 standard solutions containing 0.002, 0.003 and 0.004 mg. of uric acid per ml. respectively were prepared. Three working standards equivalent to 0.004, 0.006 and 0.008 mg. of uric acid were obtained by using 2 ml. of each standard solution. These 2 ml. portions were treated in the same manner as the 2 ml. aliquots of the 1:10 tungstic acid filtrate.

The optical density readings for the unknown were corrected for sample and reagent blank readings and the mg. of uric acid produced per gm. of liver (wet wt.) per hour were calculated. The data on xanthine oxidase activity were also calculated on the basis of dry weight, liver nitrogen and total liver.

Preparation of reagents used in measurement of enzyme activities. Succinic dehydrogenase and cytochrome oxidase reagents:

1. 0.1M KH_2PO_4 (Buffer salt, Fisher Scientific Co., St. Louis, Mo.): 13.6 gm. of KH_2PO_4 were dissolved in distilled water and diluted to 1 liter.
2. 0.1M Phosphate buffer, pH 7.4: 39.5 ml. of 0.1N NaOH was added to 50 ml. of 0.1M KH_2PO_4. The pH was adjusted to 7.4 by the addition of 0.1N NaOH. The buffer was stored under refrigeration.
3. 0.5M Sodium succinate, pH 7.4: 9.9 gm. of succinic acid disodium salt (Eastman Organic Chemical, Rochester, N. Y.) was dissolved in distilled water and diluted to 100 ml. The pH was adjusted to 7.4 with 0.1N NaOH, then stored under refrigeration.
4. 4×10^{-3}M $AlCl_3$: 193 mg. of $AlCl_3$ were dissolved in distilled water and diluted to 100 ml.
5. 4×10^{-3}M $CaCl_2$: 59 mg. of $CaCl_2 \cdot 2H_2O$ were dissolved in distilled water and diluted to 100 ml.
6. 0.228M Na-ascorbate, pH 7.0: 1 ml. of 0.2N NaOH was added to 40 mg. of ascorbic acid (U.S.P., Merck and Co., Rahway, N. J.) just before use.

7. Cytochrome c solutions: Cytochrome c was purchased from the Nutritional Biochemicals Corp., Cleveland, Ohio. The molecular weight was assumed to be 14,000 (Cooperstein et al., 1950). A stock solution (2.4×10^{-4}M) was prepared by dissolving 170 mg. of cytochrome c in 0.1M phosphate buffer (7.4) and diluting to 50 ml. with the buffer. The prepared stock solution was kept in 2 ml. portions in tightly stoppered small pyrex test tubes and stored in a deep freeze.

For assay purposes, the stock solution was brought to room temperature and used directly in the determination of cytochrome oxidase activity. A 1×10^{-4}M solution was prepared for the succinic dehydrogenase assay by adding equal parts of 0.1M phosphate buffer (pH 7.4) and cytochrome c stock solution.

The stock solution of cytochrome c was standardized spectrophotometrically prior to use according to the method given by Umbreit et al. (1949). The reaction mixture consisted of the following:

a. Oxidized cytochrome c

Water	1.7 ml.
0.1M Phosphate buffer, pH 7.4	1.0 ml.
Stock cytochrome c solution	0.2 ml.
0.01M $K_3Fe(CN)_6$	0.1 ml.
Final volume	3.0 ml.

b. Reduced cytochrome c
Same solution as "a" plus 0.1 to 1.0 mg. solid $Na_2S_2O_4$.

The optical density of the first reaction mixture "a" was measured with the Beckman spectrophotometer at a wave length of 550 millimicrons and a slit width of 0.02 mm. After the readings were taken, a few grains of sodium hydrosulfite were added and the extinction of the resulting mixture measured.

The concentration of cytochrome c in the stock solution was calculated according to the formula given by Umbreit and co-workers (1949, p. 214). The average concentration of the stock cytochrome c solutions for the various assays carried out in the present study was 2.07×10^{-7} moles per milliliter.

Xanthine oxidase reagents:

1. 0.0666M Phosphate buffer, pH 7.4: 26.3 ml. of 0.2N NaOH were added to 66.6 ml. of 0.1M KH_2PO_4 and diluted to 100 ml. with buffer. The pH was adjusted to 7.4 with 0.1N NaOH, then stored under refrigeration.
2. 4.02×10^{-2}M Monosodium xanthine solution: 1.53 gm. of xanthine (Fisher Scientific Co., St. Louis, Mo.) and 400 mg. of NaOH were dissolved in distilled water. Heat was used when necessary.

The solution was diluted to 250 ml. with distilled water and stored under refrigeration.

3. 10 Per cent sodium tungstate solution: 10 gm. of sodium tungstate (General Chem. Div., Allied Chemical and Dye Corp., New York, N.Y.) were dissolved in distilled water and diluted to 100 ml.
4. 2/3N H_2SO_4: 4.6 ml. of concentrated H_2SO_4 (reagent grade) were diluted to 250 ml. with distilled water.
5. Folin stock uric acid solution (0.1 per cent): This was prepared according to the procedure given by Koch and Hanke (1953, p. 521). Two-tenths of a gram of uric acid (C. P., Fisher Scientific Co., St. Louis, Mo.) was weighed on a watch glass and transferred through a dry funnel into a dry 200 ml. volumetric flask. A solution of 0.15 gm. of lithium carbonate in 30 ml. of distilled water was warmed to 60° C. and poured over the watch glass and through the funnel into the 200 ml. flask which previously had been warmed with hot tap water. When the uric acid had dissolved after about 5 minutes of shaking, the flask was cooled under tap water and 4 ml. of a 40 per cent formaldehyde (formalin) and 70 ml. of distilled water was added. Then 10 ml. of 0.5N H_2SO_4 were

added gradually with continuous shaking. The mixture was diluted to 200 ml. and stored under refrigeration in a well-stoppered bottle.

6. Standard uric acid solution: Three standard solutions were made by diluting 1, 1.5 and 2.0 ml. of the stock uric acid solution with distilled water to a final volume of 500 ml. Each standard solution contained 0.002, 0.003 and 0.004 mg. of uric acid per ml. respectively and was stored in well-stoppered bottles under refrigeration.
7. Urea solution: 50 gm. of urea (General Chem. Div., Allied Chemical and Dye Corp., New York, N.Y.) were dissolved in distilled water and diluted to 100 ml.
8. Twelve per cent sodium cyanide solution: A freshly prepared solution was used for each determination. Three grams of sodium cyanide (Fisher certified reagent, Fisher Scientific Co., St. Louis, Mo.) was dissolved in distilled water and diluted to 25 ml.
9. Phosphotungstic acid reagent: 100 gm. of sodium tungstate, 20 gm. of anhydrous disodium hydrogen phosphate and about 150 ml. of distilled water were heated together in a 500 ml. Erlenmeyer flask. Twenty-five ml. of concentrated H_2SO_4 were mixed

with 75 ml. of distilled water and the warm solution was slowly poured into the Erlenmeyer flask with continuous shaking. The contents of the flask were boiled gently for one hour using as a condenser a funnel holding a 200 ml. Erlenmeyer flask partly filled with ice water. The solution was cooled in running tap water and transferred to a 1 liter volumetric flask and diluted with distilled water. The prepared solution has a greenish yellow tint. This was stored under refrigeration.

RESULTS AND DISCUSSION

Food Intake

Increments in dietary protein levels were accompanied by decreases in voluntary food consumption as shown in Table 6. Female rats fed 15 per cent of the calories in the diet as protein voluntarily ingested less food than those given 5 per cent of the calories as protein. A similar observation had been made by Samvik (1953) on adult male rats.

The experimental diets employed during ad libitum feeding were adequate in all respects and varied only in the amount of protein. One possible explanation for the differences observed in food intake is that animals receiving the low protein diet ate more to compensate for minimal levels of essential amino acids. Another possibility is that the animals on the higher protein ration may have consumed more water and therefore ate less food than those on the low protein diet. Water was offered ad libitum with no attempts made to record water intake. Osborne and his co-workers (1927) have shown that additional water is needed by rats fed diets unusually rich

Table 6. Average daily food intake in grams per rat

Groups of rats	Experiment I	Experiment II	Experiment III	Experiment IV	Experiment V
	Period of *Ad libitum* food intake				
Rats fed 15 per cent of cal. as protein	7.0 (6.7 - 7.2)[a]	7.5 (6.5 - 8.3)	7.2 (6.4 - 8.0)	9.6 (8.8 - 10.5)	9.3 (8.0 - 10.0)
Rats fed 10 per cent of cal. as protein	7.3 (6.4 - 8.1)	--	--	--	--
Rats fed 5 per cent of cal. as protein	7.6 (6.9 - 8.4)	8.4 (7.7 - 9.5)	8.3 (7.2 - 9.4)	10.6 (9.1 - 11.4)	9.7 (8.3 - 10.7)
	Period of restricted food intake				
Sub-groups:					
A	5.0 (4.5 - 5.9)	5.0 (4.9 - 5.0)	4.7 (4.3 - 5.0)	6.0 (5.9 - 6.2)	6.1 (4.7 - 6.7)
B	4.9 (6.4 - 8.1)	4.7 (4.3 - 4.9)	4.7 (4.3 - 5.3)	6.4 (5.9 - 6.5)	6.5 (6.3 - 6.7)
C	5.3 (4.9 - 5.7)	5.2 (4.7 - 5.5)	4.9 (4.3 - 5.1)	6.6 (6.5 - 6.7)	6.2 (5.3 - 6.7)
D		5.3 (5.1 - 5.5)	5.5 (5.3 - 5.8)	7.4 (7.1 - 7.6)	6.4 (5.7 - 6.9)
E		5.6 (5.3 - 6.2)	5.6 (5.5 - 5.7)	6.8 (6.1 - 7.2)	6.9 (6.9 - 7.1)
F		5.8 (5.3 - 6.3)	7.1 (5.8 - 6.3)	7.1 (6.3 - 7.1)	6.2 (5.5 - 6.9)

[a](No.) = range.

in protein (50-70 per cent protein) in order to promote increased deamination and urea excretion necessitated by the protein increment.

Mackay and associates (1941) have provided evidence that reduced appetite in rats fed a high protein, carbohydrate-free diet was due to the protein content of the diet rather than a lack in the carbohydrate component. The authors suggested that the decrease in appetite on a protein-rich ration may be the result of an improved maintenance of the blood sugar concentration since glucose derived from protein is formed at a slower rate and is therefore more uniformly available than glucose derived from dietary carbohydrates. This explanation might apply to the observed differences in food intake in this study, i.e., animals receiving the higher protein ration were able to maintain a more constant blood glucose level than those fed the lower protein diet. According to the glucostatic theory of Mayer (1952), an elevated blood glucose level influences the food regulating mechanism of the hypothalamus which in turn decreases food intake.

In view of the observed differences in food intakes of animals receiving diets containing varying amounts of protein, it would be of interest to investigate the activity of the different groups of animals during ad libitum feeding.

Food intakes of animals in Experiments I, II and III were approximately 20 per cent lower than those of the animals used in Experiments IV and V. As indicated in Table 1, the animals used in Experiments II and III were younger than those studied in Experiments I, IV and V, the difference in average age being about 15 per cent. There was also a difference in average body weights at the beginning of the experiments of approximately 10 per cent between animals in Experiments II and III and animals in Experiments I, IV and V. These differences in ages and body weights at the beginning of the experiments may account in part for the differences noted in food consumption. Since body size influences food intake, older animals which are heavier may tend to consume more food than younger animals. Animals in Experiment I would be an exception to this explanation, however.

The mean body weight and age of the animals at the beginning of Experiment I were comparable to those of the animals in Experiments IV and V, but food intakes during Experiment I were lower than those in Experiments IV and V. Differences in environmental conditions may account for this observation. Considerable variation in the temperature of the rat laboratory were noted although not recorded during these experiments.

In Experiments I, II and III, food was removed from the cages 6 hours before each balance period. In Experiments IV and V, no balance information was collected so food was available at all times. The removal of food prior to the balance collections probably did not account for the lower food intakes in Experiments I, II and III as compared to those in Experiments IV and V because food intakes during the adjustment period were about the same as food intakes during the balance period.

Body Weight Changes and Nitrogen Balance

During ad libitum feeding, small gains in body weights were noted in all the animals fed either 15, 10 or 5 per cent of the calories in the diet as protein (Table 7). Samvik (1953) had observed that with adequate consumption of food energy, weight changes of male adult rats appeared to be related to the protein intake. The results of the present study of weight changes during unrestricted feeding failed to indicate clear-cut relationships of weight changes with either protein or caloric intakes.

When caloric restriction was imposed by reducing the energy intake to two-thirds of the voluntary intake, the animals showed steady but decreasing losses in body

Table 7. Average total caloric intake, nitrogen intake, weight change and nitrogen balance on ad libitum and restricted food intake. Experiments I, II and III

Groups of rats	Total caloric intake	Total nitrogen intake (mg.)	Total weight change (gm.)	Total nitrogen balance (mg.)
Experiment I				
Ad libitum intake				
Groups A	635.0	4015.1	- 0.3	+488.7
Groups B	668.6	2803.3	0	+359.1
Groups C	698.7	1549.6	- 2.7	+122.3
Restricted intake				
Groups A	678.0	6351.0	-18.0	-378.5
Groups B	799.8	4270.7	-24.3	-442.3
Groups C	610.2	2441.8	-21.0	-277.2
Experiment II				
Ad libitum intake				
1. Rats fed 15 per cent of cal. as protein	377.9	2379.7	+ 1.9	+241.3
2. Rats fed 5 per cent of cal. as protein	430.1	891.8	+ 2.3	+170.8
Restricted intake				
Sub-groups A	687.0	6256.2	-18.7	-256.7
Sub-groups B	638.2	3926.0	-41.0	-391.8
Sub-groups C	714.6	2290.6	-25.0	-487.0
Sub-groups D	714.0	6501.0	-12.3	-140.8
Sub-groups E	766.0	4700.4	-16.3	-133.3
Sub-groups F	806.8	2579.4	-17.0	-287.8

Table 7 (Cont'd)

Groups of rats	Total caloric intake	Total nitrogen intake (mg.)	Total weight change (gm.)	Total nitrogen balance (mg.)
Experiment III				
Ad libitum intake				
1. Rats fed 15 per cent of cal. as protein	327.1	1946.5	+ 3.6	+187.3
2. Rats fed 5 per cent of cal. as protein	383.6	816.8	+ 2.6	+147.3
Restricted intake				
Sub-groups A	105.1	937.3	+ 2.0	- 45.1
Sub-groups B	107.1	650.4	- 2.3	- 5.4
Sub-groups C	113.0	364.1	- 2.7	- 5.8
Sub-groups D	124.1	1113.6	- 0.3	- 20.2
Sub-groups E	127.7	772.6	0.0	- 12.7
Sub-groups F	139.5	447.5	- 1.5	+ 10.0

weight which reached a plateau towards the end of 30 days. These weight losses were not related to the intake of protein. It was also observed that the total weight losses incurred during the entire experiment were not related significantly to either total protein intake or total caloric intake.

A linear relationship was found between weight changes during ad libitum feeding and nitrogen balances in Experiments I and III but not in Experiment II as shown in Figure 1. No satisfactory explanation can be offered for the discrepancy in results of Experiments I and III from Experiment II.

During caloric restriction, nitrogen balances were directly related to weight changes in Experiments I and II (Figure 2). In Experiment III, weight changes during restricted feeding could not be related to nitrogen balance because the data were too few. These findings imply that the nitrogen balance of an animal may be predicted on the basis of weight changes. Such predictions may not always be accurate, however, because there were instances when weight losses occurred concomitantly with nitrogen retentions and weight gains with nitrogen losses.

During the first balance period on ad libitum feeding, the positive nitrogen retentions in Experiment II were

Figure 1. Relation of weight changes in grams per 5 days to nitrogen balance in mg. of nitrogen per rat per 5 days during *ad libitum* feeding in Experiments I, II and III

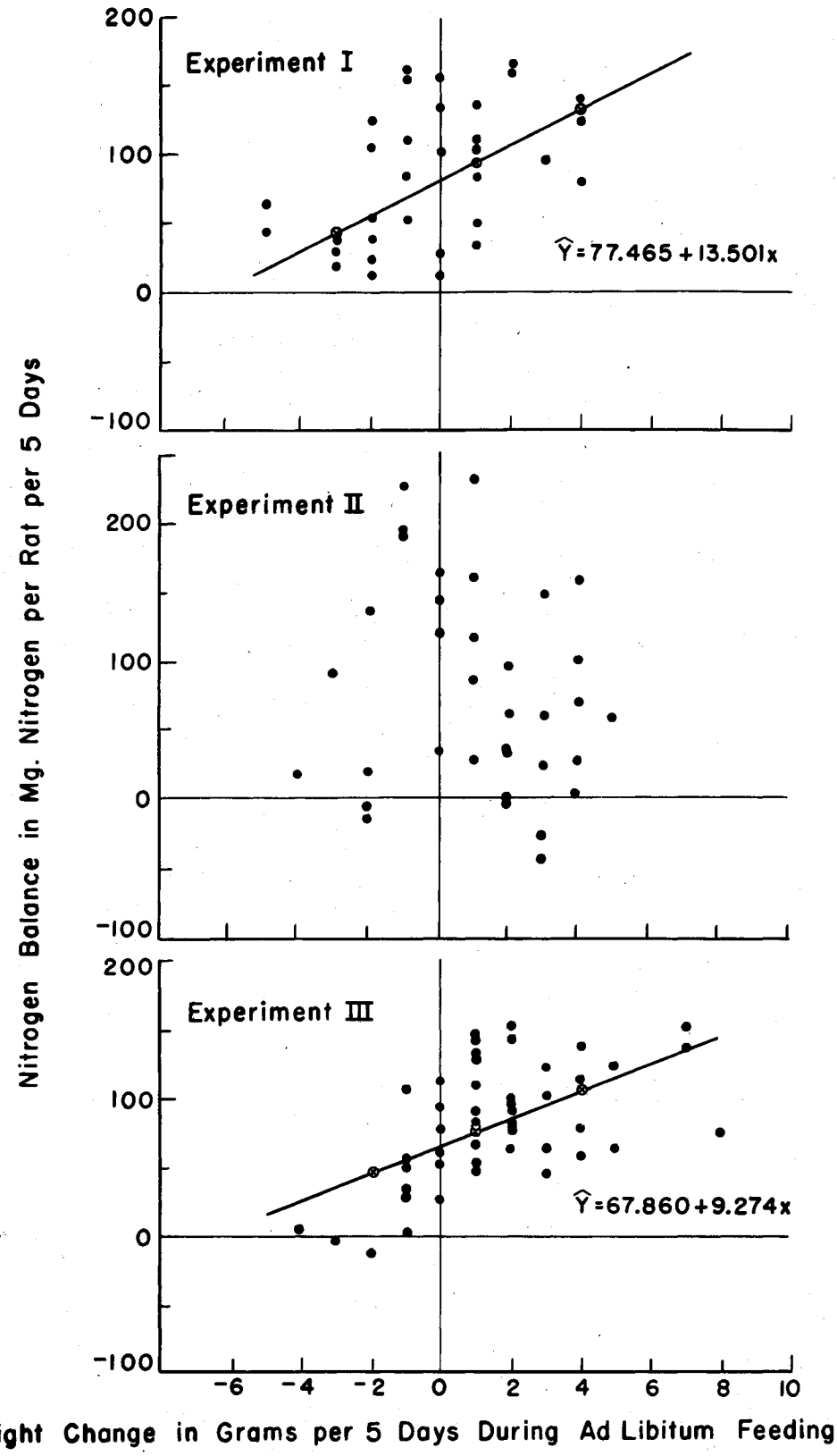
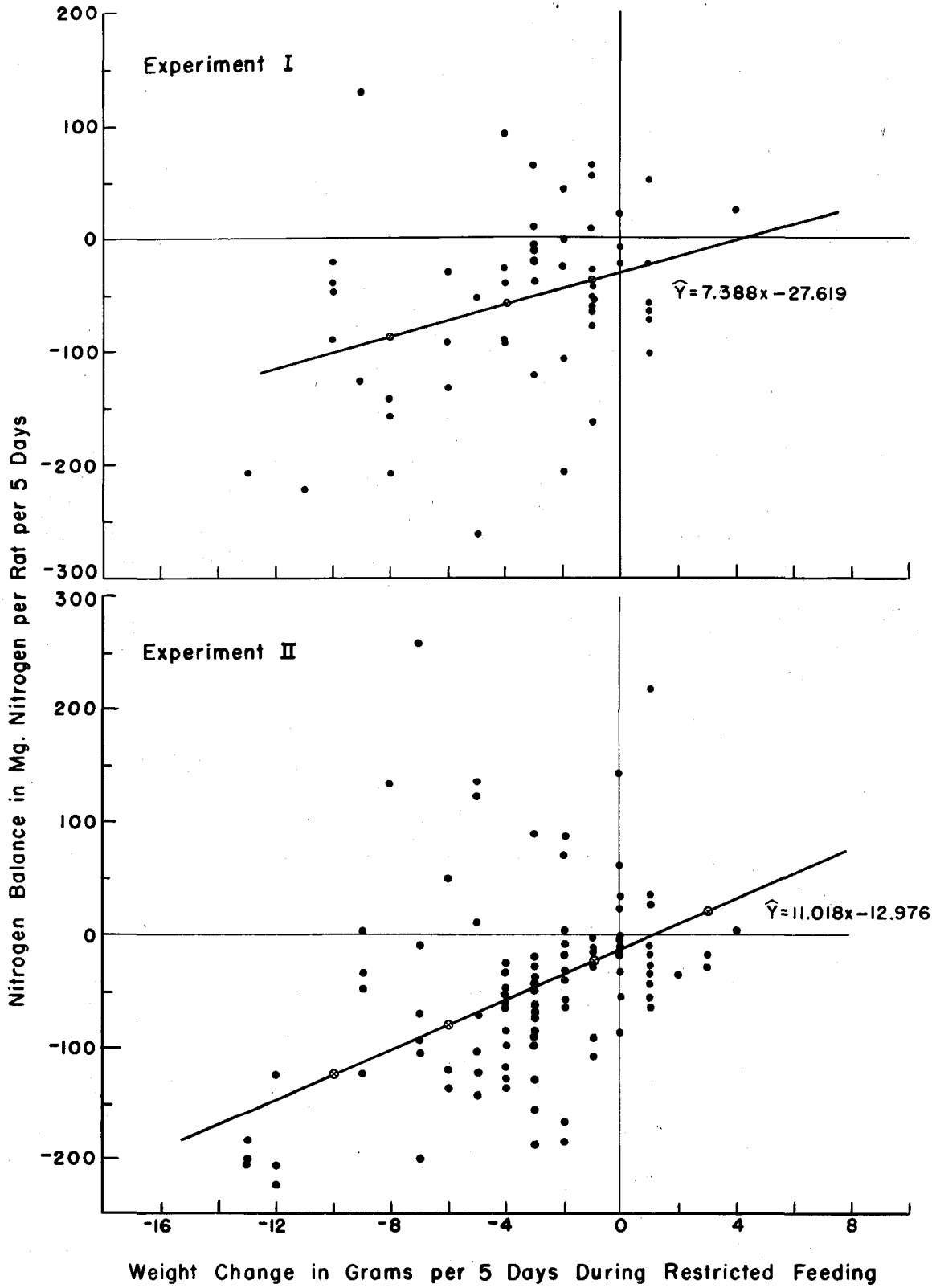

Experiment I
$\hat{Y}$=77.465+13.501x
Experiment II
Experiment III
$\hat{Y}$=67.860+9.274x
200
100
0
-100
-6
-4
-2
0
2
4
6
8
10
Nitrogen Balance in Mg. Nitrogen per Rat per 5 Days
Weight Change in Grams per 5 Days During Ad Libitum Feeding

Figure 2. Relation of weight changes in grams per 5 days to nitrogen balance in mg. of nitrogen per rat per 5 days during restricted feeding in Experiments I and II


Experiment I
Ŷ = 7.388x − 27.619
Experiment II
Ŷ = 11.018x − 12.976
Nitrogen Balance in Mg. Nitrogen per Rat per 5 Days
Weight Change in Grams per 5 Days During Restricted Feeding
200
100
0
-100
-200
-300
300
200
100
0
-100
-200
-16
-12
-8
-4
0
4
8

much higher than those obtained in Experiments I and III. There is a possibility that prior to the initiation of Experiment II, the animals may have been exposed to some unfavorable conditions such as changes in environmental temperature or humidity which caused losses in nitrogen. If this had occurred the animals would retain more nitrogen in order to compensate for losses.

