NITROGEN EXCRETION IN THE DEER IN RELATION TO AGE AND METABOLIC RATE

by

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Abstract

Total nitrogen excretion levels were measured on five male, and three female Vancouver Island Black-tailed deer (Odocoileus hemionus columbianus), which were raised in captivity in the deer unit at the University of British Columbia. They were raised from approximately three weeks of age until they had reached an adult body weight. The measurements were made at various intervals throughout the prepubertal growth period of the deer. Metabolic rate determinations were made on one of the deer, namely the doe R-5, after it had reached an adult body weight. Nitrogen balance tests were made at the same time. The method used in the nitrogen balance trials consisted of alternate periods of fasting and feeding. The ration used is described in Appendix I. It was given at one and two pound levels alternately. The procedure of fasting and feeding at different levels permitted the determination of the point of nitrogen balance, as well as that of total nitrogen excretion while feeding. The results and discussion of the nitrogen balance trials, and the distribution of nitrogen obtained, appear first, followed by that of the ntrogen excretion results observed during growth.

The point of nitrogen balance was found to occur at 16.5 to 17.3 grams of nitrogen intake per day. The crude protein requirement, calculated on this basis, was approximately

100 grams for a protein of perfect biological value.

The energy requirement of R-5 was found to be 1,300 to 1,400 Calories per day for maintenance. This energy requirement was met by the U.B.C. ration number 36-57 (Appendix I.) at the one pound level. This level also provided more than adequate amounts of nitrogen to fulfill the above protein requirement. The dietary requirement for nitrogen, based on the lowest level of nitrogen excretion obtained, was much lower than that calculated from the point of nitrogen balance. The lowest level obtained approximated the estimated endogenous total urinary nitrogen excretion level for an animal of the same body weight. It was concluded that insufficient time was allowed for nitrogen depletion, and that the true endogenous level was not obtained.

The urea nitrogen expressed as a percentage of the total nitrogen excretion reflected the status of protein nutrition. Upon fasting the percentage fell rapidly from the non-fasting level of 90 per cent to levels of less than 85 per cent. A level of less than 75 per cent was obtained in one case. When the animal was given feed the percentage immediately returned to levels of 90 per cent or more. The prompt response on the part of urea to changes in protein intake indicated that the percentage of total nitrogen made up of urea nitrogen might be of value as an index of protein nutritional status for field

studies.

The creatinine nitrogen excretion level also reflected, to a slight degree, the changes in nitrogen intake. Despite the ease in determining creatinine levels, the relatively greater constancy of creatinine excretion reduces the value of such determinations as indices of protein intake.

The ammonia nitrogen as a per cent of the total nitrogen reacted in an inverse manner to urea, and could be regarded as a check on the conclusions derived from the results with urea.

The pattern of nitrogen excretion during growth showed changes which were similar to those observed by previous investigators on the character of increase in body weight during growth. The rate of increase in total nitrogen excretion is characterized by changes in rate of increase which occur at similar times, and in a similar manner, to those of body weight.

The total creatinine nitrogen excretion increased in a regular manner during growth, from values of less than 100 milligrams per day, to values of between 400 and 600 milligrams. This is in agreement with the results of previous investigators, who have stated that creatinine excretion reflects the size of the "active body mass."

The total nitrogen excretion showed a trend toward reduced levels at approximately three months of age. This reduction coincides with a major change in the growth rate, which is associated with the appearance of puberty. The reduction may indicate increased retention of nitrogen at this time, although the same result could be caused by reduced nitrogen intake.

The total nitrogen excretion during growth greatly exceeded the estimated endogenous excretion level, for all body weights, as a result of the high plane of nutrition enjoyed by the deer throughout the growing period. Because the level of total nitrogen excretion reflects the level of nitrogen intake once the maintenance and growth requirements have been surpassed, it is difficult to interpret the level of excretion obtained in terms of metabolic functions. The pattern of nitrogen excretion during growth was therefore considered solely from the point of view of representing the increase in protein stores, and in the total amount of protein metabolism associated with increasing body size.

The importance of these results in terms of field studies is discussed. The lack of adequate techniques, at the present time, to enable samples to be taken from the field for the type of analyses used in this experiment, makes the appli-

cation of nutritional principles, such as the type discussed in this experiment, very difficult. However the results of experiments performed in the laboratory may be seen from the results of this experiment to be of great value in attempting to understand the relationship between the game animal and it's environment.

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Table of Contents

List of Tables

List of Figures

Introduction

Successful game management is predicated upon an adequate understanding of two complexities. These are; the environmental complex, of which the game population forms an important part, and the growth complex of the individual game animal. From an adequate understanding of these two complexities, for example, the relation between the character of the food provided by the range and the nutritional status of the game animals in residence on this range may become better understood. Then the nutritional status of the game animal may be advantageously modified, if necessary, by instigating suitable reforms in the character of the available herbage for example, and thus the better understanding of the factors which contribute to the nutritional status of the game animal has been applied to create an important and powerful game management technique.

The environmental complex consists of a great number of dynamically interrelated factors which interact with, and influence in varying degree the residing animal population. For example, factors such as the following are in a constant state of flux: (1) climate and topography (Darlington 1957); (2) changes in parasite populations (Chandler 1955, and Spencer 1938); and (3) changes in herbage as a consequence of changes in feeding habits of insects and other animals including the

game animals under consideration (Cowan 1945), chemical changes in plant composition with age and in response to climatic cycles (Cowan, Hoar and Hatter 1950), and species changes produced by climatic variations and competition within the floral population (Blaisdell and Muegger 1956, Elton 1935, Holmgreen 1956 and Hubbard 1957). The three factors mentioned form important controls on the size of residing animal populations. They present a kaleidoscope of environmental influences which effect game animals, for example, in varying degree depending on the ever-changing physiological state of the individuals. The game animals react to the changes in environmental influences by changes in viability in accordance with their physiological state and their genetic character. With changes in their physiological condition their resistance to adverse conditions tends to vary. In this manner the constant tendency of the population to increase in size is controlled and often limited. Also, for physiological reasons originating within the game population, changes in selectivity towards browse species arise (Cowan 1945 and Swank 1956) . Thus the changing characteristics of the animal population form new sets of stimuli for the floral population. The net result is a continual and often unpredictable variation in the composition and availability of browse species of herbage for herbivorous game animals (Dietz 1958 and Lauckhart 1957). Because of the complexity of the relationship between the animal and it's environment sound field observations

are essential for accurate assessment of environmental or range conditions.

The carrying capacity of the range for a particular game species depends on the ability of the environment to supply the needs of the individual animals. Aside from suitable temperatures, humidity, and topography which may determine whether an animal will or can enter and remain in a certain area, the most important character of an animal's environment is its ability to satisfy the game animal's primary need, that of taking in nutrient. More precisely, it must supply the nutritional requirements for maintenance and growth of the individual animals. In order to establish these requirements and thus to appreciate fully the significance of the range conditions, it is necessary to study the growth complex of the game animal.

Maintenance of a population of animals and maintenance and growth of an individual animal are both problems of balance between the rate of synthesis of new body material and the rate of its destruction. The effective reduction in population numbers by the death of old adult animals is a continuous and inevitable occurrence, regardless of the extent of the favourable conditions for survival. This must be compensated for by the addition of new individuals if a stable or increasingly large population is to be obtained. The successful conception, growth to birth, growth through lactation to maturity, and

continued maintenance for a suitable reproductive period, of a certain constant fraction of the population ensures the compensation required. Maintenance of the adult body of an organism is also a matter of continued compensation, by synthesis or growth, for a continuous natural loss. Protoplasm is thermodynamically unstable and is continually disintegrating (Brody 1945). This process is facilitated by the presence of many reversible enzyme systems which are capable of speeding natural catabolic processes (Harper 1961). Uncontrolled destruction of protoplasm will continue if unopposed until irreversible changes take place and death ensues. This catabolism must be compensated for or reversed by anabolic processes if the body form and function, and indeed life itself, is to be preserved. The reversal in the natural release of energy and associated protoplasmic breakdown of body material is accomplished by means of "free energy" which is liberated as the release of configurational energy from ingested materials, and which is trapped and coupled or entrained into the synthetic processes to make them thermodynamically possible. In effect, the lower energy state is transferred from the autocatalytic products to the cell materials in accordance with the second law of thermodynamics. Also units of structure must be supplied by the same means to replace those that are completely destroyed or lost to the body. If ingested material becomes unavailable, material from less important parts of the cell or

body may be used for the same purpose. This alternative will eventually lead to serious complications as more and more essential constituents are sacrificed to preserve the most essential ones. During early growth the synthetic processes exceed the replacement requirements and the animal gains body mass. During the adult phase of life a balance is usually maintained resulting in a constant weight. The animal gradually loses its ability to balance catabolic forces and inevitably they predominate and cause death. The duration and extent of anabolism is apparently under endocrine control, which is in turn determined by the genetic complex. The potentials instilled into the animal by these means, however, are seldom realized due to the confounding effects of environmental factors which prevent the full expression of the genetic complex. The dependence on ingested material as a source of energy and building units for biosynthesis explains the importance of nutrition in controlling the extent and rate of synthesis. The major portion of protoplasm, excluding water, has been well established to be protein. Biosynthesis of protoplasm is therefore largely a process of protein synthesis. The game range must therefore be capable of supplying structural units for protein synthesis that can be made available to the game animal, and also materials from which enough free energy can be liberated and similarly be made available, to permit protein synthesis. Of course other essential dietary constituents are needed to

make this protein synthesis possible such as vitamins and minerals, and also of course other materials to provide for the synthesis of the non-protein parts of body tissue protoplasm. Establishing these needs quantitatively provides the necessary complimentary information to that of the character of the environment to enable successful assessment of the adequacy of range conditions in terms of carrying capacity. Once the relationship between range conditions and carrying capacity has been established, purposeful management of range conditions in terms of the game it supports is possible.

There have been put forward several approaches to the problem of assessing the adequacy of the range in providing the essential nutrient for good nutritional status in game animals.

One of these approaches has been the study of range herbage in terms of population density, species present, and of chemical analysis of various species as to quality, quantity, and availability determined by feeding trials, of nutrients normally considered essential to animal maintenance and growth. These studies provide much data which must be interpreted in terms of calculated theoretical requirements of game animals (Bissell and Strong 1955, Bissell et al. 1955, Cook 1954, Dietz 1958, Einarsen 1946, Gordon and Sampson 1939, Smith 1950, Smith 1952, Smith 1959, and Swank 1956).

Another approach has been what might be termed a study of the biology of the game animals found in particular ranges under consideration. The "performance" of the animals is "rated" in terms of the degree to which they achieve or fail to achieve certain limits in various parameters such as those concerned with body confirmation, size and weight, digestive capacity, and certain characters of blood chemistry and bone marrow, for example (Bandy et al. 1955, Cowan 1945, Einarsen 1946, Forbes et al. 1941, 1946, French 1955, Kitts et al. 1956, Leopold et al. 1951, Nichol 1938, Rosen and Bischoff 1952, and Svihla et al. 1955). The performance or extent to which certain parameters are developed is often difficult to measure accurately, for example, the bulk of an animal as a parameter associated with nutritional status. However, indirect methods are being developed, such as the ratio of heart or chest girth to the thigh length, as a measure of bulf changes with respect to the relatively short term stable thigh length. Once obtained, the measures of various parameters may be compared with those determined on animals; of "ideal" nutritional status, of different localities, or of different species.

A third approach has been the experimental determination of nutritional requirements for certain of the above parameters, or others such as the time of antler appearance, or maximum growth rate, using empirical feeding methods with "ideal"

diets, range herbage diets, or diets used with varying degrees of success in domestic animals (Cowan et al. 1955, Nichol 1938).

The lines of demarcation between these studies are not precise as they all represent different ways of approaching the same central problems, also outlined previously. However, they do represent clearly different lines of approach though the methods and results sometimes intermingle.

A fourth approach, exemplified by the present experiment, entails an attempt to determine the requirement for one specific nutrient, namely protein, during growth and following puberty, for good nutritional status, by direct measurements of metabolism on the individual game animal. This approach has been used extensively on humans, many laboratory animals, and also extensively on animals associated with agricultural production (Albanese 1959, Allison 1951, Blaxter and Mitchell 1948, Blaxter and Wood, 1956, Block 1956, Butcher and Harris 1957, Bricker et al. 1945, Greaves and Scott, 1960, Majumdar 1960, Mitchell 1924, 1926, 1929, 1948, and 1950, Munro 1951, Murlin et al. 1946, and Waterlow et al. 1959). A review of the literature shows less frequent application of this method to game animals.

The evolution of the present knowledge of metabolism and nutrition is based on the firm foundations of modern chemistry

which were laid down toward the close of the 18th Century. The gradual establishment of a deeper understanding of metabolism and of the relation between nutrition and metabolism necessitated the recession and eventual dissolution of two traditional and popular schools of thought, both strengthened by generations of acceptance and one of them an heirloom from ancient philosophic giants. The first of these was the phlogiston theory invented by J. Becher during the 17th Century (Mendel 1923). This theory restricted earlier attempts to understand the true nature of respiration, due to its peculiar requirements. The second was the hypothesis of a single fundamental aliment present in a cryptic form in all foods. This theory was put forward by Hippocrates in the 4th Century B.C. (Mendel 1923), and seemingly verified by Galen in the 2nd Century A.D. (Sahyun 1948), and as late as 1833 by William Beaumont (Beaumont 1833) with the observation that digestive processes in the stomach led to the formation of a similar looking material whether the ingesta were predominantely starchy or "albuminous".

The phlogiston theory was destroyed by the works of Black, Scheele, Priestly, Cavendish, Rutherford and Lavoisier (Bourne 1953) who uncovered the true nature of combustion and laid the foundation for modern chemistry. Their great contributions hinged on the fact that they learned to recognize atmospheric CO_2, O_2, and finally H_2, and to observe the changes in amounts of these during combustion. They also observed

similar changes associated with animal respiration, and during these experiments the presence of nitrogen was indicated. D. Rutherford (Bourne) is credited with the discovery and first isolation of atmospheric nitrogen in 1772. He called it azote because of abundant proof of its inability to support respiration or combustion. Lavoisier made the most important and culminating contribution with regard to biochemical and nutritional studies, in the observation that the process of respiration in animals is equivalent to other combustion processes in terms of heat production associated with a certain amount of O_2 consumption and CO_2 production.

During the 18th Century and much of the 19th Century, protein was being discovered to be widely distributed in nature. Gelatin had been prepared from bones since the time of Boyle (1627-1691) (Sahyun 1948). Gluten was isolated by Becarri (1682-1766) (Sahyun) from flour. Zein was prepared by J. Gorham early in the 19th Century (Gorham 1821). P. Bertholet in 1786 reported that he found nitrogen to be a constant constituent of all animal tissues (Bourne). Fourcroy in 1789 showed three kinds of animal products (Bourne) on the basis of their nitrogen concentration and laid foundations for the study of protein by noting the similarity of gluten and flesh. J. Gay-Lussac (1778-1850) helped to perfect nitrogen analysis and he found all seeds to contain a principle "abounding in azote" (McCollum 1939). Nitrogen analysis was slow and complicated until the time of

the Danish chemist J. Kjeldahl (1849-1900), who developed the relatively rapid and very accurate ammonia method (Hawk 1954, and the discoveries made before him concerning the amount and distribution of nitrogen represent a great amount of painstaking effort. In 1818 Braconnot studied the proteins of legumes and found a similarity in all (Bourne). Proust (1754-1876) improved the isolation of gelatin, and isolated a white compound from cheese (Bourne) similar to that which, a year later Braconnot, who also started the acid hydrolysis of protein, obtained from a sulphuric acid digest of muscle fibre and wool (Sahyun). Braconnot termed this product leucine. Besides nitrogen, phosphorus and sulphur were also found to be common to all protein by these and other investigators.

G. Mulder in 1838 and 1839 probably provided the greatest initial impetus for protein research with his efforts to unify thought on the albumin-like or azotized materials being discovered one by one up until this time (Mulder 1838). He emphasized protein in nutrition as being primarily important. He called the albumen-like materials protein, and he showed the similarity of plant and animal protein, holding this fact as the explanation for the as yet inexplicable nutritive value of plant material to animals. His views and influence served however to strengthen the age-old belief in a single fundamental aliment by providing protein as this single aliment. This idea

had the unfortunate long term effect of causing a separation, in the minds of physiologists, between respiration, and the relations pertaining to its continuance, and body metabolism or nitrogen metabolism and its associated phenomena. Respiration was to be thought of as being controlled largely by the amount of available oxygen, and to be a special mechanism for heat production and maintenance of body temperature. While nitrogen or protein metabolism was to be thought of as largely catabolism to provide energy for muscular work.

Magendie, at the beginning of the 19th Century, founded the study of nutrition by demonstrating the unlike nutritive value of the then recognized three types of foodstuffs, later designated: saccharina, oleosa and albuminosa, by Prout (Sahyun). He made a clear distinction between nitrogenous and non-nitrogenous foods and he demonstrated that the only source of nitrogen for the animal body was nitrogen containing foods (Bourne). However, this result was not entirely accepted until almost 100 years later when Krogh in 1906 gave incontestible proof in his exhaustive series of respiratory experiments (Krogh 1906). Magendic's work supplemented the work of Lavoisier and of Depretz and Dulong, who studied respiration for the Academie de Science in 1839 and saw respiration as the sole source of body heat (Bourne), to give the foundation for the modern theories of metabolism.

In 1844 Boussingault invented the balance technique (Bourne). He studied the relation between C,H,N, and O of the maintenance diet and of the urine, feces and milk. He re-affirmed the absolute essentiallity of nitrogen in the diet. Carbohydrate and fat were still considered to be catabolized by oxygen solely for the production of body heat. Barral (1819-1884) was the first to use this method on humans (Bourne). Along with this work came further attempts to quantitate metabolism. In 1849 Regnault and Reisit (Brody 1945 and McCollum 1939) devised a closed circuit respiratory apparatus and were the first to determine R.Q.'s. They also furthered the enumciation of Bergman in 1845 (Brody) of the increase in basal metabolic rate with the increase in surface area relative to weight. The age of calorimeters is said to have begun with their heat production studies.

Justus von Liebig (1803-1873) is often regarded as the central figure in the early history of protein theory. The importance of his work is primarily that of organizing and greatly stimulating future thought (Liebig 1843). By bringing the principles of organic chemistry, which he was also developing, to biology, he laid the foundations for the study of intermediary metabolism. He stressed the importance of noting the chemical change between food and metabolic end products as the key to understanding energy changes regardless of the unknown details of the metabolic paths utilized. His work led to the

verification of the principle of the conservation of energy in the metabolism of living organisms. He extended the nitrogen balance technique to studies of nitrogen equilibrium. However, his very dominant pronouncements on protein as the plastic principle and as the only source of energy for muscular activity served to preserve the separation in thought of respiration, and carbohydrate and fat metabolism, from the "primarily important" protein metabolism. Though the methods and the knowledge necessary for the approach to modern day understanding of metabolism were becoming more and more abundant the necessary steps could not be taken until the essential unity of carbohydrate, fat, and protein metabolism was accepted.

In 1852, F. Bidder and C. Schmidt (McCollum 1939) published results of nitrogen balance work using refined techniques, showing an accurate accounting of food nitrogen in urine and feces of adult cats.

The nitrogen balance technique became a powerful instrument in the hands of C. Voit (1831-1908), and von Pettenkoffer, who in 1866 showed that protein was not metabolized primarily for energy (Brody and Sahyun). Voit showed that "circulating protein" did not have to enter into the body structure in order to be catabolized, contrary to the insistence of von Liebig. Pettenkoffer confirmed that protein was not the primary energy source, Voit introduced many ideas and observa-

tions which hold today. He introduced the idea that the function of food protein was to replace body protein which was inevitably broken down to a small degree during its operation. He also demonstrated a time lag in adjustment of the body from a high plane protein diet to a low plane, suggesting two types of protein metabolism and two types of body protein, namely; "organ" or stable structural protein with little availability for metabolic needs, and loosely bound reserve protein in cells and "circulating".

Rubner is credited with confirming the first law of thermodynamics as applied to animal metabolism (Brody). He, like Voit, discovered many aspects of animal nutrition which still hold. Examples of these are: observation and quantitation of S.D.A., first observed by Sanctorius (Chittenden 1907) and later by Bidder and Schmidt (Chittenden), observation of differences in dietary protein availability with different protein sources, using refined fecal nitrogen analysis, confirmation of "surface law"; and observation that efficiency of growth decreases with decreasing growth rate.

Outside of continental Europe, Lawes and Gilbert (Bourne) at the research station in Rothamsted, Great Britain, showed the agricultural world the importance of nitrogenous foods for producing non-nitrogenous bodies such as starch and cellulose.

Before the turn of the century, E. Smith (Bourne) in England forecast a major development toward the deeper understanding of protein metabolism by demonstrating two important facts; namely, he saw that not all urea came from food, but that almost all did provided the protoplasmic mass was kept uniform. If the bulk increased the urea output represented the food nitrogen minus the amount of tissue nitrogen gained. If it decreased, the amount of urea nitrogen lost was represented by an equivalent increase over that produced by the food. Associated with the realization that the primary function of protein in the diet was not for energy, came the gradual realization that accepted dietary recommendations of more than 100 grams of protein for the average human male was unnecessarily high. Values such as 118 grams set by Voit were based on empirical observations of current diets rather than on experimentation. In 1890 Hirschfeld (Bourne) in Berlin, started this movement, showing that there was no increase in nitrogen excretion with heavy exercise, provided the caloric intake was large enough to supply the body's need. Earlier workers had not grasped the importance of having an energy balance and nitrogen balance simultaneously in determining the overall picture of nitrogen balance. Also, many diet recommendations were based on observations of diet of working man. In 1895 Atwater (Sahyun) stated the energy yielding functions of food as an alternative to building tissue, or as

the sole function, or both, depending on the food and on the body's needs. In 1907, Chittenden saw high levels of protein intake as potentially dangerous to body health (Chittenden 1907). He felt that even though it was of prime nutritional importance, protein was not needed in greater amounts than carbohydrates.

During this evolution of ideas concerning the true purpose and fate of dietary proteins, a parallel field of study was continuing which was to be of great importance in understanding the mechanisms of protein nutrition and metabolism. This study concerned the chemical structure of protein and the metabolic significance of this structure. The observation that protein subjected to hydrolytic action by boiling acid was decomposed into relatively simple crystalline substances was made near the beginning of the 19th Century. Wollaston (Vickery 1931) is credited with the discovery of the first true amino acid, namely, cystine, in 1810 though Vanguelin and Robiquet had identified asparagine in 1806 (Bourne). He called it cystic oxide due to its occurrence in urinary calculi. Proust is credited with leucine in 1819, Braconnet with glycine in 1820 and so on until by 1903, 18 amino acids were known (Vickery).

The 20th Century, therefore, began with an environment of knowledge of protein nutrition and metabolism which had

four important aspects. First, protein though capable of yielding energy was not utilized primarily for energy for muscular activity. Second, a large intake of protein relative to carbohydrate or fat was not considered necessary or desirable. Third, there were positive indications that protein served two purposes in the body, implying therefore two types of protein metabolism, one an energy producing function, and the other a tissue building function. Also, the level of protein catabolism could be estimated fairly accurately by the level of urea nitrogen in the urine. Fourth, there was abundant evidence that though protein from all sources was fundamentally similar it contained a wide range of qualitative diversity of chemical structure or arrangement. Also there was a growing belief that the key to understanding protein nutrition and metabolism lay in the availability and handling by the body of amino acids. Otto Folin of Munich, in 1905, demonstrated clearly the dichotomy in protein metabolism (Folin 1905). By quantitative and qualitative analyses of human urinary nitrogen excretion he demonstrated the existence of an irreducible level of nitrogen excretion of characteristic composition and constancy on a low or nitrogen free diet. He further showed that elevation in urinary nitrogen excretion by elevating the dietary protein level, was accompanied by an increase in the relative importance of urea nitrogen. Urea was known at this time to be solely associated with the deamination

of amino acids. He suggested that the lower level of nitrogen excretion which he termed endogenous excretion represented the breakdown of body structure and that dietary protein in excess of the amount needed to balance this was used for energy, and ultimately gave rise to the exogenous nitrogen of the urine.

Preliminary establishment of a quantitative relationship between energy production at a basal level, and total endogenous nitrogen excretion was obtained by Terroine and Sorg-Matter in 1927 (Terroine 1927). They found an approximately constant ratio of 2.3 to 2.9 milligrams of total nitrogen excreted per Calorie of energy produced using mature animals of different species. They also found that fluctuations in endogenous excretion paralleled those in basal energy metabolism. Smuts, in 1935, with a more definitive approach, found an average ratio of 1.99 milligrams of endogenous urinous nitrogen per Calorie per day (Smuts 1935). Improvements in his method were; using urinary nitrogen only, which constituted a more refined technique, and measuring the metabolic rate on the same individuals used for nitrogen excretion measurements. He also used a wider range in weight. His results confirmed those of Terroine and Sorg-Matter. The higher value (2.3 to 2.9 milligrams) reported by the latter authors included the endogenous fecal nitrogen and as stated above represented the total nitrogen excretion exclusive of respiratory and sweat losses.

Much of the difficulty in establishing the true nature of protein metabolism and the energy required for protein synthesis has been and is due to the difficulty in obtaining basal conditions. Activity, and many other factors, both dietary and physiological, increase protein catabolism and energy production beyond basal levels. However, once established, the basal level provides an excellent reference point for studying the increments caused by these various factors. Studies of this nature add greatly to the understanding of metabolic events that lead to the minimal or basal metabolism.

During the early part of the 20th Century great impetus was provided for the chemical aspect of protein metabolism study by the brilliant analytical work of E. Fischer (Fischer 1914), A. Kossel (Kossel 1900), Van Slyke (Sahyun), and many other physiologists and biochemists who gradually demonstrated that protein was broken down to the amino acid level during digestion. Also amino acids were demonstrated to be absorbed into the portal circulation following digestion, and finally it was shown that animals could synthesize blood protein from an ingesta of amino acids. In 1901, O. Cohneim (Cohneim 1901) showed that amino acids were formed in ereptic digestion in the intestinal mucosa. At the same time, F. Kutscher and J. Seemann (Kutscher et al. 1902) found them in chyme, and O. Loewi (Sahyun) showed that amino acids of digestion could produce nitrogen equilibrium. The nitrogen requirement of

animals therefore was shown to be for nitrogen in the form of amino acids.

The first proof of the nutritive importance of qualitative differences in protein was shown by E. Willcock and Hopkins (Sahyun) with the demonstration in 1906 of the indispensibility of tryptophane for maintenance in mice. There has since been a great amount of work done on the amino acid requirements of animals extending up until the present time. T. Osborne and L. Mendel in 1912 showed that lysine was indispensable for growth in rats, though proteins deficient in this amino acid would support life. Thus, the qualitative differences in amino acid requirements for growth and maintenance were demonstrated. In 1915 they enunciated the "law of minimum" and in 1919 Osborne developed the procedure of estimating the biological value of food protein using growth as the criterion (Osborne and Mendel, 1912, 1915, and Osborne et al 1919). This latter method was a modification of the method of K. Thomas, (Chittenden 1907) a student of Rubner who first measured the amount of various protein sources required to produce nitrogen equilibrium on himself, in cognisance of the necessity of defining the state of the animal due to the different associated nitrogen requirements.