Groups of rats fed 15 per cent of the calories as protein retained more nitrogen than those on the lower intakes of protein during ad libitum feeding (Table 7). Figures 3 and 4 show that the magnitude of nitrogen retentions was greater when the protein intake was high (rats in Group A, Experiment I; rats in groups A, B and C, Experiment II) than when the protein intake was low (rats in group C, Experiment I; rats in groups D, E and F, Experiment II). In Experiment I, nitrogen retentions and intakes by animals in group B were intermediate between those of rats in groups A and C.

When the energy intakes were restricted to two-thirds of the ad libitum food consumptions, nitrogen retentions decreased abruptly and negative nitrogen balances resulted. The magnitude of the negativity of nitrogen balance during the first period immediately following caloric restriction appeared to be dependent on the protein intake prior to

Figure 3. Nitrogen balance per 100-gram rat per 5-day periods during *ad libitum* and restricted feeding in Experiment I

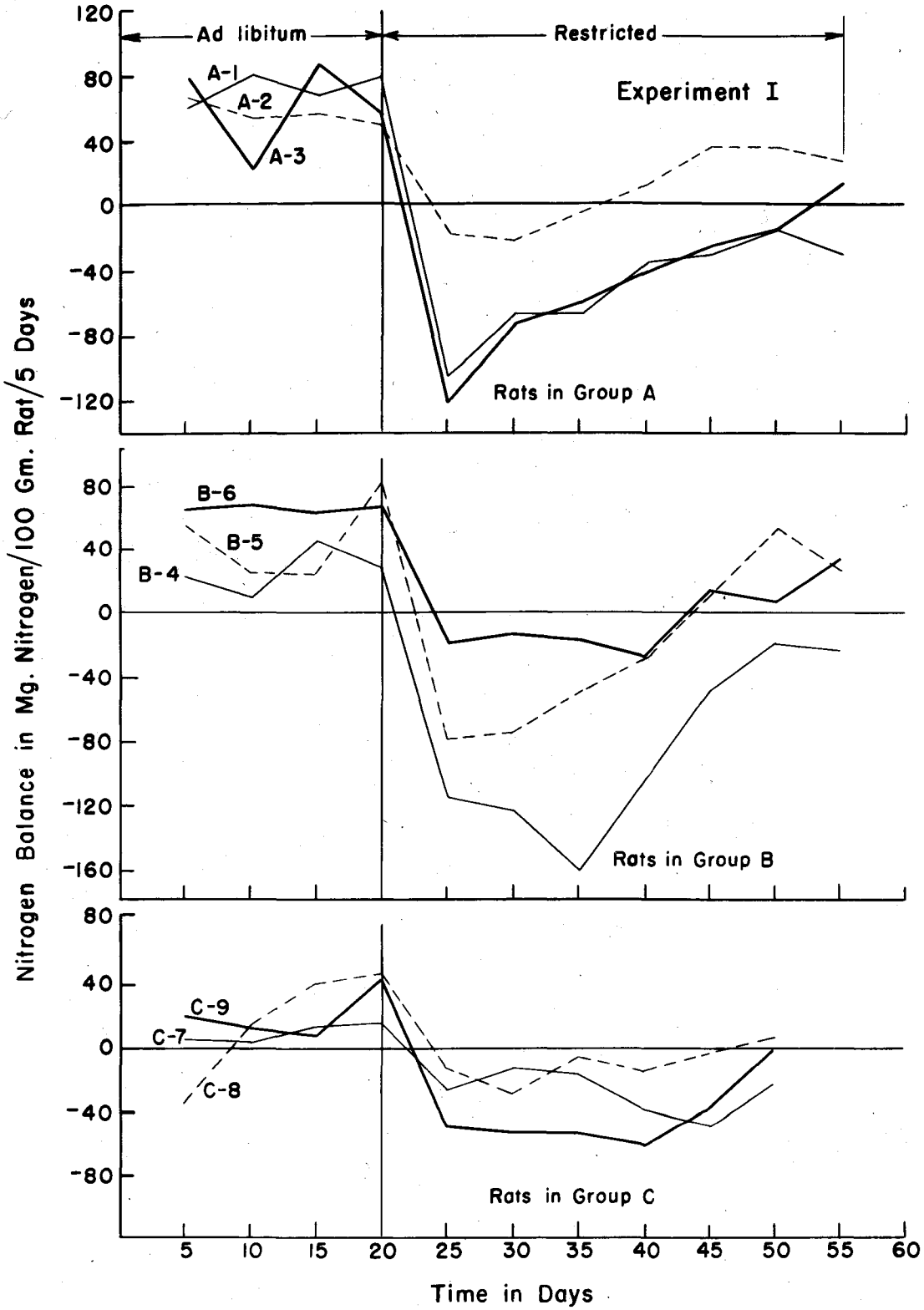
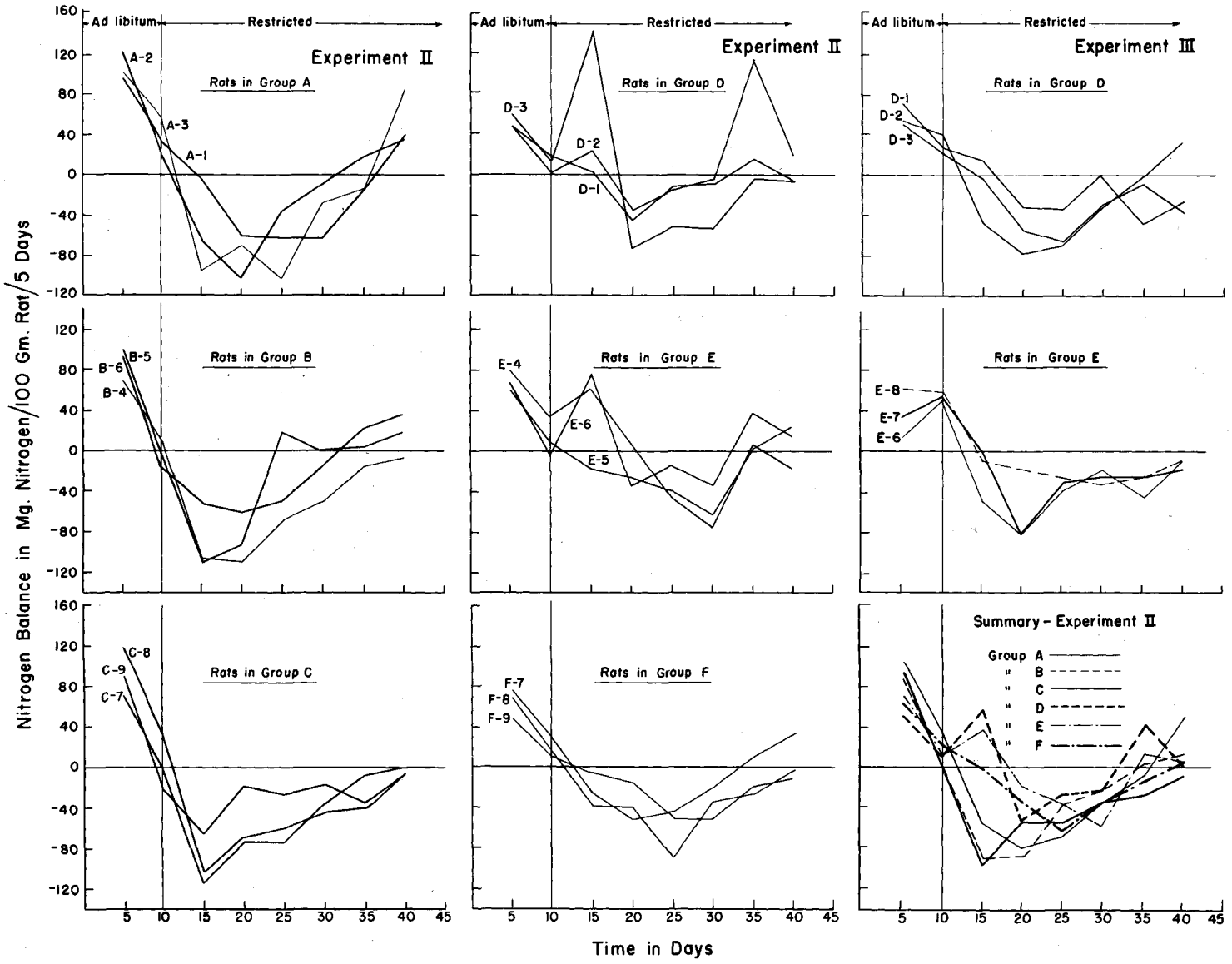

Ad libitum
Restricted
Experiment I
A-1
A-2
A-3
Rats in Group A
B-6
B-5
B-4
Rats in Group B
C-9
C-7
C-8
Rats in Group C
Nitrogen Balance in Mg. Nitrogen/100 Gm. Rat/5 Days
Time in Days
120
80
40
0
-40
-80
-120
-160
5
10
15
20
25
30
35
40
45
50
55
60

Figure 4. Nitrogen balance per 100-gram rat per 5-day periods during *ad libitum* and restricted feeding in Experiments II and III


Ad libitum
Restricted
Experiment II
Rats in Group A
A-2
A-3
A-1
Experiment II
Rats in Group D
D-3
D-2
D-1
Experiment III
Rats in Group D
D-1
D-2
D-3
Rats in Group B
B-6
B-5
B-4
Rats in Group E
E-4
E-6
E-5
Rats in Group E
E-8
E-7
E-6
Rats in Group C
C-8
C-9
C-7
Rats in Group F
F-7
F-8
F-9
Summary - Experiment II
Group A
" B
" C
" D
" E
" F
Nitrogen Balance in Mg. Nitrogen/100 Gm. Rat/5 Days
Time in Days

restricted feeding. The negative nitrogen balances observed in rats previously fed 5 per cent of the calories as protein (rats in group C, Experiment I; groups D, E and F in Experiments II and III) were less pronounced than those noted in animals which consumed 10 per cent (rats in group B, Experiment I) or 15 per cent of the calories as protein (rats in Group A, Experiment I; groups A, B and C in Experiment II).

Groups of animals fed 5 per cent of the calories as protein on unrestricted feeding and subsequently offered either 3 times or 2 times as much protein but only two-thirds as much food energy (groups D and E, Experiment II) were in positive nitrogen balance in the first period of caloric restriction. These observations are in accord with those made by Rosenthal and Allison (1951) on normal dogs. They noted that dogs whose initial body protein reserves were low responded to moderate amounts of protein during caloric restriction by a short period of positive nitrogen balance followed by a steadily increasing negative nitrogen balance. In the present study, the liver nitrogen but not the carcass nitrogen of the animals consuming the low protein diet on ad libitum food intake was significantly lower than that of animals fed the high protein diet.

Continuous nitrogen balance data based on 5-day periods were collected in Experiment III for animals in sub-groups D and E in order to verify the results obtained in Experiment II. Nitrogen balance information on these groups of animals in Experiment III (Figure 4) showed that the animals were in equilibrium during the first 5 days following caloric restriction.

When the nitrogen balances for successive periods in Experiment I during caloric restriction were added, the total nitrogen loss appeared to be independent of the protein intakes of the animals. However, there was a tendency for animals maintained on the low protein intake to excrete less nitrogen than animals on the two higher protein intakes. Thomson and Munro (1955) have shown a linear relationship between carbohydrate intake and nitrogen balance. In the present study, the carbohydrate content of the low protein diet was higher than that of the high protein diet.

In Experiment II, a statistically significant difference in total nitrogen lost during caloric restriction was obtained between the groups of animals which received 15 per cent (sub-groups A, B and C) and 5 per cent of the energy value of the diets as protein (sub-groups D, E and F). A summary of these data is shown in Figure 4. The

significant difference was due largely to the animals in groups D and E which were in positive nitrogen balance when caloric restriction was first imposed. It should be pointed out that rats in sub-groups C and F received about the same amount of nitrogen during restricted feeding. Rats in sub-groups B and E received about twice as much nitrogen and those in sub-groups A and D received about three times as much nitrogen as sub-groups C and F.

Several factors may be operating to spare nitrogen during caloric restriction in animals previously offered the low protein diet. It is possible that the total nitrogen losses incurred during caloric restriction may have reflected limited nitrogen reserves in the animals offered the low amounts of dietary protein on unrestricted feeding. Also, the protein intake during caloric restriction was larger in groups D and E than during ad libitum feeding and this may have prevented large losses of nitrogen.

No statistically significant differences were found between nitrogen losses of groups of animals on the three different protein intakes during caloric restriction, i.e.. sub-groups A and D vs. B and E vs. C and F, although the mean nitrogen loss tended to decrease with increasing nitrogen intake. Results reported in the literature indicated that restricting the energy intake below the critical caloric intake resulted in an impaired utilization of dietary protein for

tissue synthesis or maintenance (Bosshardt et al., 1946; Benditt and Humphreys et al., 1948; Cox et al., 1953).

Higher nitrogen intakes during ad libitum feeding resulted in higher nitrogen losses after restriction of food energy than lower nitrogen intakes during the ad libitum period, when nitrogen intake was similar in both groups after restriction. When nitrogen intake was maintained at the same level during both ad libitum and restricted feeding, caloric restriction determined the nitrogen loss.

The animals approached nitrogen equilibrium toward the end of a 30-day period of restricted feeding. In Experiment I, rats A-1, B-4 and C-6 which failed to re-attain nitrogen equilibrium showed lung infections at autopsy. Rat D-2 in Experiment II lost only small amounts of nitrogen during the period of restricted feeding and was retaining nitrogen after 20 days. At autopsy, this animal had unusually large amounts of visible fat in the viscera. This fat reserve must have spared body nitrogen losses when energy intake was reduced.

At the end of caloric restriction in Experiment III, most of the animals had not quite achieved nitrogen equilibrium. Perhaps the period of restriction needed

to be extended to 35 days instead of the 30 days used in the experiment.

In the present study, most of the animals were able to adjust to a limited caloric intake as reflected by their re-attainment of nitrogen equilibrium after restricted feeding for 30 or 35 days. At this point, it is worthwhile to recall Mitchell's (1944) definition of "nutritional adaptation":

> If an animal in equilibrium with its food supply (meaning a well-nourished animal) is subjected to nutritional stress, such as an inadequate (or excessive) supply of one or more of the essential nutrients, the animal will react in such a way as to minimize, as far as possible, or to undo entirely the effects of the nutritional stress.

In view of the fact that the problem of protein-calorie interrelationship has been investigated under a wide variety of experimental conditions, the present findings will apply only to the specific experimental situations adopted in this study. All the original data obtained in the present investigation are given in the Appendix.

Liver Protein and Enzyme Activity

Since the liver is capable of combining various metabolic activities with protein storage, this organ was investigated under the present experimental conditions.

Weights of livers obtained from stock control animals and groups of animals which had been fed ad libitum either 15 per cent or 5 per cent of the calories in the diet as protein did not differ significantly (Table 8).

The total nitrogen content of the livers of stock control animals was significantly higher than that of rats fed 15 per cent of their calories as protein from lactalbumin except in Experiment IV. The average liver weights of the stock animals in Experiment III and V (Series III) tended to be larger than those of rats fed lactalbumin and may account in part for the difference in total nitrogen. The total nitrogen content of the livers of rats maintained on diets containing 5 per cent of the calories as protein was somewhat lower than that of rats fed a higher level of protein.

In order to facilitate the discussion, diets supplying 15 per cent of the calories in the diet will be referred to hereafter as the high protein diet and those providing 5 per cent of the energy value of the diet will be referred to as the low protein diet.

In Experiments III and V (Series III), the percentage of protein in the livers of animals maintained on the high protein diet was greater than the protein concentration in the livers of the stock control rats (Table 9). The livers of these experimental animals were smaller than

Table 8. Average liver weights and liver nitrogen of rats on ad libitum and restricted food intake

Experiment no.	Liver weights						Mg. nitrogen/liver					
	I Gm.	II Gm.	III Gm.	IV Gm.	V Series III Gm.	V Series IV Gm.	I	II	III	IV	V Series III	V Series IV
Stock animals	--	--	6.5	6.4	6.9	6.4	--	--	233	219	239	237
Ad libitum intake												
1. Rats fed 15 per cent of cal. as protein	--	--	5.4	6.6	6.0	6.6	--	--	198	214	211	218
2. Rats fed 5 per cent of cal. as protein	--	--	5.9	6.3	6.8	5.9	--	--	187	194	201	189
Restricted intake												
Sub-groups												
A	4.7	4.6	3.9	4.4	4.7	4.6	171	161	158	171	182	176
B	4.2	4.1	4.1	4.8	4.7	--	162	151	151	173	175	--
C	4.5	4.0	4.2	4.8	4.3	4.6	164	148	152	176	161	171
D	--	4.5	4.3	5.5	4.7	4.4	--	164	172	202	176	171
E	--	4.4	4.1	4.6	4.8	--	--	158	162	172	183	--
F	--	4.7	4.8	4.4	4.3	4.4	--	162	176	174	162	162

Table 9. Average percentage of liver protein of rats on *ad libitum* and restricted food intake

Experiment no.	Percentage of protein					
	I	II	III	IV	V	
Groups of rats					Series III	Series IV
Stock animals	--	--	22.65	21.59	21.57	22.22
Ad libitum intake						
1. Rats fed 15 per cent of cal. as protein	--	--	23.24	20.50	22.15	20.60
2. Rats fed 5 per cent of cal. as protein	--	--	20.02	19.28	18.59	19.17
Restricted intake						
Sub-groups						
A	22.87	22.09	25.45	24.14	24.54	24.17
B	23.87	23.15	22.76	22.79	23.47	--
C	22.81	23.50	22.55	22.99	23.67	23.11
D	--	22.75	25.10	23.12	24.08	24.32
E	--	22.67	24.63	23.63	23.87	--
F	--	21.78	23.16	22.83	23.71	22.81

the livers of the stock control animals. In Experiments IV and V (Series IV), the percentage of protein in the livers of stock control animals was higher than the percentage of protein in the livers of rats fed the high protein diet. In this case, the livers of the control animals were smaller than those of the experimental animals. It, therefore, appears that the protein concentration in the livers of the stock control animals and the animals fed the high protein diet is a function of liver size.

The percentage of protein in the livers of animals fed the low protein diet was significantly smaller than that of stock controls or of animals fed the high protein diet. Since the liver weights of animals fed ad libitum did not vary significantly, the decrease in percentage of liver protein on the low protein diet may be associated with differences in other liver components. Increased deposition of liver glycogen and/or fat may have accompanied the loss in liver protein. Attention is called to the fact that this group of animals consumed more food than the group of rats fed the high protein diet. Such increased caloric intake might account for accumulation of appreciable quantities of glycogen or fat.

Livers were reduced considerably in size after caloric restriction regardless of the amount of protein in the

diets offered to the animals. The marked losses in the weights of the liver appear to be associated with changes in liver composition incurred by the reduction in food energy intakes. The total nitrogen content of the livers was markedly decreased (Table 8) and the per cent of protein in the liver increased (Table 9).

In Experiment I, animals maintained on an intermediate intake of protein (rats in group B) appeared to have a higher percentage of liver protein than rats consuming higher or lower amounts of protein. The livers of the rats in group B were also smaller than the livers of the animals in the other 2 groups of animals. Otherwise, the three different protein intakes during caloric restriction did not influence the percentage of protein in the liver significantly. These findings are in agreement with those obtained by Samvik (1953).

In Experiment II, the percentage of protein in the livers of rats in sub-group A were lower than the percentage of protein in the livers of rats in sub-groups B and C. The animals in sub-group A were maintained on the high intake of protein during both ad libitum and restricted feeding while rats in sub-groups B and C were fed two-thirds or one-third as much protein in the diet respectively on reduced food energy intake. The livers

of rats in sub-group A were larger than livers of rats in sub-groups B and C which may account for the lower protein concentration per unit of liver. Reduction in the protein intake did not appear to influence the percentage of protein in the livers of animals in sub-groups B and C. These results are not in agreement with those obtained in Experiments III, IV and V.

At the end of caloric restriction in Experiments III, IV and V, the percentages of protein in the livers of the animals fed different amounts of dietary protein were significantly higher than the percentage in the livers of the stock controls and animals fed ad libitum. The maintenance of a constant high intake of dietary protein by the animals in sub-groups A resulted in a greater rise in the percentage of liver protein than when the protein intake was progressively reduced (rats in sub-groups B and C). The differences in the percentage of liver protein between sub-groups A, B and C were statistically highly significant only because the values obtained for sub-group A were high.

Successive increments in the dietary protein intake during caloric restriction were associated with corresponding increases in the percentage of protein in the livers of rats in sub-groups D and E (Experiments II, III, IV and V). Animals in sub-group F maintained a constant low

intake of protein during periods of both ad libitum and restricted feeding. Increasing intakes of dietary protein influenced the percentage of liver protein at the 5 per cent level of statistical significance. The data also indicate that the intermediate protein intake was as effective as the high protein intake in increasing the concentration of protein per unit of liver.

When animals were grouped according to their protein intakes during the period of caloric restriction (sub-groups A and D vs. B and E vs. C and F), it was found that the influence of the protein intakes on the liver protein was highly significant. The average percentage of protein in the livers of rats in sub-groups A and D was higher than that of rats in sub-groups B and E and lowest in the livers of rats in sub-groups C and F.

As a consequence of caloric restriction, stores of glycogen or fat appear to have been withdrawn from the liver at a faster rate than protein in order to meet the body needs for energy. This may account for the increase in protein concentration since the percentage of moisture in the hepatic tissues was approximately 70 per cent in the livers of both ad libitum and restricted animals.

Reduction in liver weights of rats subjected to protein depletion or inanition have been reported by other

workers (Kosterlitz, 1949; Wainio et al., 1953). Harrison and Long (1945) have noted that glycogen was the most important factor determining liver weights because water was retained along with glycogen.

The weights of the livers were not significantly related to terminal body weights of the animals at autopsy. Keys and his associates (1950) have indicated that the relative as well as the absolute decreases in liver weights in cases of chronic undernutrition and starvation seem to exceed that of the human body as a whole.