Rose in the period from 1935 to 1938 (Rose 1955, and West et al 1957) brought the many experiments on amino acid requirements together with tests using purified amino acids. This

brilliant series of experiments showed that; of nineteen amino acids required for body synthesis nine are essential as preformed dietary constituents for maintenance and growth, five of the rest are semi-essential as preformed amino acids in the diet depending on the physiological state of the experimental animal and on its dietary character, and the remainder are non-essential as preformed amino acids in diet. He also obtained values for the amount of each needed under different conditions. In the light of present day knowledge of carbohydrates and fat metabolism, the requirement of the animal for amino acids rather than preformed polypeptide nuclei for synthesis of body protein provides an explanation of the mechanism for the protein sparing effect of carbohydrate and fat. However, the requirement for nine preformed amino acids places a limit on the protein sparing effect and on Rubner's isodynamic law which suggested complete caloric interchangeability. The synthesis of non-essential amino acids from carbohydrate illustrates a similar dichotomy in carbohydrate metabolism to that of protein metabolism and helps to explain the nonspecificity of the source of non-essential nitrogen.

Schoenheimer in 1939 (Schoenheimer 1942) and later, found a complete interchange of amino acids and parts of amino acids, between body protein, and dietary protein via the amino acid metabolic pool of temporarily free amino acids. This showed that nitrogen of exogenous and endogenous metabolism was indis-

tinguishable on a physical basis. However, as Mitchell showed in 1955 (Albanese 1959, Maynard 1956) there was still a constant amount of protein catabolism which was independent of protein intake. Schoenheimer's results showed that metabolites involved in various reactions are in a dynamic equilibrium of interchange and that metabolic reactions represent trends. Thus dietary material may still serve two independent purposes. Since 1949 the development and application of new procedures has permitted the uncovering of a great deal of information on the complex interrelated factors which affect amino acid requirements as well as general nutritional requirements. Factors such as species and individual differences in protein structure, which is to a great extent due to variations in amounts of enzymes, differences in the type of tissue currently under construction, the source of non-essential nitrogen, the balance among essential amino acids, the quantity and quality of the non-nitrogenous ingesta, and the physiological state of the animal are all interrelated and exert some influence on the nitrogen requirement of the animal. All of these minutiae do not invalidate the general laws of metabolic rate and waste per unit surface area arrived at by Brody and others before 1949, which show that the endogenous nitrogen excretion and basal energy production are related to the biologically active size of an animal and that as the size of the animal increases the endogenous excretion and energy production are elevated by decreasing

increments. The equation set forth by Brody and Klieber is: Calories of basal energy production = 70.5 x (Weight in kilograms to the power 0.75) for the relation between biological size and caloric requirement (Brody 1945, Klieber 1932). This equation shows that for a one percent increase in body weight in kilograms there is a 0.66 percent increase in energy requirement. Because of the relation established by Smuts the above equation can be used to estimate the minimum requirement for a biologically perfect protein. Thus minimum protein requirement in pounds = $\frac{2 \times 70 \times W_{kg.}^{0.7} \times 6.25}{1000 \times 454}$.
The figure 6.25 arises from the fact that the average nitrogen content of body protein is sixteen percent. The protein requirement thus estimated is inaccurate due to the fact that part of the nitrogen of endogenous excretion is associated with creatinine, creatine and uric acid. Application of the minimum protein requirement to actual dietary protein is complicated by the great difficulty in estimating the true biological value of the dietary protein. Once obtained it is relatively easy to correct the minimum protein requirement for a perfect protein to that of the dietary protein.

The accepted pattern of nitrogen metabolism in monogastric animals presumably applies to ruminants except for modifications brought about by the synthesis of microbial protein in the rumen and by the loss of nitrogenous materials from

the rumen by direct absorption (Lewis 1957). The rumen is a swelling in the digestive tract for delay in the passage of crude fibre. It is functionally a fermentation vat supporting a large population of protozoa and bacteria which degrade dietary protein and carbohydrate to short chain volatile fatty acids. These acids and ammonia from the degradation of protein are rapidly absorbed from the rumen. Part of the ammonia, following normal detoxification by the liver, is recycled via the saliva into the rumen. However, nitrogen removed from the rumen this way, even though it is recycled, must be incorporated into bacterial or protozoal cell protein or it is soon lost as exogenous nitrogen excretion. The degree of loss depends on; the extent of degradation as unaffected protein may be utilized in a similar way to the mono-gastric animals after it passes on from the rumen into the abomasum and small intestine, and on the rate of degradation, as this determines the amount of released ammonia which can be utilized by the microflora and the amount which can be recycled as urea. Also protein synthesis by the microflora depends on an adequate supply of carbohydrate to the rumen to provide adequate energy and carbon chain units for utilization of ammonia. Once utilized in the synthesis of microbial protein nitrogen is available to the host by digestion of the continuous passage of excess microflora along the digestive tract to the abomasum and small intestine. The nutritive value of microbial protein has been

analysed by feeding experiments with microbial preparations with resultant true digestibility values of approximately 70 and biological values of approximately 80 (Annison 1959). These results show a good but not outstanding feeding value.

Though there is a great deal of material in the literature regarding empirical feeding trials with deer, there is very little concerning the determination of protein requirement from metabolic studies. The material presented here is derived from measurements made on nitrogen metabolism during metabolic studies on individual animals. The animals studied are Vancouver Island genotype of black-tailed deer, Odocoileus hemionus columbianus (Richardson).

Methods and Materials

Animals

Eight coastal or Vancouver Island black-tailed deer, Odocoileus, hemionus, columbianus, (Vancouver Island genotype), of which three were female, were captured near Courtenay, B.C. in early June, 1960. They were approximately three weeks of age and in apparently good physical condition. Nothing is known of their nutritional history prior to capture nor of the nutritional history of their respective dams. They were taken directly to the University of B. C. and housed in a special wild ungulate unit. This unit is described in the Journal of Wildlife Management (Wood, Nordan, and Cowan 1961). They were housed in individual pens and coded R-1 to R-8. In accordance with the methods described in the above reference, for the care and management used in raising deer in captivity, the fawns were immediately placed on a regimen of evaporated milk mixed in warm water. After six weeks, or when the animals had reached a weight of twelve to fifteen pounds, they were weaned to a dry ration. (see Appendix 1) After five months they were switched from the weaning ration to an adult ration. (see Appendix 1)

At approximately one month intervals, excluding the month of November, and extending from July to December 1960, and in some cases January 1961, total metabolism studies of

twenty-four hours' duration each were performed on the growing deer as part of a separate experiment. The final trial on each deer was of three days' duration. All of these studies were done in a specially constructed respiration calorimeter which contained a galvanized iron animal cage, (see Appendix V). The cage was constructed to facilitate the collection of feces and urine. The urine and feces samples thus obtained were frozen for storage. Advantage was taken of the urine collection from this separate experiment to study the nitrogen metabolism of growing black-tailed deer. The results of this study represent the first part of the present experiment. They are also of value in establishing and proving the methods used.

The second part of the experiment consisted of three combined nitrogen balance and metabolism trials, performed on one white-tailed deer (Odocoileus virginianus ocrurus) and on one black-tailed deer during the animal's residence in the respiration calorimeter. The first was on P-1, an adult white-tailed doe; and the second and third were on R-5, an adult black-tailed doe, both already on the adult ration. The first of these trials was started on October 16th, and was stopped after four days for technical reasons. It is thus incomplete. The second and third trials were started on January 15th and March 12th, and lasted 13 and 11 days respectively. Feces and urine were collected daily as in the three day trials during the growth period.

The feeding schedule used during these trials consisted of an initial starvation period, a controlled feed intake test period, and a final starvation period. Free access to drinking water was provided throughout. The initial starvation period in the second and third trial lasted four days. This length of time was necessary to ensure complete passage of dietary material through the digestive tract of the ruminant. Also, due to the lack of incoming carbohydrate or roughage to the rumen, the metabolic activity of the rumen microorganisms is markedly decreased causing a decreased absorption of metabolic by-products of microbial origin. During this food-free period the level of urinary nitrogen was expected to drop in curvilinear fashion to a new lowered level associated with the maintenance catabolism of protein. The object of the controlled feed intake test period was to measure increments or decrements in nitrogen excretion at various levels of feed intake. Re-alimentation was begun with one pound of adult ration. If this amount was all consumed within twenty-four hours two pounds were given on the second day. If this amount was also well consumed, for example, up to 75%, one pound was again given and the feed level was alternated between these two values during each day of a five day test period. The feed was well consumed at the one pound level and fairly well consumed at the two pound level. However, in the third trial, R-5 consumed one pound of feed only after it had

been repeatedly presented for three days, and consumed slightly more than one pound on the fourth day when offered two pounds. The feeding period was therefore terminated at this point. Following the realimentation period, a second starvation period, comparable to the first, was instituted to allow verification of the resting urinary nitrogen level following a measured feed intake. The two levels of feed intake used in these trials were selected according to the weight of the does. The weight of the white-tailed doe was 203 pounds, and that of the black-tailed doe was approximately 100 pounds or 454 kilograms, during these experimental periods. The general relationship between basal energy requirement, and adult, non-pregnant, non -lactating body weight, for homeotherms, has been stated previously to be:

$$\text{Basal metabolic Calories} = 70.5 \times \text{Weight in kilograms}^{0.75}$$

The more accurate power of 0.73 was used (Brody 1945), and the total digestible energy requirement for basal metabolism was calculated to be 1,336 and 1,142 Calories per day for the deers R-5 and P-1. If the caloric requirement for maintenance metabolism is assumed to be twice the basal requirement, as Brody and others have suggested, the maintenance requirement of the two does would be 2,672 and 2,248 Calories per day. The adult deer ration contained approximately 1,300 Calories of digestible energy per pound. Therefore, the feed intake was set to

alternate between the basal energy requirement and the maintenance energy requirement.

According to the relationship between basal energy expenditure and endogenous urinary nitrogen excretion, also stated previously, the requirement for endogenous nitrogen metabolism for R-5 was approximately 2.3 grams of nitrogen per day, and for P-1 it was 3.8 grams per day. The adult deer ration contained approximately 2.49% nitrogen or 11.3 grams of nitrogen per pound. The nitrogen intake was therefore well above the endogenous requirement.

During the second and third nitrogen balance trials the water intake was measured four times daily to the nearest gram. Insensible water loss, which included a fraction evaporated from the feces, on standing following defecation and until collection, was measured three times daily to the nearest milliliter.

In accordance with the normal functioning of the respiration calorimeter, measurements of methane, carbon dioxide, and heat production (by calculation), and oxygen consumption were continuously recorded throughout all the trials.

Sample Collection

The illustration, included in Appendix V, shows the animal cage which was used to confine the experimental animals within the calorimeter during the metabolic trials. The provisions built into the cage to facilitate sample collection are shown.

Fine wire mesh screening, attached to a frame for support, and placed immediately below the thick galvanized iron mesh floor of the animal cage, furnished the means for both fecal collection and separation of feces from urine. The screen was slightly larger in overall dimensions than the cage floor to prevent losses. The frame of the screen was arranged to slide on side tracks secured to the cage support members. The screen could thus be withdrawn entirely from the calorimeter to facilitate fecal collection. On removal of the screen, fecal material was brushed carefully into a collecting pan, weighed, and then frozen for storage.

A galvanized iron tray shaped into a shallow four-sided funnel, which drained acentrally through a short piece of pipe, was used for collecting urine. The drain was near the front of the calorimeter to facilitate handling of collecting flasks. Urine was collected under a thin film of mineral oil in narrow-necked flasks of approximately five liters capacity.

Upon removal of a urine sample, a new flask could be quickly put in the old one's place to prevent loss of urine. A large pyrex laboratory funnel was placed in the neck of each flask to prevent spillage of urine. A pad of fibre glass wool set in the funnel prevented contamination of the urine with detritus of fecal or epidermal origin. Following collection the volume of the urine sample was measured to the nearest milliliter, and the specific gravity was measured by weighing a five milliliter aliquot on an analytical balance. The urine was then frozen for storage. The urine tray was arranged to slide in the same manner as the fecal screen, being the same size as the screen and fitting immediately below it, to facilitate cleaning. Chaff brushed from the tray was collected separately from the feces. This was primarily of epidermal origin. The tray was washed down with distilled water and dried with paper towels during the last two balance trials. Both the tray and the wire screen were steam cleaned after each collection period during the growth studies. This could not be done with the continuing regime of the nitrogen balance tests.

The food and drinking water pans in the animal cage were offset toward the front of the calorimeter to prevent undue spillage onto the collection media.

Sample Analysis

As mentioned previously, the urine samples were measured as to volume and specific gravity immediately upon removal at the end of each twenty-four hour period. In the second and third balance trials the pH was also recorded. The pH was never greater than 7.21 and therefore no acid was added. In most cases aliquots were taken immediately for analysis and the remainder of the samples were frozen for storage. This procedure was preferred since it was felt that precipitates formed during the freeze-thaw process might contribute to analytical error.

The urine was analysed for total nitrogen by the Kjeldahl micro method using steam distillation (Consolazio 1951). Two grams of copper sulphate and dibasic potassium phosphate mixture in 3:1 ratio were used as the digestion catalyst. A two percent borate solution, with brom cresol green and methyl red added as mixed indicator, was used to trap the ambient or dissolved ammonia during distillation. The trapped ammonia was titrated directly with N/14 hydrochloric acid, made from a stock solution of constant boiling acid. The constant boiling acid was distilled at 109°C. and at a barometric pressure of 758.8 mm. Hg. The normality of the solution used for titration was selected to facilitate subsequent calculations.

The distribution of the total nitrogen among its components: urea, ammonia, creatine and creatinine, was determined. Urea and ammonia content in the urine samples was determined using the Conway microdiffusion method (Conway 1957). A urease preparation was obtained from finely ground Jack bean meal, in accordance with the method of Conway. For the urea determination urine and buffered urease were placed in the outer well, and after the units were sealed, the two materials were mixed by tilting the unit. After mixing they were left standing at room temperature for one-half hour. Following this the lid was slid to one side and saturated potassium carbonate was added to the outer well. The lid was quickly replaced and the units were again left standing at room temperature for three to four hours. Liberated ammonia from the above processes was trapped in the boric acid solution described above, placed in the center well initially. The trapped ammonia was titrated as above, using N/28 hydrochloric acid made by dilution from N/14. Pre-formed ammonia was determined by repeating the process without the addition of enzyme. The reliability of the Conway method was established using reagents of known nitrogen concentration. Variations in the times used for the various steps had no effect on the reliability of the method.

The alkaline picrate method of Folin (Consolazio 1951) was used to determine the creatine and creatinine content

of the urine. The creatinine picrate concentration following one-half hour for color development, was determined photometrically using a Coleman spectrophotometer, model 11A. A standard curve was prepared relating transmittance to known amounts of creatinine. The results are presented in Appendix VI. Those samples intended for determination of pre-formed creatinine were made alkaline with ten percent sodium hydroxide, with no previous treatment. Those samples intended for determination of creatine indirectly by conversion of creatine to creatinine were autoclaved with picrate for more than one-half hour, at 121°C. and 15 pounds pressure, before being made alkaline. The same treatment as for pre-formed creatinine followed this after the flask was allowed to cool to room temperature. Pre-formed creatinine was then subtracted from the total creatinine present to determine the newly converted creatinine. Since this process is considered to be only eighty percent complete in all cases, (Consolazio 1951), the amount of newly formed creatinine was multiplied by a correction factor of 1.16.

Results and Discussion

Choice of Methods

In order to understand fully the relationship between the animal body and its ingested foodstuffs, it is necessary, at the outset, to measure and analyse the following: the changes which occur in the feed and in the animal after the feed has been taken into the digestive tract, factors associated with the absorption of a fraction of the ingested feed, and the changes which occur in the absorbed nutrient and in the animal body following absorption. The first two are accomplished by quantitation and chemical analysis of feed and feces, accompanied by methods of determining feces of metabolic origin. The third aspect is part of the greater study of all of the activities and characteristics of living cells and tissues and the nutritionist draws upon entire fields of study, such as the field of biochemistry or physiology, all of which are converging on this same central area, for innumerable methods and procedures of study. The study of nutrition itself, therefore, becomes one of unlimited scope with indefinite boundaries. However, for practical purposes, the study of nutrition uses more limited parts of all three aspects to assess the nutritive state of the animal body in relation to its feed intake relying primarily on studies of growth, metabolic rate, respiratory quotient, urinalysis and body composition changes to assess the internal

changes. Body composition studies, though extremely useful, are often unavailable, as was the case in this experiment, because they involve slaughter and post morten analysis of the animal under investigation. However, if they cannot be performed, they may at least be estimated in part from the results of the other studies combined. Fecal and feed analysis were used in this experiment to establish the behavior of the animal towards the feed used. Urinalyses and metabolic studies were also used to study the metabolic reactions to the absorbed nutrient. Since protein metabolism was of primary concern the urinalyses consisted of nitrogen analyses. The nitrogen balance method was used to establish the nutritive requirement for protein and the fasting catabolism of protein in the animal. Though the nitrogen balance method has built-in errors (Allison 1951, Albanese 1959, Darke 1960, Wallace 1959 and Waterlow 1960), it is, in principle, one of the best methods available to study the extent of in vivo protein synthesis and catabolism. Also, if the point of nitrogen equilibrium can be estimated for certain well defined conditions, it provides an excellent baseline for estimating the dietary protein requirement and the character of protein metabolism under other well defined conditions such as range conditions for deer.

It is characteristic of the study of nutrition that

single measurements of changes in the body during feeding or fasting do not often represent single processes, not do they in fact often pin down the cause of a change to a single agency. This is so for two important reasons. First, the measurement itself often does not provide the means of differentiating the cause regardless of interpretation. For example, weight loss may be due to a loss of water or it may be equally due to a catabolism of body fat. Secondly, in most of the bodily changes there are usually two or more causes for a change in a single parameter. For example, weight loss is often both water loss and catabolism of body material participating together in an unknown ration. In order to overcome such ambiguities it is necessary to employ several methods simultaneously. Several measurements of concurrent activities associated with a body change provide pieces of information which tend to point to a single highly probable cause or to a definite set of such causes for a single event. By a process largely of elimination of many possibilities a unified result may be obtained. However, even the most exacting experimental procedures give results which bear limited interpretation. Also, it is most characteristic of nutritional experiments that experimental results apply to the conditions of the experiment, both known and unknown, and can only apply to other more general conditions with proper modifications of interpretation. Nutritional experiments are therefore fraught with difficulties, and they test the

ingenuity of the experimenter as to the choice of methods and techniques best suited to reveal the illusive causes of the measured change. The several methods used in this experiment were chosen with these views and aims in mind and are accompanied by several methods of interpretation of the data.

Nitrogen Excretion in Adult Deer

General Discussion of Nitrogen Excretion Results in the Light of Presently Held Theories of Protein Metabolism

The results of the three nitrogen balance trials are presented in Tables I to VI. The first trial, using the white-tailed doe, P-1, was halted due to technical difficulties with the calorimeter, and therefore does not constitute a completed nitrogen balance experiment. Included with the data for the nitrogen relations are the data for the changes in dry matter, in Table I, the water balance, in Table IV, and oxygen uptake, in Table VII. The results of the two complete nitrogen balance trials on the black-tailed doe R-5 are shown graphically in Figures 1 and 2. These figures include the total urinary nitrogen excretion throughout the experimental periods. They also include the total nitrogen intake and true digestible intake during each day of the test feeding period. Several other parameters of the nitrogen excretion obtained during these trials are shown in Figures 3 to 6.

The urinary nitrogen excretion of both the white-

tailed and black-tailed does appeared to be characterized by occasional sudden large increases which occurred within a 24 hour collecting period. Following the elevated level, the excretion decreased to that obtained on previous days. These increments were unexpected, and remain, for the most part unexplained. They seemed to occur at just the wrong times, from a theoretical point of view, as in the case of the collection following the fourth feeding day in the last trial. For example, in Trial III, on the ninth day, a large amount of nitrogen was excreted (21 grams). This was not accompanied by a large excess in caloric output (1,260 Calories) and occurred at a time of theoretical maximum nitrogen depletion in the balance trial and in the overall seasonal variation which normally occurs in these animals. These large increases could be due to variations in urea retention at the renal level as has been shown with other ruminants (Schmidt-Nielsen 1957, 1958). However, if renal regulation had played a significant role in the excretion pattern of these experiments, much lower levels of excretion would have been displayed during the periods of restricted intake. In order to accurately assess the status of renal regulation of urea, plasma urea concentrations would have to be carried out throughout the tests. Blood urea determinations were made at various times, on all of the black-tailed deer, during their residence in the deer enclosure. In order to secure fresh whole blood for this purpose advantage had to

be taken of immobilization procedures on adult deer due to their extreme refractile nature. These opportunities were necessarily rare due to the undesirable side effects resulting from this treatment. In March 1962, R-5 had a urea nitrogen value of 33.2 milligrams per 100 milliliters of whole blood. In April 1962, R-5 had a value of 25.0 milligrams per 100 milliliters. These values compare favourably with those obtained with cattle, sheep and goats (Spector 1956). The values for these animals are 6-27, 13-28, and 8-20 milligrams of urea nitrogen per 100 milliliters of blood respectively. The values for R-5 are representative of the highest levels in these ranges. This is understandable considering the high level of nutritional status maintained in this deer.

The second balance experiment began with one of these high levels of excretion. A level of 25.4 grams of nitrogen, or more than twice the level obtained during the next two collection periods, was obtained on the second day of residence. However, this may be explained partly by the fact that urine was retained during the first day of residence in the calorimeter. Though there is no record of the amount of urine released during the last day of residence in the deer pen, this apparent urine retention must to some extent represent actual storage of urine in the bladder and the elevation in nitrogen level in the first urine collection, on the second

day of residence, must represent nitrogen eliminated in part to the bladder during the first day in the calorimeter. The retention was presumed to be associated with stimulation caused by the transference of the animal from the deer enclosure to the laboratory and calorimeter (Fulton 1946).

The total nitrogen excretion decreased markedly in the white-tailed deer, upon feed restriction, from a level of 23.1 grams immediately following withdrawal of feed, to a level of about 9 grams per day. Upon resumption of feeding an unexpectedly early and large increase of 43.2 grams resulted. Even if the large 24-hour excesses are discounted, however, it can be seen that the fasting decrease in nitrogen excretion was not great in any of the trials and that the nitrogen excretion levelled off at a rather high level in all cases. This result in general differs in part from the results obtained by previous authors with both monogastric animals and ruminants. Lower values of between 0.04 and 0.05 grams of nitrogen per kilogram of body weight were obtained with various ruminants (Bricker, Kinsman and Mitchell, 1945, Hutchinson and Morris 1936, Majumdar 1960, Mitchell 1929) and similar values for various monogastric animals (Bricker, Kinsman and Mitchell) in accordance with the general relationship between endogenous nitrogen excretion and body weight (Brody 1945). The decrease in total nitrogen excretion, expected and obtained to a degree,

is caused by changes in the labile protein stores which have a characteristic turnover (Allison 1951, 1953, Block 1956, Tuttle 1959, and Wallace 1959). These stores are fully maintained only during periods of high plane protein intake with otherwise adequate diet, and at this time they have a characteristic high rate of turnover and consequently are associated with a high level of nitrogen catabolism and nitrogen excretion. On fasting these stores rapidly decrease in size because of their high rate of activity. As this happens the areas of highest turnover are exhausted first, and at a rapid rate, and consequently, with the reduction of the size of protein stores the nitrogen excretion decreases proportionately. When the body protein stores are nearly exhausted, more slowly moving and important areas of body protein begin to give up amino acids. Because of their slower turnover these areas give up amino acids much more slowly and consequently in smaller volume than in the case of the high plane protein stores. As this process continues, protein catabolism, and consequently nitrogen excretion approaches a minimum level associated with vital processes essential for continued existence. This level is the endogenous level of protein metabolism. In the ruminants, despite continued fasting, there is usually present a continued level of restricted nitrogen metabolism in the rumen and continued small supplies of microbial protein are given up to the host (Annison and Lewis 1957). These additions of protein to

the intestinal tract of the host provide small supplies of nitrogen to the animal's metabolic pool by absorption of amino acids. This process prevents the constant rapid catabolism of labile protein stores from causing protein depletion as quickly as is the case with monogastric animals.

The levelling of the total nitrogen excretion to the average level of approximately 11 grams during the second trial and 6.9 grams during the third trial represents the closest relatively constant approach to the endogenous level during the respective fasting periods. There was one collection on the fourth fasting day of the second trial when the level was 3.2 grams of total nitrogen.

The expected endogenous level of nitrogen excretion may be calculated from the experimental relation to body weight of Brody, viz.

$$E.U.N. = 146W^{0.72} \qquad \text{(Brody 1945)}$$

where E.U.N. corresponds to the amount of endogenous urinary nitrogen excretion, and W corresponds to body weight in kilograms. This relation applies, however, to a non-pregnant, non-lactating, adult monogastric mammal under basal conditions. From this relation the endogenous level for the white-tailed doe at 203 pounds should be 3.80 grams per day, and for the black-tailed doe at 100 pounds it should be 2.28 grams and at 90 pounds 2.11 grams of nitrogen per day. From the relation of

Smuts of 2 milligrams of excreted total urinary nitrogen per kilocalorie of basal energy production, these levels of excretion would be associated with basal calorie outputs of approximately 1,900, 1,138 and 1,055 kilocalories per day. These values are slightly lower than those obtained using Klieber's formula for the relation between basal heat production and body weight, but are approximately 1 kilocalorie per kilogram body weight per hour (Klieber 1932). The level of 3.2 grams is therefore very close to the expected endogenous urinary total nitrogen excretion. It is important in order to discuss the dietary protein requirement of these deer to try and further interpret the elevation obtained in this experiment in the level of urinary nitrogen excretion of about 4.8 to 8.7 grams of nitrogen per day over the expected endogenous level.

Changes in the Animal which Influence Dietary Requirements

Assuming, for theoretical considerations, that the average basal energy production per day for the black-tailed deer was 1,138 Calories, and allowing an additional 50 percent increase for activity, the total energy requirement per day would be approximately 1,700 Calories. This would include an additional increment, during the feeding period, due to the specific dynamic action caused by ingested and abosrbed food.

If the 1,700 Calories of energy had to be supplied

entirely by protein catabolism, 425 grams of metabolizable protein, either from feed or body sources, would have to be utilized per day. The same amount of absorbed protein as body protein would be needed because both suffer the same losses to urine (Brody 1945, and Maynard 1956), thus providing the same amount of utilizable energy per gram to the body. This amount of protein catabolism would result in a total urinary nitrogen excretion of 68 grams per day.

However, some of the energy would be supplied by the limited amount of carbohydrate stored in muscle and in the liver, especially during the initial stages of fasting catabolism. Also, during the utilization of stored carbohydrate, some of the energy would be supplied by catabolism of body fat, and following the exhaustion of the carbohydrate supply, a large proportion would then be derived from storage fat. If all the energy was obtained by fat catabolism, 190 grams of fat would be required, and there would be a resultant weight loss of this amount per day as compared with a loss of up to 4 pounds if all was obtained by protein catabolism. The figure of 4 pounds is suggested because of the fact that body protein is associated with body water in a ratio of 1 part to 3 (Brody, and Kinney 1959). When body proteins are catabolized this water is freed, and may be completely eliminated depending on the conditions of water balance. This balance is determined on the one hand by the physio-

logical state of the animal, and on the other by the water intake. The weight loss obtained in the second trial was calculated as an arbitrary average to be approximately 2.3 pounds per day from the total weight loss of 30 pounds in 13 days. This loss was not due to observed water loss, as the water balance data shows a gain of 2.1 kilograms for the same period.

If the nitrogen excretion increment above the predicted level, consisting of 8.72 grams per day in the second trial and 4.69 grams in the third, represented the contribution of body protein to energy production, then 54.5 grams and 28.8 grams of protein respectively would be available, thus representing 218 and 115 Calories. This would leave between 1,500 and 1,600 Calories per day to be supplied by fat catabolism. This would require about 170 grams of fat. This would set the weight loss per day at 310 to 390 grams. The gaseous exchange associated with this amount of protein and fat catabolism would result in a respiratory quotient of about 0.72, a value which is characteristic of energy production derived from fat metabolism. Including the water associated with body protein, the weight loss which would occur in the first of the above two instances would be up to 388 grams per day or 0.8 pounds with a total loss of 6 pounds for seven fasting days. During the third trial the daily weight loss would be 310 grams or nearly $\frac{1}{2}$ pound and the total loss for eight fasting days would be 4 pounds. The

smaller daily loss of less than a pound compared to about 4 pounds with protein as the sole energy source reflects the caloric density of fat relative to protein. The above figures show that the observed elevation in nitrogen excretion is not actually very large from the point of view of protein catabolism for energy, due to the relative inferiority of protein as an energy fuel compared to fat.

As will be shown, the increase of 50 per cent in energy production over the calculated basal level, is too generous. The animals were placed in quiet surroundings with a relatively constant air temperature of 15°C., which is within the zone of thermo-neutrality, and with limited space in order to enforce limited physical activity. Various workers including Brody have estimated the maintenance requirement for energy to be approximately 20 to 25 per cent higher than the basal energy requirement. The maintenance requirement may be defined as the basal energy requirement plus those increments associated with activity and the specific dynamic effect of feeding. Of this figure slightly less than half is said to be due to SDA. The rest is due to the increased tonal activity of the postural muscles and to other physiological efforts associated with standing. A figure of 12 per cent of the total energy production has been stated by Kinney as the fraction used in this kind of activity, (Kinney 1959). Therefore, a figure of 15 per

cent would not seem unreasonable in the case of these deer under the conditions indicated. This would revise the figures for the total energy production of the black-tailed deer to about 1,340 Calories per day. The revised figure for the body fat catabolism requirement would be about 130 grams per day and the total weight loss would be lowered only slightly to about 10 pounds in 30 days. The R.Q. would remain nearly the same. The water liberation associated with the above activities would be up to 360 grams per day, inclusive of the water production associated with fat oxidation.

The basal metabolic rates obtained during the three metabolic trials are recorded in Table VI. They were computed from the amount of oxygen consumed during the three test periods per day of 6, 10, and 5 hours respectively, starting at about 4 P.M. each day, with about an hour required between each period for flushing out the chamber. The oxygen consumption was calculated from data made on the percentage oxygen decrement which occurred during each test period. The oxygen content of the chamber was measured and recorded on a continuous recording apparatus which constantly sampled air from the chamber via a bleed tube that provided a circuit of air through the recorder and back to the chamber again. Even during the longest test period which was the ten hour period from 11 P.M. until 9 A.M., and with the highest rates of activity obtained with the resident

animals, the oxygen concentration did not decrease at any time to levels that could be considered to have had an influence on the metabolic rate, in accordance with standard physiological considerations (Guyton 1956). The total oxygen consumptions for each 24 hour period are shown in Table VI.

The carbon dioxide production was recorded in similar fashion to the oxygen consumption, using a continuous recorder to measure the increment in percentage carbon dioxide during each test period. Values for the respiratory quotient were calculated for some of the 24 hour periods, and the values for those obtained in the third trial are included in Table VI.

The average heat production during the second trial was 1,400 Calories per day. During the third trial it was 1,300 Calories per day. These values were calculated on the basis of the average R.Q., which was 0.82. The average was obtained largely from the values obtained in the third trial, and it agrees with the values used by previous investigators. At this R.Q. one liter of oxygen consumed represents the expenditure of 4.83 Calories. The caloric expenditure would be only slightly less with an R.Q. of 0.7. The average experimental results quoted above for the heat production obtained are only slightly higher than the values computed from the formula of Brody and Kleiber for the body weight metabolic rate relationships.

The individual values for daily heat production are however more illustrative of the changes which occurred in metabolic activity during the test periods. They also supply more information as to the character of resting metabolism in this animal by the degree of change in response to the various changes in the pattern of feeding, and by the extent of decrease during periods of inactivity and inanition. Taken singly they enable a more analytical study of the character of protein metabolism in this animal as well.

During the first period of inanition, in the second trial, the metabolic rate of R-5 dropped from a level of 1,640 on an intake of approximately 1.7 pounds standard feed as given in the pen, to a level of 1,300 Calories after 96 hours of starvation with free access to water. Following this it went lower, to 1,170 Calories on the second day of feeding, during the presumed absorption and utilization of about 3/4 of a pound of feed. The level of 1,170 is very close to the predicted value for R-5 at about 100 pounds. Following the postabsorptive state, the average caloric output of R-5 before the test feeding period is 1,330. This corresponds to a 12% increment over the predicted basal level and represents the maintenance requirement of R-5 for the above described conditions. During the second trial with R-5 the caloric output during the same period is within 1% of the basal requirement showing a very slight degree of physio-

logical activity on the part of R-5 at this time. This fact probably explains the relative indifference of the deer to the ration presented to it for several consecutive days, compared to its reaction to the ration in the first trial with R-5. Following the feeding period in the first trial the caloric output of R-5 was reduced to only a 4 per cent increase over the basal level. In the last trial however it was 10 per cent higher.

The relatively moderate metabolic rates encountered in these experiments would not be expected to necessitate large expenditures of body resources in order to replace dietary intake of calories. However, in the second trial, for example, during the feeding period, the dietary intake of calories fell short of adequacy by significant amounts. On the first feeding day it was short by 269 Calories, on the third day by 920 Calories, and on the fifth day it was short by 523 Calories. Therefore it is not entirely surprising that an exact nitrogen balance was not achieved on each feeding day, even though the total caloric discrepancy for the entire feeding period was only 1,837 Calories and could thus be satisfied by the catabolism of 204 grams of body fat, or 367 grams of body carbohydrate, and less than 367 grams of a mixture of the two. This applies especially to the first two days of the last trial where the number of calories needed from endogenous sources was more

than 1,000 Calories per day. The results of the nitrogen analysis during this feeding period are very interesting however, in that they show the increasing importance of body protein stores as a source of endogenous energy. During the first two feeding days mentioned above, body protein supplied very little energy toward rectifying the rather large deficit of caloric output. However, on the third and fourth day it suddenly rose in importance. On the third day it supplied almost one half the caloric output, and on the fourth day it supplied 99 per cent of the caloric output. This observation, together with knowledge concerning the natural history of black-tailed deer which shows that in late spring they tend to be in a state of depleted energy reserves for several reasons other than dietary, seems to indicate that R-5 had finally reached a state wherein it's body carbohydrate and fat stores were depleted sufficiently to necessitate the use of body protein as the nearly sole source of endogenous energy.

The high weight loss in the second trial is difficult to explain. It is indicative of a predominantly lean body mass type body wastage in response to a high total metabolic rate. The general elevation in the urinary nitrogen excretion level is not great enough to indicate a large participation of lean body mass, or dietary protein in the production of energy. At the same time the water balance data shows a gain of a few kilo-

grams in 13 days. This leaves fat as the only source of energy, but this should give a fairly slow rate of weight loss.

According to Kinney during early starvation which in the case of monogastric animals means from 1½ to 3 days following a good nutritional history, the mammalian body tends to lose a high proportion of lean body mass. (Kinney 1959) This lean body mass consists of labile body protein stores plus associated water. The composition of the total loss is about 80 per cent LBM and 20 per cent body fat. This loss produces approximately 2,600 calories per kilogram of weight loss. For a daily energy production of 1,700 Calories a 0.65 kilogram loss is needed. For a production of 1,400 Calories, a loss of 0.54 kilograms is needed. For 1,300 Calories, a loss of 0.50 kilograms or about 1 pound is needed. This early starvation type of composition of loss in weight holds until the storage carbohydrate has been used up. The extent of the carbohydrate stores and thus of this type of loss is dependent on the previous nutritional history of the animal, and on its physiological age and condition which influence the use to which nutrients are put. The carbohydrates store in a 70 kilogram mammal of good previous nutritional history average, about 200 grams, which is capable of supplying only 800 Calories by itself. Following the use of this "emergency" energy store the composition of the loss changes to approximately 50 per cent lean

tissue and 50 per cent depot fat. This type of tissue combination contributes about 5,000 Calories per kilogram on catabolism. It would thus produce a daily weight loss of 0.34 kilograms to satisfy an energy requirement of 1,700 Calories per day. For 1,400 Calories, a 0.28 kilogram loss would be required, and for 1,300 Calories, a 0.26 kilogram loss or nearly half a pound would be required. The rate of weight loss per day is thus indicative of the type of tissue being catabolized. This is so, provided there are no overriding endocrinal or other physiological functions, for example, body temperature regulation in an adverse temperature gradient between animal and environment, which demand a differential loss of body water. Unfortunately no method was available for determining daily weight fluctuation during the metabolism trials.

As the body mass is used up, both the lean tissue and the fat depots decrease in size, despite the fact that the catabolism of fat is largely designed to spare lean tissue. As the lean tissue decreases in size the basal energy production, which is closely associated with or dependent on the size of the lean body mass, inevitably decreases. There is also an inevitable reduction in endogenous nitrogen excretion because of the decreased dynamic turnover and maintenance requirement of the smaller lean mass. The reasons for the reduction of energy production and for the lowering of endogenous nitrogen excretion are

essentially the same. These factors, however, steadily lower the protein and energy requirements for energy and nitrogen equilibrium throughout the fasting period. This indirectly provides greater chance of survival for the animal during periods of nutrient restriction, especially since the process can be carried to subnormal limits with extreme body wastage. Below average maintenance requirements may be established at any level which provides continued life in an individual, even though several normal and fairly important functions are temporarily or permanently held in abeyance through wastage of tissue.

Changes in Feed Following Ingestion which Influence Dietary Requirements

The establishment of resting metabolic requirements from the measurements of fasting catabolism, permit the estimation of nutrient requirements in terms of total digestive nutrient, or T.D.N. (Morrison 1956), provided the changes which take place, following ingestion, in the particular feed under study, are determined directly from feeding experiments. In this experiment the feeding test was combined with the establishment of fasting requirements in single experimental runs. These requirements can also be expressed as metabolizable energy, or metabolizable nitrogen, and as net energy, following metabolic tests. Once these factors are established, and expressed in

terms of one or more of the above catagories, the investigator can express the test animals' requirements for natural feed such as, for example, browse species for deer. These predictions, of course, depend on chemical analyses of the browse in order to compare the content with that of the feed tested on a dry weight basis. Feeding trials are still needed, however, to show the exact requirements. This is so because factors such as digestibility cannot always be accurately predicted. Also, the effect of amino acid imbalance and chemicals which act antagonistically within the metabolic machinery, must at present be established or discovered through direct experiment. As stated previously, the use of the nitrogen balance method for determining protein requirement can be misleading. Annison considers it of little use in ruminants due to the nitrogen metabolism of the rumen organisms (Annison and Lewis 1959). However, there are, even in his treatise on ruminant metabolism, several arguments which show that useful information may be obtained from careful use and interpretation of this method. The most important of these is, that during complete starvation of ruminants, the rumen microbial activity is severely restricted within a few days. This occurs because the symbionts in the rumen need a ready source of carbohydrate for biosynthesis. The carbohydrate requirement is twofold. There is a need for carbon and for oxidative energy in order to effect microbial protein synthesis. Therefore it is necessary, as was

done in this experiment, to starve the ruminant for 4 or more days while noting the decrease in nitrogen output. Part of the care in interpretation as mentioned previously enters into the extrapolation of results to field or other conditions. In addition, although the above reasoning has shown that the nitrogen balance method is not infallible in determining the state of protein stores in an undernourished animal, on a low level diet, it is useful in detecting deficiencies in a diet. Hence the many experiments of Mitchell and others on the biological value of feed protein were conceived using the appearance of negative balance as an indication of inadequacy. The errors of the balance method in ruminants is added to those already present with monogastric animals. The ones most often mentioned are dermal loss and adult growth. Dermal loss, as the term is used here, refers to nitrogen contained in sweat and sebum. Adult growth refers to loss, and consequently continual replacement by growth, of cornified epithelial cells and hair from the integument. Nitrogen is also lost by similar means from oral surfaces and from the digestive epithelium, but this fraction is accounted for by its inclusion in the estimation of metabolic fecal nitrogen. There was an attempt made in this experiment to account for both these sources of nitrogen loss. The metabolic fecal nitrogen was calculated, as mentioned previously, on the basis of the results of Mitchell, 1943 (Maynard 1956), who stated that the amount of MFN depended on the amount of dry

matter excreted. Mitchell stated that approximately 0.2 grams of MFN are excreted per 100 grams of dry matter on a low roughage diet, while approximately 0.5 grams are excreted with 100 grams of high roughage diet. The value for the low roughage diets was used in the calculations in this experiment, in accordance with the character of the U.B.C. rations used. The adult growth obtained with R-5 in terms of hair and flakes of skin amounted to approximately one gram of dry matter per day. This material was not analysed for nitrogen content. However it would, of course indicate a level of nitrogen loss of less than one gram per day from this source. Mitchell, 1949, gave a value of 0.56 grams / meter^2 / day, which would indicate a value of somewhat less than a gram per day for R-5. Maynard stated a value of 0.7 grams of nitrogen per day for 100 pound sheep (Maynard 1956). The amount of nitrogen lost from dermal sources depends on environmental conditions and physiological state of the animal. Values of 23-141 milligrams of nitrogen per 100 milliliters of sweat have been given in the literature (Darke 1960) for humans. Under the ideal conditions provided for R-5 the amount of sweat should have been very small. Unfortunately, although the amount of insensible water loss was determined for each day of residence in the calorimeter, there was no method of separating this collection into the component released from the lungs and the component excreted from the integumental surface. Using the maximum figure for the nitro-

gen content of sweat, and assuming 50 per cent of the insensible water loss to be sweat, the maximum dermal nitrogen loss would be 0.5 grams per day using the largest volumes of water obtained in either trial. These levels are exemplified by the results shown in the second fasting period of Trial 2 where they range from 613-698 milliliters per day. In the light of the above considerations a value of one gram of nitrogen per day was considered a safe estimate of the combined dermal nitrogen loss and adult growth. The error caused by this value is indicated by the dotted line shown below the level of total urinary nitrogen excretion in Figures 1 and 2.

In using the nitrogen balance method with ruminants, one of the major problems with the conventional approach is the determination of the actual amounts of metabolic fecal nitrogen and endogenous urinary nitrogen. Mitchell found the M.F.N. to be proportional to the roughage content and dry matter content of the diet, as stated previously. Though the endogenous urinary nitrogen is presumably related to body size, as in monogastric animals, it is doubtful that the true level can be easily achieved for reasons stated previously. An unknown endogenous level of protein catabolism would also obtain among the members of the rumen microflora and microfauna. This would result in the liberation of ammonia to the rumen contents, and much of this would be absorbed directly through

the rumen wall, converted in part to urea, and recycled in part to the rumen via residual salivary flow. This ammonia or urea would remain unavailable to the bacteria until resumption of carbohydrate intake. The part not recycled would result in inevitable additions to urinary excretion.

Using Mitchell's values for the amount of metabolic fecal nitrogen on a low roughage diet the true digestibility was calculated for each feeding day. The M.F.N. varies from about 0.3 grams during the very low intake of 17.3 grams dry matter to 0.8 grams for a one pound intake and 1.3 grams on a 650 to 690 gram dry matter intake. The low intake value of 0.3 grams compares with the fecal nitrogen obtained after 3 to 4 days starvation in the second trial. However, the level remained nearer the value for the one pound intake level on starvation in the last trial, thus showing a possible slower time of passage. The high intake value of 1.3 grams compares with the values obtained immediately upon the start of all starvation periods. However, it must be recognized that much of this was contributed by the previous diet of the animals while in the deer enclosure. The previous nutritional history of R-5 is fairly uniform in terms of monthly intake. The daily average is between 0.8 pounds and one pound calculated on a 30-day basis. During December it was 0.8 pounds, and during January it was 1 pound. The daily intake itself, however,

shows a definite cycling or fluctuation ranging from 0.2 to 2.8 pounds as a maximum high value and with 1.7 pounds as a common high value. The high and low levels are usually alternated and last for 3 to 5 days. There was no relation between the amount of feed presented to the animals, in terms of total apparent mass visible to them, as the consumption does not correlate well with the amount initially present in the feed pan. The dietary level given in the feeding test period is very similar to the intake on the above mentioned ad libitum regimen. The values for the second fasting periods of the trials, in both the second and third runs, were lower than those of the initial one reflecting the smaller overall intake of the relatively short feeding periods. The values chosen to represent M.F.N. were therefore considered reasonable estimates of the true M.F.N.

The coefficient of dry matter digestibility obtained, varied from about 60 to 90 per cent. According to Schneider and Maynard the normal value for most ruminant feeds is 65 per cent. The higher values obtained here of 75 percent and 87 per cent, averages of the second and third trials are not unreasonable for two reasons. The low amount of roughage in the diet seems to reduce the passage time greatly, as the effects of previous ad libitum feeding appear for 3 to 4 days, indicating the normal passage time of up to 2 days for the

major portion of the feed. Meanwhile, the low amount of roughage reduces the amount of protection against bacterial and digestive enzyme attack. It should be borne in mind, however, that extensive bacterial attack reduces the energetic efficiency of the feeding process by raising the level of production of metabolically unavailable methane, thus making a larger amount of feed energy unavailable to the deer than would be so with less extensive attack. The levels of methane production were very small during this experiment, however, Also, Mitchell showed that the plane of nutrition affects the digestibility obtained with a given ration. While experimental animals were fully fed a value of 65 per cent could be obtained, while, at or near the maintenance level a value of 80 per cent was possible. The levels used in this experiment were near the maintenance level for energy and for protein at the one pound level. However, confusing variation occurred. In the second trial, values of about 60 per cent and 70 per cent were obtained at the 1 pound level on two occasions. In the third trial 85 per cent to 90 per cent were obtained with the level just below the 1 pound level. At nearly twice this level the value varies between 77 per cent and 91 per cent.

High digestibilities are associated with feeds with narrow nutritive ratios such as this one. Actually, the digestibility is, in many cases, only apparently lowered for the most part, as the nutritive ratio is widened. This is due to the fact

that a constant amount of M.F.N. tends to be associated with a constant amount of feed dry matter, regardless of the percentage of feed nitrogen. This is not rigid due to the fact that, for example, certain sources of protein tend to have a differential evocating effect on digestive enzyme outflow via the endocrine system. With feeds of narrow nutritive ratio, the larger amounts of nitrogen, whether it is protein which can be utilized by the rumen organisms, or it is N.P.N., does contribute to increased digestibility by stimulating bacterial attack on the higher carbohydrates of the feed. This greater utilization of the higher carbohydrates in turn makes other nutrients more available. The percent digestibility of nitrogen gave different values, and a different pattern with variation in intake level from the dry matter digestibility. The digestibility during the second trial seems to be inversely proportional to that of dry matter with high digestibility coefficients when that for dry matter is low. Contrary to the dry matter results, the nitrogen digestibility tends to agree more closely with the rule of Mitchell's in both the second and third trials, by having higher values during lower intake levels.

At the one pound level of intake with the observed digestibility of 90 per cent, the total digestible protein available to the deer is approximately 58 grams or 9.5 grams of nitrogen. According to the relation of Brody for endogenous nitrogen wastage per unit body weight, this amount is more

than enough to establish nitrogen equilibrium even with a biological value somewhat lower than 100. However, with a continuing fasting catabolism of about 11 grams of nitrogen, it would, of course, be inadequate even with a perfect biological value. The nearly similar intake in the third trial with the fasting level of about 7 grams should have been sufficient to effect nitrogen retention. Also, the two pound level of feed administration in the second trial which made between 12 and 15 grams of nitrogen available respectively, should have provided sufficient extra nitrogen for retention. The metabolizable energy at the one pound level, as will be shown later, was probably below the current maintenance requirement.

Calculation of Nitrogen Requirements from Urinary Nitrogen Excretion

Nitrogen Requirements Based on the Point of Nitrogen Balance

The behavior of the nitrogen excretion after repeated absorption of 6 and then 9 grams of metabolizable nitrogen, both as stated, being insufficient to effect immediate nitrogen equilibrium, was very interesting. At first, as expected, the absorption of 6 grams nitrogen showed a 6 gram deficit or - 6 grams nitrogen balance. Later, following absorption and probable utilization of an additional 15 grams of nitrogen, 9 grams of nitrogen caused near equilibrium of nitrogen balance. Still later, following a 12.16 gram absorption, 9 grams caused

retention. Though the two higher levels introduce complications in interpreting the results, the greater ability of the one pound level of feed to satisfy the nitrogen requirement, and thus simultaneously the energy requirement of the black-tailed deer, showed the progressive reduction in nitrogen turnover and basal metabolic rate associated with the reduction in labile nitrogen stores caused by the steady loss of nitrogen during the appearance of negative nitrogen balance. The final retention of 3.2 grams of nitrogen represents 20.0 grams of body protein, 80 Calories of stored available energy, and 80 grams of lean body mass.

The resistance to establishment of nitrogen balance on the several occasions of 2 pound feeding when, according to estimation and to the fasting level of nitrogen excretion, more than adequate nitrogen was apparently absorbed, is difficult to explain. There were two marked examples of this resistance. In the second trial following near equilibrium from absorption of 9 grams of nitrogen 12 grams failed even to equal the reduction in excretion of 9 grams. Following a negative nitrogen balance of 3 grams with an absorption of 7.8 grams of nitrogen, a further absorption of 7.89 grams of nitrogen was associated with a deficit of 13 grams of total nitrogen.

The difficulty in achieving nitrogen equilibrium may be explained, as suggested above, by the use of ingested protein

for energy. The pelleted ration used contained 2,000 Calories of total energy per pound, and 1,300 Calories of metabolizable energy per pound calculated on an average digestibility of 65 per cent. This is equivalent to 2.9 Calories per gram. The one pound level of feed gave 1,213 Calories total energy on this basis throughout the latter part of the second trial and 1,037 to 1,099 Calories in the third. With the actual amount of dry matter absorbed, however, it gave 795, 682, 910 and 896 on the 4 occasions 1 pound was consumed. Following losses in digestion, the one pound level was between 500 and 600 Calories short of the calculated 1,300 Calories per day maintenance requirement in the second trial, and 546 Calories short in the third trial. The total nitrogen loss during the 3 one pound feeding days in the second trial should have been 7.8 grams assuming a steady 11 grams of nitrogen for essential catabolism. With the lower endogenous urinary nitrogen of 7 grams per day in the third trial a total nitrogen retention of 1.72 grams should have been realized. The lower than expected loss in the second trial, of 2.6 grams reflects a lowered endogenous level during feeding, perhaps representing less nitrogen wastage for endogenous catabolism. It also reflects a smaller energy deficit. During the third trial, the lack of retention indicates an elevation in protein catabolism above the endogenous level. This indicates that the energy absorbed is inadequate. The amount was, in fact, up to 400 Calories per day short of the

calculated requirement during the last two feeding days, when the deer began to consume significant amounts of feed. Even though most of this energy could be supplied by body fat, some body or dietary protein would be expected to be used. The discrepancy of 14.28 grams between a retention of 1.72 grams, as a theoretical figure, and the actual negative balance of 16 grams nitrogen, is equal to 89.3 grams of protein. This amount of protein, from either endogenous or exogenous source would supply about 180 Calories per day. After caloric loss to urine, protein would be expected to be utilized for energy, even with adequate fat supply, for the following reasons. Though fat is already being mobilized and utilized for energy, it cannot completely replace carbohydrate or protein in this function, because it cannot supply essential two-carbon compounds needed for the operation of the citric acid cycle. These can only be supplied by metabolism of glucose, or certain of the essential amino acids which are ketogenic. Protein is therefore a nutrient of choice, to a certain extent, for energy production.

Another explanation for the inability on several occasions to establish expected nitrogen equilibrium is that, addition of foodstuffs in the ruminant may stimulate nitrogen metabolism in the rumen to the extent of increasing blood and urine nitrogen levels. Non-protein nitrogen in the feed participates actively in this stimulation. The interfering factors

are: increased absorption of ammonia and urea from the rumen to the blood stream, and increased microbial protein in the diet of the host. The amount, rate and time of appearance and duration of these effects can be anticipated only with difficulty. Despite recycling of urea via saliva, some urea and ammonia is lost to the urine simply because of mass action. The utilization of non-protein nitrogen in the rumen has the effect of increasing the biological value of the dietary protein. This in turn reduces the nitrogen wastage. At the same time, fermentation increases the nitrogen wastage by reducing dietary protein to short-chain fatty acids with the consequent release of amino acid nitrogen. Much of this released nitrogen is excreted along with the feces thus becoming unavailable to both the microflora and the host. The balance between these antagonistic functions depends on the amount of non-protein nitrogen in the diet relative to protein of good biological value. The non-protein nitrogen level in the ration used in this experiment was low.