Varying the dietary protein and caloric intakes in Experiments IV and V (Series III) had essentially the same effect on the activities of xanthine oxidase, succinic dehydrogenase and cytochrome oxidase systems in hepatic tissues as it did on the percentage of protein in the liver (Table 10).

The unit activity of xanthine oxidase was expressed in terms of mg. of uric acid produced per gm. of liver (dry weight) per hour and will be referred to hereafter simply as unit activity of xanthine oxidase. The unit activity of succinic dehydrogenase and cytochrome oxidase expressed as microliters of oxygen per mg. of liver tissue (dry weight) per hour will also be referred to as unit activity of succinic dehydrogenase or of cytochrome oxidase. Total

Table 10. Average xanthine oxidase, succinic dehydrogenase and cytochrome oxidase activites in liver tissues of rats on ad libitum and restricted food intake. Experiment IV (Series I and II) and Experiment V (Series III)

Groups	Xanthine oxidase activity		Succinic dehydrogenase activity		Cytochrome oxidase activity	
	Unit activity in mg. uric acid/gm. liver (dry wt.)/hr.	Total activity in uric acid/ liver/hr.	Unit activity succinate Q_{O_2}	Total activity in ml. O_2/liver/hr.	Unit activity ascorbate Q_{O_2}	Total in activity in ml. O_2/liver/hr.
Stock animals	5.39 (8)[a]	10.71	88.4 (8)	176.1	75.4 (4)	161.2
Ad libitum intake						
1. Rats fed 15 per cent of cal. as protein	4.74 (8)	9.75	71.3 (8)	149.2	79.7 (2)	151.8
2. Rats fed 5 per cent of cal. as protein	3.48 (8)	7.17	54.8 (8)	113.7	64.5 (2)	141.2
Restricted intake						
Sub-groups						
A	5.11 (6)	6.99	95.7 (6)	131.5	83.1 (4)	113.0
B	4.96 (6)	7.16	86.8 (6)	125.2	83.9 (3)	118.0

[a](No.) indicates number of rats represented.

Table 10 (Cont'd)

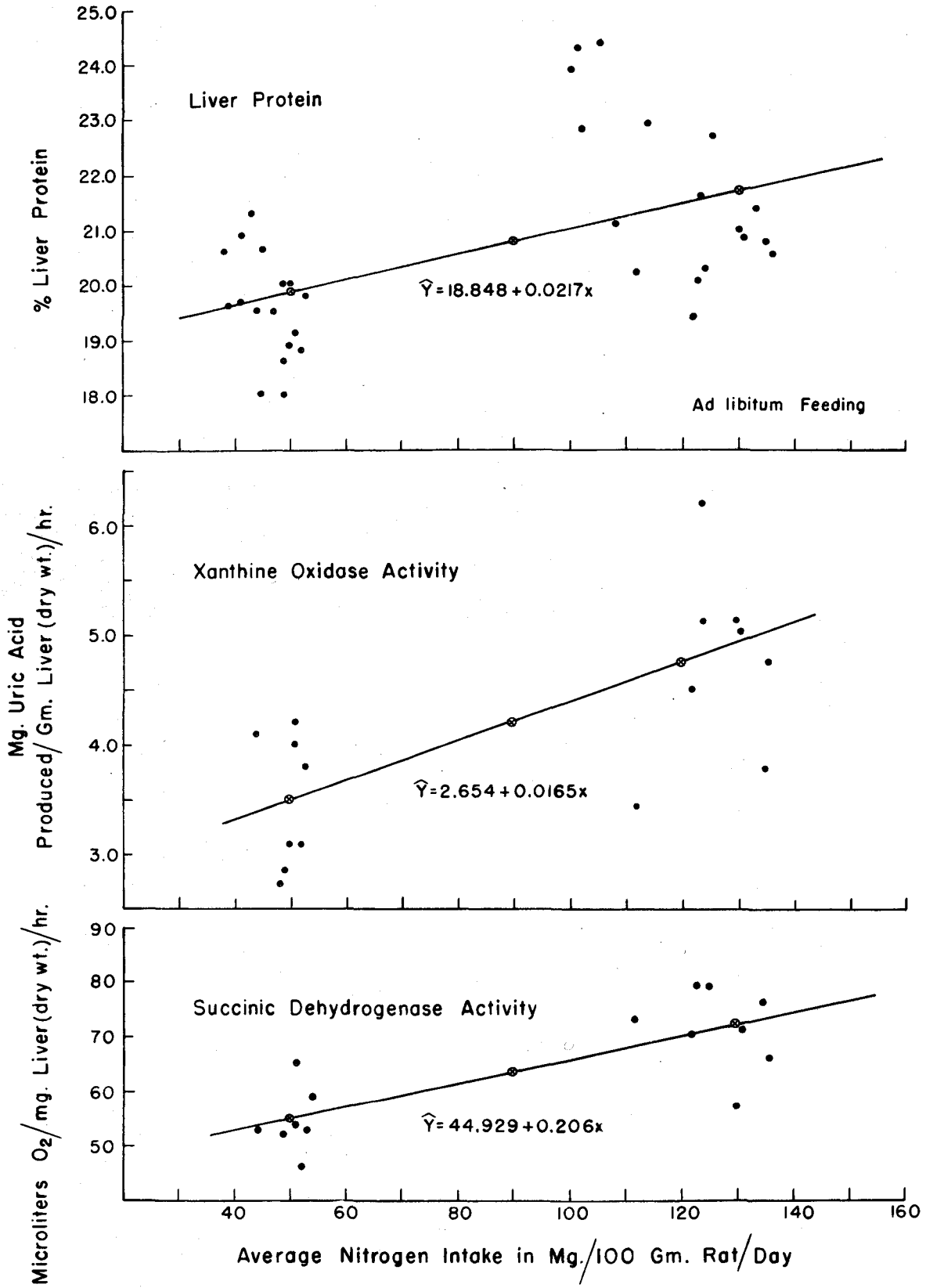
Groups	Xanthine oxidase activity		Succinic dehydrogenase activity		Cytochrome oxidase activity	
	Unit activity in mg. uric acid/gm. liver (dry wt.)/hr.	Total activity in uric acid/ liver/hr.	Unit activity succinate Q_{O_2}	Total activity in ml. O_2/liver/hr.	Unit activity ascorbate Q_{O_2}	Total in activity in ml. O_2/liver/hr.
C	5.01 (6)	6.73	81.4 (6)	113.6	71.2 (1)	98.3
D	5.80 (6)	9.23	89.3 (6)	139.7	84.1 (4)	128.2
E	5.43 (6)	7.79	94.2 (6)	134.7	78.3 (3)	115.2
F	4.78 (6)	6.71	83.2 (6)	116.6	76.6 (3)	110.0

activity will refer to total activity per liver per hour.

Both the unit and total activities of xanthine oxidase and succinic dehydrogenase enzyme systems in the liver tissues of animals fed the high protein diet during ad libitum feeding were significantly lower than those of the stock animals and greatly diminished in the livers of the animals maintained on the low protein diet. Statistical treatment of the data indicated highly significant differences between these 3 groups of animals. The heightened activities of the xanthine oxidase and succinic dehydrogenase systems in the livers of the stock control animals may reflect their protein intake which was 23 per cent of the diet. In the case of cytochrome oxidase enzyme system, the unit activity was higher in the livers of animals fed the high protein diet than in the livers of the stock control animals. However, data collected on the activity of the cytochrome oxidase system in the liver were too few to be analyzed statistically.

During ad libitum feeding, linear relationships were observed between average daily nitrogen intake expressed in mg. of nitrogen per 100-gram rat and unit activities of both xanthine oxidase and succinic dehydrogenase (Figure 5). Correlation coefficients were 0.715 and

Figure 5. Relation of per cent liver protein and of xanthine oxidase and succinic dehydrogenase activities in liver tissues to average daily nitrogen intake in mg. of nitrogen per 100-gram rat during ad libitum feeding in Experiments IV and V (Series III)


Liver Protein
% Liver Protein
25.0
24.0
23.0
22.0
21.0
20.0
19.0
18.0
Ŷ = 18.848 + 0.0217x
Ad libitum Feeding
Xanthine Oxidase Activity
Mg. Uric Acid Produced/Gm. Liver (dry wt.)/hr.
6.0
5.0
4.0
3.0
Ŷ = 2.654 + 0.0165x
Succinic Dehydrogenase Activity
Microliters O2/mg. Liver (dry wt.)/hr.
90
80
70
60
50
Ŷ = 44.929 + 0.206x
40
60
80
100
120
140
160
Average Nitrogen Intake in Mg./100 Gm. Rat/Day

0.780 respectively. The relationship observed for percentage liver protein and average daily nitrogen intake per 100-gram rat had a correlation coefficient of 0.506. The parallelism between unit enzyme activity and protein concentration per unit of liver is evident.

The unit activities of xanthine oxidase, succinic dehydrogenase and cytochrome oxidase in liver tissues of rats after a 30-day period of restricted food energy intake exceeded the control and ad libitum values. The reduction in liver size accompanied by an increase in protein concentration would account for the increased unit activities of the 3 enzyme systems studied.

During caloric restriction of the rats pre-fed the high protein diet, protein intakes which were equivalent to the absolute amounts of protein consumed ad libitum or two-thirds or one-third of this amount appeared to be equally effective in maintaining a high unit activity of xanthine oxidase in the hepatic tissues (rats in sub-groups A, B and C). Statistical analyses of the data showed that the influence of decreasing intakes of protein on liver xanthine oxidase during restricted feeding was not significant. It also appears that protein reserves in the liver may have been depleted selectively. The concentration of xanthine oxidase was maintained although the

protein concentration of the liver decreased as the protein intake was reduced. The total activity of xanthine oxidase in the livers of restricted animals was lower than that of their ad libitum controls, but did not vary with protein intakes.

In case of the animals pre-fed the low protein diet, increasing intakes of protein on restricted caloric intakes produced corresponding increments in the unit activities of xanthine oxidase. The effects of increasing intakes of protein on the unit activity of liver xanthine oxidase were statistically significant to the 5 per cent level only. The maintenance of a low protein intake during both ad libitum and restricted feeding (rats in sub-group F) showed an increase in the unit activity of xanthine oxidase over that observed on ad libitum food intake only because the livers were smaller after caloric restriction. The total activities were higher than the corresponding ad libitum value except in the livers of rats in sub-group F. Progressive increments in protein intake by rats in sub-groups D and E were associated with increasing total activity. These observations may indicate that the protein reserves in the liver were being increased.

The maintenance of a constant high intake of protein by rats in sub-group A after restricted feeding resulted in

an increased unit activity of succinic dehydrogenase as compared to the activity obtained during ad libitum feeding. Successive decrements in protein intake resulted in corresponding reductions in the unit activities of succinic dehydrogenase which was statistically significant to the 1 per cent level. The total activities of this enzyme were lower after caloric restriction than during ad libitum feeding. Decreasing protein intakes by rats in sub-groups B and C resulted in a corresponding decrease in total activities.

When the intakes of dietary protein were increased progressively (sub-groups D and E), the increase in unit activities of succinic dehydrogenase were of this enzyme highly statistically significant. The total activity after restricted food intake was higher than the ad libitum value except in the livers of rats in sub-group F. Increasing intakes of protein by rats in sub-groups D and E were accompanied by increments in the total activities above the ad libitum value.

From the data collected on liver cytochrome oxidase, there appeared to be an increase in the unit activities with progressive reductions in protein intake during caloric restriction except in the livers of rats in sub-group C. The unit activities in livers of rats in

sub-groups A and B were similar. Attention is called to the fact that the data on cytochrome oxidase activity in sub-group C represent one animal only. The total activities were lower in the livers of rats in sub-groups A, B and C than the total activity of the livers of animals fed ad libitum.

Increasing protein intakes by rats in sub-groups D and E increased the unit activities of the cytochrome oxidase system in the livers of rats over that obtained for rats fed low protein diets ad libitum. The maintenance of a constant protein intake by rats in sub-group F likewise produced an increase in the unit activity. The total activities of this enzyme system in these groups of animals were lower than the ad libitum value.

Intakes of protein during restricted feeding regardless of the protein intakes during ad libitum food consumption (rats in sub-groups A and D vs. B and E vs. C and F) were significantly related to the xanthine oxidase and succinic dehydrogenase activities of the liver. The few data obtained for cytochrome oxidase enzyme system did not permit a similar statistical treatment.

At the end of caloric restriction, direct relationships were obtained for daily nitrogen intake per 100-gram rat and both percentage protein liver and succinic dehydrogenase (Figure 6). The correlations were statistically

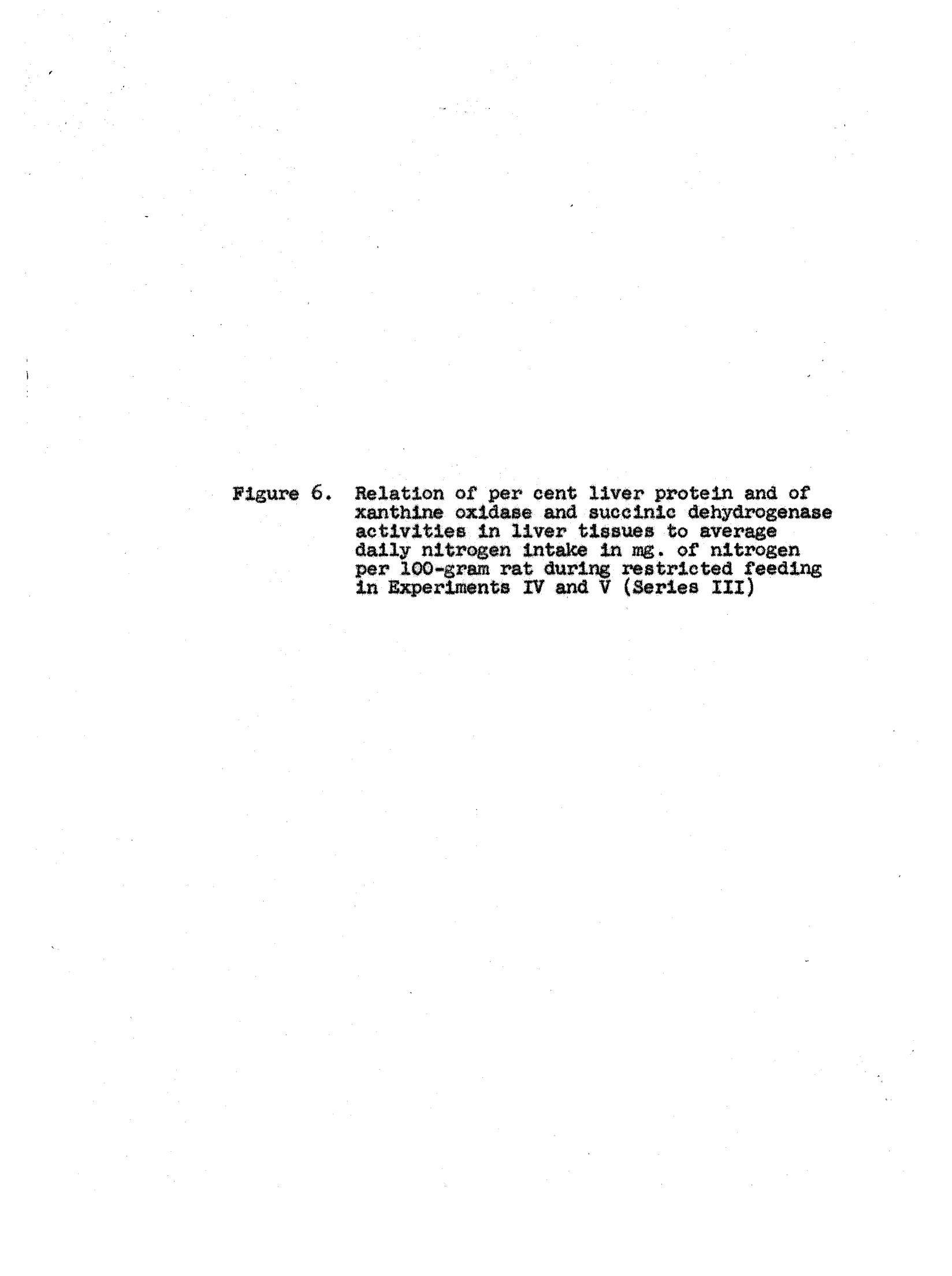
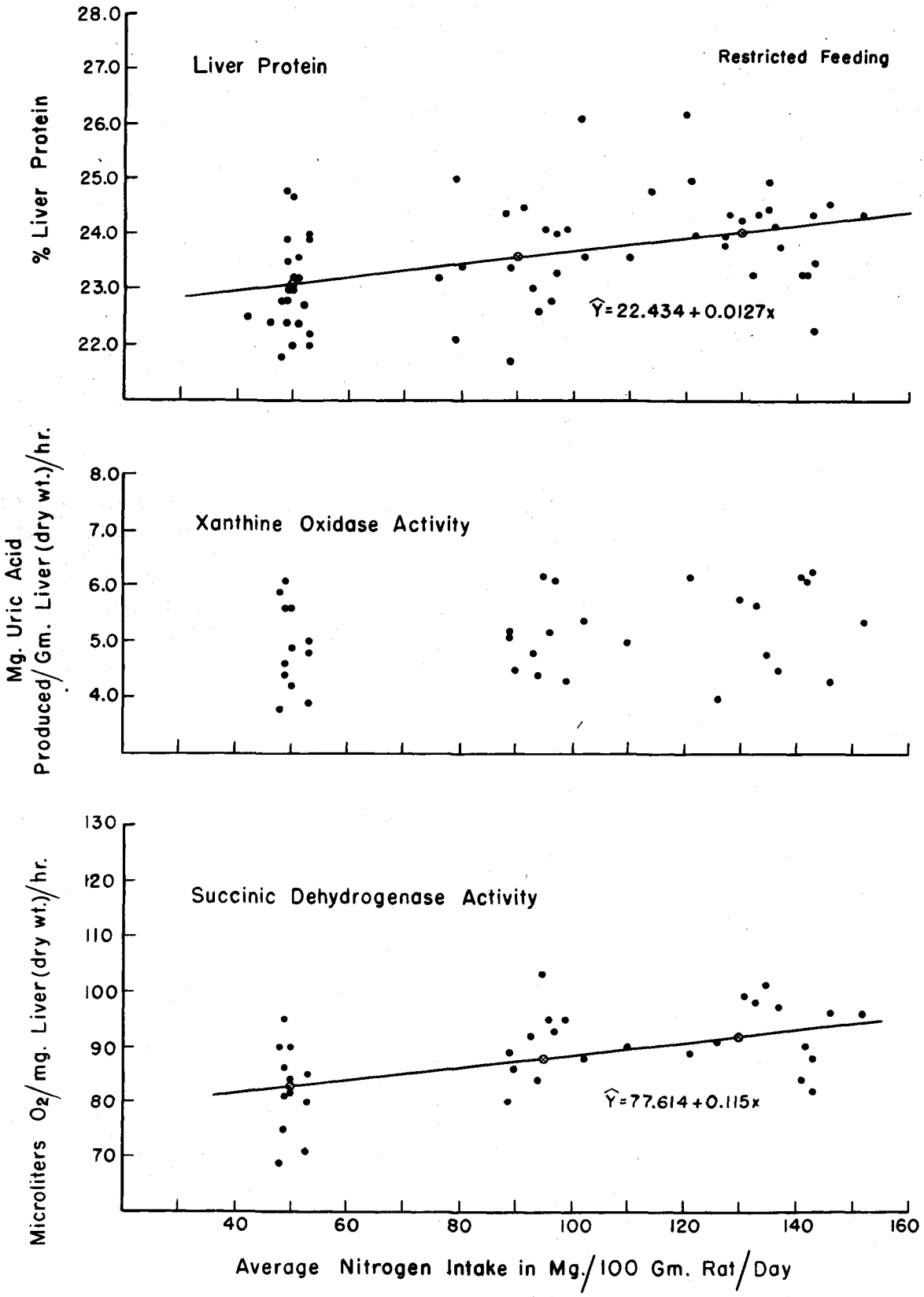


Figure 6. Relation of per cent liver protein and of xanthine oxidase and succinic dehydrogenase activities in liver tissues to average daily nitrogen intake in mg. of nitrogen per 100-gram rat during restricted feeding in Experiments IV and V (Series III)


Liver Protein
Restricted Feeding
% Liver Protein
28.0
27.0
26.0
25.0
24.0
23.0
22.0
Ŷ=22.434+0.0127x
Xanthine Oxidase Activity
Mg. Uric Acid Produced/Gm. Liver (dry wt.)/hr.
8.0
7.0
6.0
5.0
4.0
Succinic Dehydrogenase Activity
Microliters O2/mg. Liver (dry wt.)/hr.
130
120
110
100
90
80
70
Ŷ=77.614+0.115x
40
60
80
100
120
140
160
Average Nitrogen Intake in Mg./100 Gm. Rat/Day

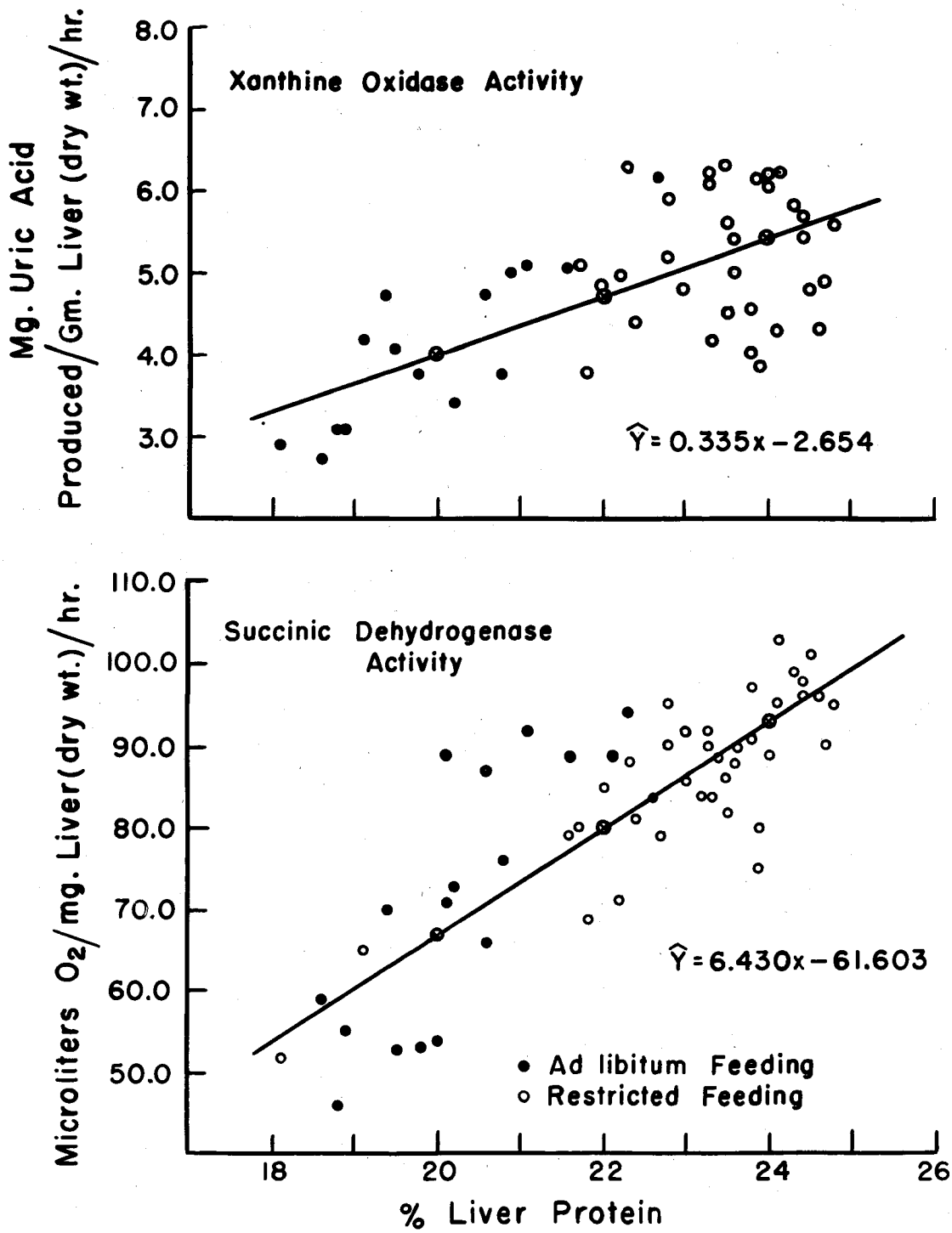
significant to the 1 per cent level with r-values of 0.459 and 0.570 respectively. The correlation obtained for xanthine oxidase activity and average nitrogen intake per 100 gram rat was significant to the 5 per cent level only.