The graphs in Figures 3, and 4, show the result of calculations of the point of nitrogen balance. These were obtained by plotting the effect of the nitrogen intake in grams, shown on the abscissa, on the nitrogen balance in grams, shown on the ordinate, and on the grams of nitrogen balance per kilogram of body weight, shown on a second ordinate, to the left of

the first. The point of intersection of the two lines with themselves and with the abscissa indicates the point of nitrogen balance. This method of obtaining the point of nitrogen balance is a modified version of the graphical method of Leitch and Duckworth (Leitch and Duckworth 1937). According to the work of Majundar (Majundar 1960), the results of this technique compare favourably with other methods of analysing nitrogen balance data. As verification of this he used this method and several others to analyse his own nitrogen balance results obtained with Jumna Pari goats of 70-94 pounds. However, other authors have stated that the nitrogen requirements calculated by the above method are higher than those obtained from values for the endogenous urinary total nitrogen excretion alone. The point of intersection in the first trial with R-5 is at about 17.3 grams, and in the second trial it is at 16.5 grams. As the following results show this value is a great deal higher than the most probable figure for the endogenous level of nitrogen excretion. The nitrogen balance index was calculated for each one pound feeding day, where $\frac{B_I - B_0}{I}$ equals the balance index, and in which; B_I represents the nitrogen balance during intake, and B_0 represents the nitrogen excretion or nitrogen balance with no intake, and I represents the nitrogen intake. The numerical value of this index appears above the one pound feed level columns on the histograms of nitrogen balance.

The index reflects the effect of absorbed nitrogen per day on the nitrogen balance and should increase numerically with constant intake. When the protein stores of an animal are full, as they are during conditions of good nutritional status, it is difficult to obtain nitrogen retention. When the protein stores are depleted, on the other hand, it is relatively easy to obtain retention even with small amounts of dietary protein. As the protein stores gradually become depleted, as is the case when the animal is under a regimen of alternate fasting and feeding, using a feed intake at about the maintenance level for the alternate periods when feed is given, the degree of retention will gradually increase even though the feed level remains constant. If the nitrogen balance per day is plotted against the absorbed nitrogen per day, a curve is obtained which reflects the change in the animal's protein stores. It also reflects the biological value of the protein used as the feed source. The nitrogen balance index of the intake on any particular day may be represented by the tangent of the curve at the point in question (Albanese 1959). The nitrogen balance index is the fraction of nitrogen retained of that absorbed; if the endogenous nitrogen excretion, represented by the excretion at zero, intake is constant and independent of nitrogen intake. The fraction represents the biological value of the feed protein. In the first trial with R-5 the nitrogen balance index increased from 0.6 to 1.1 and on the day of the third one pound level feeding a retention was obtained.

Nitrogen Requirements Based on the Endogenous Total Urinary Nitrogen Level

On the fifth day of the second nitrogen balance trial, the lowest level of nitrogen excretion during starvation was achieved. This level was obtained on the fifth day of fasting. The total nitrogen excretion at this time was 3.225 grams. This value was obtained in association with a caloric output of 1,457 Calories calculated on the basis of an estimated R.Q. of 0.82, obtained as an average value in the third trial and recommended by previous authors (Brody). The actual R.Q. was probably nearer 0.7 at this time but as mentioned previously the difference caused by using the average value instead of an actual value, which was not available in the second trial, is not great. The relationship between nitrogen excretion and calories of resting heat production is 2.21 milligrams nitrogen per calorie, which is very close indeed to the relationship between the endogenous urinary total nitrogen excretion and basal Calories of heat production known to exist between adult mammals of different species, size and age. It seems justifiable, therefore, to suggest that this value of total urinary nitrogen excretion is a fair representation of the endogenous urinary nitrogen excretion and thus the minimum nitrogen required of R-5, and therefore represents the minimum amount of nitrogen required by an adult non-pregnant female Columbian black-tailed deer.

In calculating the minimum protein requirements of

animals the value for endogenous urinary nitrogen is multiplied by a factor of 6.25. The result is then corrected for the biological value of the dietary protein under consideration, for example, if the biological value of the protein is 50, a factor of x 2 is used. The value for endogenous urinary nitrogen x 6.25 represents the minimum requirement for metabolizable protein. After this value has been corrected for the biological value of the protein being used, the new value represents the minimum requirement for digestible crude protein. Losses incurred during digestion must also be accounted for, using factors obtained from digestibility trials on the protein source being used, either on nitrogen itself or on dry matter. Once these losses have been accounted for the protein requirement in terms of the feed may be obtained. It must then be realized that the value thus obtained represents the minimum protein requirement based on the lowest level of protein catabolism, in association with the lowest level of body amino acid turnover and irreversible amino acid nitrogen loss, possibly associated with the smallest size of labile protein stores, in keeping with the maintenance of normal body form and function. Therefore, additional corrections must be made in arriving at the most desirable protein requirement, based on nitrogen balance trials, for the achievement of a high plane of nutritional status. The starting point for this process, however, remains the establishment of the levels of nitrogen excretion

obtained under basal conditions.

The digestible crude protein requirement of R-5 is calculated, on the basis of the above formula is 20.887 grams per day for R-5, a 45 kilogram adult female, non-pregnant, black-tailed deer. With an average nitrogen and dry matter digestibility of 84-85 per cent for the U.B.C. ration used (Appendix I) this would indicate a minimum requirement for this feed of 24.856 grams of dietary total crude protein per day and considering the experimentally determined value for the percentage of crude protein in this ration, namely, 15.55 per cent, a minimum feed requirement of 159.8 grams per day is indicated. This would not, of course, satisfy the caloric needs of the animal, but levels of feed which would, that is, approximately 700 grams per day, would supply more than four times the minimum total crude protein requirement as calculated by the above method. To supply sufficient digestible crude protein to satisfy the nitrogen catabolism associated with the high plane of nutrition experienced by animals such as R-5, or more specifically, to maintain a nitrogen balance is association with a nitrogen excretion of about 12 grams per day, as was found in R-5 immediately after starvation was begun, 92 grams of dietary crude protein would be needed necessitating the consumption of 600 grams of U.B.C. pelletted feed dry matter per day. This level of nitrogen requirement is reflected to a

greater degree by the previous method which used the calculation of the point of nitrogen balance to indicate the dietary requirements. The balance point of 17 grams of dietary nitrogen intake indicates a need for approximately 100 grams of protein daily for maintenance of minimal nitrogen stores alone. This result is, of course, too high for the purposes of maintaining minimal nitrogen stores and therefore the agreement between the values of the two methods is of less significance than if it had been for the maintenance of a high plane of protein nutrition. The value of 92 grams of dietary protein and 600 grams of pelletted feed obtained above by calculation from experimentally determined nitrogen excretion does, in fact, represent well the normal voluntary feed intake of R-5 and others of the black-tailed deer maintained at U.B.C., although the males commonly eat poorly during the season of rut.

There have been many nitrogen balances carried out with domestic ruminants. Recently Majundar found a value of 0.65 pounds per 1,000 pounds live weight to be the minimum protein requirement for maintenance. This result was obtained from the endogenous urinary nitrogen excretion which was 0.052 grams per kilogram live weight. The result of this experiment expressed in these terms is 0.071 grams per kilogram live weight. According to Maynard (Maynard 1936), the results of many metabolic trials with ruminants indicate a requirement for true

digestible dietary protein of 0.5 pounds, or 0.6 pounds of total digestible dietary protein, per 1,000 pounds of live body weight. These values are equal to 225 grams of true digestible protein and 280 grams of total digestible protein per 1,000 pounds of live body weight. The results of this experiment indicate a requirement of 249 grams of dietary total crude protein with a digestibility coefficient of about 85 and a biological value of 100. Using feeds of a lower biological value, as is commonly the case with domestic ruminants, the requirement of 249 grams would be correspondingly increased. No references were made in the literature pertaining to nitrogen balance experiments with wild ruminants, and therefore these results cannot be compared with values for other members of the family Cervidae. However, the similarily between the results obtained in this experiment and those obtained with domestic ruminants is reasonable.

Nitrogen Distribution

Urea

Folin investigated the changing levels of total urea and ammonia excretion with changes in nitrogen intake in humans (Folin 1905). He found the level of urea excretion dropped significantly when nitrogen intake was restricted and rose again with the return of normal nitrogen intake. Since Folin many authors have studied urea excretion in both monogastric animals and domestic ruminants and have in general corroborated Folin's

results. However, prior to the work of Schmidt-Nielsen (Schmidt-Nielsen 1957 and 1958) it was thought that the excretion of urea, formed from ambient ammonia of the blood as a detoxification measure as shown in Appendix III was effected by means of simple glomerular filtration, and the occurrence of urea retention was due to simple back diffusion at the collecting tubules. No regulation of urea excretion was postulated. The amount of urea excreted was therefore associated directly with the amount formed and the amount formed was due in turn to the amount of excess nitrogen ingested and absorbed. The requirements for the proof of tubular regulation only, are: independent rates of glomerular filtration and subsequent clearance, with the glomerular filtration rate remaining unchanged at different levels of blood urea. Schmidt-Nielsen (1957) found that these requirements were met by the camel to a great degree during periods of nitrogen restriction. Working with other ruminants as well he found that the urea clearance may be highly restricted while there was little change in glomerular filtration rate with different levels of nitrogen intake. This was so as long as there was no interference caused by changes in salt intake. The clearance rate was found to be independent of the plasma concentration of urea, but very sensitive to the level of nitrogen intake. This was especially true during growth. Schmidt-Nielsen (1958) found a qualitatively similar situation in non-ruminants such as rodents, dogs and

man, but results were quantitatively very much smaller. He proposed a counter-current multiplier system for recovery and concentration of urea during clearance, similar in functioning principle to the arrangement of blood vessels he found in extremities of singular aquatic and wading terrestrial mammals for conserving body heat at the expense of regulating the temperature of the extremities (Scholander 1955). In these systems body heat being carried to the extremities via the blood stream in the major arteries rapidly conducts to several closely applied returning vessels before it is carried far into the extremities and lost by conduction to the environment. The same functional principle, involving urea diffusion was postulated for the rete of venules surrounding the renal collecting tubules to prevent back diffusion of cleared urea.

The extensive studies of Somers (Somers 1961) show that ruminants can and do recycle urea, which has been absorbed from the rumen primarily in the form of ammonia, back into the rumen via salivary secretion. He showed that the urea content of the saliva of Merino sheep was related to the amount of circulating blood urea. He showed that the content of urea in saliva was greatest when the animal was receiving inadequate amounts of dietary protein. He also showed that a much larger amount of urea injected into the blood appeared in the urine with the animal on an adequate protein diet than on an inadequate diet. This latter result probably reflects the renal

regulation of urea found by Schmidt-Nielsen, however, as well as the salivary recycling effect.

Houpt (Houpt 1959) found that together with the above two mechanisms for urea conservation ruminants could also recycle urea by direct absorption from the blood back into the rumen.

The work of D. Lewis (Lewis 1957), which demonstrated that blood urea concentrations increase as a direct result of increased ammonia production by the rumen microflora, ties all the above results together and shows that ruminants have evolved a series of mechanisms which enable them to conserve nitrogen which would be wasted during periods of nitrogen restriction in their environment. The rumen microflora continue to deaminate amino acids despite impending shortages such as appear during the hot dry season when the nitrogen content of herbage drops to exceedingly low levels, and this deamination represents loss of nitrogen to the flora and consequently inevitably to the host. By means of these conserving mechanisms the host can restore "wasted" nitrogen to the microflora which will use it once given a "second chance" for the synthesis of microbial protein and thus essentially provide the host with amino acid nitrogen from what would have otherwise been waste nitrogen. Presumably some recycled urea may arise from endogenous host body protein catabolism providing an additional opportunity to recapture

what for monogastric animals is unquestionably wasted or lost nitrogen.

Phillipson (Phillipson 1960) has shown that there must be a certain amount of carbohydrate available to the rumen microflora in order for recycled urea to be utilized. The provision of exogenous carbon chains as well as nitrogen for the microbial synthesis of amino acids is not an unreasonable proposition. The limitation thus set on the conservation of nitrogen is, of course, that it cannot be undertaken to a significant degree under conditions of complete starvation.

The level of urea nitrogen as a percentage of the total nitrogen responds to the presence and level of protein intake quite markedly in all of the trials in this experiment. The results are shown graphically in Figure 5. During periods of inanition, the percentage urea nitrogen drops 10-15 per cent from values of 90 per cent and over to values of 77-85 per cent of the total nitrogen. The values of the second trial present the most uniform response to protein nutrition. The previous nutritional history of R-5 prior to the second trial was similar to the one pound level of feeding. The similarity of the urea percentage nitrogen after absorbing one pound, to the initial fasting level is not surprising. Upon realimentation the level rises steeply to that found preceding the fasting level, provided that the feed is accepted and consumed to the

extent of more than a fraction of a pound. This was not the case during the feeding test period of the third trial. The gradual consumption of more feed each day caused a variable increase in percentage with a dip in the curve during the first consumption of nearly one pound. During the second consumption of almost one pound, the percentage rose sharply to 95 per cent of the total nitrogen. After the brief period of consumption during the third trial, the level fell rapidly again, within twenty-four hours, to a new low of about 72 per cent. Following the longer lasting and three times larger absorption of the second trial, the level fell to less than 84 per cent, after forty-eight hours. These time relations lend support to the possibility of a fairly short passage time in the doe R-5 with the particular pelleted ration used. These data indicate that it might be possible to estimate the protein or nitrogen nutritive condition of black and white-tailed deer by the relative level of urea nitrogen excretion compared with total nitrogen excretion. Levels above 90 per cent, except for one absorption day of 7.08 grams of nitrogen, indicate recent absorption of significant amounts of nitrogen. Also, levels below 85 per cent are indicative, in this experiment, of a fasting state with respect to dietary protein.

The simplicity of the Conway micro-diffusion method for the determination of urinary and blood urea nitrogen makes

it an attractive method for research. From a theoretical point of view it would be particularly advantageous for conducting a survey of the protein nutritional status of a population of game animals. The technique itself is suitable for field conditions. However, the difficulties of obtaining urine samples from game animals under field conditions are, at the present time, practically insurmountable. Also, even if animals could be captured and confined within the necessary structure for obtaining the sample collection, it is extremely unlikely that normal samples could be taken without a lengthy period of adjustment to the new surroundings.

Ammonia

The level of urinary ammonia nitrogen behaves in an inverse manner to that of urea in this experiment. In this way, it agrees with other previous findings which have shown it to be an opposite index, to urea nitrogen, of protein nutrition. During protein starvation the level rises from a normal range of 1 to 4 per cent of total nitrogen, to values greater than 5 per cent, for example, 7.5 to 10 per cent.

Urea and ammonia fluctuate in opposite directions in part because their production is inextricably involved in acid-base regulating mechanisms. The production of urea from free ammonia is brought about by the liver as a detoxification measure. This process is shown schematically in Appendix III.

Ammonia is produced by deamination of amino acids and it circulates through the body fluids as ammonium chloride. The release of hydrochloric acid and conversion of ammonia together reverse excessive elevation of body fluid pH. The formation of urinary ammonia is brought about by the kidneys as shown in Appendix II, in order to preserve bodily fixed bases such as sodium which have combined with metabolically produced acids in order to prevent abnormal lowering of pH. The extent of the formation of ammonia is then a reflection of the metabolic acid production and of the function of the acid-base regulating mechanism in elevating body pH.

During fasting the increase in relative importance of fat metabolism tends to produce metabolic acidosis. Also, the deamination of amino acids decreases with the drift of protein metabolism to lower levels associated with protein depletion.

Creatinine and Creatine

In the series of experiments mentioned previously Folin found total creatinine excretion to be relatively constant from day to day and to be relatively independent of nitrogen intake, unlike urea excretion. There have been, since this time, many experiments the results of which support Folin's observations. Burroughs et al have said that the endogenous metabolism of Folin, which represents the summation of the

irreversible reactions of nitrogen, (see Appendix V) are exemplified by the bodily conversion of creatine to creatinine which represents an inevitable loss of nitrogen from the body (Burroughs, Burroughs and Mitchell 1940). The nitrogen distribution data of Blaxter and Wood (Blaxter and Wood 1951) indicate that about 12 per cent of the endogenous nitrogen metabolism of the growing calf on nitrogen free diet, involves the above irreversible reaction. For comparison 25 per cent involves purine metabolism and 50 per cent involves reactions terminating in urea and ammonia. Several investigators have shown that creatine is the bodily precursor of creatinine and that creatinine is the only normal urinary constituent with a significant amount of body creatine nitrogen, (Myers and Fine 1913, Schoenheimer and Block 1941). Schoenheimer showed that three amino acids were involved in the biosynthesis of creatine (Schoenheimer 1942). Glycine formed the fatty acid chain, arginine formed the guanidine nucleus, and the compound glycocyamine thus formed is methylated by transmethylation using labile methyl groups from methionine, (see Appendix IV).

Schaffer (1908) showed that creatinine excretion represented only part of endogenous nitrogen metabolism and took place primarily in muscle. Palmer, Means and Gamble (1914) showed that creatinine excretion was proportional to metabolic

rate in man. Catherwood and Stearns (1937) found creatinine excretion to be a function of body weight to the power of 0.9 where muscle mass usually dominates the endogenous nitrogen metabolism. Also the usual leanness of young animals and people removes the disturbing effect of non-metabolically functional fat weight. Macy found a similar coefficient with normal adults (Macy 1942). Talbot (1936) and Macy (1942) confirmed that creatinine was indirectly related to body weight and that it was an excellent indicator of skeletal muscle size. Talbot showed that the excretion of creatinine was similar for adult men and women upon correcting for fat differences. McCluggage (1931) found the same relation of creatinine to muscle mass in adults as children, and that with no fat interference the excretion remains constant, and falls off after the age of 65 along with decrease in BMR and muscle mass. Wuthier et al showed that serum creatinine concentration is related to carcass composition and per cent of lean body mass (Wuthier et al 1957).

De Groot et al studied the above mentioned constancy of creatinine excretion (De Groot 1960). In their opinion none of the previous investigators had paid sufficient attention to the constancy of creatinine excretion per hour or to the existence of possible variation in excretion during the day. Also Butcher and Harris had stated that since ruminants had a high level of creatine excretion, they would not exhibit a constant

creatinine excretion. De Groot showed that creatinine excretion is constant during the day and that high levels of creatinine excretion during a short interval are immediately balanced by low levels giving a constant average level per minute all day long even during periods of large creatine excretion. He showed therefore that the large levels of creatine excretion found in ruminants does not alter the constancy of creatinine excretion and also that the sum of creatine and creatinine during 24 hours is not constant as was postulated by Harding and Gaebler (1922) from theoretical considerations. The relative constancy of creatinine nitrogen excretion is shown in Figure 5.

Butcher and Harris demonstrated that creatinine may serve as an index of the total volume of expelled urine because of its relative constancy despite fairly wide variations in urine volume, and its constant excretion with time from hour to hour and day or night as shown by de Groot. There has been some work done over the years to show that the constancy of creatinine can be effected by the protein intake. In particular Hehyami (1958) showed that the level of arginine had a positive influence on the level of creatinine and creatine excretion. Dining et al (1949) found a significant variation between different species of beef steers and between individuals of the same species (Herefords) of similar weight, (300 kilograms) and age (2 years) while he found relatively constant levels within individuals in all these categories. These results are

confirmed in this experiment as far as relative constancy of creatinine excretion within individual animals is concerned, and in the light of the above considerations probably to some extent reflect differences in body composition.

According to Brinkerkink, part or perhaps all of the variation in creatine excretion is due to errors of unknown extent inherent in the alkaline picrate method. He says that the degree of conversion of creatine to newly formed creatinine in the presence of pre-formed creatinine is subject to 10-50 per cent error (Brinkerkink 1961). This is because the reaction is that of an equilibrium situation in which the degree of conversion is related to the amounts of creatine and pre-formed and newly formed creatinine as well as the other standard variables such as pH and temperature and the presence of other chromogenic materials. The results therefore depend partly on the variation in creatinine and creatine excretion, thus compounding the apparent variability of creatine excretion. Creatinine determinations on separate samples are, on the other hand, unaffected by this equilibrium situation and are according to the author fairly accurate. These results are corroborated by the present author's experience in obtaining variable results with creatine standards when these are included in urine samples of known creatine content. According to Brinkerkink, the reaction can even pass in reverse under suitable conditions such

that the pre-formed creatinine is converted to creatine and the result is an apparently smaller sum of pre-formed and new creatinine than the original pre-formed value. This result was also obtained in the present experiment upon occasion. In the light of the above considerations the significance of the creatine results during growth and following puberty are not discussed in detail.

The results of the creatinine nitrogen determinations obtained during the balance trials of this experiment appeared, in general, to support the previous statements regarding the relative independence of creatinine excretion levels and changes in nitrogen intake. The creatinine nitrogen, as a percentage of total nitrogen excretion, tended to be fairly constant during wide fluctuations in total nitrogen excretion. However, the total creatinine nitrogen excretion per day showed a slight tendency to reflect changes in nitrogen intake and excretion, (Figure 6) as de Groot had found to be the case. The slight reduction in creatinine excretion during fasting is not unreasonable because the reduction in protein stores which occurs at this time involves protein from the skeletal muscles. The reduction in creatinine excretion thus reflects a reduction in active body mass. Although the creatinine excretion showed this tendency to respond to changes in nitrogen intake within the two balance trials, they did not show a corresponding ten-

dency between trials. As shown in Figure 6, the general level of creatinine nitrogen excretion was similar at levels of total nitrogen excretion of about 14 grams in Trial II, and about 7.5 grams in Trial III. These results suggest that the conditions surrounding the determination of creatinine excretion must be carefully standardized when attempting to interpret them in terms of the characteristic creatinine excretion level of an individual animal. There were no measurements available on the constancy of creatinine excretion per hour as the samples of urine were collected only once in each 24 hour period.

Creatinine nitrogen concentration per milliliter of urine showed a tendency to decrease in regular fashion as the total volume of urine flow increased. Although the results of these determinations differed quantitatively between the two trials with R-5, they showed evidence of being of possible value as an index of urine volume, as Butcher and Harris have suggested. For example, in the second trial, the concentration of creatinine nitrogen was 2.3 milligrams per milliliter when the urine output was 412 milliliters, 2 milligrams with 658 milliliters and 1.7 milligrams with 1,272 milliliters. There were intermediate values between these. In the third trial, however, the values were 1.2 milligrams with 370 milliliters of urine output, 1 milligram with 385 milliliters, 0.8 milligrams with 577 milliliters and 0.3 with 1,646 milliliters.

Values for this relationship would have to be obtained from a larger amount of data, and under normal dietary conditions to eliminate the interfering effects caused by fluctuations in nitrogen stores, in order to determine the actual value of creatinine nitrogen as an index of total urinary output for an individual animal. If the level of creatinine nitrogen was found to be reliable as an index of urine volume it would provide a valuable tool for determining the total nitrogen output using a small sample of deer urine. As is the case with the urea analysis described above, the ease of conducting the creatinine determination makes it a good method for field work, but the problem of obtaining even small samples of urine under field conditions remains.

Nitrogen Excretion during Growth

Total Urinary Nitrogen Excretion

The results of total urinary nitrogen excretion obtained during the growing period on the three female and five male black-tailed deer are shown in Tables VII to XI, along with the nitrogen distribution data. The results are shown in graphic form in Figures 7 to 11.

The increase in total nitrogen excretion with the increase in body weight is shown on arithmetic grid in Figure 7 and on arithlog grid in Figure 8. The total nitrogen

excretion, as illustrated in Figure 7, can be seen to take the form of a curve which increases with ever decreasing increments, or with negative acceleration, as growth progresses. This curve is similar is gross form to that often used to express the average increase in body size with age during the later, or negative acceleration phase, of animal growth.

The same data expressed in arithlog form is more illustrative of the character of the changes which occured in total nitrogen excretion during the growing period of the deer (Figure 8). The data expressed in this manner shows that the pattern of change in total nitrogen excretion consists of several phases of different constant percentage rates of increase. These phases are separated by "breaks" or inflections in the curve which indicate the areas of rather abrupt change in the rate of increase. The relatively small amount of data available shows at least two, and possibly three, of these phases. In accordance with the method of expression used in Figure 8, that is, the log of the grams of total nitrogen excretion per day as a function of the change in body weight in kilograms, the rate of increase in total nitrogen excretion may be stated in terms of a power value of body weight. For example, in the first phase, which extends from a body weight of about 4 kilograms to about 8 kilograms the rate of increase in total nitrogen excretion with increasing body weight is proportional to the power 4.0

of body weight. This means that for a 1 per cent increase in body weight, there will be an increase in total nitrogen excretion on the order of about 4 per cent. If the rate of increase in total nitrogen excretion following the body weight of 8 kilograms is expressed in terms of a single constant value, the rate of increase is proportional to the 1.08 power of body weight. However, the data appear to suggest the presence of two separate phases following the body weight of 8 kilograms. The first of these extends over a period of growth from 8 kilograms of body weight to about 22 kilograms. The rate of increase in magnitude of total nitrogen excretion during this period is proportional to the 1.06 pwer of body weight. The second phase extends from 22 kilograms to over 35 kilograms of body weight and the rate of increase during this period is proportional to 0.73 power of body weight. Because of the relatively small amount of data available, and of the hazards involved in attaching significance to values of total nitrogen excretion without considering changing body composition, metabolic rate, and nitrogen and caloric intake simultaneously, statistical analysis was not employed in arriving at these values. They therefore represent approximations of changes in magnitude of nitrogen excretion during growth. However, the illumination of the pattern of the change in magnitude of total nitrogen excretion, even if largely of a qualitative nature, can serve in an attempt to understand the underlying mechanisms involved

in bringing these changes about.

The type of change in rate of increase in size of nitrogen output, illustrated by the above results with growing deer, is very interesting because it resembles the changes in growth rate obtained with advancing age, or increase in body size, when growth data are handled in the above manner. Brody (1945) and many other investigators since, have shown that the rate of increase in body size is dependant on the growth already achieved during the pre-pubertal period of growth. Following puberty the growth rate appears to be a function of the size yet to be achieved in terms of the adult body weight. The first phase is characterized by positive acceleration, while the second is characterized by negative acceleration. The percentage rate of increase in body size is markedly different in the two phases of growth, and when the data for the body weight, expressed logarithmically, are considered as a function of increasing age, a major inflection occurs in the curve at the point of puberty in similar fashion to the inflections demonstrated by the nitrogen excretion data above. When other parameters associated with metabolic function are treated in this fashion as a function of increasing age or body size they also tend to follow a similar pattern, for example, the change in endogenous nitrogen excretion, or in basal metabolic rate with advancing age, (Brody 1945).

The points at which the two inflections in the nitrogen excretion curve occur, in terms of age and body weight, correspond to the ages and weights wherein the value for the rate of growth of black-tailed deer is found to change (Cowan and Wood 1955). The first inflection coincides with the change in growth rate which occurs at 14 days in the data of Cowan and Wood, however, and therefore only agrees with the body weight data and not with the age. The lack of agreement in age probably reflects a less rapid growth rate during the first weeks following capture on the part of the deer in the present experiment, whereas the deer in the 1955 experiment were raised in captivity from birth and presumably had access to a higher plane of nutrition during the period of extremely rapid and important weight gain. Also, as stated previously, the exact date of birth is not known for the deer used in the present experiment. The second inflection occurs at approximately 100 days of age in this experiment. This agrees in terms of both age and body weight with the growth curve date of Cowan and Wood. It was further established by Cowan and Wood that the inflection in the growth curve at this age and weight is associated with the appearance of puberty, as signalled by the appearance of naked antler buds and the demonstration of fertility in two individuals. The agreement between the nitrogen curve and the growth curve in terms of body weight is of greater significance than that of age, as suggested earlier due to

the fact that "physiological aging" is primarily dependent upon environmental influences, especially those affecting nutritional status, and not upon the passage of time itself.

The exact values of nitrogen excretion obtained during normal feeding periods depends to a very large extent upon the degree of nitrogen intake once the maintenance and growth requirements have been met and surpassed. Therefore, it is reasonable to assume that fluctuations in the excretion level obtained in this experiment would be in part due to fluctuations in intake during growth. However, the pattern of excretion obtained is more suggestive of changes in physiological condition associated with growth, in the same way that the changing pattern of growth rate is so affected, due to the regular manner in which it appears to change in magnitude as growth progresses. For example, it would be expected that the level of nitrogen excretion should increase as the animal grows larger. It is also reasonable to expect that the level of nitrogen excretion would increase at a lesser rate when the general metabolic activity and growth rate of the animal assumes a reduced rate of increase following an inflection in the growth curve.

The increase in nitrogen excretion with age illustrated in Figures 9 and 10 provide useful information for the illumination of the underlying metabolic mechanisms which

influence the level of nitrogen excretion during growth. The data in these figures show a marked tendency for a reduction in output at the age of three months. This is especially evident in the data from the male deer. The excretion rises again within a short period of time. This result is indicative of a short period of increased nitrogen retention. It would be reasonable to expect certain periods of increased retention during growth when greater needs of protein for tissue formation arise. The point of puberty is an example of a critical period of physiological and structural re-organization wherein such an increased need for dietary protein would arise. At this time the growth in muscle is emphasized, and the protein anabolic effect of androgens and estrogens is well established.

The total nitrogen excretion per kilogram of body weight is fairly uniform throughout the entire growing period. This is a reasonable result because of the fact that during the growing period the muscular mass dominates the nitrogen excretion picture to a certain extent. During the growing period the build-up of fat storage depots is very slight until the rate of growth begins to decrease markedly. However, the large elevation of the nitrogen excretion over the estimated endogenous level based on body weight shows that the level of excretion is controlled largely by the nitrogen intake.

The predicted endogenous level of excretion is shown in

Figure 8. This high elevation, which represents an increase over the endogenous level of about 1,000 per cent, would seem to suggest that the pattern of nitrogen excretion during growth, as it was obtained in this experiment, more truly reflects the behavior of the animals tested, in terms of nitrogen intake, than the actual growth requirements. However, it should be reiterated that the results also represent the degree of protein catabolism associated with growth under ideal conditions of nutritional status, and thus with the development and preservation of an ideal level of protein reserves.

Creatinine and Creatine Nitrogen Excretion

The increase in creatinine nitrogen excretion with the increase in body weight is shown in Figure 11. The level of excretion increases in a linear manner with the increase in body weight. This result agrees with theoretical considerations (Albanese 1959). As mentioned previously, the increase in body weight is largely represented by active body mass during the period of growth. The creatinine nitrogen excretion represents unavoidable nitrogen loss from this fraction of body material, and should, therefore, be a representative index of the increase in body weight during this period. Also, it is not influenced by the degree of nitrogen intake to the extent that the total nitrogen excretion is affected. The creatinine nitrogen excretion per kilogram of body weight was uniform throughout

the growing period of all the individuals and was similar in value from one individual to another. The

The creatine nitrogen excretion was very variable and ranged from values of 10 to 150 milligrams. These values are similar to those quoted for humans. The creatine nitrogen excretion tended to reach peak values with each individual, and in both sexes, at about 120 to 135 days and with body weights of between 16 and 22 kilograms. This peak excretion coincides with the establishment of puberty.

Tables and Figures

TABLE I. Dry Matter Relations

Date	D.M. Intake in Gr.	Fecal D.M. in Gr.	Total Dig. Nutrient (appar.*) in Gr.	% Dig. D.M.	True Dig. Nutrient* in Gr.	True % Dig. D.M.	Dig. Cals.	Total Cals.
			Trial 1 October (P-1)					
17								
18								
19		no						
20	421.4	data	-	-	-	-	-	-
			Trial 2 January (R-5)					
15/16		—						
16/17		—						
17/18		72.9						
18/19		10.1						
19/20	322.0	15.5						
20/21	690.4	59.8	262.2	81.8	272.2	84	759.8	
21/22	421.4	68.3	522.1	75.5	532.1	77	1,513.8	
22/23	657.0	147.3	274.1	65.1	284.1	67	794.6	1,222
23/24	421.4	66.4	590.6	89.9	600.6	91	1,713.9	
24/25		186.6	234.8	55.7	244.8	58	681.5	1,222
25/26		45.9						
26/27		6.9						
27/28		11.9						
			Trial 3 March (R-5)					
12/13		68.5						
13/14		56.2						
14/15		64.3						
15/16		25.0						
16/17	17.3	none						
17/18	142.2	13.2	4.1	42.2	14.1	82	118.9	
18/19	378.8	31.2	110.0	77.4	120.0	85	319.0	
19/20	357.7	64.7	314.1	79.8	324.1	85	910.6	1,098
20/21		49.1	308.6	86.3	318.6	89	896.1	1,037
21/22		22.5						
22/23		18.0						

TABLE II. Nitrogen Digestibility Data

Date	Feed N.	Metabolic Fecal N.	Fecal N.	Appar. Dig. N.	True Dig. N.	Digestibility % or Coefficient (true)	True Dig. N./kg. Body Wt.
			Trial I October (P-1)				
16/17							
17/18							
18/19							
19/20	10.48						
			Trial 2 January (R-5)				
15/16			-				
16/17			2.30				
17/18			0.32				
18/19			0.51				
19/20	8.01		2.05				
20/21	17.18	0.64	2.34	5.67	6.31	79	0.139
21/22	10.48	1.38	3.43	13.75	15.13	88	0.334
22/23	16.35	0.84	1.91	8.57	9.41	90	0.207
23/24	10.48	1.31	5.50	10.85	12.16	74	0.268
24/25		0.84	1.82	8.66	9.50	91	0.209
25/26			0.23				
26/27			0.46				
27/28			-				
			Trial 3 March (R-5)				
12/13							
13/14							
14/15							
15/16							
16/17	0.43						
17/18	3.54	0.346	0.383	0.047	0.08	19	0.002
18/19	9.42	0.284	1.095	2.445	2.73	77	0.060
19/20	8.90	0.758	2.349	7.081	7.83	83	0.173
20/21		0.715	1.723	7.177	7.89	89	0.174
21/22			0.734				
22/23			0.628				

TABLE III. Nitrogen Balance Data

Date	True Dig. N. in G.	Urinary N. in G.	N. Balance IN G.	N. Bal./Kg. b. wt. IN G./Kg.	Gr. b. Prot. Used for Energy	Cal. Equiv. of this amt. Body Prot.	Cals. Req'd. from Body CHO &/or Fat
		Trial 1 October (P-1)					
16/17		23.124	-23.124	0.509	144.5	612.7	
17/18		8.806	- 8.806	0.194	55.0	233.3	
18/19		9.200	- 9.200	0.202	57.5	243.8	
19/20	0.28	43.221	-43.221	0.953	271.2	1,145.0	
		Trial 2 January (R-5)					
15/16		—	—	—	—	—	1,636.5
16/17		25.384	-25.384	0.560	158.6	672.6	219.6
17/18		12.096	-12.096	0.267	75.6	320.4	1,010.0
18/19		12.929	-12.929	0.285	80.8	342.6	1,104.6
19/20	6.31	3.225	- 3.225	0.071	20.2	85.5	1,229.1
20/21	15.31	11.719	- 5.409	0.119	33.8	143.1	269.0
21/22	9.41	15.531	- 0.221	0.005	6.8	53.0	80.7
22/23	12.16	10.252	- 0.842	0.019	7.7	21.2	919.8
23/24	9.50	14.537	- 2.377	0.052	14.9	63.6	44.4
24/25		6.343	+ 3.157	0.070	19.7	84.8	523.2
25/26		8.900	- 8.900	0.196	55.7	235.8	978.9
26/27		11.501	-11.501	0.254	71.9	304.7	899.0
27/28		10.413	-10.413	0.230	65.1	275.9	959.5
		Trial 3 March (R-5)					
12/13		6.002	- 6.002	0.0132	37.5	159.0	1,388.3
13/14		6.674	- 6.674	0.147	42.7	176.9	1,096.4
14/15		17.702	-17.702	0.390	111.0	469.0	538.3
15/16		6.720	- 6.720	0.148	43.0	178.1	946.2
16/17	0.08	8.488	- 8.488	0.187	53.1	224.9	775.5
17/18	2.73	6.834	- 6.754	0.149	42.2	180.2	950.8
18/19	7.83	8.068	- 5.338	0.118	33.4	140.5	955.5
19/20	7.89	10.836	- 3.006	0.066	18.8	80.0	110.0
20/21		21.000	-13.110	0.289	82.1	347.4	16.8
21/22		7.032	- 7.032	0.155	43.9	186.3	1,000.7
22/23		6.437	- 6.437	0.142	40.2	170.6	972.9

TABLE IV. Water Balance Data

Date	Water from Feed	Drinking Water	Metabolic Water from Feed	Insensible Water Loss	Fecal Water Loss	Urine Water Loss	Water Balance
		Trial 1 October (P-1)					
16/17		2,600				887	+1,713
17/18		1,300				428	+ 872
18/19		572		no	no	406	+ 166
19/20	32.2	1,828	-	data	data	1,882	- 29
		Trial 2 January (R-5)					
15/16		696		490	-	-	+ 206
16/17		700		343	89	962	- 694
17/18		514		435	19	828	- 768
18/19		1,664		434	36	1,443	- 249
19/20	24.6	1,172		14	7	258	- 918
20/21	52.8	1,424	163	168	117	1,235	+ 120
21/22	32.2	1,606	319	282	194	1,686	- 205
22/23	50.7	2,053	171	224	97	1,099	+ 855
23/24	32.2	2.471	360	698	58	2,126	- 19
24/25		495	147	613	58	450	- 479
25/26		410		619	4	394	- 607
26/27		705		627	22	634	- 578
27/28		632		437	28	590	- 423
		Trial 3 March (R- 5)					
12/13		63		142	64	377	- 520
13/14		1,209		493	7	335	+ 374
14/15		832		597	36	837	- 638
15/16		446		680	60	382	- 676
16/17	1.3	179		367	none	346	- 535
17/18	10.9	1,990	9	357	-	372	+1,281
18/19	29.0	754	72	318	62	551	- 76
19/20	27.3	1,493	194	628	103	1,598	- 615
20/21		310	191	420	37	1,120	-1,076
21/22		320		216	-	361	- 257
22/23		400		242	29	296	- 167

TABLE V. Nitrogen Distribution Data

Date	Total Nitrogen	Nitrogen Distribution								Total %
		Urea N.	% T.N.	Amm. N.	% T.N.	Cr' N.	% T.N.	Cr" N.	% T.N.	
		Trial 1 October (P-1)								
16/17	23.360	20.990	90	0.086	0.4	0.530	2.3	0.162	0.7	92.9
17/18	8.806	8.211	93	0.044	0.5	0.283	3.2	0.023	0.3	97.2
18/19	9.200	7.618	82	0.194	2.1	0.315	3.4	0.023	0.3	85.6
19/20	43.221	41.507	96	0.199	0.5	1.446	3.3	0.069	0.2	100.0
		Trial 2 January (R-5)								
16/17	25.385	24.001	95	0.734	2.9	0.601	2.4	0.049	0.2	100.0
17/18	12.096	10.292	85	0.913	7.5	0.438	3.6	0.153	1.3	97.4
18/19	12.929	10.884	84	1.299	10.1	0.570	4.4	0.176	1.4	100.0
19/20	3.225	2.713	84	0.274	8.5	0.145	4.5	0.002	0.1	97.2
20/21	11.719	10.147	87	0.889	7.6	0.595	5.1	0.089	0.8	100.0
21/22	15.531	14.640	94	0.254	1.7	0.566	3.7	0.071	0.5	100.0
22/23	10.252	9.746	95	0.124	1.2	0.358	3.5	0.025	0.2	100.0
23/24	14.537	13.788	95	0.217	1.5	0.524	3.6	0.000	0.0	99.9
24/25	6.343	5.725	90	0.115	1.8	0.246	3.9	0.016	0.3	96.3
25/26	8.899	8.281	93	0.196	2.2	0.257	2.9	0.015	0.2	98.4
26/27	11.501	9.950	87	0.431	3.8	0.371	3.2	0.064	0.5	94.0
27/28	10.413	8.682	83	0.621	6.0	0.361	3.5	0.050	0.48	93.3
		Trial 3 March (R-5)								
12/13	6.002	5.421	90	0.421	0.7	0.213	3.5	0.004	0.1	94.6
13/14	6.674	5.985	90	0.068	1.1	0.076	1.1	0.019	0.3	92.2
14/15	17.702	14.785	84	0.518	2.9	0.144	0.8	0.064	0.4	87.6
15/16	6.720	5.193	77	0.516	7.7	0.366	5.4	0.137	2.0	92.4
16/17	8.488	6.703	79	0.641	7.6	0.467	5.5	0.061	0.7	92.8
17/18	6.834	5.754	84	0.609	8.9	0.448	6.6	0.023	0.3	100.0
18/19	8.068	6.847	85	0.740	9.2	0.473	5.9	0.008	0.1	100.0
19/20	10.836	9.033	83	1.007	9.3	0.612	5.7	0.183	1.7	100.0
20/21	21.000	19.926	95	0.372	1.8	0.687	3.3	0.000	0.0	99.9
21/22	7.032	5.047	72	0.246	3.5	0.390	5.4	0.002	0.0	80.9
22/23	6.437	5.687	88	0.361	5.6	0.334	5.2	0.055	0.9	99.9

TABLE VI. Metabolic Rate Determination Data, and Required Changes in Body Composition at the R.Q. of 0.82

Date	O_2 Consumption in Liters	Value of O_2 in Cals at R.Q. of 0.82	Grams of Body Fat for NPN Cals	Grams of Body CHO for NPN Cals	Pounds of Total Wt. Loss Prot. - Ass. Water	Prot. Alone
		Trial 1 October (P-1)				
		no data				
		Trial 2 January (R-5)				
16/17	339.18	1,636.5	100.7	13.2		
17/18	252.76	1,219.6	75.1	15.0	724.5	248.7
18/19	275.73	1,330.4	81.9	16.4	400.7	173.9
19/20	302.00	1,457.2	89.7	17.9	430.8	188.4
20/21	272.45	1,314.6	80.9	16.2	177.9	117.3
21/22	242.91	1,172.0	72.1	14.4	221.7	120.3
22/23	331.53	1,599.6	98.5	19.7	145.4	125.0
23/24	359.73	1,736.0	106.9	21.4	159.1	136.0
24/25	377.49	1,821.4	112.1	22.4	194.1	149.4
25/26	269.17	1,298.7	80.0	16.0	174.8	115.7
26/27	251.76	1,214.7	74.8	17.0	314.6	147.5
26/27	249.48	1,203.7	74.1	16.8	378.5	162.8
27/28	256.04	1,235.4	76.0	17.2	353.6	158.3
		Trial 3 March (R-5)				
12/13	320.68	1,547.3	95.2	19.0	264.2	151.7
13/14	263.89	1,273.3	78.4	15.7	264.9	136.8
14/15	188.04	907.3	55.9	11.2	511.1	178.1
15/16	233.02	1,124.3	69.2	13.8	255.0	126.0
16/17	207.33	1,000.4	61.6	12.3	286.3	127.0
17/18	237.78	1,147.3	70.6	16.1	255.5	128.8
18/19	293.35	1,415.4	87.1	17.4	238.1	137.9
19/20	228.20	1,101.1	67.8	13.5	156.5	100.0
10/21	261.16	1,260.1	77.6	15.5	421.5	175.2
21/22	246.01	1,187.0	73.1	14.6	263.3	131.6
22/23	246.01	1,143.5	70.4	14.1	245.3	124.7

TABLE VII. Nitrogen Excretion During Growth

Date	Age in Days (appr.)	Weight in Kg.	Volume of Urine in cc.	Time Interval Taken for Trial (hours)	Total Nitrogen Excreted in Urine Gr./dy	Ammonia Nitrogen		Urea Nitrogen		Creatine Nitrogen		Creatinine Nitrogen		Total Nitrogen Expressed as N./Kg./dy	Creatinine Nitrogen Expressed as N./Kg./dy
						%T.N.	Total in Gr.	%T.N.	Total in Gr.	%T.N.	Total in Gr.	%T.N.	Total in Gr.		
R-4: Male Black-tailed Deer															
1960															
July 24/25	35	6.8	430	21.5	4.728	3.04	1.440	28.5	1.350	0.64	0.031	1.44	0.068	0.695	0.001
Aug. 4/5	45	8.2	400	18	9.552	1.20	0.115	96.4	9.208	1.15	0.110	1.26	0.120	1.165	0.015
17/18	55	9.3	657	18	8.064	7.22	0.582	94.6	7.629	1.33	0.107	1.82	0.147	0.867	0.016
Aug. 31-Sept. 1	70	-	562	18	6.792	9.70	0.659	90.3	6.133	-	-	-	-	-	-
Sept 15/16	85	13.8	518	17.3	9.408	5.94	0.559	68.7	6.463	0.93	0.087	1.54	0.145	0.682	0.016
Oct. 20/21	122	19.3	862	12.2	-	-	0.944	-	-	-	-	-	0.301	-	0.016
Dec. 2/3	165	27.2	1,369	24	16.339	2.13	0.348	90.7	14.819	0.87	0.142	5.36	0.876	0.601	0.032
3/4	"	"	1,100	24	13.877	3.06	0.425	89.9	12.475	-	-	-	-	0.510	-
4/4	"	"	420	12	10.104	2.47	0.324	88.3	8.922	0.89	0.090	9.25	0.935	0.371	0.034
4/5	"	"	560	12	14.232	2.52	0.359	52.7	7.500	-	-	-	-	0.523	-
R-6: Male Black-tailed Deer															
1960															
July 26/27	35	8.6	460	-	3.936	11.30	0.445	-	-	-	-	-	-	0.457	-
Aug. 25/26	65	11.6	742	18	7.200	1.96	0.141	94.3	6.790	1.42	0.102	2.27	0.163	0.621	0.014
Sept. 6/7	80	14.7	442	22	8.265	3.31	0.273	94.5	7.802	0.61	0.050	1.36	0.112	0.562	0.008
20/21	92	17.9	773	19.3	9.600	3.14	0.301	95.6	9.178	0.96	0.092	2.49	0.239	0.536	0.013
Dec. 6/6	170	30.8	850	12.5	20.760	0.58	0.120	-	-	0.11	0.023	2.04	0.424	0.674	0.014
7/7	"	"	910	12	22.495	0.84	0.189	94.7	21.303	0.81	0.182	1.84	0.414	0.730	0.013
7/8	"	"	780	12	15.912	2.66	0.423	-	-	0.63	0.100	2.14	0.341	0.517	0.011
8/8	"	"	620	12	12.096	1.64	0.198	-	-	0.77	0.093	2.39	0.289	0.393	0.009
8/9	"	"	780	12	14.424	2.96	0.427	97.2	14.020	0.17	0.025	2.63	0.379	0.468	0.012

TABLE VIII. Nitrogen Excretion During Growth

Date	Age in Days (appr.)	Weight in Kg.	Volume of Urine in cc.	Time Interval Taken for Trial (hours)	Total Nitrogen Excreted in Urine Gr./dy	Ammonia Nitrogen		Urea Nitrogen		Creatine Nitrogen		Creatinine Nitrogen		Total Nitrogen Expressed as N./Kg./dy	Creatinine Nitrogen Expressed as N./Kg./dy
						%T.N.	Total in Gr.	%T.N.	Total in Gr.	%T.N.	Total in Gr.	%T.N.	Total in Gr.		
R-7: Male Black-tailed Deer															
1960															
July 29/30	42	8.6	330	21	4.944	2.83	0.140	-	-	-	-	-	-	-	-
Aug. 10/11	52	10.7	428	16.5	7.224	2.39	0.173	93.7	6.769	1.86	0.134	2.01	0.145	0.464	0.014
23/24	65	12.2	312	18.1	7.512	6.67	0.501	33.3	2.501	-	-	2.04	0.153	0.464	0.013
R-8: Male Black-tailed Deer															
1960															
Aug. 1/2	40	11.6	554	19	6.264	3.59	0.228	55.1	3.450	1.50	0.094	2.26	0.142	0.540	0.012
11/12	50	-	592	18	-	-	0.543	-	0.154	-	0.154	-	0.207	-	-
24/25	65	15.5	617	18.5	1.920	3.13	0.060	52.0	0.960	7.77	0.149	10.99	0.211	0.124	0.014
Sept. 12/13	85	20.0	537	17.7	9.648	3.12	0.310	68.1	6.570	1.24	0.120	2.35	0.190	0.356	0.011
23/24	95	19.1	556	16	9.408	8.59	0.808	87.3	8.213	1.00	0.094	3.19	0.300	0.493	0.016
Nov. 1/2	135	18.8	790	10.8	-	-	-	-	-	-	0.931	-	0.405	-	0.022
Dec. 19/19	185	36.3	1,220	13	-	0.10	0.203	-	20.260	-	-	-	-	0.634	-
19/20	"	"	1,040	11	23.112	1.25	0.289	90.9	21.009	0.98	0.226	2.03	0.469	0.637	0.013
20/20	"	"	1,620	12	16.200	1.00	0.162	95.5	15.471	10.48	0.340	2.81	0.577	0.466	0.016
20/21	"	"	1,860	12	20.046	1.28	0.257	100.0	20.046	0.10	0.016	2.54	0.509	0.552	0.014
21/22	"	"	1,180	12	21.672	1.49	0.323	96.1	20.872	0.86	0.186	2.12	0.459	0.597	0.013

TABLE IX. Nitrogen Excretion During Growth

Date	Age in Days (appr.)	Weight in Kg.	Volume of Urine in cc.	Time Interval Taken for Trial (hours)	Total Nitrogen Excreted in Urine Gr./dy	Ammonia Nitrogen %T.N.	Ammonia Nitrogen Total in Gr.	Urea Nitrogen %T.N.	Urea Nitrogen Total in Gr.	Creatine Nitrogen %T.N.	Creatine Nitrogen Total in Gr.	Creatinine Nitrogen %T.N.	Creatinine Nitrogen Total in Gr.	Total Nitrogen Expressed as N./Kg./dy	Creatinine Nitrogen Expressed as N./Kg./dy
			R-9: Male Black-tailed Deer												
1960															
July - -	ca. 30	3.6	300	21	2.352	9.77	0.230	100.0		-	-	1.48	0.035	0.653	0.010
Aug. 2/3	40	4.1	325	17.7	3.209	0.55	0.018	77.2	2.477	0.97	0.032	1.43	0.046	0.782	0.011
29/30	70	7.8	365	19.5	5.736	7.51	0.431	-	-	2.73	0.157	1.57	0.090	0.735	0.012
Sept. 13/14	85	10.4	365	18.3	-	-	-	-	-	-	0.052	-	0.130	-	0.013
Oct. 18/19	120	15.9	592	17	10.560	6.00	0.634	75.3	7.952	1.32	0.139	1.67	0.176	0.664	0.011
Nov. 29/30	165	23.1	373	12	12.698	2.12	0.269	77.1	9.790	0.75	0.095	1.61	0.204	0.550	0.009
1961															
Jan. 16/17	214	27.7	860	24	7.608	2.01	0.153	91.0	6.923	1.27	0.097	5.68	0.432	0.275	0.016
17/18	"	"	450	24	8.472	2.08	0.176	94.4	7.988	0.56	0.047	2.92	0.247	0.306	0.009
18/19	"	"	160	24	12.504	-	-	88.2	11.029	0.34	0.043	4.94	0.618	0.451	0.223

TABLE X. Nitrogen Excretion During Growth

Date	Age in Days (appr.)	Weight in Kg.	Volume of Urine in cc.	Time Interval Taken for Trial (hours)	Total Nitrogen Excreted in Urine Gr./dy	Ammonia Nitrogen		Urea Nitrogen		Creatine Nitrogen		Creatinine Nitrogen		Total Nitrogen Expressed as N./Kg./dy	Creatinine Nitrogen Expressed as N./Kg./dy
						%T.N.	Total in Gr.	%T.N.	Total in Gr.	%T.N.	Total in Gr.	%T.N.	Total in Gr.		
R-1: Female Black-tailed Deer															
1960															
July 21/22	30	7.3	370	21	2.112	1.55	0.033	65.8	1.389	0.50	0.011	4.37	0.092	0.289	0.126
Aug. 3/4	45	8.6	379	18	5.232	3.36	0.176	75.2	3.934	1.34	0.070	2.01	0.105	0.608	0.012
16/17	55	9.8	620	18	7.128	9.70	0.691	91.3	6.508	0.96	0.068	1.62	0.115	0.727	0.012
30/31	70	12.5	718	18.5	3.600	18.4	0.662	88.4	3.182	0.50	0.018	1.79	0.064	0.288	0.005
Sept 1/2	70	-	-	18	-	5.69	-	92.7	-	1.34	-	2.39	-	-	-
14/15	85	15.0	576	18.6	5.808	9.83	0.571	68.3	3.967	1.72	0.100	1.72	0.100	0.387	0.007
22/23	90	-	372	18	3.384	19.03	0.644	-	-	-	-	6.88	0.233	-	-
Oct. 19/20	120	20.0	1,285	16.5	12.936	5.59	0.723	-	-	3.25	0.420	1.76	0.228	6.47	0.114
Nov. 30-Dec. 1	165	-	588	11.6	6.768	1.20	0.081	90.5	6.125	-	-	-	-	-	-
1961															
Jan. 11/11	204	29.5	420	12	10.488	0.87	0.091	91.7	9.617	0.61	0.064	2.26	0.237	0.356	0.008
11/12	"	"	1,020	12	16.608	1.21	0.201	-	-	0.45	0.075	3.36	0.558	0.563	0.019
12/12	"	"	770	12	17.544	0.38	0.067	-	-	0.057	0.100	2.35	0.412	0.595	0.014
12/13	"	"	880	24	17.424	0.99	0.172	-	-	-	-	-	-	0.591	-
13/13	"	"	820	12	15.936	0.92	0.147	-	-	-	-	2.40	0.382	0.540	0.013
13/14	"	"	840	12	20.328	0.32	0.077	81.6	16.588	0.68	0.138	2.21	0.449	0.689	0.015