The activities of xanthine oxidase and succinic dehydrogenase in hepatic tissues during ad libitum and restricted feeding were directly related to the percentage of liver protein (Figure 7). The correlation coefficients for xanthine oxidase activity and for succinic dehydrogenase activities were 0.646 and 0.821 respectively.

The percentage of protein and the activities of xanthine oxidase and succinic dehydrogenase enzyme systems in liver tissues were all dependent on the intake of dietary protein. The linear relationships between percentage of liver protein and both xanthine oxidase and succinic dehydrogenase activities strengthen the assumption that liver enzyme activities are dependent on the protein concentration of the liver. These findings provide further support to Miller's (1948, 1950) thesis that changes in enzyme activity represent changes in the amount of enzyme protein per se.

Although the percentage of liver protein was directly related to the average daily nitrogen intake, a significant but inverse relationship existed between the percentage

Figure 7. Relation of xanthine oxidase and succinic dehydrogenase activities in liver tissues to per cent liver protein during *ad libitum* and restricted feeding in Experiments IV and V (Series III)

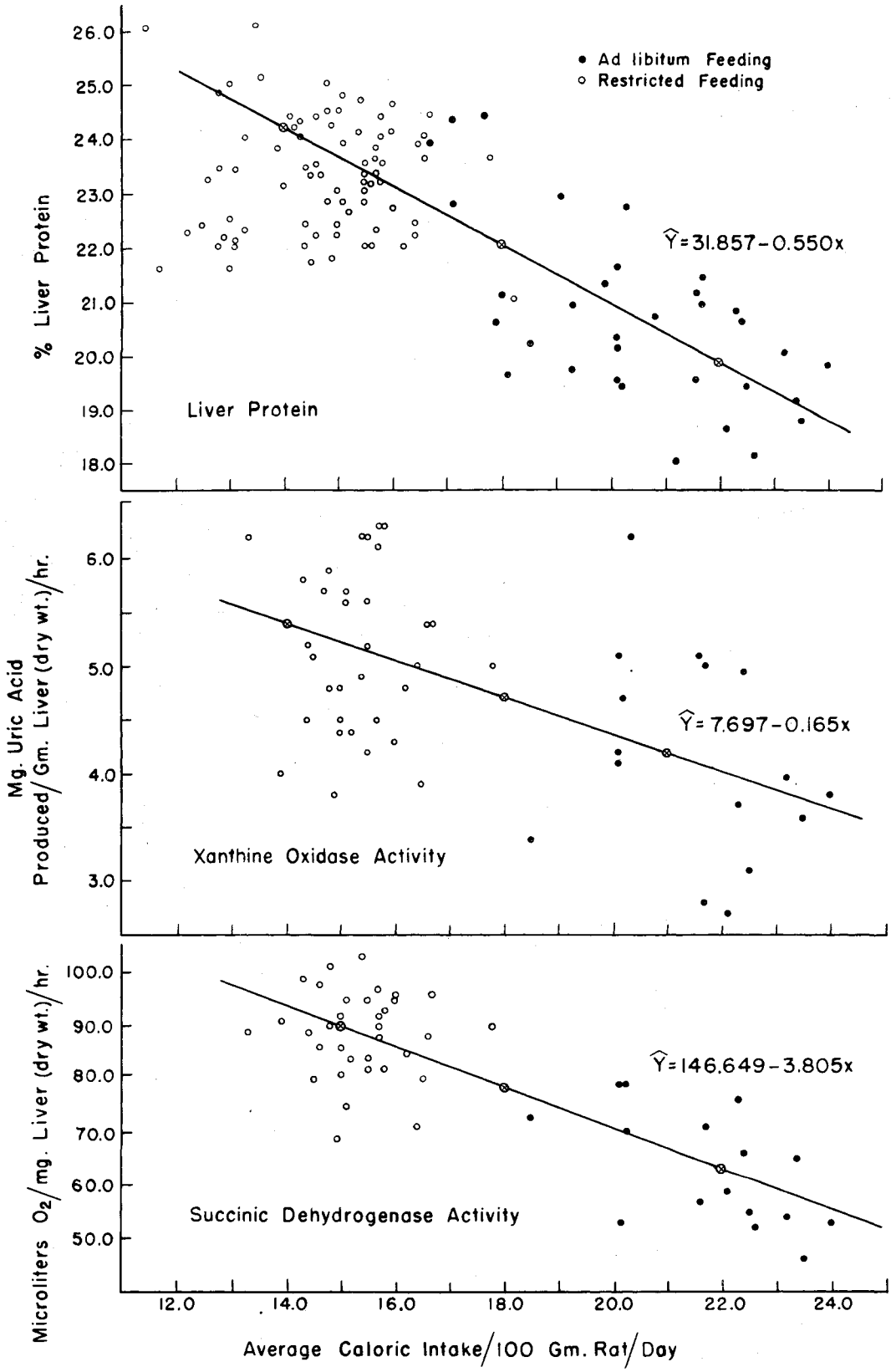

Xanthine Oxidase Activity
Mg. Uric Acid Produced/Gm. Liver (dry wt.)/hr.
8.0
7.0
6.0
5.0
4.0
3.0
Ŷ = 0.335x − 2.654
Succinic Dehydrogenase Activity
Microliters O2/mg. Liver (dry wt.)/hr.
110.0
100.0
90.0
80.0
70.0
60.0
50.0
Ŷ = 6.430x − 61.603
● Ad libitum Feeding
○ Restricted Feeding
18
20
22
24
26
% Liver Protein

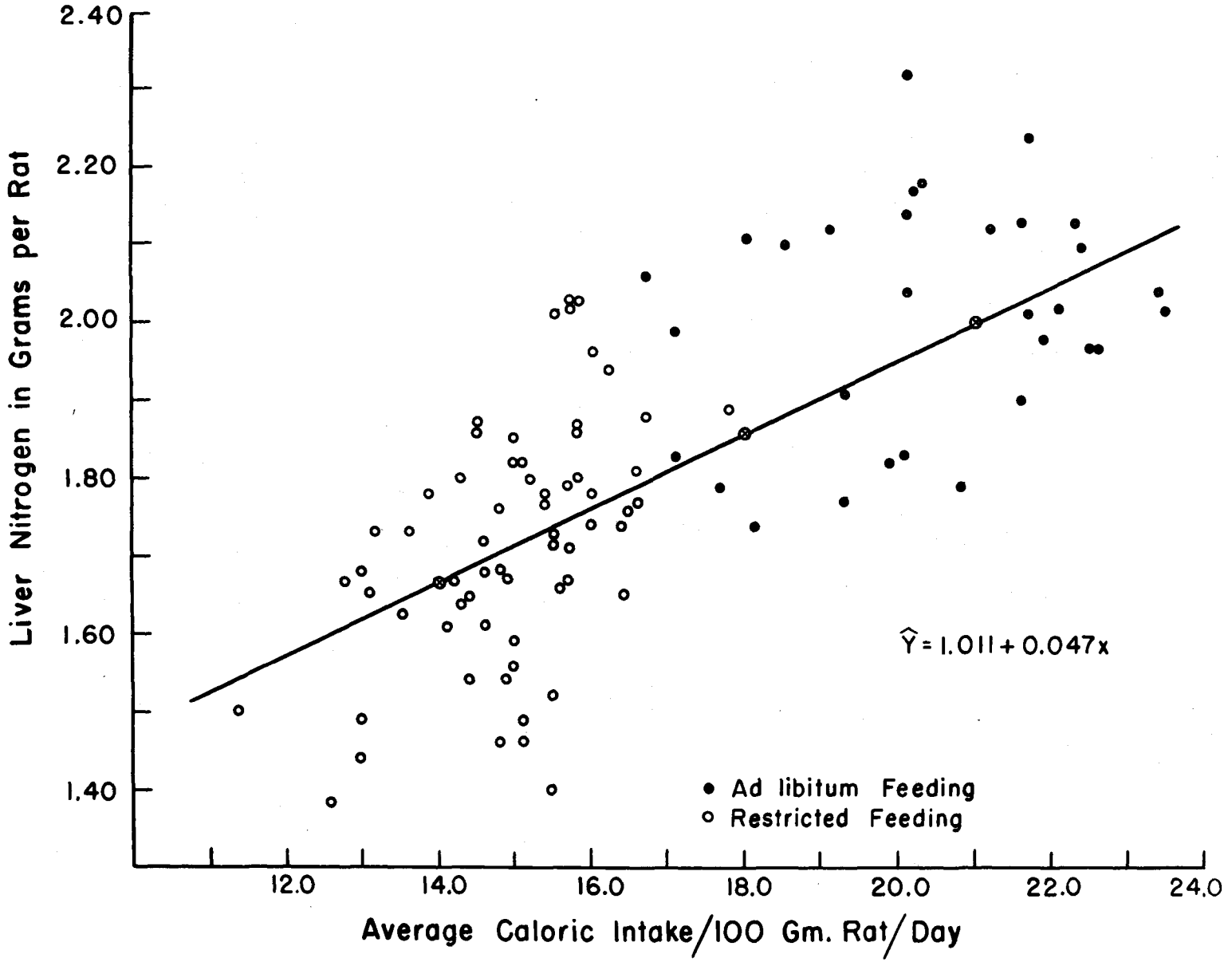
of liver protein and the average daily caloric intake per 100-gram rat during ad libitum and restricted feeding (Figure 8). The correlation coefficient (r) was -0.868. On ad libitum intakes of dietary food energy, the concentration of protein per unit of liver was low because the livers probably contained appreciable stores of glycogen or fat.

Both xanthine oxidase and succinic dehydrogenase activities in the liver were also inversely related to the average daily caloric intake per 100-gram rat during ad libitum and restricted feeding (Figure 8). The correlation coefficients (r) were -0.541 and -0.823 for the activities of xanthine oxidase and succinic dehydrogenase respectively. Therefore, variations in food energy intakes influenced liver enzyme activities and the percentage of liver protein in a similar manner.

Figure 9 shows that the total liver nitrogen is linearly related to the average daily caloric intake per 100-gram rat. The relationship was highly significant, $r = 0.709$. This observation is in agreement with the finding of Munro and Naismith (1953) that the amount of protein per liver was positively influenced by increments in food energy intake. Under the present experimental conditions, increasing intakes of calories were associated

Figure 8. Relation of per cent liver protein and of xanthine oxidase and succinic dehydrogenase activities in liver tissues to average daily caloric intake per 100-gram rat during *ad libitum* and restricted feeding in Experiments IV and V (Series III)


• Ad libitum Feeding
○ Restricted Feeding
% Liver Protein
Liver Protein
Ŷ=31.857-0.550x
Mg. Uric Acid Produced/Gm. Liver (dry wt.)/hr.
Xanthine Oxidase Activity
Ŷ=7.697-0.165x
Microliters O2/mg. Liver (dry wt.)/hr.
Succinic Dehydrogenase Activity
Ŷ=146.649-3.805x
Average Caloric Intake/100 Gm. Rat/Day


2.40
2.20
2.00
1.80
1.60
1.40
Liver Nitrogen in Grams per Rat
$\hat{Y} = 1.011 + 0.047x$
• Ad libitum Feeding
○ Restricted Feeding
12.0
14.0
16.0
18.0
20.0
22.0
24.0
Average Caloric Intake/100 Gm. Rat/Day

with higher total liver nitrogen content because the livers also increased in size.

Whether the unit activity of the enzyme systems studied was expressed on a wet weight basis, or dry weight basis or per mg. liver nitrogen, the same relationships hold for enzyme activity and caloric intake, nitrogen intake, and percentage of liver protein during ad libitum and restricted feeding.

Carcass Nitrogen

The carcass weights and total nitrogen content of the carcasses of the stock control and animals fed ad libitum were not significantly different (Table 11). After restricted feeding, there was a loss of both carcass weight and carcass nitrogen. The mean total carcass nitrogen tended to reflect nitrogen intake during caloric restriction.

No significant differences were noted in the percentage of protein in the carcasses of the different groups of animals maintained on varying protein intakes during ad libitum and restricted feeding (Table 12). The percentage of carcass nitrogen was not related to either the protein intake or caloric intake. Interaction between protein and

Table 11. Average carcass weights[a] and carcass nitrogen of rats on *ad libitum* and restricted food intake

	Carcass weights				Carcass nitrogen				
Experiment No.	I	II	III	V	I	II	III	IV	V
Groups of rats	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
Stock animals	--	--	151.8	155.7	--	--	5.40	5.55	5.80
Ad libitum intake									
1. Rats fed 15 per cent of cal. as protein	--	--	153.4	162.2	--	--	5.51	6.10	5.74
2. Rats fed 5 per cent of cal. as protein	--	--	157.1	157.0	--	--	5.57	6.01	5.64
Restricted intake									
Sub-groups									
A	145.0	146.7	144.8	153.8	5.17	5.02	5.22	5.72	5.48
B	141.0	142.8	137.4	--	5.14	4.95	5.09	5.77	5.60
C	140.5	142.1	129.1	153.2	5.08	4.74	4.84	5.84	5.40
D	--	152.8	150.7	155.7	--	5.07	5.33	6.07	5.53
E	--	146.6	144.6	--	--	4.99	5.22	5.70	5.38
F	--	149.8	143.1	148.8	--	5.01	4.97	5.76	5.20

[a]No carcass weights recorded in Experiment IV.

Table 12. Average percentage of carcass protein[a] of rats on ad libitum and restricted food intake

Experiment No.	Per cent protein			
	I	II	III	V[b]
Groups of rats				
Stock animals	--	--	22.27	21.86
Ad libitum intake				
1. Rats fed 15 per cent of cal. as protein	--	--	22.48	22.21
2. Rats fed 5 per cent of cal. as protein	--	--	22.18	22.13
Restricted intake				
Sub-groups				
A	22.29	21.40	22.51	21.93
B	22.85	21.66	23.19	--
C	22.62	20.84	23.43	22.44
D	--	20.79	22.09	22.00
E	--	20.29	22.60	--
F	--	20.92	21.80	21.32

[a]No carcass weights recorded in Experiments IV and V (Series III).

[b]Includes Series IV only.

calories may have prevented changes in the protein concentration per unit of carcass.

Samvik (1953) had observed a linear relationship between the dietary intake of protein and the percentage of protein in the carcass of male adults rat fed varying amounts of protein and calories. One possible explanation for the discrepancy between her experiment and the present one is the difference in the sex of the animals studied. Female rats have a greater inherent tendency to accumulate body fat than male rats. The presence of appreciable and varying quantities of body fat in female rats may have counteracted the influence of dietary protein intake on the protein concentration in the carcasses of these animals when given different amounts of dietary protein and calories.

Body Fat and Protein Metabolism

The body stores of fat represent a potential source of energy. Under conditions of caloric deprivation, the energy needs of animals are met in part by tissue fat. When the fat reserves become depleted, tissue protein is burned for energy. Therefore, the course of protein

metabolism during caloric inadequacy will depend upon the amount of body fat.

In the present study, the amounts of fat in the carcasses of eviscerated animals were measured by a specific gravity method and by volumetric estimations of the separated fat layer in the acid digest of the carcass. The data obtained did not indicate any relationship with varying intakes of dietary protein and calories.

Records were made of the amounts of visible fat in the viscera of the animals at autopsy. In Experiment I, there were no indications of unusual amounts of visible fat at the end of the experimental period. In Experiment II, there appeared to be appreciable quantities of visible fat in all the animals. Following the period of ad libitum feeding, animals in Experiment III had unusually large quantities of visible fat regardless of the intake of protein. At the end of caloric restriction, animals previously fed diets supplying 5 per cent of the calories as protein had definitely more visible fat than animals maintained on the high protein diet. The animals studied in Experiments IV and V did not have large amounts of visible fat in the viscera at autopsy. However, volumetric estimates of carcass fat indicated appreciable amounts of fat. These animals were older than the animals used in

Experiments II and III. Large quantities of visceral fat may be associated with young adult animals. The distribution of body fat in the animals studied in Experiments IV and V may have changed so that the amount of fat in the subcutaneous fat depot was larger than the amount of visceral fat.

Reed and his co-workers (1930) have found that in a normal female rat 50 per cent of the total storage fat is located in the subcutaneous fat depot and 20 per cent in the genital fat depot. The rest of the body fat is distributed in the mesenteric, intermuscular and omental fat depots.

Specific gravity measurements were made on the clipped eviscerated carcasses of stock control animals and animals fed ad libitum in Experiments III and V (Series IV). Neither the specific gravity nor the percentage of body fat derived from specific gravity data were significantly different in these two groups of animals (Table 13). Following caloric restriction, there was a significant difference in specific gravity and percentage body fat between groups of animals previously fed 15 and 5 per cent of the calories as protein in Experiment III, but not in Experiment V (Series IV). No significant differences were found in specific gravity and percentage of fat in the

Table 13. Average percentages of body fat derived from specific gravity measurements and from estimated volumes of fat of eviscerated carcasses. Experiments III and V (Series IV)

Groups of rats	Experiment III			Experiment V (Series IV)		
	Specific gravity		Estimated ml. of fat/100-gm. carcass	Specific gravity		Estimated ml. of fat/100-gm. carcass
	Gms/ml.	Calculated body fat in per cent		Gms/ml.	Calculated body fat in per cent	
Stock animals	1.061 (6)[a]	17.4	8.9	1.062 (3)	17.1	7.9
Ad libitum intake						
1. Rats fed 15 per cent of cal. as protein	1.066 (6)	15.1	8.0	1.058 (3)	18.6	8.3
2. Rats fed 5 per cent of cal. as protein	1.062 (6)	17.1	8.0	1.055 (3)	20.4	8.5
Restricted intake						
Sub-groups: A	1.062 (2)	16.7	7.6	1.055 (3)	20.4	9.9
B	1.069 (3)	13.6	4.2	--	--	--
C	1.067 (3)	14.5	4.2	1.059 (3)	18.3	7.3
D	1.056 (3)	19.8	7.8	1.053 (3)	21.1	8.9
E	1.060 (3)	17.8	8.0	--	--	--
F	1.056 (2)	19.5	6.3	1.066 (3)	15.0	7.4

[a]Number of animals represented.

carcasses of the animals fed on the different intakes of protein during caloric restriction. The percentage of fat derived from specific gravity measurements was not related to the average daily caloric intake per 100-gram rat.

The percentage of fat derived from estimated volumes of carcass fat in Experiments II, III and IV but not in Experiment V indicated that at the end of caloric restriction, animals pre-fed the low protein diet (rats in sub-groups D, E and F) appeared to have more fat in their carcasses than the animals fed the high protein intake (rats in sub-groups A, B and C). The difference in the amounts of fat in the carcasses of these two groups of animals may be a reflection of the higher food intake of animals fed the low protein diet in contrast to the lower food intake of animals maintained on the high protein intake. Similar observations were made by Samvik (1953) that male albino rats maintained on diets supplying 5 per cent of the calories as protein appeared to have more but not significantly more amounts of fat in the carcass than animals fed the higher intakes of protein. In Experiment V of the present study, the amount of fat in the carcass of all animals after restricted feeding were about the same.

Since no carcass weights were recorded in Experiments IV and V (Series III), the data obtained on estimated

volumes of carcass fat were expressed in terms of the ratio of ml. of fat to terminal body weight x 100 for purposes of comparison. These conversions were justified because the carcass weights were directly related to the terminal body weights. The ratio of fat volume to terminal body weight x 100 was not related to the caloric or protein intake during either ad libitum or restricted feeding.

Information on the activity as well as the basal metabolic activity of the animals during the experimental period may help clarify the findings on body fat under the conditions used in the present study.

Hemoglobin Concentration in the Blood

The presence of an adequate supply of dietary protein is essential for the maintenance of a normal concentration of hemoglobin in the blood. According to Orten and Orten (1943), the synthesis of hemoglobin is dependent upon the amounts of protein in the diet.

The hemoglobin concentrations expressed in gram per cent at the end of Experiments I and II are summarized in Table 14. The influence of the different intakes of dietary protein during caloric restriction on the percentage of hemoglobin in the blood of the different groups of

Table 14. Hemoglobin levels in grams per 100 grams of blood of rats at the end of Experiments I and II

Experiment I		Experiment II			
Rat number	Gm. per cent hemoglobin	Rat number	Gm. per cent hemoglobin	Rat number	Gm. per cent hemoglobin
A-1	12.81	A-1	11.02	D-1	12.31
A-2	12.35	A-2	12.09	D-2	12.77
A-3	13.42	A-3	11.90	D-3	13.29
Average	12.86	Average	11.67	Average	12.79
B-4	13.19	B-4	12.58	E-4	11.57
B-5	12.90	B-5	12.01	E-5	12.33
B-6	12.17	B-6	12.25	E-6	12.14
Average	12.75	Average	12.28	Average	12.01
C-7	12.83	C-7	11.65	F-7	11.22
C-8	11.19	C-8	12.67	F-8	12.77
C-9	13.24	C-9	12.00	F-9	12.39
Average	12.42	Average	12.11	Average	12.14
Total average:	12.68	Total average:	12.17		

animals was not significant. No data were obtained on the hemoglobin concentration in the blood of stock control and animals fed ad libitum for purposes of comparison with normal or control values.

SUMMARY AND CONCLUSIONS

The question is often raised as to when the need for calories takes precedence over that for protein at minimal intakes of both food energy and protein. The present investigation is an attempt to relate varying intakes of dietary protein and calories to nitrogen utilization and body composition of adult female albino rats.

Groups of animals were given different amounts of protein as lactalbumin during ad libitum feeding. This was followed by a period of caloric restriction to two-thirds of the ad libitum intake and accompanied by the same, or more, or less protein than consumed ad libitum. Five experiments were conducted to obtain information about nitrogen balance, carcass and liver nitrogen, body fat and hemoglobin concentration in the blood of the rats. The activities of xanthine oxidase, succinic dehydrogenase and cytochrome oxidase systems in liver tissues were also studied. Daily records of food consumption and of body weights during each experiment were kept.