TABLE XI. Nitrogen Excretion During Growth

Date	Age in Days (appr.)	Weight in Kg.	Volume of Urine in cc.	Time Interval Taken for Trial (hours)	Total Nitrogen Excreted in Urine Gr./dy	Ammonia Nitrogen		Urea Nitrogen		Creatine Nitrogen		Creatinine Nitrogen		Total Nitrogen Expressed as N./Kg./dy	Creatinine Nitrogen Expressed as N./Kg./dy
						%T.N.	Total in Gr.	%T.N.	Total in Gr.	%T.N.	Total in Gr.	%T.N.	Total in Gr.		
R-5: Female Black-tailed Deer															
1960															
July 28/29	42	8.9	440	20.5	5.640	-	-	84.7	4.777	1.19	0.067	2.29	0.013	0.637	0.015
Aug. 9/10	52	10.2	413	19.8	7.464	4.61	0.344	91.2	6.807	1.03	0.077	2.28	0.170	0.732	0.017
19/20	62	12.7	428	19.8	7.776	1.56	0.121	97.4	7.574	0.01	0.008	0.98	0.076	0.612	0.006
Sept. 7/8	80	-	647	19.6	6.312	-	-	96.5	-	0.28	0.018	2.17	0.137	-	-
Oct. 13/14	115	-	1,260	24	13.142	0.59	0.078	-	-	-	-	-	-	-	-
24/25	125	-	884	12	18.120	1.68	0.304	95.9	12.603	0.11	0.014	3.42	0.449	-	-
26/27	"	22.7	676	12	12.816	1.95	0.250	98.3	17.812	-	-	-	-	0.565	-
27/28	130	22.7	706	12	16.680	1.71	0.285	95.7	12.265	0.84	0.108	2.28	0.292	0.565	0.013
Dec. 12/12	175	29.0	680	12	16.704	0.61	0.101	97.8	16.313	0.36	0.060	1.83	0.305	0.735	0.013
12/13	"	"	1,280	12	16.242	-	-	97.5	16.286	0.49	0.079	2.73	0.444	0.576	0.015
13/14	"	"	1,260	24	-	-	-	-	-	-	-	-	0.260	0.560	0.009
14/14	"	"	420	12	10.496	-	-	-	-	0.64	0.067	2.75	0.289	0.372	0.010
14/15	"	"	680	12	20.692	-	-	-	-	0.25	0.052	2.92	0.604	0.714	0.021
R-12: Female Black-tailed Deer															
1960															
July 25/26	35	7.5	650	20	7.032	1.13		44.3	2.590	-	-	-	-	0.938	-
Aug. 8/9	50	-	820	16	6.072	-		-	-	1.46	0.087	2.19	0.013	-	-
18/19	60	10.1	491	18	-	5.91		-	-	-	-	-	-	0.411	-
21/22	65	10.8	778	18	4.152	3.83		78.4	1.045	-	-	-	-	-	-
Sept. 1/2	70	13.0	791	19	6.600	9.86		46.3	2.080	-	-	-	-	0.508	-
19/20	90	-	847	19	-	-		-	-	-	0.112	-	0.203	-	-

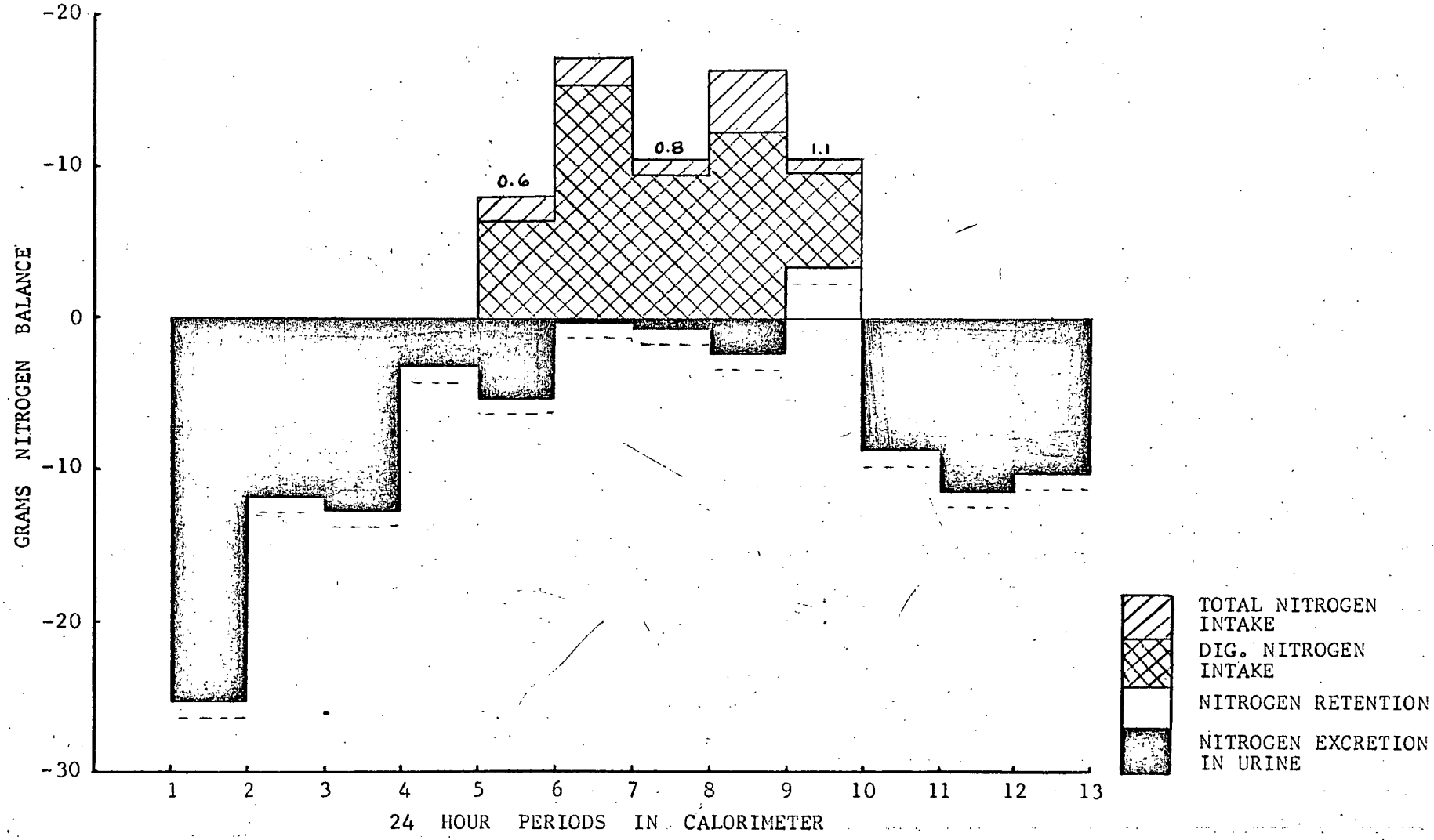


Fig. 1. Course of nitrogen balance obtained on R-5 in Trial II.

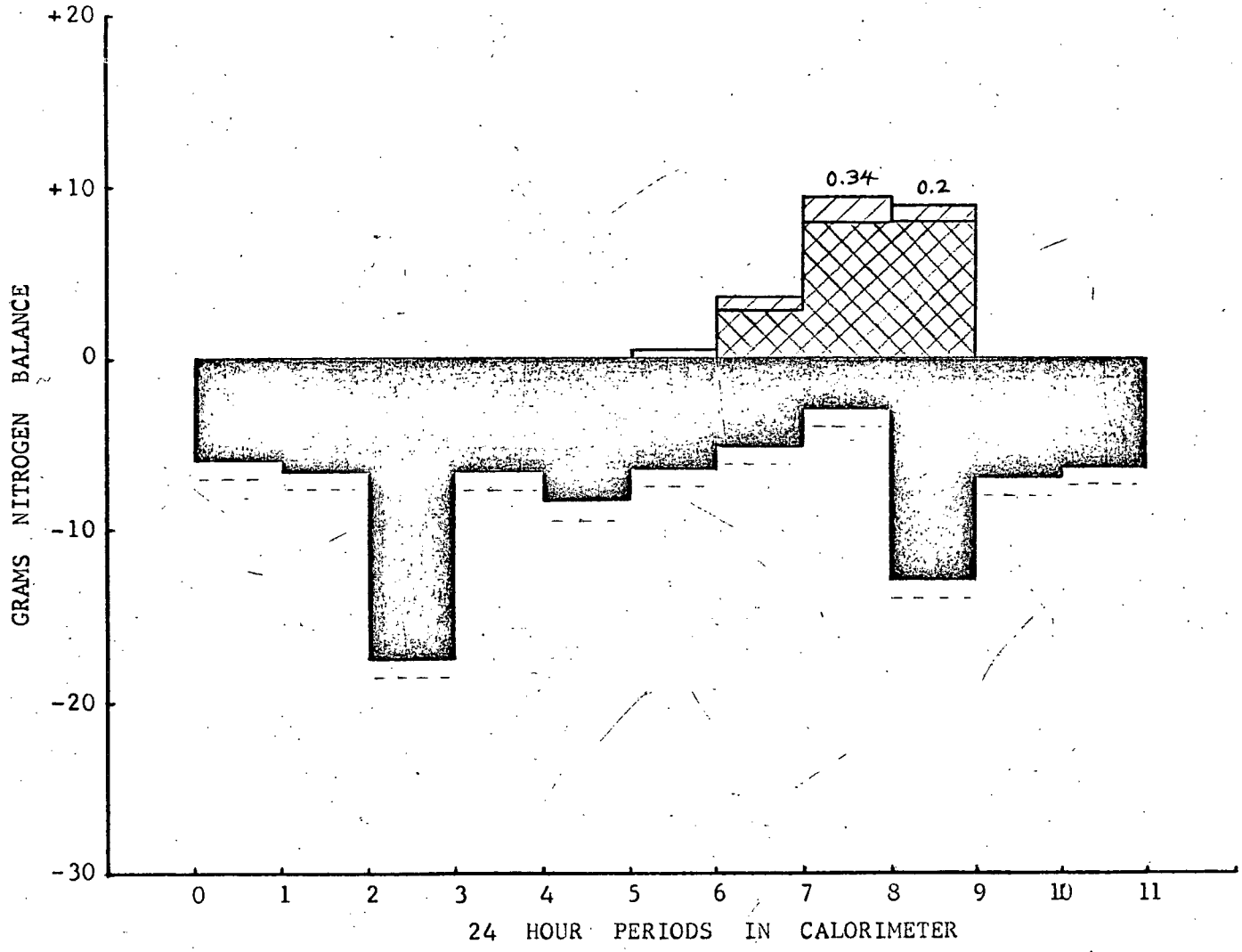


Fig. 2. Course of nitrogen balance obtained on R-5 in Trial III.

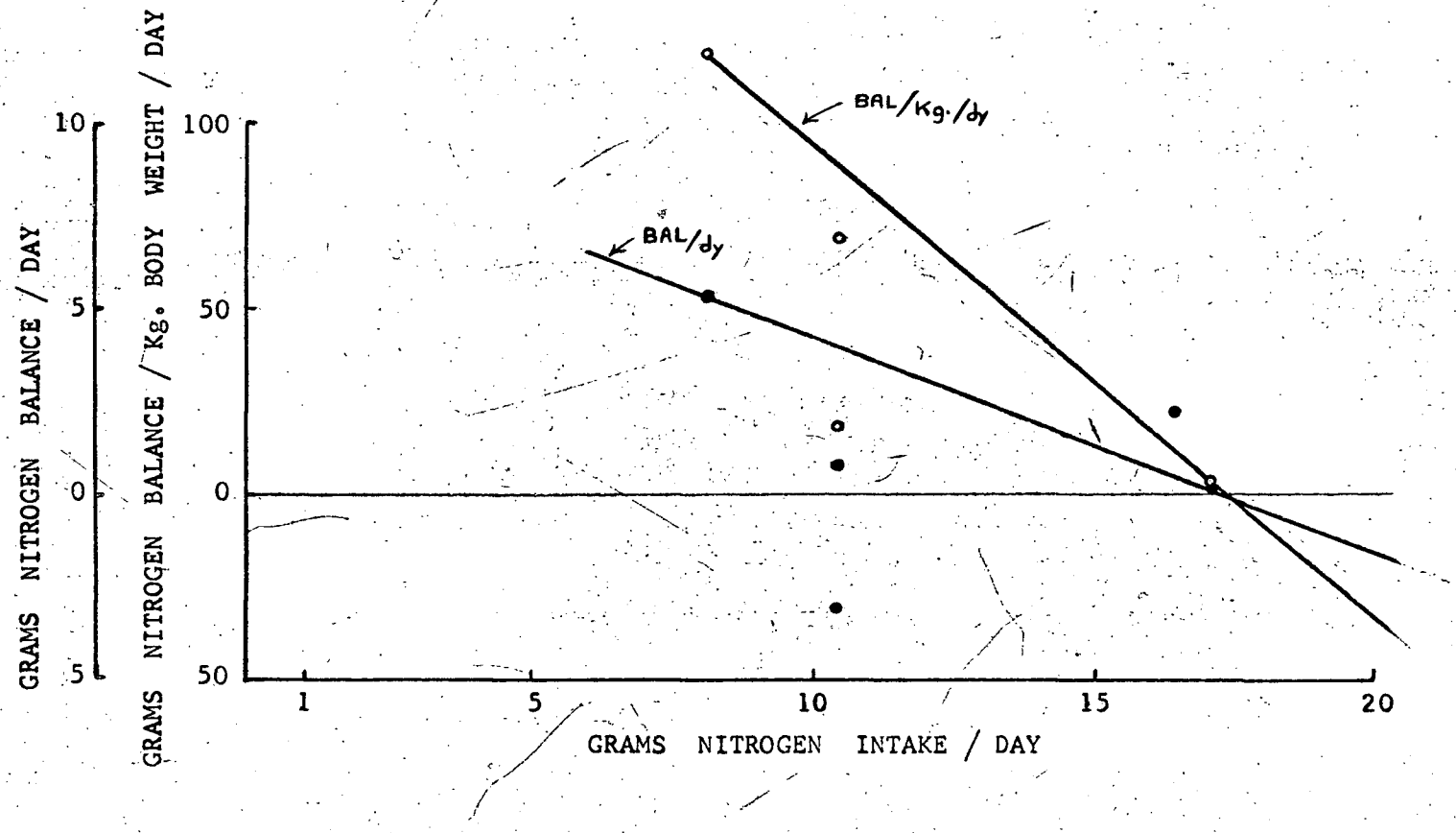


Fig. 3. Illustration of the point of nitrogen balance in Trial II.

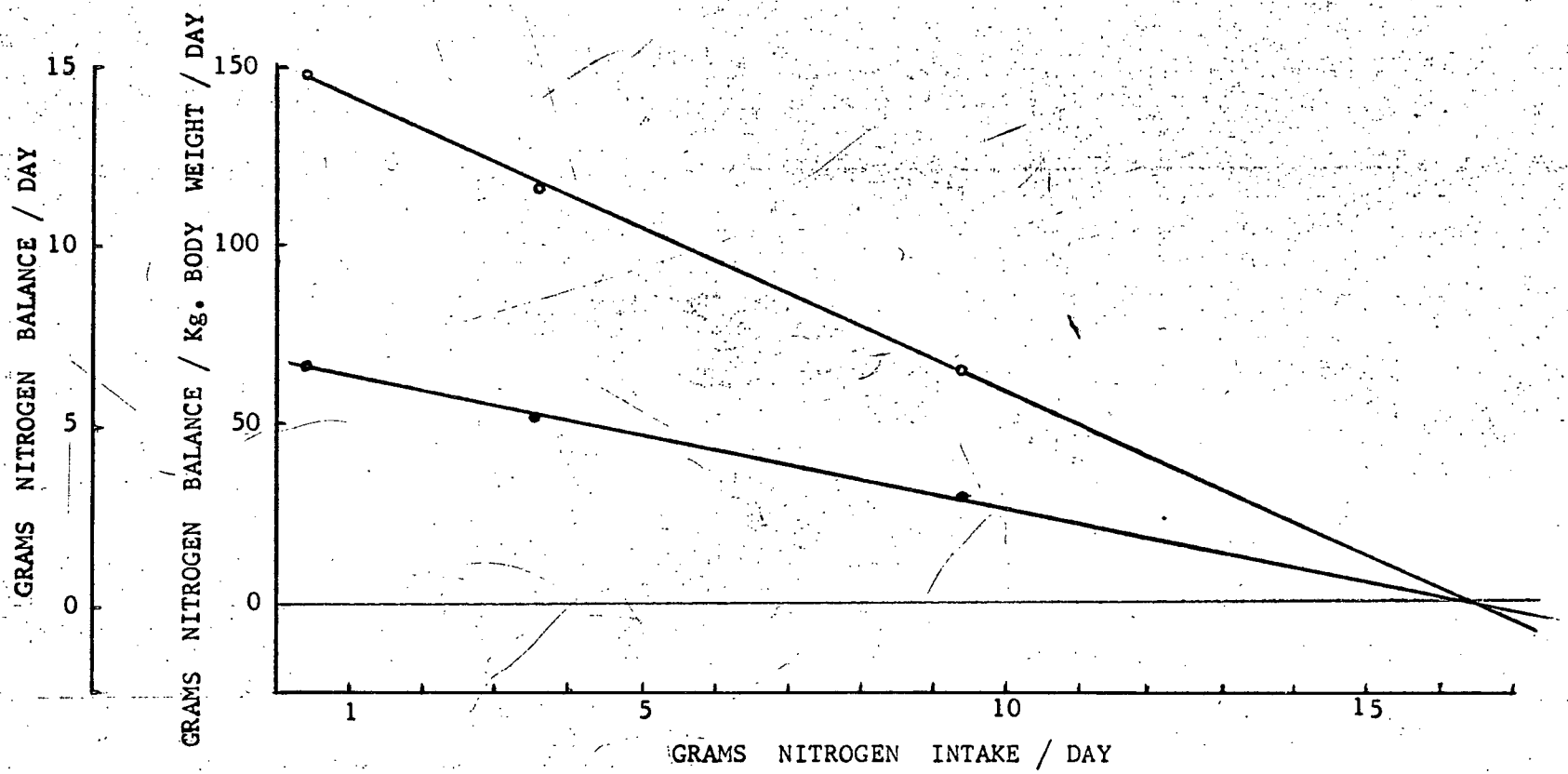


Fig. 4. Illustration of the point of nitrogen balance in Trial III.

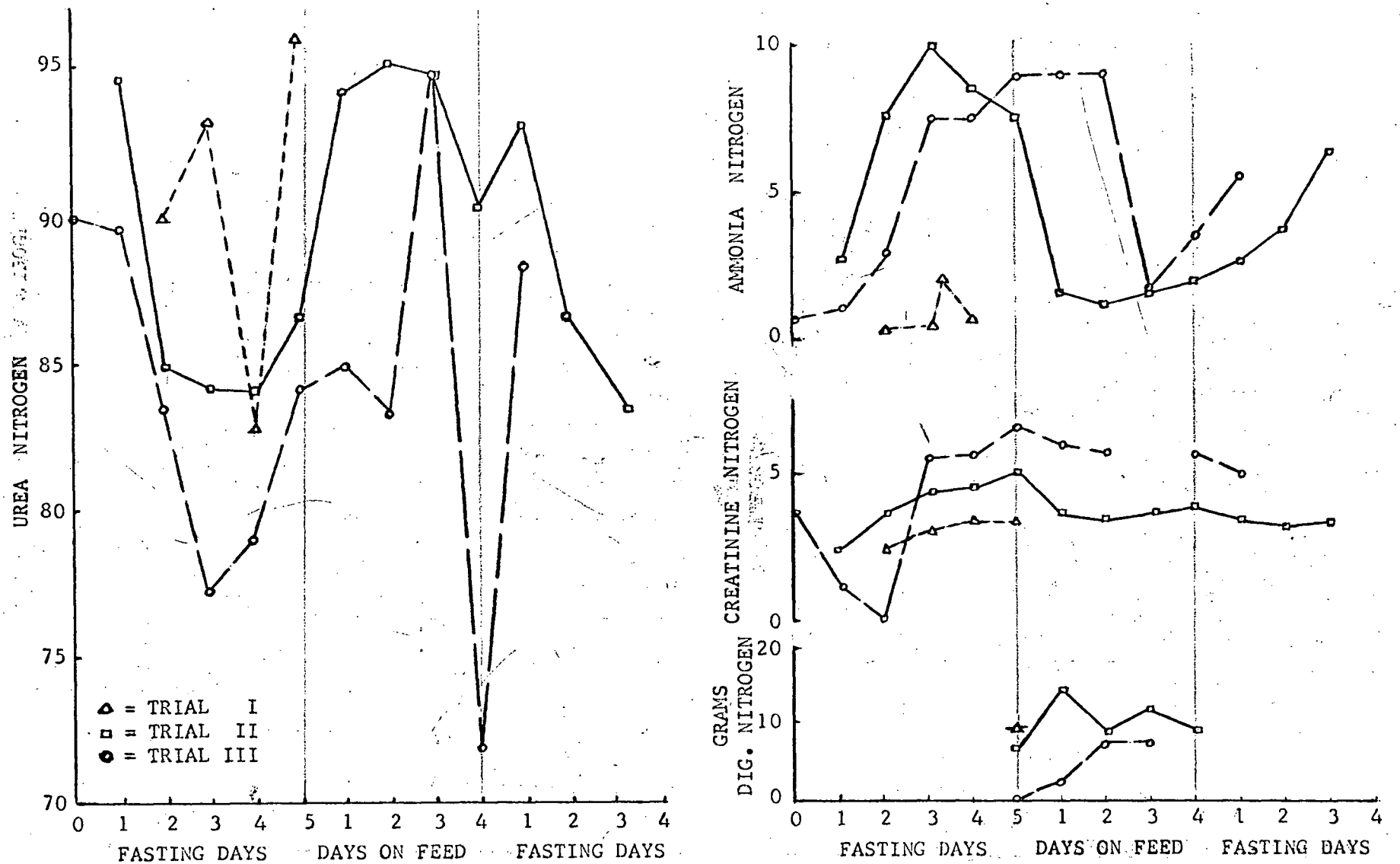


Fig. 5. Changes in nitrogen distribution during the balance trials, showing the changes in per cent total nitrogen of urea, ammonia, and creatinine nitrogen, during periods of fasting and of feeding. The amount of digestible nitrogen intake, in grams, is shown in the lower right hand corner.

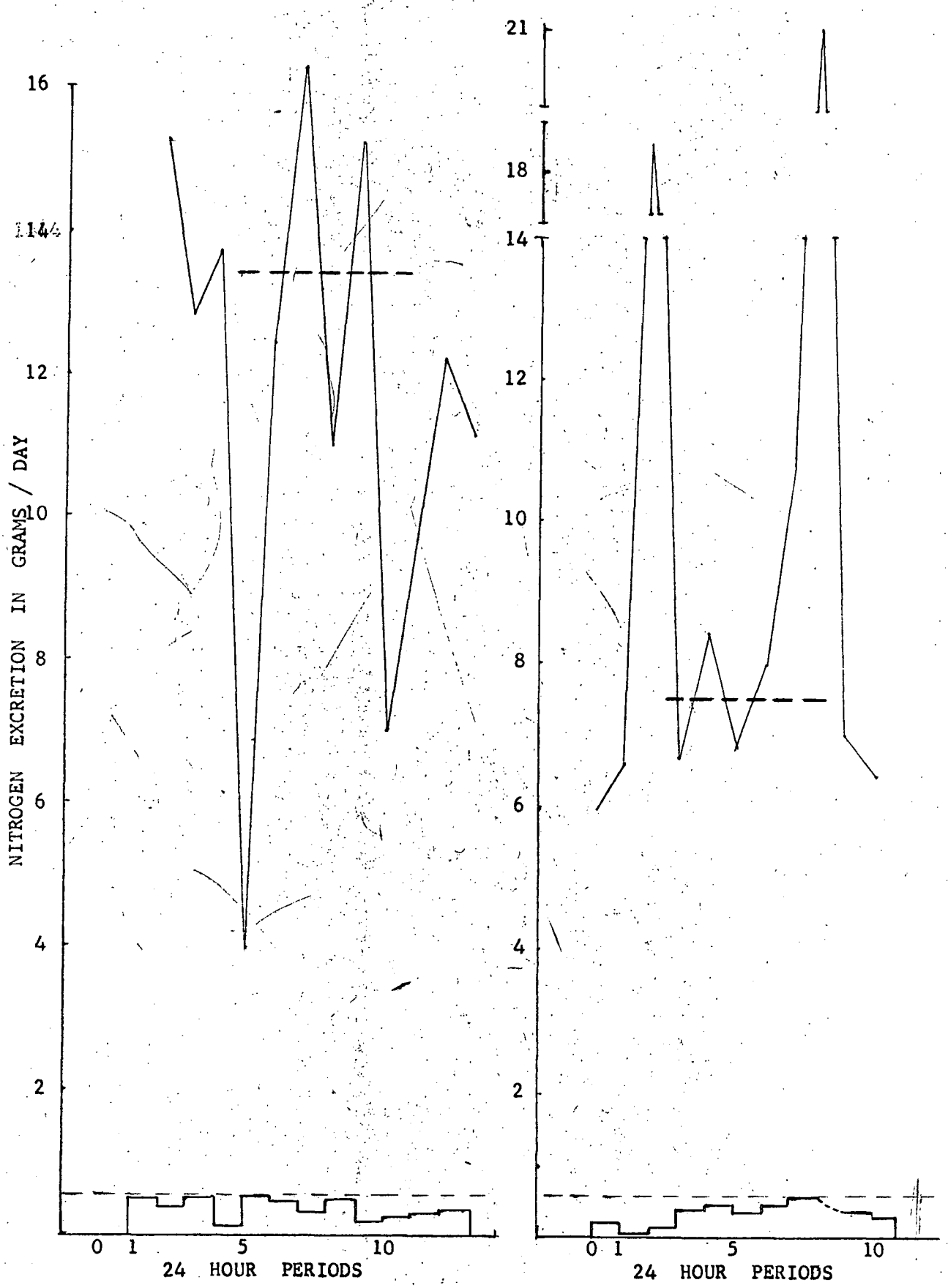


Fig.6. The change in creatinine excretion, with changing levels of total nitrogen excretion, during the balance trials.

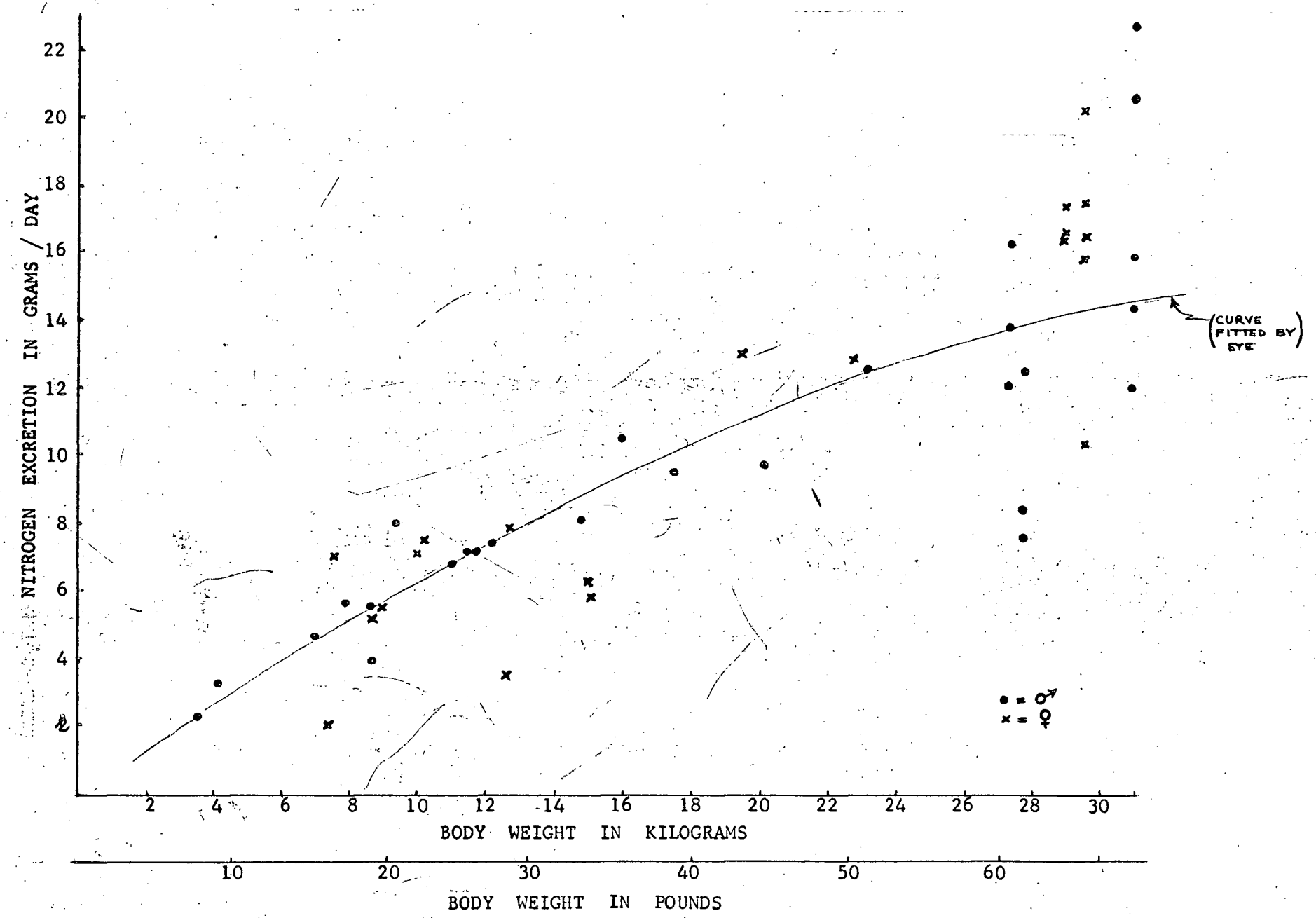


Fig. 7. The cumulative change in total nitrogen excretion with increasing body size, in growing black-tailed deer.

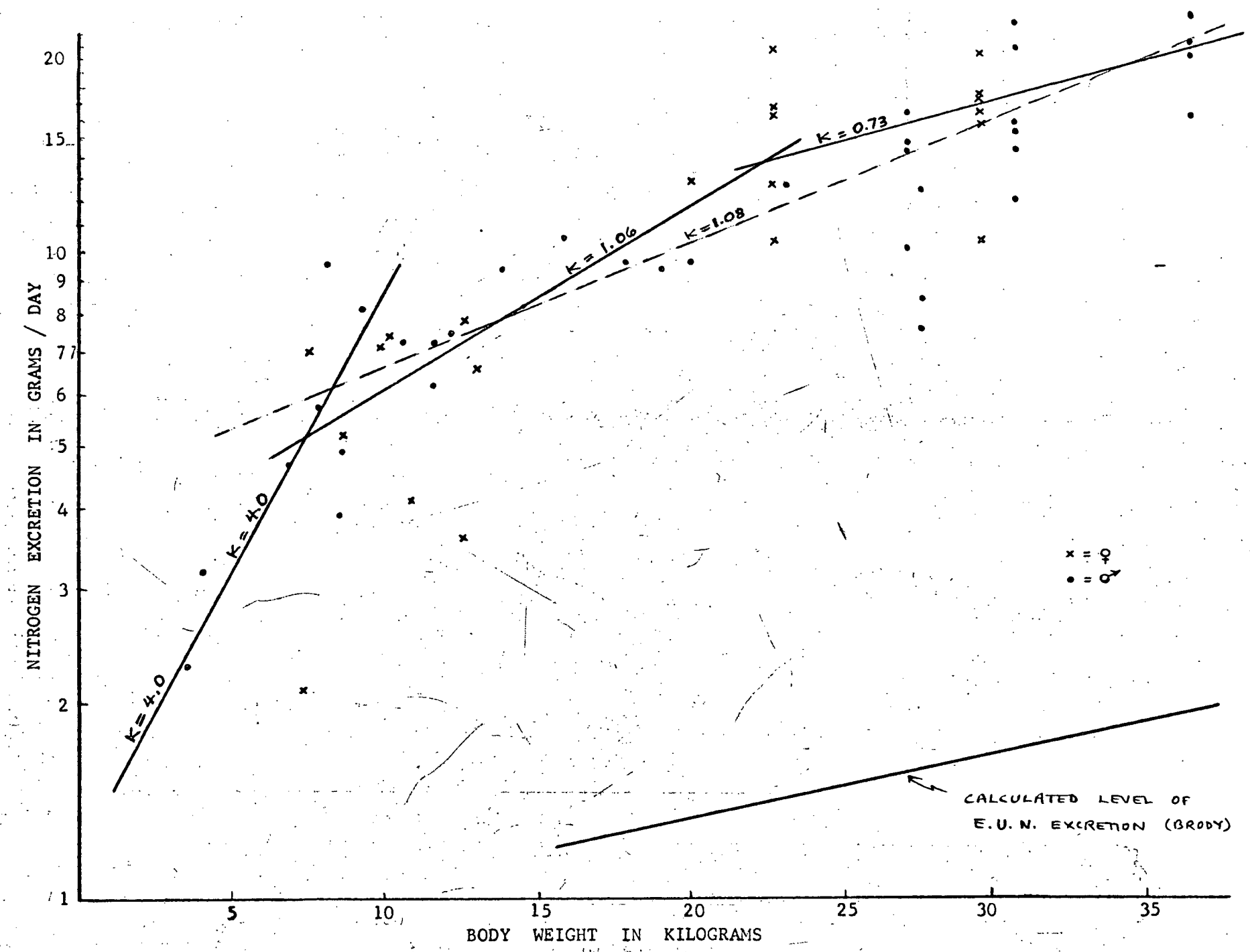


Fig. 8. The change in rate of increase in magnitude of total nitrogen excretion with increase in body size, in growing black-tailed deer.

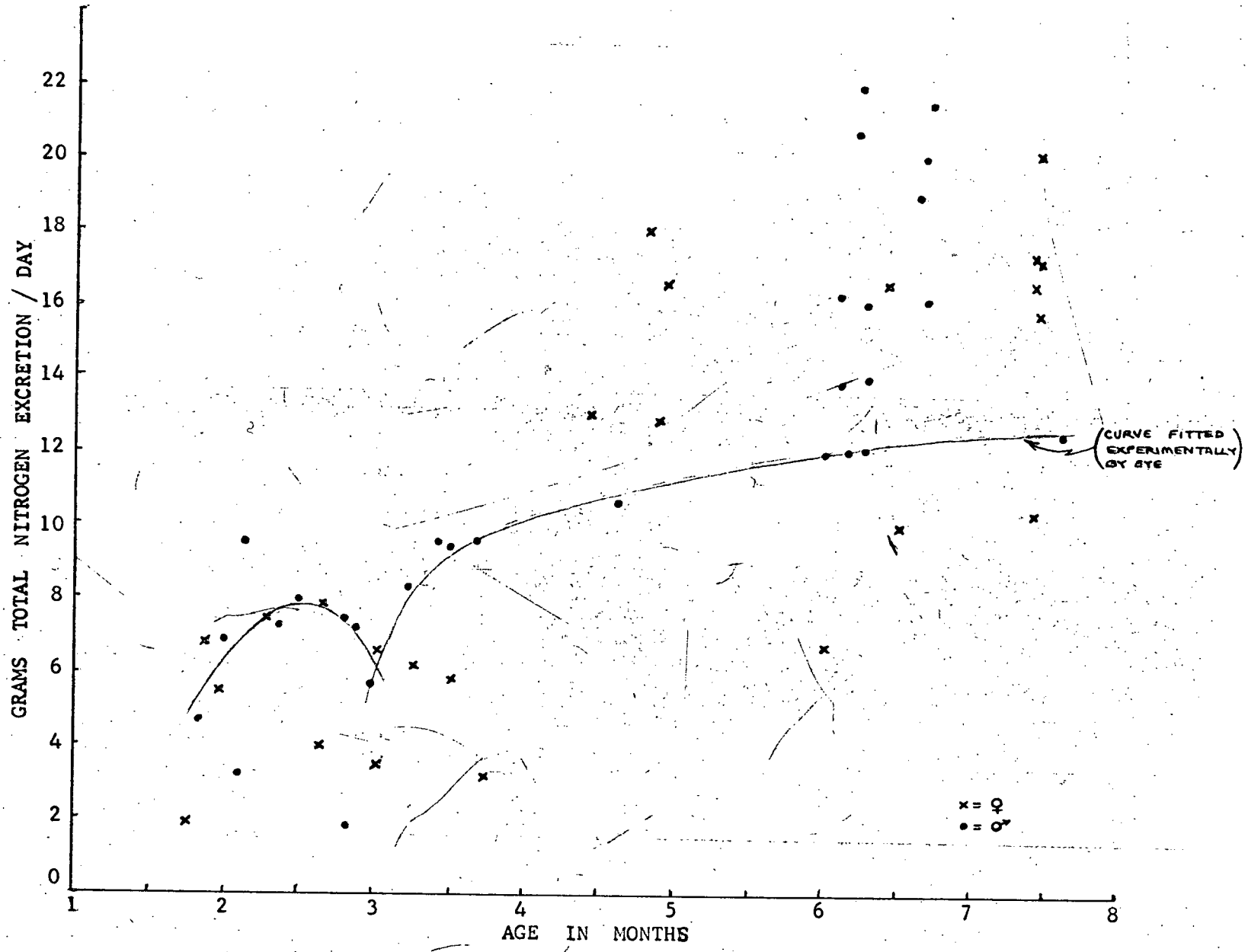


Fig. 9. The cumulative change in total nitrogen excretion with increase in age, in growing black-tailed deer.

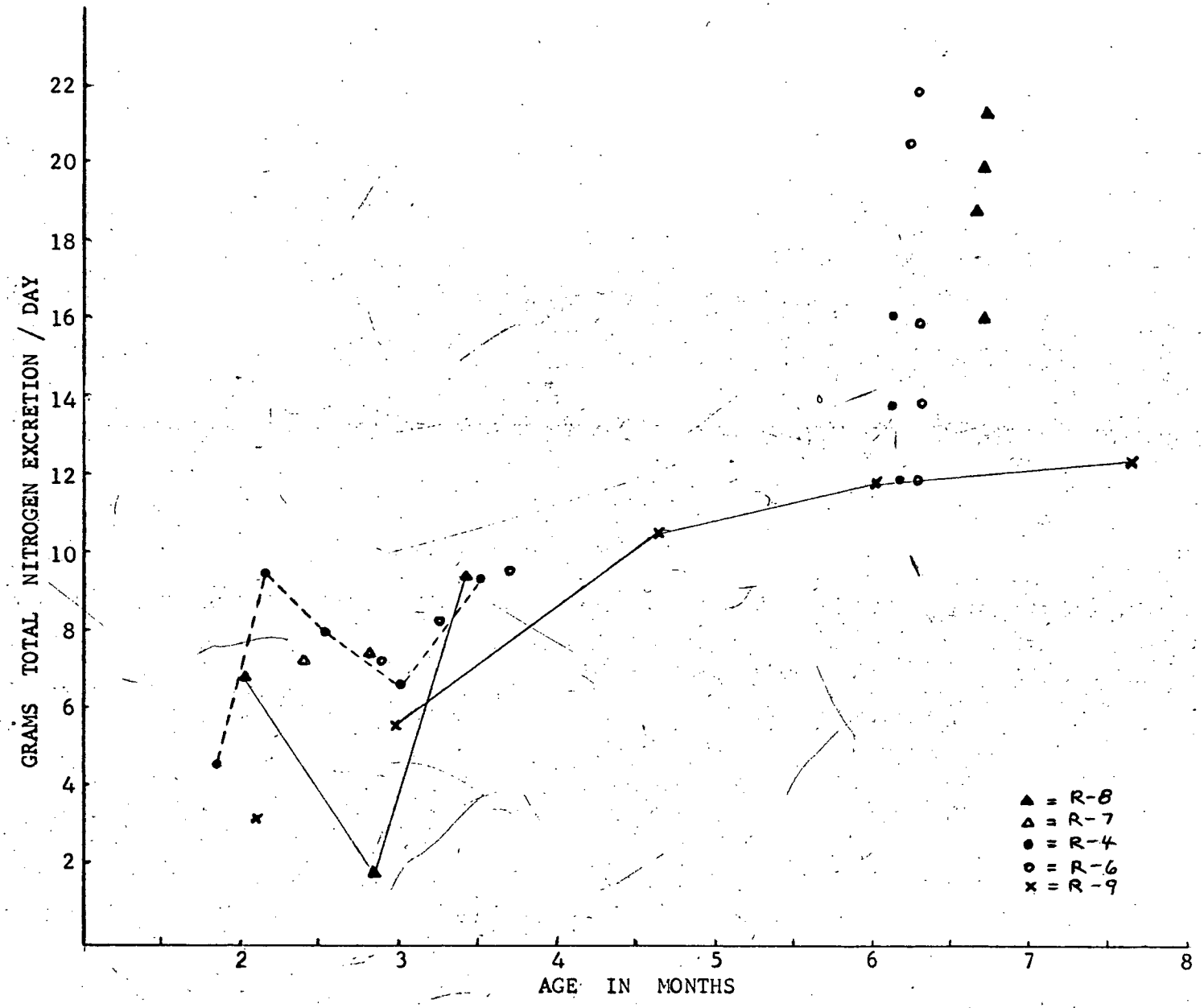


Fig. 10. The cumulative change in total nitrogen excretion with increase in age, in growing male black-tailed deer.

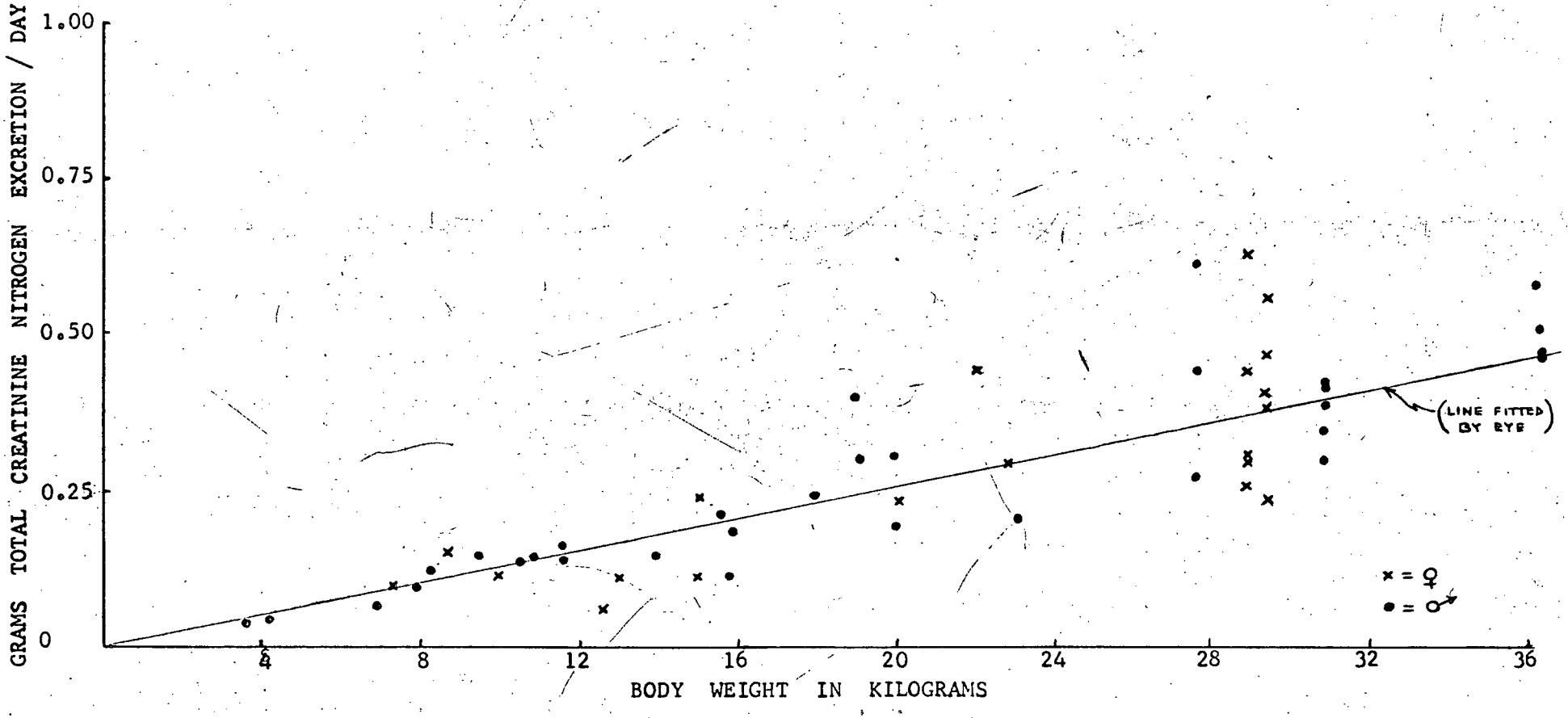


Fig. 11. The cumulative change in total creatinine excretion with increase in body size, in growing black-tailed deer.

Conclusion

The crude protein requirement of R-5 has been calculated, on the basis of the experimental value obtained for the endogenous level of total urinary nitrogen excretion to be approximately 25 grams. This value is taken as the most accurate estimate of the minimum protein requirement, for a protein of perfect biological value and 85 per cent digestibility, for a representative of the species. The values were obtained on a 100 pound doe and, therefore, comparative values from a male of the species would be of interest. The crude protein requirement based on the point of nitrogen balance indicates a much larger requirement. This latter estimate is taken as a representative value for the maintenance of ideal nutritional status with regard to protein nutrition.

Although there are limitations to the nitrogen balance method as a method of determining nitrogen requirements, the results are of considerable value because they are based on direct measurements of the character and extent of nitrogen metabolism in an experimental animal. Measurements on the character of nitrogen metabolism on an animal such as the one used in this experiment are of particular significance because they are related to the extent of nitrogen turnover associated with an animal which has a large amount

of protein reserves. They are indicative of the extent, and character of nitrogen intake required to produce a high plane of protein nutritional status in a similar animal. This requirement may be stated in quantitative terms once the degree of similarity between the experimental animal, and the second animal under consideration, has been thoroughly established. Therefore, it is possible in principle to extend the results obtained from experiments such as the present one to animals under range conditions,,provided the relationship between the experimental animal and the game animals is properly understood.

Measurements of nitrogen excretion made during the growing period have been found, in this experiment, to be indicative of the nature of nitrogen requirements associated with the rate and character of growth. The same application of these results to range animals, as discussed above for trials on adult animals, is theoretically possible.

The measurement of nitrogen excretion under varying conditions of growth, and of metabolic rate, can thus be seen to be of value in providing complementary information to that obtained in field studies, for the greater understanding of the significance of field conditions in terms of the successful management of game animals.

Appendix I

Formulation for University of British Columbia Deer Weaning Ration. No. 36-S-60.

Constituent	Pounds per ton	Pounds per 100
Ground No. 5 feed wheat	660	33.00
Ground oat groats	260	13.00
Ground wheat bran	200	10.00
Ground yellow corn	200	10.00
Fish meal (70%) herring	200	10.00
Soybean meal (50%)	100	5.00
Skim milk powder (spray)	200	10.00
Dehydrated grass	100	5.00
Dicalcium phosphate	10	0.50
Iodized salt	15	0.75
Stabilized animal fat	50	2.50
Vitamin pre-mix	5	0.25
	2,000	100.00

Formulation for University of British Columbia Adult Ration for Deer. No. 36-57.

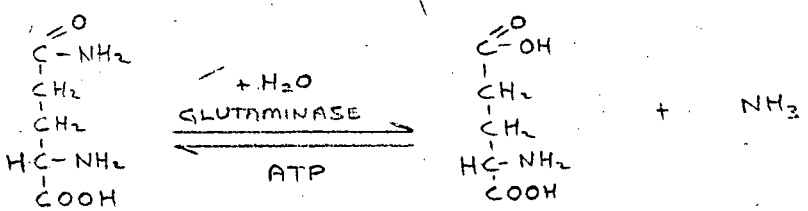
Constituent	Pounds per ton	Pounds per 100
Ground yellow corn	600	30.00
Ground No. 5 feed wheat	250	12.50
Ground wheat bran	275	13.75
Molasses (cane)	150	7.50
Ground beet pulp	200	10.00
Dehydrated grass meal	200	10.00
Soybean meal (50%)	175	8.75
Fish meal (70%) herring	110	5.50
Steamed bone meal	20	1.00
Iodized salt	20	1.00
	2,000	100.00

Appendix II

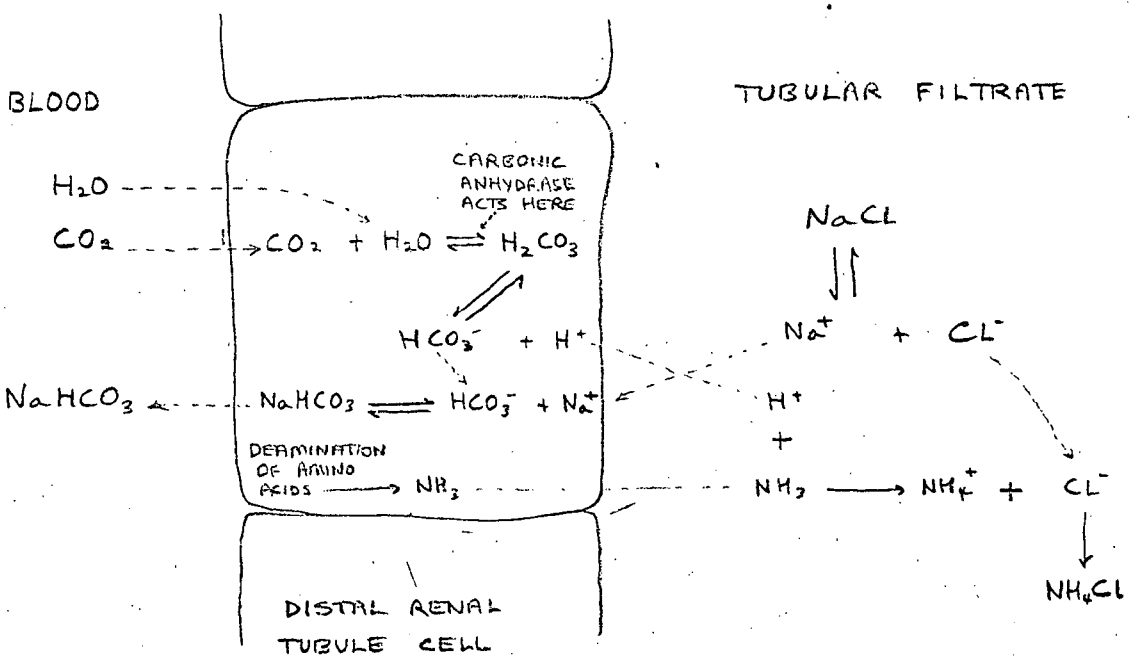
Ammonia formation

The amino acids serve as the source of blood and urinary ammonia. The production of ammonia within the gastrointestinal tract by the action of intestinal bacteria on nitrogenous substances accounts for the high ammonia content of the portal blood. The kidney also produces ammonia and adds it to the blood of the renal vein. Oxidative deamination of amino acids in the liver adds a further fraction depending upon the surplus of amino acids not taken up in protein synthesis. Most of this ammonia is converted to urea within the liver as shown in Appendix III. However, a small remainder and an important portion produced by the kidney tubule cells is excreted into the urine. This process is an important mechanism for the conservation of fixed base, see below.

Glutamine is the most important amino acid source of ammonia from deamination, as follows:

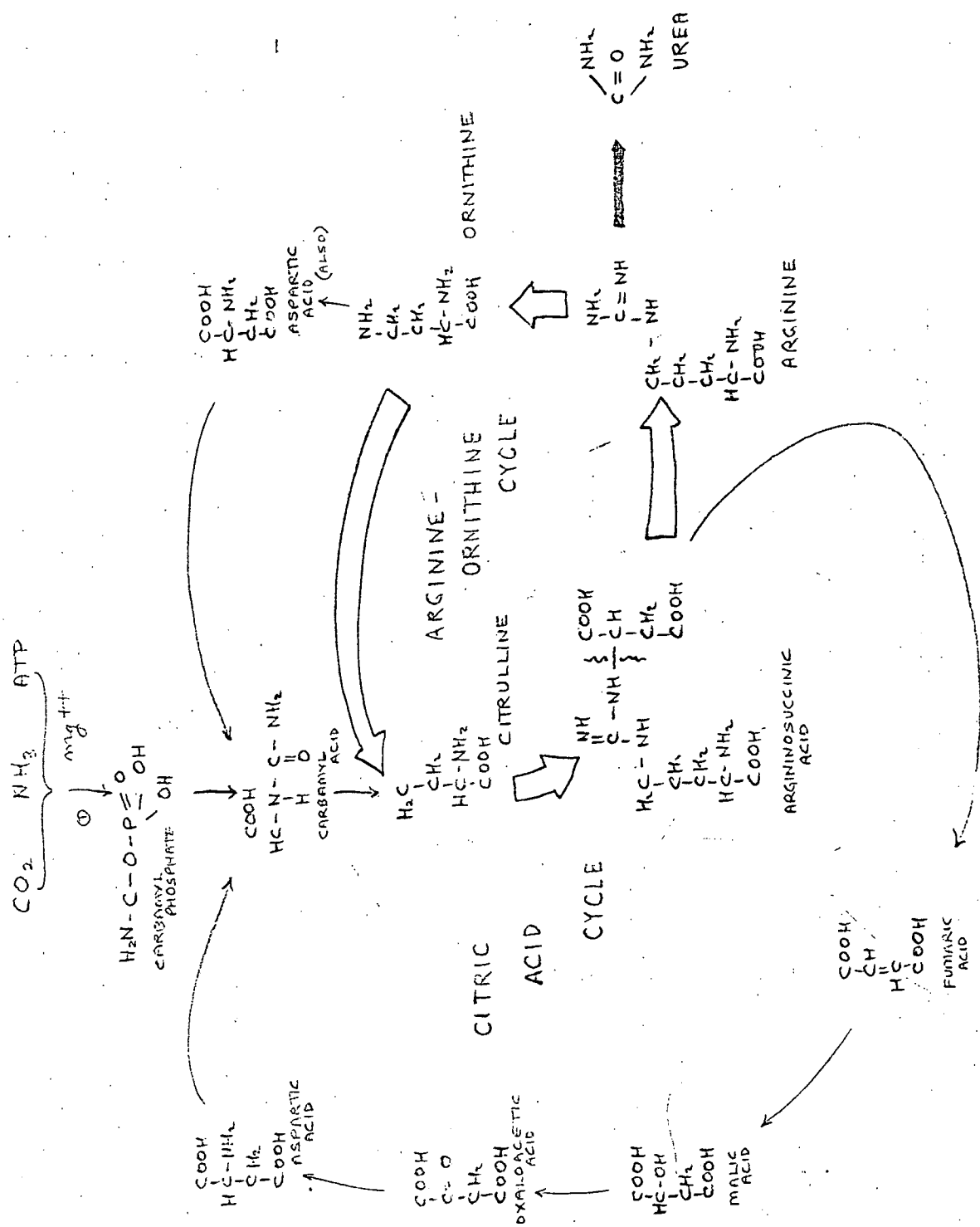


A diagram is shown below of the mechanism for the elimination of hydrogen ions by combination with ammonia within the tubule cell or in the tubular filtrate.