In the first experiment, diets supplying 15, 10 and 5 per cent of the calories in the diet as protein were fed ad libitum to each of 3 groups of rats until nitrogen

equilibrium was achieved. At these three intakes of protein, the daily nitrogen intake per rat was equivalent to approximately 200, 140 and 70 mg. The energy intakes of the animals in each group were reduced to two-thirds of the voluntary food consumption without altering the absolute amount of protein intake and the feeding was continued until nitrogen equilibrium was re-attained.

The plan of feeding was modified in the succeeding 4 experiments. During ad libitum feeding, diets supplying 15 or 5 per cent of the calories as protein were fed to each of two groups of animals for 20 days. At the end of this period, each group of rats was divided into 3 sub-groups. For the succeeding 30 days, sub-groups A, B and C which were pre-fed 15 per cent of their calories as protein received the same, two-thirds or one-third as much protein in diets supplying two-thirds as much food energy. Sub-groups D, E and F which were pre-fed 5 per cent of their calories as protein received the same, twice or three times as much protein in diets supplying two-thirds as much food energy. Sub-groups A and D, B and E, and C and F received approximately the same nitrogen intakes.

Stock animals were sacrificed at the beginning of each of the last 3 experiments to serve as a control group. At the end of ad libitum feeding, animals representing each group were also sacrificed.

Nitrogen retention increased with increments in dietary protein intake when food consumption was unrestricted. After caloric restriction was imposed, the nitrogen losses occurred regardless of the intake of dietary protein. However, nitrogen equilibrium was re-attained within 30 to 35 days of restricted feeding. Rats in sub-groups D and E were in nitrogen equilibrium or retaining nitrogen during the first five days of caloric restriction but this was followed by nitrogen losses. The nitrogen balance picture was complicated by the fact that animals receiving the low protein diet during ad libitum feeding voluntarily consumed more food than animals fed the high protein diet. This may have been a compensatory response to minimal amounts of essential amino acids.

The magnitude of the negative nitrogen balance immediately following caloric restriction was less for animals fed the low intake of protein than for animals previously conditioned to a high protein intake. The difference in the nitrogen losses of the two groups of animals pre-fed two different levels of protein during ad libitum feeding may be a reflection of nitrogen reserves in the animals. After restriction of food intake and with similar nitrogen intakes, higher nitrogen losses occurred in animals pre-fed 15 per cent of their calories as protein than in those pre-fed 5 per cent of their calories as protein. If, on

the other hand, nitrogen intake was maintained at the same amount during both ad libitum and restricted feeding, caloric restriction determined nitrogen loss.

Ad libitum food consumption was associated with small gains in body weights and restricted feeding, with weight losses. Weight changes during ad libitum and restricted feeding were linearly related to nitrogen balance although not significantly related to the intake of either calories or proteins. Hence, interaction between protein and calories may have influenced both weight changes and nitrogen balance.

The weights of the livers of the stock control animals and animals fed ad libitum were not significantly different. There was a considerable reduction in the weights of the livers at the end of restricted feeding regardless of the protein intake. The marked loss in liver weights was accompanied by a decrease in the total nitrogen content of the liver as a consequence of a reduction in liver size. Although the total nitrogen in the liver was related directly to protein intake during ad libitum feeding, no such relationship existed after restriction of food energy.

The percentage of protein in the liver of the ad libitum-fed animals was directly related to the intake of dietary protein. An increase in the deposition of glycogen and/or fat in the liver may have accompanied the small but

significant loss in liver protein when the low protein diet was fed.

As a consequence of limited intakes of food energy, the percentage of protein in the livers of the animals was increased significantly. The marked reduction in the size of the livers may be associated with correspondingly greater losses in liver glycogen or liver fat or both than in protein, hence the concentration of protein per unit of liver increased. The effects of caloric restriction on the percentage of protein in the liver is clearly indicated by the inverse relationship between the percentage of liver protein and the average daily caloric intake per 100-gram rat.

Increments or decrements in the dietary protein intake during restricted feeding were accompanied by corresponding increases or decreases in the protein concentration per unit of liver. The influence of protein intake was highly significant and independent of the protein intake of the animals prior to caloric restriction.

The effects of varying the intake of dietary protein and calories on the activities of xanthine oxidase, succinic dehydrogenase and cytochrome oxidase systems in hepatic tissues were essentially similar to the effects on the percentage of protein in the liver. Since enzymes are proteins themselves, enzyme activity is dependent on the concentration of enzyme protein in the liver. The

unit activities of both xanthine oxidase and succinic dehydrogenase in the liver were directly related to the protein concentration of the liver.

The unit and total activities of xanthine oxidase and succinic dehydrogenase were related directly to protein intake during ad libitum feeding. Following restriction of food intake, the unit activities of xanthine oxidase and succinic dehydrogenase increased.

The unit activities of xanthine oxidase in sub-groups A, B and C were not different from each other although protein intakes varied. On the other hand, the unit activities of xanthine oxidase increased in sub-groups D, E and F with increasing protein intake. The total xanthine oxidase activities were lower during caloric restriction than during ad libitum feeding except in sub-groups D and E which were given larger amounts of protein after food intake was restricted than before restriction. The unit succinic dehydrogenase activity decreased significantly with successive decreases in protein intake and increased with increments in protein intake during the period of restricted food intake. The total succinic dehydrogenase activity was lower during restricted food intake than during ad libitum feeding except sub-groups D and E which were fed larger amounts of protein after restricted food intake than

before restriction. Caloric restriction increased the unit activity and decreased the total activity of cytochrome oxidase. Increments in the protein intake above the ad libitum intake appeared to increase the unit activity of cytochrome oxidase while decrements in protein intake appeared not to affect the unit activity of this lower enzyme system.

The data obtained on body fat were variable and no significant relationships with changes in dietary intake of protein or calories were observed. Although animals pre-fed 5 per cent of their calories as protein tended to be fatter after 30 days of restricted feeding than those pre-fed 15 per cent of their calories as protein. This may be attributed partly to the inherent tendency of female rats to accumulate appreciable quantities of body fat which were not affected by the food restriction in the present experiment.

Neither the concentration of nitrogen in the carcasses nor the hemoglobin concentration in the blood of rats after caloric restriction was significantly influenced by the intake of protein during restricted feeding. The decrease in the total carcass nitrogen after caloric restriction is attributed to a loss in body weight.

It may be concluded from the results obtained under the present experimental conditions that protein intake

prior to caloric restriction influences nitrogen loss only when the protein intake is varied simultaneously with the reduced intake of food energy. However, if the protein intake is maintained, caloric restriction determines nitrogen loss. The per cent of protein in the liver and the activity of both xanthine oxidase and succinic dehydrogenase are related directly to the protein intake and indirectly to the intake of food energy. Animals voluntarily consume more food when the level of protein in the diet is 5 per cent than when it is 15 per cent. Total carcass nitrogen and total liver nitrogen are related to the intake of food energy. During caloric restriction varying intakes of protein are not related to either total carcass nitrogen or total liver nitrogen.

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APPENDIX

Table A. Caloric intake, nitrogen intake, weight changes and nitrogen balance per 5-day period on ad libitum and restricted food intake

Experiment I

Rat number	A-1				A-2			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	149.7	959.0	-2	+120.7	171.5	1097.6	0	+130.9
2	151.5	970.6	-1	+159.2	156.5	1002.3	-2	+102.9
3	173.4	1082.1	+4	+135.4	161.5	1008.9	+1	+109.2
4	159.7	997.6	0	+155.1	169.7	1059.6	0	+ 97.2
Total	634.3	4009.3	+1	+570.4	659.2	4168.4	-1	+440.2
Average	158.6	1002.3	+0.3	+142.6	164.8	1042.1	-0.3	+110.1
Restricted intake								
5	104.0	973.0	-2	-206.7	133.3	1245.5	-6	- 31.6
6[a]	"	975.0	-9	-129.0	"	1247.5	-4	- 39.9
7	"	"	-3	-122.1	"	"	-3	- 7.3
8	"	"	-1	- 65.1	"	"	+4	+ 21.6
9	"	"	-1	- 54.5	"	"	-1	+ 64.2
10	"	"	-4	- 28.0	"	"	-3	+ 63.7
11	"	"	-1	- 51.7	"	"	+1	+ 50.7
Total	728.0	6823.0	-21	-657.1	933.1	8730.5	-12	+121.4

[a]Fresh mixture of vitamin.

Table A (Cont'd)

Rat number	A-3				B-4			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	162.9	1042.8	-1	+150.5	146.6	620.3	-5	+ 43.5
2	132.9	852.2	-3	+ 38.0	136.9	580.2	-3	+ 18.3
3	158.8	992.0	+2	+162.9	158.9	658.8	-1	+ 82.1
4	157.0	980.7	+1	+104.2	147.5	612.0	-2	+ 53.7
Total	611.6	3867.7	-1	+455.6	589.9	2471.3	-11	+197.6
Average	152.9	966.9	-0.3	+113.9	147.5	617.8	-2.8	+ 49.4
Restricted intake								
5	101.7	952.1	-11	-222.6	95.8	609.8	-13	-206.6
6[a]	"	954.0	-6	-131.2	"	611.8	-8	-208.9
7	"	"	-2	-106.8	"	"	-5	-260.4
8	"	"	+1	- 72.1	"	"	-1	-166.4
9	"	"	-1	- 41.5	"	"	-1	- 76.5
10	"	"	-2	- 26.5	"	"	-6	- 31.0
11	"	"	0	+ 24.2	"	"	-1	- 37.9
Total	711.9	6676.1	-21	-576.5	670.6	4280.6	-35	-987.7

Table A (Cont'd)

Rat number	B-5				B-6			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	177.2	748.4	- 1	+108.5	191.9	809.5	+ 4	+125.3
2	153.9	650.9	- 1	+ 50.0	180.0	759.8	+ 1	+134.4
3	163.0	675.7	+ 1	+ 46.7	191.4	791.9	+ 4	+123.1
4	174.5	740.6	+ 2	+158.5	184.1	761.9	+ 1	+133.2
Total	668.6	2815.6	+ 1	+363.7	747.4	3123.1	+10	+516.0
Average	167.2	703.9	+ 0.3	+ 90.9	186.9	780.8	+ 3.3	+129.0
Restricted intake								
5	111.7	710.0	- 8	-155.5	127.7	810.2	-10	- 39.7
6[a]	"	712.5	- 8	-141.3	"	812.1	- 4	- 28.2
7	"	"	- 4	- 93.5	"	"	- 2	- 36.2
8	"	"	+ 1	- 57.2	"	"	0	- 57.0
9	"	"	+ 1	+ 21.3	"	"	0	+ 21.5
10	"	"	- 4	+ 91.5	"	"	- 1	+ 8.4
11	"	"	- 2	+ 44.4	"	"	- 1	+ 55.3
Total	781.9	4985.0	-24	-290.3	893.9	5682.8	-18	- 75.9

Table A (Cont'd)

Rat number	C-7				C-8			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	173.8	386.8	- 2	+ 11.1	164.1	365.7	- 5	- 67.0
2	154.9	345.7	0	+ 6.9	197.3	437.9	+ 1	+ 31.1
3	152.6	338.4	- 2	+ 23.2	206.1	453.9	+ 4	+ 78.5
4	152.6	338.4	- 3	+ 29.0	203.3	448.0	+ 3	+ 94.2
Total	633.9	1409.3	- 7	+ 70.2	770.8	1705.5	+ 3	+136.8
Average	158.5	352.3	1.8	+ 17.6	192.7	426.4	+ 0.8	+ 34.2
Restricted intake								
5	112.9	380.1	-10	- 46.1	131.4	440.7	-10	- 23.3
6[a]	"	382.0	- 3	- 20.5	"	442.7	- 5	- 53.3
7	"	"	- 4	- 27.8	"	"	- 3	- 10.7
8	"	"	+ 1	- 65.6	"	"	- 1	- 25.8
9	"	"	0	- 80.1	"	"	0	- 7.8
10	"	"	- 3	- 36.0	"	"	- 3	+ 8.3
11[b]								
Total	677.4	2290.1	-19	-276.1	788.4	2654.2	-22	-112.6

[b]Period omitted due to lack of diet.

Table A (Cont'd)

Rat number		C-9		
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake				
1	186.2	413.9	- 2	+ 38.3
2	167.8	373.8	0	+ 23.7
3	160.9	356.3	- 3	+ 14.9
4	176.6	390.2	+ 1	+ 82.9
Total	691.5	1534.2	- 4	+159.8
Average	172.9	383.6	- 1.0	+ 40.0
Restricted intake				
5	117.6	395.2	-10	- 89.6
6	"	397.2	- 6	- 93.6
7	"	"	- 4	- 90.2
8	"	"	+ 1	-101.2
9	"	"	- 1	- 65.7
10	"	"	- 2	- 2.5
11[b]				
Total	705.6	2381.2	-22	-442.8

Table A (Cont'd)

Experiment II

Rat number	A-1				A-2			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1[c]	213.9	1345.5	- 1	+226.5	228.4	1436.5	+ 1	+276.6
2	179.7	1132.2	+ 2	+ 63.0	161.5	1018.4	+ 2	+ 33.8
Total	393.6	2477.7	+ 1	+289.5	389.9	2454.9	+ 3	+310.4
Average	196.8	1238.9	+ 0.5	+144.8	195.0	1227.5	+ 1.5	+155.2
Restricted intake								
3	117.5	1069.9	+ 1	- 10.4	115.3	1049.5	-12	-121.6
4	"	"	- 4	-117.2	"	"	- 3	-188.0
5	"	"	- 6	-118.3	"	"	- 2	- 63.6
6	"	"	- 4	-115.6	"	"	- 2	- 17.1
7	"	"	0	- 31.5	"	"	+ 1	+ 28.0
8	"	"	- 2	+ 70.0	"	"	0	+ 61.3
Total	705.0	6419.4	-15	- 32.3	691.8	6297.0	-18	-301.0

[c]Period 1: A 6-day period instead of a 5-day period due to technical error.

Table A (Cont'd)

Rat number	A-3				B-4			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1[c]	204.3	1285.8	- 1	+233.5	191.6	1206.1	- 2	+165.9
2	172.5	1086.7	+ 4	+101.4	143.3	904.7	- 2	+ 19.0
Total	376.8	2372.5	+ 3	+334.9	334.9	2110.8	- 4	+184.9
Average	188.4	1186.3	+ 1.5	+167.5	167.5	1055.4	- 2.0	+ 92.5
Restricted intake								
3	110.7	1008.7	-13	-182.9	98.0	603.6	-12	-206.8
4	"	"	- 3	-127.9	"	"	- 7	-200.3
5	"	"	- 2	-184.5	"	"	- 5	-122.9
6	"	"	- 3	- 50.9	"	"	- 3	- 86.3
7	"	"	- 2	- 31.5	"	"	- 4	- 25.7
8	"	"	0	+141.0	"	"	- 1	- 13.5
Total	664.2	6052.2	-23	-436.7	588.0	3621.6	-32	-655.5

Table A (Cont'd)

Rat number	B-5				B-6			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1[c]	204.3	1285.8	- 1	+233.9	196.6	1237.4	+ 1	+198.9
2	167.4	1055.4	+ 2	- 2.6	163.8	1032.6	+ 3	- 28.6
Total	371.7	2341.2	+ 1	+231.3	360.4	2270.0	+ 4	+170.3
Average	185.9	1170.6	+ 1.5	+115.7	180.2	1135.0	+ 2.0	+ 85.2
Restricted intake								
3	111.7	686.6	-13	-204.2	109.4	672.8	- 7	- 93.6
4	"	"	- 2	-167.7	"	"	- 1	-108.5
5	"	"	- 4	+ 29.8	"	"	- 3	- 88.6
6	"	"	0	- 1.0	"	"	- 1	- 27.1
7	"	"	- 2	+ 5.6	"	"	- 2	+ 40.0
8	"	"	0	+ 33.6	"	"	0	+ 61.7
Total	670.2	4119.6	-21	-303.9	656.4	4036.8	-14	-216.1

Table A (Cont'd)

Rat number	C-7				C-8			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1[c]	193.8	1220.3	0	+169.0	217.9	1371.1	- 1	+272.3
2	161.5	1018.4	+ 2	- 5.6	185.6	1169.2	+ 5	+ 60.0
Total	355.3	2238.7	+ 2	+163.4	403.5	2540.3	+ 4	+332.3
Average	177.7	1119.4	+ 1.0	+ 81.7	201.8	1270.2	+ 2.0	+166.2
Restricted intake								
3	108.3	348.1	-12	-222.1	122.2	391.4	-13	-199.6
4	"	"	- 4	-135.0	"	"	- 4	-125.6
5	"	"	- 6	-135.0	"	"	- 7	-104.3
6	"	"	- 2	- 64.7	"	"	- 3	- 73.0
7	"	"	- 1	- 13.6	"	"	- 3	- 68.3
8	"	"	- 1	- 4.2	"	"	+ 1	- 11.3
Total	649.8	2088.6	-26	-574.6	733.2	2348.4	-29	-582.1

Table A (Cont'd)

Rat number	C-9				D-1			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1[c]	226.6	1425.1	0	+199.3	225.4	467.0	- 3	+109.0
2	188.4	1186.2	+ 3	- 44.2	181.2	377.0	+ 2	+ 32.7
Total	415.0	2611.3	+ 3	+155.1	406.6	844.0	- 1	+141.7
Average	207.5	1305.7	+ 1.5	+ 77.6	203.3	422.0	- 0.5	+ 70.9
Restricted intake								
3	126.8	405.8	- 9	-122.4	117.5	1069.9	- 9	+ 2.0
4	"	"	- 3	- 31.9	"	"	0	- 86.6
5	"	"	- 4	- 47.5	"	"	- 1	- 26.1
6	"	"	- 4	- 34.6	"	"	- 3	- 19.5
7	"	"	0	- 55.2	"	"	+ 3	+ 29.0
8	"	"	0	- 13.7	"	"	+ 1	- 16.3
Total	760.8	2434.8	-20	-305.3	705.0	6419.4	- 9	-117.5

Table A (Cont'd)

Rat number	D-2				D-3			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1°	223.6	463.2	+ 1	+105.0	229.6	475.4	0	+142.3
2	197.8	410.7	+ 4	+ 2.3	168.3	350.8	- 2	+ 17.0
Total	421.4	873.9	+ 5	+107.3	397.9	826.2	- 2	+159.3
Average	210.7	437.0	+ 2.5	+ 53.7	199.0	413.1	- 1.0	+ 79.7
Restricted intake								
3	124.3	1131.1	- 6	+ 49.3	115.3	1049.5	- 7	+259.7
4	"	"	- 3	- 69.4	"	"	- 8	-132.0
5	"	"	+ 1	- 27.7	"	"	- 2	- 87.1
6	"	"	- 2	- 9.3	"	"	- 3	- 89.3
7	"	"	+ 1	-216.6	"	"	0	- 5.9
8	"	"	+ 1	+ 34.7	"	"	0	- 11.4
Total	745.8	6786.6	- 8	-239.0	691.8	6297.0	-20	- 66.0

Table A (Cont'd)

Rat number	E-4				E-5			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1[c]	262.8	542.9	+ 4	+187.0	242.5	501.6	+ 1	+140.5
2	213.4	442.6	+ 4	+ 70.4	172.0	358.3	- 4	+ 17.4
Total	476.2	985.5	+ 8	+257.4	414.5	859.9	- 3	+157.9
Average	238.1	492.8	+ 4.0	+128.7	207.3	430.0	- 1.5	+ 78.9
Restricted intake								
3	141.4	866.4	- 5	+122.6	120.8	741.9	- 9	- 33.2
4	"	"	- 5	+ 11.3	"	"	- 3	- 47.6
5	"	"	- 1	- 89.5	"	"	- 3	- 65.8
6	"	"	- 5	-141.1	"	"	- 5	-104.3
7	"	"	0	+ 8.5	"	"	+ 4	+ 2.3
8	"	"	+ 1	- 34.1	"	"	- 3	+ 39.7
Total	848.4	5198.4	-15	-122.3	724.8	4451.4	-19	-208.9

Table A (Cont'd)

Rat number		E-6				F-7		
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1[c]	238.3	493.2	+ 4	+151.3	267.8	553.2	+ 3	+180.3
2	170.6	355.4	- 2	- 6.5	215.3	446.4	+ 3	+ 62.4
Total	408.9	848.6	+ 2	+144.8	483.1	999.6	+ 6	+242.7
Average	204.5	424.3	+ 1.0	+ 72.4	241.6	499.8	+ 3.0	+121.4
Restricted intake								
3	120.8	741.9	- 5	+135.1	145.2	463.6	- 9	- 46.3
4	"	"	- 4	- 60.3	"	"	- 3	- 95.6
5	"	"	- 3	- 28.0	"	"	- 4	- 84.5
6	"	"	- 4	- 64.0	"	"	- 3	- 37.9
7	"	"	+ 1	+ 62.7	"	"	+ 3	+ 16.6
8	"	"	0	+ 23.2	"	"	+ 1	+ 62.9
Total	724.8	4451.4	-15	+ 68.7	871.2	2781.6	-15	-184.8

Table A (Cont'd)

Rat number		F-8				F-9		
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1[c]	235.6	487.6	+ 1	+154.2	248.9	514.8	+ 2	+116.3
2	174.7	363.9	0	+ 32.6	203.3	422.0	+ 3	+ 22.9
Total	410.3	851.5	+ 1	+186.8	452.2	936.8	+ 5	+139.2
Average	205.2	425.8	+ 0.5	+ 93.4	226.1	468.4	+ 2.5	+ 69.6
Restricted intake								
3	122.2	391.4	- 7	- 68.2	136.0	434.7	- 7	- 9.8
4	"	"	- 5	- 70.1	"	"	- 4	- 29.8
5	"	"	- 3	-155.5	"	"	- 4	- 95.8
6	"	"	- 4	- 54.9	"	"	- 4	- 96.0
7	"	"	+ 1	- 42.7	"	"	+ 2	- 34.6
8	"	"	- 1	- 3.0	"	"	0	- 18.1
Total	733.2	2348.4	-19	-394.4	816.0	2608.2	-17	-284.1

Table A (Cont'd)

Experiment III

Rat number	A-2				A-3			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	165.6	986.9	+ 2	+152.3	141.5	844.5	- 1	+ 55.3
2	172.4	1024.7	+ 1	+146.8	158.8	946.6	+ 2	+144.1
Total	338.0	2011.6	+ 3	+299.1	300.3	1791.1	+ 1	+199.4
Average	169.0	1005.8	+ 1.5	+149.6	150.2	895.5	+ 0.5	+ 99.7
Restricted intake								
8[d]	113.0	1007.2	+ 3	- 3.5	97.2	867.4	+ 1	- 86.6
Rat number	A-4				A-5			
Ad libitum intake								
1	161.1	960.0	+ 3	+ 64.8	158.8	946.6	+ 8	+ 74.1
2	187.9	1115.4	+ 5	+123.0	138.8	827.3	- 4	+ 4.6
Total	349.0	2075.4	+ 8	+187.8	297.6	1773.9	+ 4	+ 78.7
Average	174.5	1037.7	+ 4.0	+ 93.9	148.8	887.0	+ 2.0	+ 39.4
Rats A-4 and A-5 sacrificed after ad libitum feeding								

[d]Period 8: represents sixth period on restricted feeding.