BLOOD
TUBULAR FILTRATE
H_2O
CO_2
CARBONIC ANHYDRASE ACTS HERE
$CO_2 + H_2O \rightleftharpoons H_2CO_3$
$HCO_3^- + H^+$
$NaHCO_3$
$NaHCO_3 \rightleftharpoons HCO_3^- + Na^+$
DEAMINATION OF AMINO ACIDS
NH_3
NaCL
$Na^+ + CL^-$
H^+
+
$NH_3 \longrightarrow NH_4^+ + CL^-$
NH_4Cl
DISTAL RENAL TUBULE CELL

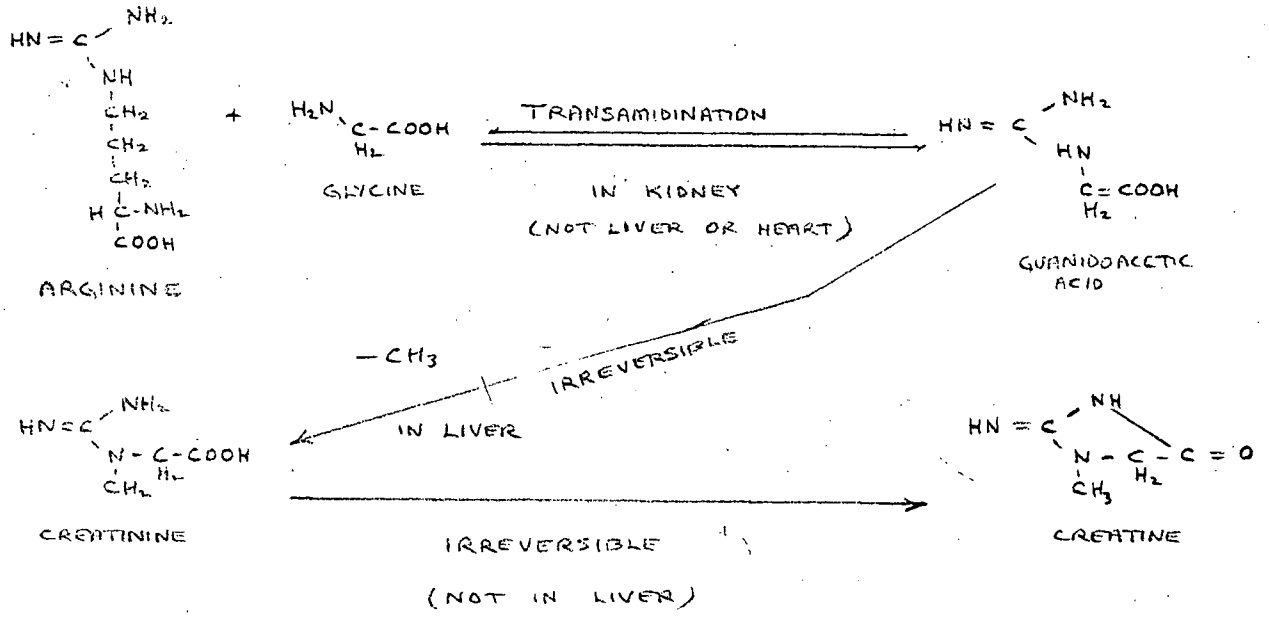
Appendix III.

A schematic representation of the bodily formation of urea is shown on the following page. Blood ammonia enters into the series of reactions which lead ultimately to the formation of urea by combining with CO_2 and phosphate to form carbamyl phosphate. This process represents an activation of ammonia which is necessary in order to enable it to enter the arginine-ornithine cycle. The arginine-ornithine cycle consists of a series of reactions connected with urea formation the most important of which is the integration with a segment of citric acid cycle. This integration provides a catalytic return of aspartic acid, formed during the formation of arginine, to the beginning of the cycle, where it reacts with carbamyl phosphate to facilitate the entrance of the carbamyl group to the arginine-ornithine cycle. The aspartic acid molecule thus acts as a transfer agent for the carbamyl group. There are three other amino acids directly connected with urea formation. These are arginine, ornithine and citrulline. Aspartic acid is formed in two ways in association with urea formation. It is readily available by the transamination of oxaloacetic acid from the citric acid cycle, and is also formed from ornithine. Ornithine and citrulline arise from arginine during the actual formation of urea. The formation of arginine from argininosuccinic acid results in the splitting off of fumaric acid which then enters the citric acid cycle, thus linking the citric acid cycle with the arginine-ornithine cycle.


CO2
NH3
ATP
Mg++
CARBAMYL PHOSPHATE
CARBAMYL ACID
ASPARTIC ACID (ALSO)
ORNITHINE
UREA
ARGININE
ARGININE - ORNITHINE CYCLE
CITRULLINE
ARGININOSUCCINIC ACID
ASPARTIC ACID
OXALOACETIC ACID
MALIC ACID
FUMARIC ACID
CITRIC ACID CYCLE

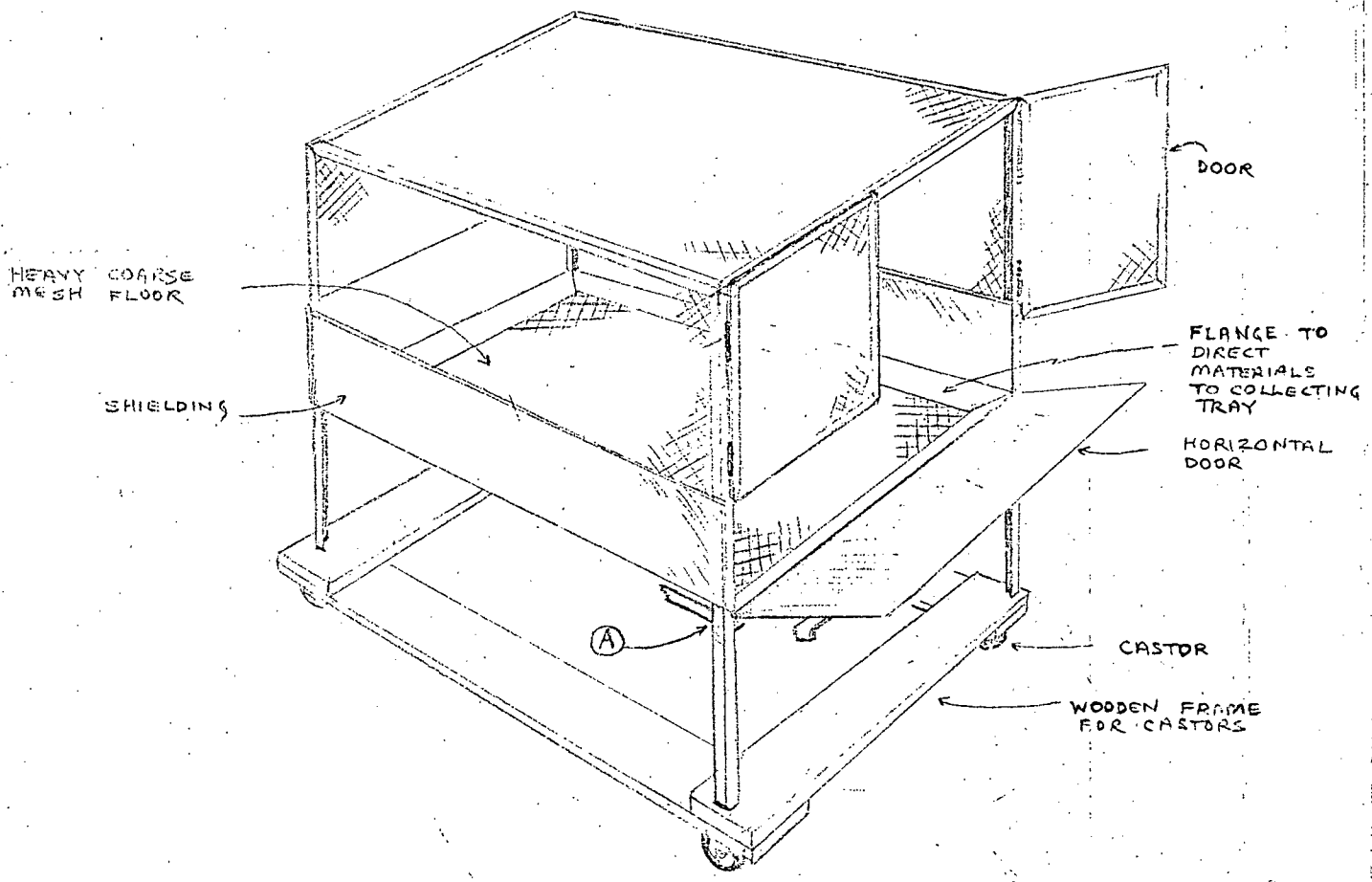
Appendix IV.

Creatinine and Creatine Formation

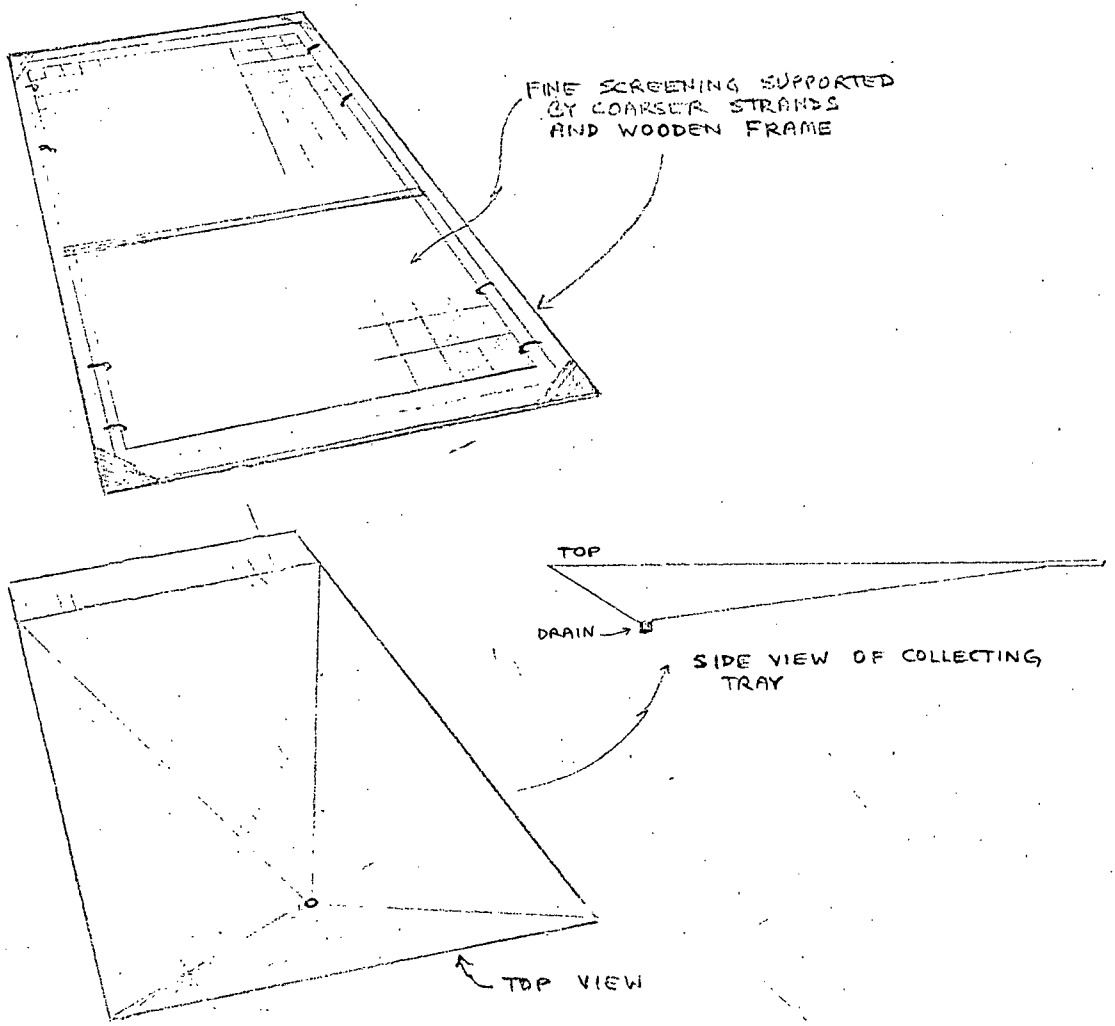


The formation of creatinine and creatine is outlined schematically above. Creatinine is formed from guanidoacetic acid by the removal of a single methyl group. This action takes place in the liver and the creatinine thus formed is transported to the tissues. Creatine is produced in the tissues for the purpose of high energy bond storage, and therefore to facilitate energy metabolism. Because creatine remains where it is formed, and is of value to the tissues rather than being a waste product, it is not as easily lost to the urine as creatinine.

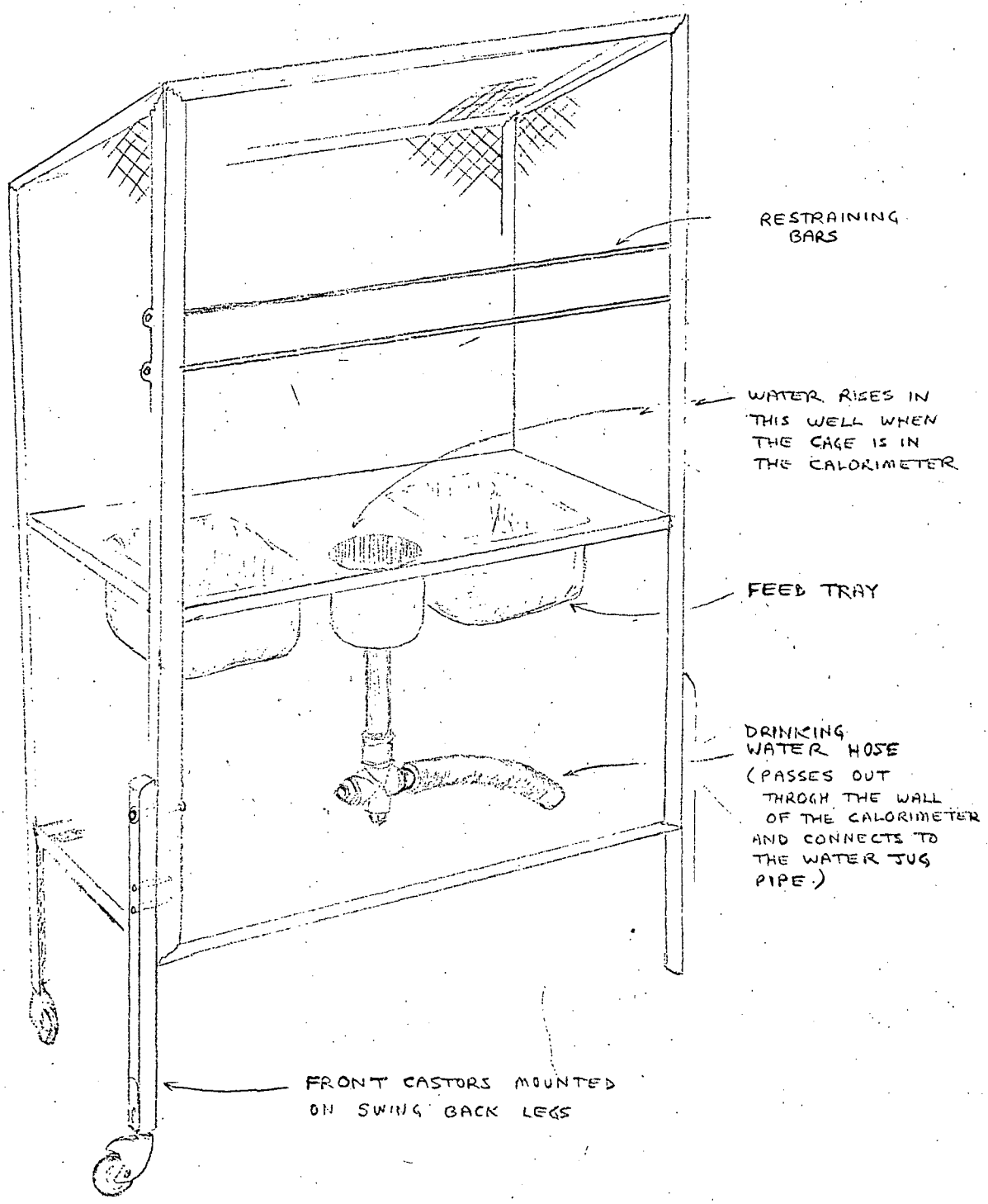
Appendix V. Large Apparati



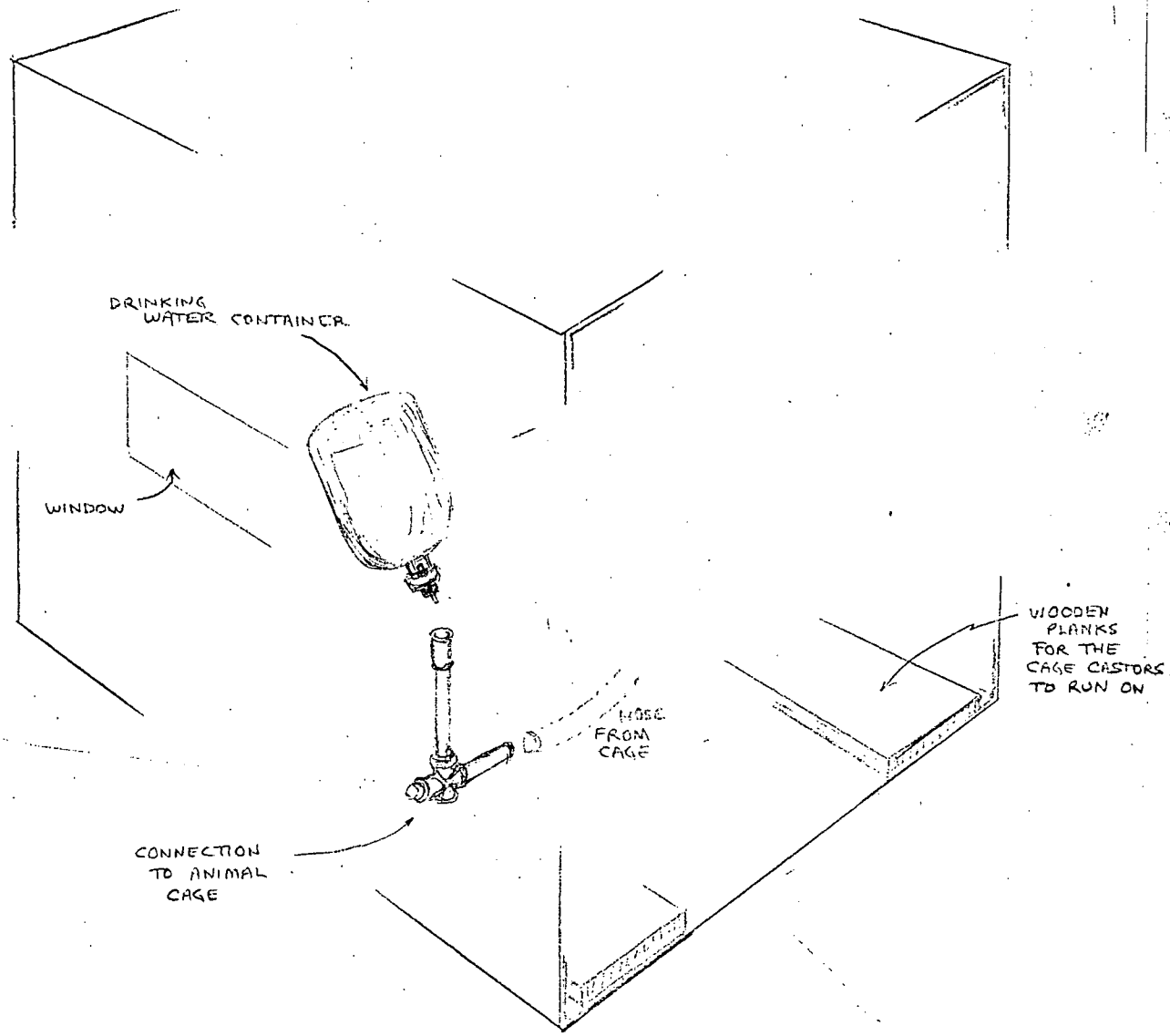
Above is shown a sketch of the cage used to restrain the experimental animals during the metabolic trials. The fecal screen and urine tray were placed on tracks below the floor of the cage, at A. The doors could be shut to restrain the animal while the front of the cage, shown on page 132 was being prepared. The entire cage was placed within a large respiration calorimeter, outlined on page 132a.



The fecal screen, A., and the urine tray, B., are shown in the above sketches.

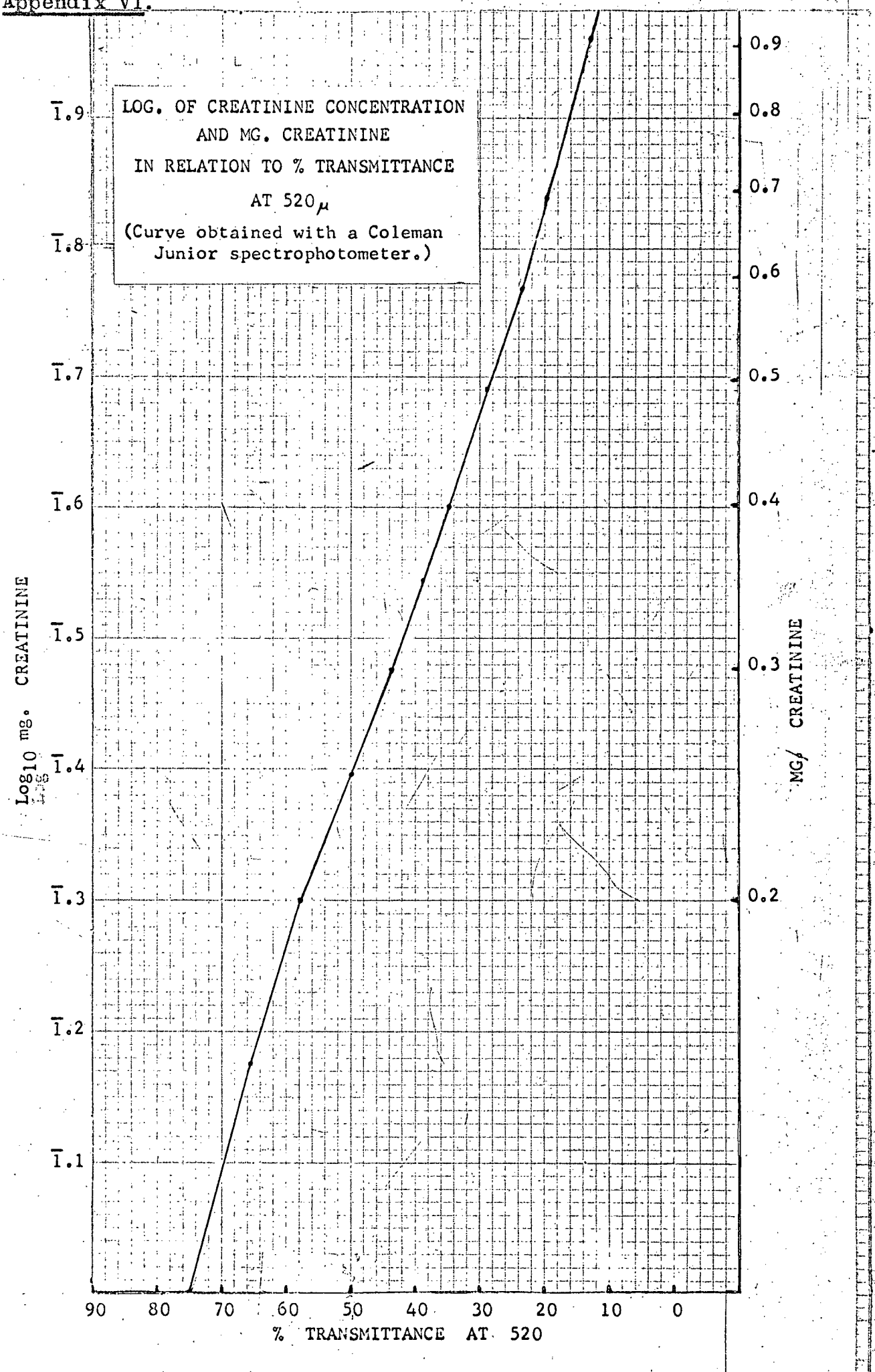


The front end of the animal-cage is shown above. It is fastened to the cage, page 130, during metabolic trials and it supplies water and feed to the animal ad. libitum.



The above sketch is that of an outline of the respiration calorimeter. The cage is moved into the calorimeter on castors which run on the wooden planks so indicated. The water hose from the cage is then attached to the water supply as indicated at A. The levels of water are adjusted such that as the animal drinks, the supply is renewed.

Appendix VI.


LOG. OF CREATININE CONCENTRATION
AND MG. CREATININE
IN RELATION TO % TRANSMITTANCE
AT 520μ
(Curve obtained with a Coleman
Junior spectrophotometer.)
Log10 mg. CREATININE
MG% CREATININE
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
90 80 70 60 50 40 30 20 10 0
% TRANSMITTANCE AT 520

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