Table A (Cont'd)

Rat number		B-6				B-7		
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	174.7	1040.6	+ 2	+ 83.9	152.4	909.0	+ 4	+ 59.2
2	186.1	1104.7	+ 1	+ 85.6	139.2	820.9	0	+ 96.6
Total	360.8	2145.3	+ 3	+169.5	291.6	1729.9	+ 4	+155.8
Average	180.4	1072.7	+ 1.5	+ 84.8	145.8	865.0	+ 2.0	+ 77.9
Restricted intake								
8[d]	120.8	732.3	- 1	- 43.3	98.0	595.8	- 5	- 72.2
Rat number		B-8				B-9		
Ad libitum intake								
1	146.1	871.4	+ 4	+137.8	155.6	927.8	+ 2	+ 80.0
2	160.2	951.8	+ 1	+ 65.4	160.6	954.8	+ 1	+143.1
Total	306.3	1823.2	+ 5	+203.2	316.2	1882.6	+ 3	+223.1
Average	153.2	911.6	+ 2.5	+101.6	158.1	941.3	+ 1.5	+111.6
Rat B-9 sacrificed after ad libitum feeding								
Restricted intake								
8[d]	102.6	623.1	- 1	- 45.3				

Table A (Cont'd)

Rat number		B-10				C-11		
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	175.6	1046.0	+ 2	+ 63.9	167.0	994.9	+ 4	+ 75.9
2	180.2	1070.3	+ 2	+ 95.2	181.5	1078.0	+ 7	+151.8
Total	355.8	2116.3	+ 4	+159.1	348.5	2072.9	+11	+227.7
Average	177.9	1058.2	+ 2.0	+ 79.6	174.3	1036.5	+ 5.5	+113.9
Rat B-10 sacrificed after ad libitum feeding								
Restricted intake								
8d					117.6	378.6	- 1	- 59.9
Rat number		C-12				C-13		
Ad libitum intake								
1	175.2	1043.3	+ 2	+ 71.2	139.7	833.7	- 1	+ 34.5
2	183.4	1088.7	+ 1	+ 45.7	157.0	933.3	+ 2	+ 99.8
Total	358.6	2132.0	+ 3	+116.9	296.7	1767.0	+ 1	+134.3
Average	179.3	1066.0	+ 1.5	+ 58.5	148.4	883.5	+ 0.5	+ 67.2
Restricted intake								
8d	122.2	393.1	- 2	- 48.5	99.1	320.6	- 5	- 66.1

Table A (Cont'd)

Rat number	C-14				C-15			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	170.2	1013.8	0	+111.9	154.2	919.7	0	+ 77.0
2	167.9	998.0	+ 1	+133.4	167.9	998.0	0	+144.7
Total	338.1	2011.8	+ 1	+245.3	322.1	1917.7	0	+221.7
Average	169.1	1005.9	+ 0.5	+122.7	161.1	958.9	0	+110.9
Rats C-14 and C-15 sacrificed after ad libitum feeding								
Rat number	D-1				D-2			
Ad libitum intake								
1	206.5	441.2	+ 7	+135.0	184.4	394.0	+ 3	+ 99.7
2	194.1	412.0	0	+ 50.7	182.1	387.1	+ 2	+ 77.0
Total	400.6	853.2	+ 7	+185.7	366.5	781.1	+ 5	+176.7
Average	200.3	426.6	+ 3.5	+ 92.9	183.3	390.6	+ 2.5	+ 88.4
Restricted intake								
3	131.1	1166.8	- 5	+ 31.3	119.8	1066.8	- 8	- 89.9
4[e]	"	1166.9	- 3	- 60.6	"	1066.9	- 5	-137.0
5	"	"	- 1	- 64.3	"	"	- 1	-120.5
6	"	"	+ 1	+ 1.8	"	"	+ 1	- 55.7
7	"	"	- 2	- 88.4	"	"	+ 3	- 0.2
8	"	"	0	- 49.6	"	"	0	+ 59.4
Total	786.6	7001.3	-10	-229.8	718.8	6401.3	-10	-343.9

[e]Fresh vitamin mixture.

Table A (Cont'd)

Rat number	D-3				D-4			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	183.9	393.0	+ 2	+ 99.7	200.5	427.7	+ 1	+ 54.4
2	198.2	420.5	+ 3	+ 44.8	195.0	413.9	+ 1	+ 84.1
Total	382.1	813.5	+ 5	+144.5	395.5	841.6	+ 2	+138.5
Average	191.1	406.8	+ 2.5	+ 72.3	197.8	420.8	+ 1.0	+ 69.3
Rat D-4 sacrificed after ad libitum feeding								
Restricted intake								
3	124.3	1106.9	- 9	- 5.5				
4e	"	1107.0	- 5	-106.6				
5	"	"	0	-124.2				
6	"	"	- 3	- 58.1				
7	"	"	- 2	- 19.3				
8	"	"	- 1	- 70.5				
Total	745.8	6641.9	-20	-384.2				

Rat number	D-5				E-6			
Ad libitum intake								
1	179.8	384.3	- 1	+ 49.6	177.9	380.5	0	+ 27.5
2	149.8	320.2	- 1	+ 0.3	202.8	430.1	+ 3	+ 98.6
Total	329.6	704.5	- 2	+ 49.9	380.7	810.6	+ 3	+126.1
Average	164.8	352.3	- 1.0	+ 25.0	190.4	405.3	+ 1.5	+ 63.1
Rat D-5 sacrificed after ad libitum feeding								

Table A (Cont'd)

Rat number	D-5				E-6			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Restricted intake								
3					125.4	758.9	- 9	- 91.5
4e					"	759.0	- 4	-141.9
5					"	"	- 1	- 67.6
6					"	"	- 4	- 33.4
7					"	"	+ 1	- 76.9
8					"	"	+ 1	+ 15.3
Total					752.4	4553.9	-16	-396.0

Rat number	E-7				E-8			
Ad libitum intake								
1	195.5	417.1	+ 2	+ 67.9	195.0	416.1	+ 4	+112.1
2	201.0	426.3	+ 1	+109.2	189.5	402.4	+ 1	+109.4
Total	396.5	843.4	+ 3	+177.1	384.5	818.5	+ 5	+221.5
Average	198.3	421.7	+ 1.5	+ 88.6	192.3	409.3	+ 2.5	+110.8
Restricted intake								
3	130.0	786.2	- 6	- 4.2	127.7	772.5	- 6	- 12.6
4e	"	"	- 6	-162.9	"	772.6	- 3	- 28.2
5	"	"	- 1	- 60.7	"	"	- 3	- 43.8
6	"	"	0	- 50.7	"	"	0	- 54.8
7	"	"	- 3	- 50.0	"	"	- 1	- 44.3
8	"	"	0	- 35.8	"	"	- 1	- 17.5
Total	780.0	4717.2	-16	-364.3	766.2	4635.5	-14	-201.2

Table A (Cont'd)

Rat number	E-9				E-10			
Period	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)	Caloric intake	Nitrogen intake (mg.)	Weight changes (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	159.5	342.0	- 1	+ 27.6	207.0	441.2	+ 2	+ 69.6
2	174.3	370.7	+ 1	+ 90.3	173.8	370.0	- 3	- 3.8
Total	333.8	712.7	0	+117.9	380.8	811.2	- 1	+ 65.8
Average	166.9	356.4	0	+ 59.0	190.4	405.6	- 0.5	+ 32.9
Rats E-9 and E-10 sacrificed after ad libitum feeding								
Rat number	F-12				F-13			
Ad libitum intake								
1	208.8	445.0	+ 3	+121.7	201.9	430.6	+ 2	+100.3
2	223.6	473.2	+ 1	+128.5	196.4	416.8	+ 5	+ 63.3
Total	432.4	918.2	+ 4	+250.2	398.3	847.4	+ 7	+163.6
Average	216.2	459.1	+ 2.0	+125.1	199.2	423.7	+ 3.5	+ 81.8
Restricted intake								
8[d]	145.2	465.6	- 2	+ 44.5	133.7	429.3	- 1	- 64.5
Rat number	F-14				F-15			
Ad libitum intake								
1	198.2	422.9	+ 2	+ 94.1	224.5	477.8	- 1	+105.3
2	161.4	344.1	- 2	- 13.8	205.1	435.0	0	+ 58.5
Total	359.6	767.0	0	+ 80.3	429.6	912.8	- 1	+163.8
Average	179.8	383.5	0	+ 40.2	214.8	456.4	- 0.5	+ 81.9
Rats F-14 and F-15 sacrificed after ad libitum feeding								

Table B. Average body weights and nitrogen balance in milligrams nitrogen per 100-gram rat per 5-day period on ad libitum and restricted food intake

Experiment I

Rat number	A-1		A-2		A-3	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Ad libitum intake						
1	201	+ 60.1	197	+ 66.4	192	+ 78.4
2	198	+ 80.4	196	+ 52.5	188	+ 20.2
3	200	+ 67.7	195	+ 56.0	189	+ 86.2
4	201	+ 77.2	196	+ 49.6	189	+ 55.2
Average	200	+ 71.4	196	+ 56.1	190	+ 60.0
Restricted intake						
5	194	-106.6	192	- 16.5	183	-121.6
6	184	- 70.1	186	- 21.5	175	- 75.0
7	178	- 68.6	184	- 3.9	171	- 62.5
8	175	- 37.2	182	+ 11.9	168	- 42.9
9	174	- 31.3	183	+ 35.1	169	- 24.5
10	172	- 16.3	182	+ 35.0	168	- 15.8
11	170	- 30.4	182	+ 27.9	168	+ 14.4

Table B (Cont'd)

Rat number	B-4		B-5		B-6	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Ad libitum intake						
1	192	+ 22.6	200	+ 54.2	197	+ 63.6
2	189	+ 9.7	198	+ 25.3	200	+ 67.2
3	187	+ 43.9	198	+ 23.6	202	+ 60.9
4	185	+ 29.0	200	+ 79.2	204	+ 65.3
Average	188	+ 26.3	199	+ 45.6	201	+ 64.3
Restricted intake						
5	178	-116.1	195	- 79.7	198	- 20.1
6	167	-125.1	187	- 75.6	192	- 14.7
7	162	-160.8	181	- 51.7	189	- 19.2
8	156	-106.7	179	- 31.9	186	- 30.6
9	154	- 49.6	179	+ 11.9	187	+ 11.5
10	152	- 20.4	178	+ 51.4	186	+ 4.5
11	150	- 25.3	176	+ 25.2	186	+ 29.8

Table B (Cont'd)

Rat number	C-7		C-8		C-9	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Ad libitum intake						
1	187	+ 5.9	192	- 34.9	193	+ 19.8
2	186	+ 3.7	196	+ 15.9	192	+ 12.4
3	185	+ 12.6	199	+ 39.5	190	+ 7.8
4	183	+ 15.9	202	+ 46.6	189	+ 43.8
Average	185	+ 9.5	197	+ 67.1	191	+ 21.0
Restricted intake						
5	179	- 25.8	198	- 11.8	184	- 48.7
6	174	- 11.8	191	- 27.9	176	- 53.2
7	171	- 16.3	187	- 5.7	171	- 52.8
8	168	- 39.1	184	- 14.0	168	- 60.2
9	166	- 48.2	182	- 4.3	168	- 39.1
10	165	- 21.8	181	+ 4.6	166	- 1.5

Table B (Cont'd)

Experiment II

Rat number	A-1		A-2		A-3		B-4	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	202	+ 93.4	192	+120.0	192	+101.4	201	+ 68.8
2	204	+ 30.9	194	+ 17.4	193	+ 52.6	199	+ 9.6
Average	203	+ 62.2	193	+ 68.7	191	+ 77.0	200	+ 39.2
Restricted intake								
3	199	- 5.2	188	- 64.7	187	- 97.8	191	-108.3
4	192	- 61.0	181	-103.9	181	- 70.7	182	-110.1
5	187	- 63.3	178	- 35.7	177	-104.2	176	- 69.8
6	181	- 63.9	176	- 9.7	175	- 29.1	171	- 50.5
7	180	- 17.5	176	+ 15.9	173	- 18.2	168	- 15.3
8	180	+ 38.9	175	+ 35.0	172	+ 82.0	166	- 8.1

Rat number	B-5		B-6		C-7		C-8	
Ad libitum intake								
1	193	+101.0	180	+ 92.1	197	+ 71.5	191	+118.8
2	192	- 1.4	183	- 15.6	197	- 2.9	194	+ 31.0
Average	191	+ 49.8	182	+ 38.3	197	+ 34.3	193	+ 74.9

Table B (Cont'd)

Rat number	B-5		B-6		C-7		C-8	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Restricted intake								
3	186	-109.8	180	- 52.0	192	-115.7	189	-105.6
4	180	- 93.2	178	- 60.9	184	- 73.4	181	- 69.4
5	176	+ 16.9	174	- 50.9	179	- 75.4	175	- 59.6
6	173	- 0.6	172	- 15.8	175	- 36.9	173	- 42.2
7	173	+ 3.2	171	+ 23.4	173	- 7.9	168	- 40.7
8	173	+ 19.4	171	+ 36.1	173	- 2.4	168	- 6.7
Rat number	C-9		D-1		D-2		D-3	
Ad libitum intake								
1	187	+ 89.1	202	+ 45.0	193	+ 45.3	187	+ 63.4
2	189	- 23.4	202	+ 16.2	195	+ 1.2	185	+ 9.2
Average	188	+ 32.9	202	+ 30.6	194	+ 23.3	186	+ 36.3
Restricted intake								
3	185	- 66.2	198	+ 1.0	193	+ 25.6	181	+143.5
4	181	- 17.7	193	- 44.9	188	- 36.9	181	- 72.9
5	176	- 27.0	191	- 13.6	186	- 14.9	167	- 52.2
6	174	- 19.9	190	- 10.3	186	- 5.0	165	- 54.1
7	172	- 32.1	191	- 15.2	187	+115.8	164	- 3.6
8	171	- 8.0	192	- 8.5	187	+ 18.6	164	- 7.0

Table B (Cont'd)

Rat number	E-4		E-5		E-6		F-7	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	197	+ 79.1	187	+ 62.6	187	+ 67.4	197	+ 76.3
2	201	+ 35.0	187	+ 9.3	189	- 3.5	201	+ 31.1
Average	199	+ 57.1	187	+ 36.0	188	+ 32.0	199	+ 53.7
Restricted intake								
3	200	+ 61.3	181	- 18.4	185	+ 73.0	197	- 23.5
4	196	+ 5.8	174	- 27.4	180	- 33.5	191	- 50.1
5	192	- 46.6	169	- 38.9	176	- 15.9	187	- 45.2
6	188	- 75.1	166	- 62.8	172	- 37.2	184	- 20.6
7	186	+ 4.6	166	+ 1.4	172	+ 36.4	185	+ 9.0
8	187	- 18.2	166	+ 23.9	173	+ 13.4	187	+ 33.6

Rat number	F-8		F-9	
Ad libitum intake				
1	190	+ 67.6	199	+ 48.7
2	190	+ 17.2	202	+ 11.3
Average	190	+ 42.4	201	+ 30.0

Table B (Cont'd)

Rat number	F-8		F-9	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Restricted intake				
3	185	- 36.9	199	- 4.9
4	180	- 38.9	195	- 15.3
5	174	- 89.4	190	- 50.4
6	171	- 32.1	187	- 51.3
7	170	- 25.1	186	- 18.6
8	170	- 1.8	187	- 9.6

Table B (Cont'd)

Experiment III

Rat number	A-2		A-3		A-4[a]		A-5[a]	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	199	+ 76.6	193	+ 28.7	193	+ 33.6	174	+ 42.6
2	195	+ 75.3	195	+ 73.9	197	+162.4	176	+ 2.6
Average	197	+ 76.0	194	+ 51.3	195	+ 98.0	175	+ 22.6
Restricted intake								
8	175	- 2.0	173	- 50.1				
Rat number	B-6		B-7		B-8		B-9[a]	
Ad libitum intake								
1	213	+ 39.4	181	+ 32.7	180	+ 76.5	187	+ 42.8
2	216	+ 39.6	183	+ 52.8	183	+ 35.7	188	+ 76.1
Average	215	+ 39.5	182	+ 42.8	182	+ 56.1	186	+ 59.5
Restricted intake								
8	184	- 23.5	155	- 46.6	158	- 28.6		

[a]Sacrificed following ad libitum feeding.

Table B (Cont'd)

Rat number	B-10[a]		C-11		C-12		C-13	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	185	+ 34.6	179	+ 42.4	201	+ 35.4	183	+ 18.9
2	187	+ 50.9	184	+ 82.5	203	+ 22.5	185	+ 54.0
Average	186	+ 42.8	182	+ 62.5	202	+ 29.0	184	+ 36.5
Restricted intake								
8			156	- 38.4	170	- 28.5	152	- 43.5

Rat number	C-14[a]		C-15[a]	
Ad libitum intake				
1	202	+ 55.4	181	+ 42.6
2	201	+ 66.4	184	+ 41.9
Average	200	+ 60.9	183	+ 42.3

Rat number	D-1		D-2		D-3		D-4[a]	
Ad libitum intake								
1	191	+ 70.7	189	+ 52.8	199	+ 50.1	207	+ 26.3
2	195	+ 26.0	191	+ 40.3	203	+ 22.1	207	+ 40.6
Average	193	+ 48.4	190	+ 46.6	201	+ 36.1	207	+ 33.5

Table B (Cont'd)

Rat number	D-1		D-2		D-3		D-4[a]	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Restricted intake								
3	192	+ 16.3	186	- 48.3	199	- 2.8		
4	187	- 32.4	178	- 77.0	193	- 55.2		
5	185	- 34.7	175	- 68.8	189	- 65.7		
6	185	+ 0.98	175	- 31.9	187	- 31.1		
7	184	- 48.0	175	- 0.12	184	- 10.5		
8	184	- 27.0	178	+ 33.4	183	- 38.5		
Rat number	D-5[a]		E-6		E-7		E-8	
Ad libitum intake								
1	187	+ 26.5	187	+ 14.7	205	+ 33.1	181	+ 61.9
2	186	+ 0.2	188	+ 52.4	207	+ 52.8	184	+ 59.5
Average	187	+ 13.4	188	+ 33.6	206	+ 43.0	183	+ 60.7
Restricted intake								
3			184	- 49.7	204	- 2.1	182	- 7.0
4			178	- 79.7	198	- 82.3	178	- 15.9
5			174	- 38.9	194	- 31.3	175	- 25.0
6			174	- 19.2	193	- 26.3	172	- 31.9
7			171	- 45.0	191	- 26.2	171	- 25.9
8			172	- 8.9	190	- 18.8	170	- 10.3

Table B (Cont'd)

Rat number	E-9[a]		E-10[a]		F-12		F-13	
Period	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)	Weight (gm.)	Nitrogen balance (mg.)
Ad libitum intake								
1	182	+ 15.1	192	+ 36.2	199	+ 61.1	189	+ 53.1
2	183	+ 49.4	190	+ 2.0	202	+ 63.6	190	+ 33.1
Average	183	+ 32.3	191	+ 19.1	201	+ 62.4	190	+ 43.1
Restricted intake								
8					184	+ 24.2	171	- 37.7

Rat number	F-14[a]		F-15[a]	
Ad libitum intake				
1	187	+ 50.3	204	+ 51.6
2	186	- 7.4	204	+ 28.7
Average	187	+ 21.5	204	+ 40.2

Table C. Daily average caloric intake and nitrogen intake per rat and per 100-gram rat on ad libitum and restricted food intake

Experiment IV (Series I and II)

Rat no.	Ad libitum intake				Restricted intake			
	Caloric intake		Nitrogen intake (mg.)		Caloric intake		Nitrogen intake (mg.)	
	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat
Series I								
A-1	41.9	18.7	253.4	113.1	27.6	13.3	250.8	121.2
A-2	42.3	19.1	256.2	115.4	28.0	13.9	254.9	126.2
A-3[a]	40.0	18.5	242.5	111.7				
Series II								
A-1	40.5	21.0	245.2	127.1	26.7	14.6	242.7	132.6
A-2	40.5	19.4	245.2	117.3	26.7	14.3	242.7	130.5
A-3[a]	45.1	21.6	272.6	130.4				
Average					27.2	14.0	247.8	127.6
Series I								
B-4	44.1	20.0	267.1	120.9	29.6	14.6	182.9	90.1
B-5	44.6	19.9	269.8	120.5	29.6	14.5	182.9	89.2
B-6[a]	41.4	20.2	250.7	122.3				
Series II								
B-4	40.5	20.6	245.2	124.5	26.9	14.4	166.2	88.9
B-5	44.6	21.0	269.8	127.3	29.6	15.2	182.9	93.8
B-6[a]	47.8	22.4	289.0	135.7				
Average					29.0	14.7	178.7	90.5

[a]Animals sacrificed after ad libitum feeding.

Table C (Cont'd)

	Ad libitum intake				Restricted intake			
	Caloric intake		Nitrogen intake (mg.)		Caloric intake		Nitrogen intake (mg.)	
Rat no.	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat
Series I								
C-7	41.9	19.1	253.4	115.7	30.0	14.9	97.1	48.3
C-8	45.5	20.0	275.3	120.8	30.9	15.1	100.0	48.8
C-9[a]	47.8	21.7	289.0	131.4				
Series II								
C-7	45.5	21.1	275.3	127.5	30.9	15.0	100.0	48.6
C-8	44.1	20.6	267.1	124.8	30.0	15.0	97.1	48.6
C-9[a]	45.1	22.3	272.6	134.9				
Average	43.5[b]	20.4[b]	263.3[b]	123.4[b]	30.4	15.0	98.6	48.6
Series I								
D-1	52.6	22.9	115.3	50.1	34.4	15.5	312.1	141.2
D-2	51.2	24.0	112.3	52.7	33.5	15.7	303.9	142.7
D-3[a]	46.6	22.1	102.3	48.5				
Series II								
D-1	51.2	25.2	112.3	55.3	33.5	15.8	303.9	143.3
D-2	49.3	23.8	108.3	52.3	32.1	15.7	291.6	142.3
D-3[a]	50.7	24.0	111.3	52.8				
Average					33.3	15.7	302.9	142.4

[b]Average of rats A, B and C on 15 per cent of the calories as protein during ad libitum feeding.

Table C (Cont'd)

Rat no.	Ad libitum intake: Caloric intake, Per rat	Ad libitum intake: Caloric intake, Per 100-gm. rat	Ad libitum intake: Nitrogen intake (mg.), Per rat	Ad libitum intake: Nitrogen intake (mg.), Per 100-gm. rat	Restricted intake: Caloric intake, Per rat	Restricted intake: Caloric intake, Per 100-gm. rat	Restricted intake: Nitrogen intake (mg.), Per rat	Restricted intake: Nitrogen intake (mg.), Per 100-gm. rat
Series I								
E-4	49.3	22.8	108.3	50.1	32.4	15.8	199.6	97.4
E-5	42.0	20.3	92.4	44.6	27.8	15.0	171.7	92.8
E-6[a]	51.6	23.5	113.3	51.5				
Series II								
E-4	46.6	23.9	102.3	52.5	30.6	15.7	188.5	96.7
E-5	49.8	22.7	109.3	49.9	32.8	15.4	202.4	95.0
E-6[a]	42.9	20.1	94.4	44.3				
Average					30.9	15.5	190.6	95.5
Series I								
F-7	50.3	22.6	110.3	49.7	33.7	16.5	108.8	53.4
F-8	51.2	23.3	112.3	51.0	34.1	16.2	110.3	52.5
F-9[a]	47.5	23.2	104.3	50.9				
Series II								
F-7	51.2	23.2	112.3	50.8	34.1	16.4	110.3	53.0
F-8	43.8	22.5	96.4	49.4	29.0	15.5	94.2	50.1
F-9[a]	48.4	22.5	106.3	49.5				
Average	48.7[c]	22.9[c]	106.9[c]	50.3[c]	32.7	16.2	105.9	52.3

[c]Average of rats D, E and F on 5 per cent of the calories as protein during ad libitum feeding.

Table C (Cont'd)

Experiment V (Series III and IV)

Rat no.	Ad libitum intake: Caloric intake, Per rat	Ad libitum intake: Caloric intake, Per 100-gm. rat	Ad libitum intake: Nitrogen intake (mg.), Per rat	Ad libitum intake: Nitrogen intake (mg.), Per 100-gm. rat	Restricted intake: Caloric intake, Per rat	Restricted intake: Caloric intake, Per 100-gm. rat	Restricted intake: Nitrogen intake (mg.), Per rat	Restricted intake: Nitrogen intake (mg.), Per 100-gm. rat
Series III								
A-1[d]	41.0	20.3	251.9	124.7				
A-2	41.0	20.1	251.9	123.5				
Series IV								
A-1	42.3	20.1	260.2	123.3				
A-2	41.0	21.7	251.9	133.3				
A-3	39.6	20.1	243.6	123.6				
Average[e]	42.0	20.5	257.5	126.2				
Series III								
D-3[f]	48.9	23.4	105.7	50.6				
D-4	47.0	22.6	101.8	48.9				
Series IV								
D-4	46.1	21.9	99.8	47.3				
D-5	41.0	21.6	89.0	46.9				
D-6	38.7	20.8	84.1	45.2				
Average[g]	44.1	21.9	95.7	47.4				

[d]Rats A received 15 per cent of cal. as protein, sacrificed after ad libitum feeding.

[e]Includes rats A, B and C on ad libitum intake.

[f]Rats D received 5 per cent of cal. as protein, sacrificed after ad libitum feeding.

[g]Includes rats D, E and F on ad libitum intake.

Table C (Cont'd)

Rat no.	Ad libitum intake				Restricted intake			
	Caloric intake		Nitrogen intake (mg.)		Caloric intake		Nitrogen intake (mg.)	
	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat	Per rat	Per 100-gm. rat
Series III								
A-1	41.4	19.4	254.7	119.0	27.6	14.8	251.3	135.1
A-2	45.5	22.6	279.7	139.1	30.3	16.0	275.8	145.9
Series IV								
A-1	39.1	19.4	240.8	119.2	25.8	14.2	234.9	129.8
A-2	42.3	20.8	260.2	127.6	28.0	14.8	255.4	135.1
A-3	41.0	20.3	251.9	124.7	27.1	14.5	247.2	132.2
Average	41.7	20.5	257.5	125.9	27.8	14.9	252.9	135.6
Series III								
B-3	45.5	21.9	279.7	134.5	30.6	16.6	188.5	102.4
B-4	43.2	21.1	265.8	129.7	28.7	15.5	177.3	95.8
Average	44.4	21.5	272.8	132.1	29.6	16.1	182.9	99.1
Series III								
C-5	43.2	19.7	265.8	120.8	29.0	15.5	93.8	49.9
C-6	36.4	18.8	224.1	115.5	24.4	15.1	79.2	48.9
Series IV								
C-4	42.3	20.6	260.2	127.0	28.6	15.7	92.3	50.7
C-5	43.2	20.6	265.8	126.6	29.0	15.5	93.8	49.9
C-6	45.5	21.6	279.7	132.6	30.9	16.0	99.6	51.6
Average	42.1	20.3	259.1	124.5	28.4	15.6	91.7	50.2

Table C (Cont'd)

Rat no.	Ad libitum intake: Caloric intake, Per rat	Ad libitum intake: Caloric intake, Per 100-gm. rat	Ad libitum intake: Nitrogen intake (mg.), Per rat	Ad libitum intake: Nitrogen intake (mg.), Per 100-gm. rat	Restricted intake: Caloric intake, Per rat	Restricted intake: Caloric intake, Per 100-gm. rat	Restricted intake: Nitrogen intake (mg.), Per rat	Restricted intake: Nitrogen intake (mg.), Per 100-gm. rat
Series III								
D-1	47.5	22.6	102.7	48.9	31.2	16.7	284.0	151.9
D-2	42.9	21.8	92.9	47.2	28.0	15.7	255.4	137.3
Series IV								
D-1	39.2	19.6	85.1	42.6	25.8	14.1	234.9	128.4
D-2	47.9	23.4	103.7	50.6	31.2	15.8	284.0	143.4
D-3	42.0	21.5	91.0	46.7	27.6	14.9	251.3	135.8
Average	43.9	21.8	95.1	47.2	28.8	15.4	261.9	139.4
Series III								
E-3	45.6	21.8	98.8	47.3	30.1	16.0	185.7	89.0
E-4	49.3	24.9	106.7	53.9	32.4	17.8	199.6	109.7
Average	47.5	23.4	102.8	50.6	31.2	16.9	192.7	104.4
Series III								
F-5	43.8	20.3	94.9	43.9	29.0	15.4	93.8	49.6
F-6	38.3	19.9	83.2	43.3	25.4	14.8	82.1	48.0
Series IV								
F-4	40.1	20.5	87.1	44.4	26.7	15.5	86.5	50.0
F-5	47.5	22.8	102.7	49.4	31.8	16.6	102.5	53.4
F-6	44.7	22.5	96.9	48.7	30.0	16.4	96.7	52.8
Average	42.9	21.2	93.0	45.9	28.6	15.7	92.3	50.8

Table D. Liver and carcass nitrogen of rats

Experiment I

	Liver nitrogen				Carcass nitrogen			
Rat no.	Wt. of liver in gms.	Mgs. of nitrogen	Gms. of protein	Per cent protein	Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
A-1	4.9	172.31	1.08	22.04	141.2	4.85	30.30	21.46
A-2	5.2	185.61	1.16	22.31	152.5	5.48	34.25	22.46
A-3	4.0	155.36	0.97	24.25	141.2	5.19	32.41	22.95
Average	4.7			22.87	146.9			22.29
B-4	4.1	151.35	0.95	23.17	122.1	4.63	28.92	23.69
B-5	4.1	163.25	1.02	24.88	146.4	5.34	33.40	22.81
B-6	4.5	170.08	1.06	23.56	154.5	5.45	34.07	22.05
Average	4.2			23.87	141.0			22.85
C-7	4.1	153.29	0.96	23.41	136.9	5.04	31.51	23.02
C-8	5.0	173.42	1.08	21.60	148.0	5.23	32.68	22.08
C-9	4.4	165.09	1.03	23.41	136.6	4.98	31.10	22.76
Average	4.5			22.81	140.5			22.62

Table D (Cont'd)

Experiment II

Rat no.	Liver nitrogen				Carcass nitrogen			
	Wt. of liver in gms.	Mgs. of nitrogen	Gms. of protein	Per cent protein	Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
A-1	4.8	169.32	1.06	22.04	151.5	5.08	31.75	20.96
A-2	4.4	155.61	0.97	22.11	146.5	5.01	31.34	21.39
A-3	4.5	159.24	1.00	22.11	142.0	4.97	31.04	21.86
Average	4.6			22.09	146.7			21.40
B-4	4.1	151.88	0.95	23.15	139.3	4.97	31.04	22.28
B-5	4.4	155.68	0.97	22.37	146.5	5.07	31.67	21.62
B-6	3.8	145.43	0.91	23.92	142.5	4.80	30.03	21.07
Average	4.1			23.15	142.8			21.66
C-7	4.0	144.24	0.90	22.84	144.0	5.05	31.53	21.90
C-8	3.9	148.42	0.93	24.10	138.5	4.39	27.46	19.83
C-9	4.0	150.69	0.94	23.55	143.7	4.78	29.87	20.78
Average	4.0			23.00	142.1			20.84
D-1	4.4	158.10	0.99	22.45	164.0	5.40	33.77	20.59
D-2	5.0	180.57	1.13	22.58	157.0	5.02	31.36	19.97
D-3	4.1	152.27	0.95	23.22	137.3	4.79	29.96	21.82
Average	4.5			22.75	152.8			20.79

Table D (Cont'd)

	Liver nitrogen				Carcass nitrogen			
Rat no.	Wt. of liver in gms.	Mgs. of nitrogen	Gms. of protein	Per cent protein	Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
E-4	4.6	164.42	1.03	22.35	155.4	5.24	32.72	21.06
E-5	4.4	157.71	0.99	22.41	139.0	4.81	30.07	21.63
E-6	4.1	152.46	0.95	23.25	145.3	4.93	30.79	21.19
Average	4.4			22.67	146.6			21.29
F-7	5.0	176.09	1.10	22.02	153.5	5.16	32.24	21.00
F-8	4.2	149.19	0.93	22.19	140.0	4.73	29.58	21.13
F-9	4.8	162.18	1.01	22.13	156.0	5.15	32.16	20.62
Average	4.7			21.78	149.8			20.92

Table D (Cont'd)

Experiment III

Rat no.	Liver nitrogen: Wt. of liver in gms.	Mgs. of Nitrogen	Gms. of protein	Per cent protein	Carcass nitrogen: Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
Stock animals								
S-1	7.4	251.54	1.57	21.22	169.0	5.90	36.89	21.83
S-2	6.5	234.66	1.47	22.61	158.5	5.57	34.82	21.97
S-3	5.9	222.27	1.39	23.56	153.0	5.49	34.29	22.41
S-4	6.2	226.38	1.41	22.74	144.5	5.12	32.01	22.15
S-5	6.6	232.33	1.45	21.97	143.5	5.14	32.14	22.39
S-6	6.1	232.39	1.45	23.77	142.0	5.20	32.47	22.87
Average	6.5			22.65	151.8			22.27
Ad libitum intake								
1. Rats fed 15 per cent of cal. as protein								
A-4	6.2	209.43	1.31	21.13	163.5	5.41	33.83	20.69
A-5	5.0	182.90	1.14	22.80	142.5	5.23	32.67	22.92
B-9	5.1	199.19	1.24	24.31	153.5	5.62	35.12	22.88
B-10	5.8	212.71	1.33	22.93	151.5	5.45	34.09	22.50
C-14	5.4	206.05	1.29	23.89	162.0	5.91	36.94	22.80
C-15	4.6	179.05	1.12	24.35	147.5	5.45	34.05	23.09
Average	5.4			23.24	153.4			22.48
2. Rats fed 5 per cent of cal. as protein								
D-4	6.1	191.31	1.20	19.67	172.5	6.10	38.12	22.10
D-5	5.5	186.43	1.13	20.55	151.0	5.43	33.95	22.49
E-9	5.5	173.53	1.08	19.64	150.5	5.32	33.28	22.11

Table D (Cont'd)

	Liver nitrogen				Carcass nitrogen			
Rat no.	Wt. of liver in gms.	Mgs. of nitrogen	Gms. of protein	Per cent protein	Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
E-10	5.3	181.53	1.13	21.32	152.5	5.57	34.83	22.84
F-14	5.3	177.45	1.11	20.94	152.5	5.25	32.83	21.53
F-15	7.4	212.40	1.33	17.97	163.5	5.76	36.00	22.02
Average	5.9			20.02	157.1			22.18
Restricted intake								
A-2	4.2	166.78	1.04	24.76	148.0	5.28	32.97	22.28
A-3	3.6	149.89	0.94	26.11	141.5	5.15	32.17	22.73
Average	3.9			25.45	144.8			22.51
B-6	4.4	165.28	1.03	23.41	156.1	5.67	35.43	22.70
B-7	3.7	137.60	0.86	23.24	126.0	4.75	29.69	23.56
B-8	4.3	148.94	0.93	21.63	130.0	4.85	30.30	23.31
Average	4.1			22.76	137.4			23.19
C-11	4.0	145.84	0.91	22.75	124.0	4.74	29.64	23.90
C-12	4.6	164.91	1.03	22.39	139.8	5.21	32.56	23.29
C-13	4.0	144.10	0.90	22.50	123.5	4.56	28.51	23.09
Average	4.2			22.55	129.1			23.43

Table D (Cont'd)

Rat no.	Liver nitrogen				Carcass nitrogen			
	Wt. of liver in gms.	Mgs. of nitrogen	Gms. of protein	Per cent protein	Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
D-1	4.7	180.30	1.13	24.04	152.5	5.33	33.29	21.83
D-2	3.9	163.03	1.02	26.15	150.0	5.30	33.11	22.07
D-3	4.3	173.25	1.08	25.12	149.5	5.35	33.42	22.36
Average	4.3			25.10	150.7			22.09
E-6	4.1	160.62	1.00	24.39	141.0	5.10	31.89	22.62
E-7	4.2	167.64	1.05	25.00	154.7	5.51	34.44	22.26
E-8	4.0	156.26	0.98	24.50	138.0	5.06	31.65	22.93
Average	4.1			24.63	144.6			22.60
F-12	5.0	186.00	1.16	23.20	150.7	4.81	30.06	19.95
F-13	4.5	165.89	1.04	23.11	135.5	5.13	32.05	23.65
Average	4.8			23.16	143.1			21.80

Table D (Cont'd)

Experiment IV

Rat no.	Liver nitrogen: Wt. of liver in gms.	Liver nitrogen: Mgs. of nitrogen	Liver nitrogen: Gms. of protein	Liver nitrogen: Per cent protein	Carcass nitrogen[a]: Wt. of carcass in gms.	Carcass nitrogen[a]: Gms. of nitrogen	Carcass nitrogen[a]: Gms. of protein	Carcass nitrogen[a]: Per cent protein
Stock animals								
S-1	6.2	213.73	1.34	21.61		5.28	33.00	
S-2	6.3	221.03	1.38	21.90		5.71	35.69	
S-3	6.2	228.95	1.43	23.06		5.48	34.25	
S-4	5.3	189.50	1.18	22.26		5.34	33.38	
S-5	6.9	226.95	1.42	20.58		5.99	37.44	
S-6	7.2	232.20	1.45	20.14		5.49	34.31	
Average	6.4			21.59				
Ad libitum intake								
1. Rats fed 15 per cent of cal. as protein								
Series I								
A-3	6.5	209.11	1.31	20.15		6.36	39.75	
B-6	7.0	217.21	1.36	19.43		5.97	37.31	
C-9	6.7	223.98	1.40	20.90		6.27	39.19	
Series II								
A-3	6.3	212.69	1.33	21.11		5.97	37.31	
B-6	6.4	210.94	1.32	20.63		5.97	37.31	
C-9	6.4	212.67	1.33	20.78		6.03	37.69	
Average	6.6			20.50				

[a]No record of carcass weights.

Table D (Cont'd)

Rat no.	Liver nitrogen: Wt. of liver in gms.	Liver nitrogen: Mgs. of nitrogen	Liver nitrogen: Gms. of protein	Liver nitrogen: Per cent protein	Carcass nitrogen[a]: Wt. of carcass in gms.	Carcass nitrogen[a]: Gms. of nitrogen	Carcass nitrogen[a]: Gms. of protein	Carcass nitrogen[a]: Per cent protein
2. Rats fed 5 per cent of cal. as protein								
Series I								
D-3	6.6	197.01	1.23	18.64		6.19	38.69	
E-6	6.7	202.14	1.26	18.81		5.98	37.38	
F-9	5.8	185.02	1.16	20.00		6.00	37.50	
Series II								
D-3	6.3	200.28	1.25	19.84		5.82	36.38	
E-6	5.9	183.25	1.15	19.49		5.96	37.25	
F-9	6.5	197.47	1.23	18.92		6.10	38.13	
Average	6.3			19.28				
Restricted intake								
Series I								
A-1	4.5	173.30	1.08	24.00		5.93	37.06	
A-2	4.7	178.46	1.12	23.83		6.02	37.63	
Series II								
A-1	4.3	167.87	1.05	24.42		5.25	32.81	
A-2	4.2	163.51	1.02	24.29		5.96	37.25	
Average	4.4			24.14				
Series I								
B-4	4.6	172.45	1.08	23.48		6.13	38.31	
B-5	5.4	186.46	1.17	21.67		6.06	37.88	

Table D (Cont'd)

Rat no.	Liver nitrogen				Carcass nitrogen[a]			
	Wt. of liver in gms.	Mgs. of nitrogen	Gms. of protein	Per cent protein	Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
Series II								
B-4	4.1	153.71	0.96	23.41		5.45	34.06	
B-5	5.0	180.35	1.13	22.60		5.44	34.00	
Average	4.8			22.79				
Series I								
C-7	4.4	153.91	0.96	21.82		5.81	36.31	
C-8	4.6	182.39	1.14	24.78		5.78	36.13	
Series II								
C-7	5.0	184.50	1.15	23.00		5.87	36.69	
C-8	5.1	181.71	1.14	22.35		5.88	36.75	
Average	4.8			22.99				
Series I								
D-1	5.4	200.93	1.26	23.33		6.45	40.31	
D-2	5.7	202.52	1.27	22.28		6.10	38.13	
Series II								
D-1	5.4	203.26	1.27	23.52		5.98	37.38	
D-2	5.4	201.37	1.26	23.33		5.76	36.00	
Average	5.5			23.12				

Table D (Cont'd)

Rat no.	Liver nitrogen: Wt. of liver in gms.	Liver nitrogen: Mgs. of nitrogen	Liver nitrogen: Gms. of protein	Liver nitrogen: Per cent protein	Carcass nitrogen[a]: Wt. of carcass in gms.	Carcass nitrogen[a]: Gms. of nitrogen	Carcass nitrogen[a]: Gms. of protein	Carcass nitrogen[a]: Per cent protein
Series I								
E-4	4.7	180.10	1.13	24.04		5.92	37.00	
E-5	4.3	158.89	0.99	23.02		5.47	34.19	
Series II								
E-4	4.8	179.42	1.12	23.33		5.38	33.63	
E-5	4.6	177.97	1.11	24.13		6.01	37.56	
Average	4.6			23.63				
Series I								
F-7	4.6	176.00	1.10	23.91		5.83	36.44	
F-8	5.5	194.15	1.21	22.00		5.93	37.06	
Series II								
F-7	4.9	173.95	1.09	22.24		5.94	37.13	
F-8	4.1	152.32	0.95	23.17		5.32	33.25	
Average	418			22.83				

Table D (Cont'd)

Experiment V

Rat no.	Liver nitrogen: Wt. of liver in gms.	Mgs. of nitrogen	Gms. of protein	Per cent protein	Carcass nitrogen[b]: Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
Stock animals								
Series III								
S-1	7.3	257.53	1.61	22.05		6.24	38.98	
S-2	6.5	219.66	1.37	21.08		5.42	33.90	
Series IV								
S-1	6.5	242.94	1.52	23.38	170.5	6.03	37.67	22.09
S-2	6.4	237.41	1.48	23.13	150.0	4.97	31.04	20.69
S-3	6.2	200.34	1.25	20.16	146.5	5.34	33.41	22.81
Average	6.6			21.96	155.7			21.86
Ad libitum intake								
1. Rats fed 15 per cent of cal. as protein								
Series III								
A-1	6.0	218.18	1.36	22.67		5.57	34.80	
A-2	5.9	204.10	1.28	21.62		5.84	36.52	
Series IV								
A-1	7.2	231.54	1.45	20.14	175.5	6.09	38.06	21.69
A-2	5.9	200.81	1.26	21.36	151.0	5.42	33.89	22.44
A-3	6.6	214.40	1.34	20.30	160.0	5.76	35.98	22.49
Average	6.3			21.22	162.2			22.21

[b]No record of carcass weights in Series III.

Table D (Cont'd)

Rat no.	Liver nitrogen: Wt. of liver in gms.	Mgs. of nitrogen	Gms. of protein	Per cent protein	Carcass nitrogen[b]: Wt. of carcass in gms.	Gms. of nitrogen	Gms. of protein	Per cent protein
2. Rats fed 5 per cent of cal. as protein								
Series III								
D-3	6.7	204.47	1.28	19.07		5.51	34.45	
D-4	6.8	196.96	1.23	18.10		6.04	37.74	
Series IV								
D-4	6.1	197.74	1.24	20.26	169.0	5.96	37.25	22.04
D-5	6.1	190.48	1.19	19.51	152.5	5.42	33.90	22.23
D-6	5.4	178.85	1.12	20.74	149.5	5.29	33.06	22.11
Average	6.2			19.54	157.0			22.13
Restricted intake								
Series III								
A-1	4.3	168.61	1.05	24.51		5.60	35.03	
A-2	5.0	196.47	1.23	24.56		5.59	34.94	
Series IV								
A-1	4.3	166.61	1.04	24.19	149.5	5.38	33.63	22.49
A-2	4.4	175.71	1.10	25.00	157.5	5.35	33.44	21.23
A-3	5.0	186.59	1.17	23.32	154.5	5.45	34.08	22.06
Average	4.6			24.32	153.8			21.93

Table D (Cont'd)

Rat no.	Liver nitrogen: Wt. of liver in gms.	Liver nitrogen: Mgs. of nitrogen	Liver nitrogen: Gms. of protein	Liver nitrogen: Per cent protein	Carcass nitrogen[b]: Wt. of carcass in gms.	Carcass nitrogen[b]: Gms. of nitrogen	Carcass nitrogen[b]: Gms. of protein	Carcass nitrogen[b]: Per cent protein
Series III								
B-3	4.7	177.22	1.11	23.57		5.55	34.67	
B-4	4.7	171.98	1.07	22.77		5.66	35.37	
Average	4.7			23.17				
Series III								
C-5	4.6	172.51	1.08	23.48		5.54	34.64	
C-6	3.9	149.32	0.93	23.85		4.98	31.10	
Series IV								
C-4	4.4	166.53	1.04	23.64	148.0	5.44	33.97	22.95
C-5	4.7	173.00	1.08	22.98	153.5	5.53	34.58	22.53
C-6	4.8	174.38	1.09	22.71	158.0	5.52	34.50	21.84
Average	4.5			23.33	153.2			22.44
Series III								
D-1	4.8	187.92	1.17	24.38		5.73	35.79	
D-2	4.5	170.66	1.07	23.78		5.48	34.25	
Series IV								
D-1	4.1	160.77	1.00	24.39	149.5	5.42	33.90	22.68
D-2	4.8	186.80	1.17	24.38	164.0	5.67	35.45	21.62
D-3	4.3	166.50	1.04	24.19	153.5	5.33	33.29	21.69
Average	4.5			24.22	155.7			22.00

Table D (Cont'd)

Rat no.	Liver nitrogen: Wt. of liver in gms.	Liver nitrogen: Mgs. of nitrogen	Liver nitrogen: Gms. of protein	Liver nitrogen: Per cent protein	Carcass nitrogen[b]: Wt. of carcass in gms.	Carcass nitrogen[b]: Gms. of nitrogen	Carcass nitrogen[b]: Gms. of protein	Carcass nitrogen[b]: Per cent protein
Series III								
E-3	4.6	177.77	1.11	24.13		5.52	34.51	
E-4	5.0	188.61	1.18	23.60		5.23	32.68	
Average	4.8			23.87				
Series III								
F-5	4.5	177.26	1.11	24.67		5.58	34.84	
F-6	4.0	145.79	0.91	22.75		5.11	31.91	
Series IV								
F-4	4.0	140.33	0.88	22.00	142.0	5.05	31.56	22.23
F-5	4.7	181.00	1.13	24.04	155.0	5.06	31.64	20.41
F-6[c]	4.6	165.17	1.03	22.39				
Average	4.4			23.17	148.5			

[c]No carcass sample.

Table E. Enzyme activities in liver tissues of rats on ad libitum and restricted food intake. Experiment IV (Series I and II and Experiment V (Series III)

Xanthine oxidase activity

Rat no.	Unit activity: Mg. uric acid/gm. liver (wet wt.)/hr.	Unit activity: Mg. uric acid/gm. liver (dry wt.)/hr.	Unit activity: Mg. uric acid/mg. liver nitrogen/hr.	Total activity: Mg. uric acid/liver/hr.
Stock animals				
Series I and II				
S-1	1.50	5.00	.044	9.30
S-2	1.63	5.34	.046	10.27
S-3	1.90	6.02	.051	11.75
S-4	1.98	6.38	.055	10.49
S-5	1.52	4.89	.046	10.47
S-6	1.66	5.36	.052	11.96
Series III				
S-1	1.69	5.55	.048	12.36
S-2	1.39	4.57	.041	9.06
Average	1.66	5.39	.048	10.71
Ad libitum intake				
1. Rats fed 15 per cent of cal. as protein				
Series I				
A-3	1.14	3.41	.035	7.42
B-6	1.51	4.73	.049	10.59
C-9	1.61	5.03	.048	10.79

Table E (Cont'd)

Rat no.	Unit activity: Mg. uric acid/gm. liver (wet wt.)/hr.	Unit activity: Mg. uric acid/gm. liver (dry wt.)/hr.	Unit activity: Mg. uric acid/mg. liver nitrogen/hr.	Total activity: Mg. uric acid/liver/hr.
Series II				
A-3	1.81	5.09	.054	11.39
B-6	1.44	4.72	.044	9.21
C-9	1.14	3.73	.034	7.28
Series III				
A-1	1.94	6.15	.053	11.63
A-2	1.65	5.07	.048	9.72
Average	1.53	4.74	.046	9.75
2. Rats fed 5 per cent of cal. as protein				
Series I				
D-3	0.89	2.73	.030	5.85
E-6	1.00	3.13	.033	6.72
F-9	1.33	3.97	.042	7.72
Series II				
D-3	1.27	3.80	.040	8.02
E-6	1.22	4.08	.039	7.22
F-9	0.99	3.08	.032	6.40
Series III				
D-3	1.32	4.19	.043	8.84
D-4	0.97	2.85	.033	6.58
Average	1.12	3.48	.037	7.17

Table E (Cont'd)

Rat no.	Unit activity: Mg. uric acid/gm. liver (wet wt.)/hr.	Unit activity: Mg. uric acid/gm. liver (dry wt.)/hr.	Unit activity: Mg. uric acid/mg. liver nitrogen/hr.	Total activity: Mg. uric acid/liver/hr.
Restricted intake				
Series I				
A-1	1.88	6.17	.049	8.47
A-2	1.22	4.00	.032	5.73
Series II				
A-1	1.73	5.67	.044	7.44
A-2	1.83	5.79	.047	7.67
Series III				
A-1	1.48	4.77	.038	6.36
A-2	1.26	4.26	.032	6.28
Average	1.57	5.11	.040	6.99
Series I				
B-4	1.40	4.52	.037	6.44
B-5	1.54	5.06	.045	8.33
Series II				
B-4	1.60	5.17	.043	6.57
B-5	1.36	4.39	.038	6.81
Series III				
B-3	1.61	5.35	.043	7.54
B-4	1.55	5.24	.042	7.27
Average	1.51	4.96	.041	7.16
Series I				
C-7	1.23	3.78	.035	5.41
C-8	1.71	5.59	.043	7.85

Table E (Cont'd)

Rat no.	Unit activity: Mg. uric acid/gm. liver (wet wt.)/hr.	Unit activity: Mg. uric acid/gm. liver (dry wt.)/hr.	Unit activity: Mg. uric acid/mg. liver nitrogen/hr.	Total activity: Mg. uric acid/liver/hr.
Series II				
C-7	1.36	4.55	.037	6.82
C-8	1.33	4.35	.037	6.77
Series III				
C-5	1.69	5.63	.045	7.77
C-6	1.58	6.14	.041	5.73
Average	1.48	5.01	.040	6.73
Series I				
D-1	1.89	6.21	.051	10.22
D-2	1.91	6.27	.034	10.90
Series II				
D-1	1.94	6.26	.046	10.48
D-2	1.80	6.11	.049	9.73
Series III				
D-1	1.63	5.43	.042	7.82
D-2	1.39	4.54	.037	6.23
Average	1.76	5.80	.043	9.23
Series I				
E-4	1.87	6.12	.049	8.78
E-5	1.47	4.81	.040	6.30
Series II				
E-4	1.90	6.13	.051	9.12
E-5	1.93	6.23	.050	8.88

Table E (Cont'd)

Rat no.	Unit activity: Mg. uric acid/gm. liver (wet wt.)/hr.	Unit activity: Mg. uric acid/gm. liver (dry wt.)/hr.	Unit activity: Mg. uric acid/mg. liver nitrogen/hr.	Total activity: Mg. uric acid/liver/hr.
Series III				
E-3	1.34	4.32	.035	6.16
E-4	1.50	4.98	.040	7.48
Average	1.67	5.43	.044	7.79
Series I				
F-7	1.16	3.92	.030	5.32
F-8	1.46	4.78	.041	8.02
Series II				
F-7	1.57	4.97	.044	7.67
F-8	1.30	4.20	.035	5.34
Series III				
F-5	1.49	4.89	.038	6.71
F-6	1.80	5.89	.049	7.18
Average	1.46	4.78	.040	6.71

Table E (Cont'd)

Succinic dehydrogenase activity

Rat no.	Unit activity: Succinate Q_{O_2} [a]	Unit activity: Succinate Q_{O_2} (N) [b]	Total activity: Ml. of O_2/liver/hr.
Stock animals			
Series II and III			
S-1	88.8	772.1	165.1
S-2	76.9	656.9	147.7
S-3	90.0	766.3	175.8
S-4	94.3	820.0	154.9
S-5	87.3	823.6	186.7
S-6	89.3	858.7	199.3
Series III			
S-1	88.9	766.4	197.9
S-2	92.0	828.8	182.3
Average	88.4	786.6	176.1
Ad libitum intake			
1. Rats fed 15 per cent of cal. as protein			
Series I			
A-3	73.0	760.4	159.0
B-6	69.7	718.6	163.5
C-9	70.7	679.8	158.7

[a] Microliters of O_2 per mg. liver tissue (dry wt.) per hour.

[b] Microliters of O_2 per mg. liver nitrogen per hour.

Table E (Cont'd)

Rat no.	Unit activity: Succinate Q_{O_2} [a]	Unit activity: Succinate Q_{O_2} (N) [b]	Total activity: Ml. of O_2/liver/hr.
Series II			
A-3	57.1	601.1	127.7
B-6	66.1	641.7	135.4
C-9	76.3	700.0	148.9
Series III			
A-1	78.8	685.2	148.9
A-2	79.1	746.2	151.7
Average	71.3	691.6	149.2
2. Rats fed 5 per cent of cal. as protein			
Series I			
D-3	58.8	639.1	126.1
E-6	46.5	494.7	99.7
F-9	54.2	602.2	105.3
Series II			
D-3	53.5	563.2	112.9
E-6	53.2	511.5	94.2
F-9	54.7	575.8	113.8
Series III			
D-3	65.4	674.2	138.0
D-4	51.8	609.4	119.8
Average	54.8	583.8	113.7

Table E (Cont'd)

Rat no.	Unit activity: Succinate Q_{O_2} [a]	Unit activity: Succinate Q_{O_2} (N) [b]	Total activity: Ml. of O_2/liver/hr.
Restricted intake			
Series I			
A-1	89.3	708.7	122.5
A-2	90.7	731.5	130.1
Series II			
A-1	97.9	764.8	128.4
A-2	99.4	808.1	131.5
Series III			
A-1	100.8	800.0	134.4
A-2	96.3	724.1	142.0
Average	95.7	756.2	131.5
Series I			
B-4	86.5	714.9	123.3
B-5	79.6	704.4	131.1
Series II			
B-4	88.7	733.0	112.7
B-5	83.6	720.7	129.6
Series III			
B-3	87.5	694.4	123.4
B-4	94.7	763.7	131.3
Average	86.8	721.9	125.2
Series I			
C-7	69.4	642.6	99.2
C-8	95.0	730.8	133.3

Table E (Cont'd)

Rat no.	Unit activity: Succinate Q_{O_2} [a]	Unit activity: Succinate Q_{O_2} (N) [b]	Total activity: Ml. of O_2/liver/hr.
Series II			
C-7	85.6	695.9	128.4
C-8	81.3	694.9	126.5
Series III			
C-5	82.1	656.8	113.3
C-6	75.2	541.0	80.7
Average	81.4	660.3	113.6
Series I			
D-1	83.8	686.9	138.0
D-2	87.9	757.8	152.9
Series II			
D-1	81.7	675.2	136.8
D-2	90.4	717.5	144.0
Series III			
D-1	95.7	730.5	137.8
D-2	96.6	771.8	132.5
Average	89.3	723.3	139.7
Series I			
E-4	92.9	737.3	133.2
E-5	91.5	756.2	120.0
Series II			
E-4	92.3	762.8	137.3
E-5	103.1	824.8	147.0

Table E (Cont'd)

Rat no.	Unit activity: Succinate Q_{O_2} [a]	Unit activity: Succinate Q_{O_2} (N) [b]	Total activity: Ml. of O_2/liver/hr.
Series III			
E-3	95.4	763.2	136.0
E-4	89.8	712.7	134.7
Average	94.2	759.5	134.7
Series I			
F-7	79.5	611.5	107.9
F-8	84.7	730.2	142.1
Series II			
F-7	71.0	628.3	109.6
F-8	84.1	700.8	106.9
Series III			
F-5	90.0	697.7	123.5
F-6	89.6	746.7	109.3
Average	83.2	685.9	116.6

Table E (Cont'd)

Cytochrome oxidase activity

Rat no.	Unit activity: Ascorbate Q_{O_2}[c]	Unit activity: Ascorbate Q_{O_2} (N)[d]	Total activity: Ml. of O_2/liver/hr.
Stock animals			
Series II			
S-5	78.6	741.5	168.1
S-6	62.6	601.9	139.7
Series III			
S-1	78.7	678.4	175.2
S-2	81.6	735.1	161.7
Average	75.4	689.2	161.2
Ad libitum intake			
1. Rats fed 15 per cent of cal. as protein			
Series III			
A-1	80.2	697.4	151.6
A-2	79.3	748.1	152.1
Average	79.7	722.8	151.8

[c]Microliters of O_2 per mg. liver tissue (dry wt.) per hour.

[d]Microliters of O_2 per mg. liver nitrogen per hour.

Table E (Cont'd)

Rat no.	Unit activity: Ascorbate Q_{O_2} [c]	Unit activity: Ascorbate Q_{O_2} (N) [d]	Total activity: Ml. of O_2/liver/hr.
2. Rats fed 5 per cent of cal. as protein			
Series III			
D-3	76.9	792.8	162.2
D-4	52.0	611.8	120.2
Average	64.5	702.3	141.2
Restricted intake			
Series II			
A-1	86.3	674.2	113.2
A-2	82.9	668.5	109.7
Series III			
A-1	81.9	650.0	109.2
A-2	81.2	610.5	119.8
Average	83.1	650.8	113.0
Series II			
B-4	90.1	744.6	114.5
Series III			
B-3	91.8	728.6	129.4
B-4	79.7	642.7	110.5
Average	83.9	705.3	118.1

Table E (Cont'd)

Rat no.	Unit activity: Ascorbate Q_{O_2} [c]	Unit activity: Ascorbate Q_{O_2} (N)[d]	Total activity: Ml. of O_2/liver/hr.
Series III			
C-5	71.2	569.6	98.3
C-6	58.2[e]	419.1	62.5
Average			
Series II			
D-1	88.8	733.9	148.7
D-2	85.4	677.8	136.0
Series III			
D-1	80.5	619.2	115.9
D-2	81.6	658.1	112.0
Average	84.1	672.2	128.2
Series II			
E-4	80.1	667.5	119.2
Series III			
E-3	79.5	636.0	113.4
E-4	75.3	597.6	113.0
Average	78.3	633.7	115.2
Series II			
F-8	79.4	696.5	135.4
Series III			
F-5	72.7	563.6	99.7
F-6	77.8	648.3	94.9
Average	76.6	636.1	110.0

[e]Poor homogenization. Not averaged.

Table F. Percentage of body fat derived from specific gravity measurements and from estimated volumes of fat of eviscerated carcasses together with the ratio of fat volumes to terminal body weights

Experiment III

Rat no.	Specific gravity data: Grams per ml.	Specific gravity data: Calculated percentage of body fat	Estimated volumes of fat: Ml. per carcass	Estimated volumes of fat: Ml. per 100-gm. carcass	Estimated volumes of fat: Ratio to terminal body wt. x 100
Stock animals					
S-1	1.058	18.8	20	11.8	9.4
S-2	1.057	19.3	15	9.5	7.5
S-3	1.063	16.4	13	8.5	6.8
S-4	1.053	21.2	14	9.7	7.7
S-5	1.067	14.5	12	8.4	6.5
S-6	1.068	14.1	8	5.6	4.5
Average	1.061	17.4		8.9	7.1
Ad libitum intake 1. Rats fed 15 per cent of cal. as protein					
A-4	1.062	16.9	12	7.3	6.0
A-5	1.063	16.4	10	7.0	5.7
B-9	1.059	18.3	10	6.5	5.3
B-10	1.067	14.5	10	6.6	5.3
C-14	1.069	13.6	10	6.2	5.0
C-15	1.075	10.8	8	5.4	4.4
Average	1.066	15.1		8.0	5.3

Table F (Cont'd)

Rat no.	Specific gravity data: Grams per ml.	Specific gravity data: Calculated percentage of body fat	Estimated volumes of fat: Ml. per carcass	Estimated volumes of fat: Ml. per 100-gm. carcass	Estimated volumes of fat: Ratio to terminal body wt. x 100
2. Rats fed 5 per cent of cal. as protein					
D-4	1.058	18.8	15	8.7	7.1
D-5	1.063	16.4	11	7.3	5.9
E-9	1.060	17.8	15	10.0	8.1
E-10	1.067	14.5	10	6.6	5.4
F-14	1.059	18.3	12	7.9	6.4
F-15	1.062	16.9	12	7.3	6.0
Average	1.062	17.1		8.0	6.4
Restricted intake					
A-2	1.061	17.4	12	8.1	6.7
A-3	1.064	15.9	10	7.1	5.9
Average	1.062	16.7		7.6	6.3
B-6	1.074	11.3	5	3.2	2.7
B-7	1.072	12.2	5	4.0	3.2
B-8	1.061	17.3	7	5.4	4.4
Average	1.069	13.6		4.2	3.4

Table F (Cont'd)

Rat no.	Specific gravity data: Grams per ml.	Specific gravity data: Calculated percentage of body fat	Estimated volumes of fat: Ml. per carcass	Estimated volumes of fat: Ml. per 100-gm. carcass	Estimated volumes of fat: Ratio to terminal body wt. x 100
C-11	1.073	11.7	4	3.2	2.6
C-12	1.064	15.9	5	3.6	2.9
C-13	1.064	15.9	7	5.7	4.5
Average	1.067	14.5		4.2	3.3
D-1	1.055	20.2	12	7.9	6.4
D-2	1.049	23.2	15	10.0	8.2
D-3	1.064	15.9	8	5.4	4.4
Average	1.056	19.8		7.8	6.3
E-6	1.064	15.9	12	8.5	7.0
E-7	1.055	20.2	13	8.4	6.9
E-8	1.061	17.4	10	7.2	6.0
Average	1.060	17.8		8.0	6.6
F-12	1.059	18.3	10	6.6	5.4
F-13	1.054	20.7	8	5.9	4.8
Average	1.056	19.5		6.3	5.1

Table F (Cont'd)

Experiment V (Series IV)

Rat no.	Specific gravity data: Grams per ml.	Specific gravity data: Calculated percentage of body fat	Estimated volumes of fat: Ml. per carcass	Estimated volumes of fat: Ml. per 100-gm. carcass	Estimated volumes of fat: Ratio to terminal body wt. x 100
Stock animals					
S-1	1.062	16.9	14	8.2	6.4
S-2	1.056	19.8	15	9.7	7.8
S-3	1.067	14.5	9	5.8	4.8
Average	1.062	17.1	12.7	7.9	6.3
Ad libitum intake					
1. Rats fed 15 per cent of cal. as protein					
A-1	1.059	18.3	16	8.8	7.4
A-2	1.063	16.4	10	6.3	5.4
A-3	1.053	21.2	16	9.7	8.0
Average	1.058	18.6	13.5	8.3	6.9
2. Rats fed 5 per cent of cal. as protein					
D-4	1.053	21.2	16	9.2	7.7
D-5	1.051	22.2	14	9.5	7.4
D-6	1.060	17.8	10	6.7	5.4
Average	1.055	20.4	13.3	8.5	6.8

Table F (Cont'd)

Rat no.	Specific gravity data: Grams per ml.	Specific gravity data: Calculated percentage of body fat	Estimated volumes of fat: Ml. per carcass	Estimated volumes of fat: Ml. per 100-gm. carcass	Estimated volumes of fat: Ratio to terminal body wt. x 100
Restricted intake					
A-1	1.056	19.8	11	7.4	6.0
A-2	1.050	22.7	18	11.4	9.4
A-3	1.058	18.8	17	11.0	9.0
Average	1.055	20.4	15.3	9.9	8.1
C-4	1.057	19.3	7	4.7	3.9
C-5	1.066	15.0	10	6.5	5.3
C-6	1.054	20.7	17	10.8	8.9
Average	1.059	18.3	11.3	7.3	6.0
D-1	1.056	19.8	9	6.0	4.9
D-2	1.052	21.7	17	10.4	8.5
D-3	1.052	21.7	16	10.4	10.0
Average	1.053	21.1	14.0	8.9	7.8
F-4	1.064	15.9	11	7.7	6.4
F-5	1.070	13.1	12	7.7	6.3
F-6	1.064	15.9	10	6.7	5.6
Average	1.066	15.0	11.0	7.4	6.1

Table G. Percentage of body fat derived from estimated volumes of fat eviscerated carcasses together with the ratio of fat volumes to terminal body weights

Experiment II

Rat no.	Estimated volumes of fat: Ml. per carcass	Ml. per 100-gm. carcass	Ratio of ml. to terminal body wt. x 100	Rat no.	Estimated volumes of fat: Ml. per carcass	Ml. per 100-gm. carcass	Ratio of ml. to terminal body wt. x 100
A-1	10.0	6.6	5.6	D-1	23.0	14.0	11.9
A-2	10.0	6.8	5.7	D-2	10.0	6.4	5.3
A-3	10.0	7.0	5.8	D-3	9.0	6.6	5.5
Average	10.0	6.8	5.7	Average	14.0	9.0	7.6
B-4	10.0	7.2	6.1	E-4	20.0	12.9	10.6
B-5	10.0	6.8	5.9	E-5	16.0	11.5	9.7
B-6	13.0	9.2	7.6	E-6	12.0	8.3	7.0
Average	11.0	7.7	6.5	Average	16.0	10.9	9.1
C-7	10.0	6.9	5.8	F-7	20.0	13.0	10.7
C-8	8.0	5.8	4.7	F-8	18.0	12.9	10.6
C-9	8.0	5.6	4.7	F-9	20.0	12.8	10.7
Average	8.7	6.1	5.1	Average	19.3	12.9	10.7

Table H. Estimated volumes of fat obtained from eviscerated carcasses and their ratio to terminal body weights[a]

Experiment IV (Series I and II)

Rat no.	Ml. of fat per carcass	Ratio to terminal body wt. x 100
Stock animals		
S-1	10	5.0
S-2	14	7.1
S-3	10	4.6
S-4	17	9.2
S-5	10	5.4
S-6	15	7.4
Average	12.7	6.5
Ad libitum intake		
1. Rats fed 15 per cent of cal. as protein		
Series I		
A-3	10	4.6
B-6	10	4.7
C-9	15	6.6
Series II		
A-3	15	7.0
B-6	20	9.4
C-9	16	7.8
Average	14.3	6.7
2. Rats fed 5 per cent of cal. as protein		
Series I		
D-3	12	5.7
E-6	20	8.9
F-9	10	4.8

[a]No carcass weights recorded.

Table H (Cont'd)

Rat no.	Ml. of fat per carcass	Ratio to terminal body wt. x 100
Series II		
D-3	8	3.8
E-6	3[b]	1.5
F-9	13	6.1
Average	12.7	5.9
Restricted intake		
Series I		
A-1	24	11.3
A-2	15	7.5
Series II		
A-1	17	9.2
A-2	10	5.3
Average	16.5	8.3
Series I		
B-4	14	6.8
B-5	11	5.4
Series II		
B-4	19	10.1
B-5	15	7.6
Average	14.8	7.5
Series I		
C-7	22	10.9
C-8	18	8.7
Series II		
C-7	18	8.7
C-8	16	8.0
Average	18.5	9.1

[b]Not included in average figure.

Table H (Cont'd)

Rat no.	Ml. of fat per carcass	Ratio to terminal body wt. x 100
Series I		
D-1	22	9.8
D-2	24	11.2
Series II		
D-1	25	8.6
D-2	17	8.3
Average	22	9.5
Series I		
E-4	20	9.5
E-5	27	14.8
Series II		
E-4	22	11.3
E-5	10	4.7
Average	19.7	10.1
Series I		
F-7	22	10.8
F-8	17	8.1
Series II		
F-7	21	10.0
F-8	23	12.1
Average	20.6	10.3
Experiment V (Series III)		
Stock animals		
S-1	19	8.4
S-2	13	6.8
Average	16.0	7.6

Table H (Cont'd)

Rat no.	Ml. of fat per carcass	Ratio to terminal body wt. x 100
Ad libitum intake		
1. Rats fed 15 per cent of cal. as protein		
A-1	17	8.1
A-2	16	7.9
Average	16.5	8.0
2. Rats fed 5 per cent of cal. as protein		
D-3	18	8.3
D-4	14	6.6
Average	16.0	7.5
Restricted intake		
A-1	9	4.8
A-2	11	5.9
Average	10	5.4
B-3	9	4.8
B-4	8	4.3
Average	8.5	4.6
C-5	12	6.5
C-6	7	4.3
Average	9.5	5.4
D-1	8	4.3
D-2	5	2.8
Average	6.5	3.6
E-3	10	5.3
E-4	7	3.8
Average	8.5	4.6

Table H (Cont'd)

Rat no.	Ml. of fat per carcass	Ratio to terminal body wt. x 100
F-5	13	6.8
F-6	9	5.3
Average	11.0	6.6