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THE TURNOVER OF 75-SELENIUM - SELENOMETHIONINE AS AN INDICATOR OF THE STATUS OF PROTEIN METABOLISM IN REINDEER (RANGIFER TARANDUS)

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THE TURNOVER OF 75-SELENIUM-SELENOMETHIONINE AS AN INDICATOR OF THE STATUS OF PROTEIN METABOLISM IN REINDEER (RANGIFER TARANDUS)

A

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THE TURNOVER OF 75SELENIUM-SELENOMETHIONINE AS AN INDICATOR OF THE STATUS OF PROTEIN METABOLISM IN REINDEER (RANGIFER TARANDUS)

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ABSTRACT

The turnover of a single injection of ^{75}Se-selenomethionine (^{75}SeM), a radio-labeled seleno-analog of the amino acid methionine was used to estimate protein turnover, the irreversible loss of protein nitrogen, in reindeer. ^{75}Se-selenomethionine turnover was measured in nine adult female reindeer grazing on natural forage during winter (November-April) and summer (July- August). ^{75}Se-selenomethionine turnover was two to four times higher during summer than during the winter months. Seasonal changes in ^{75}SeM turnover were believed to be due primarily to seasonal changes in protein and/or methionine intake.

The relationship between the intakes of protein and methionine and the turnover of ^{75}SeM was determined in ten pen-fed reindeer. Reindeer consumed one of three rations containing 3, 11, or 18 percent crude protein. This resulted in daily crude protein intakes of 1.6, 5.1, or 8.2 g per kg $^{0.75}$ b.w. and daily methionine intakes of 0.01, 0.06, or 0.12 g per kg $^{0.75}$ b.w. ^{75}Se-selenomethionine turnover was four times higher for reindeer with high protein and methionine intakes than those reindeer consuming low levels of these nutrients. High positive correlations

were found between ^{75}SeM turnover and the intake of crude protein and methionine.

The method of using ^{75}SeM as an indicator of protein turnover showed a good empirical relation, but application to other biological conditions should be accompanied by calibration trials.

D E D I C A T E D

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LITERATURE REVIEW

Explanation of terms.

There are a few terms used tnroughout this thesis that should be clarified at the beginning. The nomenclature is consistent with that adopted by the International Commision on Radiation Units and Measurements (ICRU) task group (Brownell, et al., 1968).

Fractional Turnover Rate (k). The fraction of tracer leaving the tracee pool per unit time. If isotopic effects are negligible, then the fractional turnover rate of the tracer is equal to the fraction of tracee leaving the metabolic pool per unit time.

Irreversible Nitrogen Loss (INL). That nitrogen eliminated from the body after being released from body proteins via protein degradation reactions.

Reutilization of Amino Acids. The reincorporation of amino acids released during protein degradation into protein synthesis reactions.

Transfer Rate (TR). The amount of tracee entering or leaving the metabolic pool per unit time. This is calculated by the equation TR = k times total metabolic pool size.

An overview of protein turnover.

The early studies by Schoenheimer and Rittenberg (1942) showed that proteins in mammalian cells are continually being degraded and replaced. This process of synthesis and breakdown of body proteins is a major component of nitrogen metabolism in animals (Reeds and Lobley, 1980). Protein turnover is a result of the sum of the irreversible loss rates of protein nitrogen from all of the individual protein compartments in the body (Millward and Garlick, 1972). The rate at which body proteins are turned over is dependent upon the relative rates of protein synthesis, net protein deposition, protein degradation and amino acid reutilization in protein resynthesis (Figure A).

Protein synthesis is usually measured by monitoring the uptake of continuously infused radio-labeled amino acids into various tissues (Simon et al., 1982; Bryant and Smith, 1982; Reeds et al., 1980; Lobley et al., 1980; Nicholas et al., 1977). Protein degradation rate has often been measured as the difference between the rates of protein synthesis and net protein deposition (Millward et al., 1975), which can be determined directly by time series chemical analysis of animal tissues.

Byers (1982) suggests that degradation rate may be a good indicator of protein turnover. The protein degradation rate of a particular protein pool has been measured by following the disappearance of a radio-labeled amino acid from

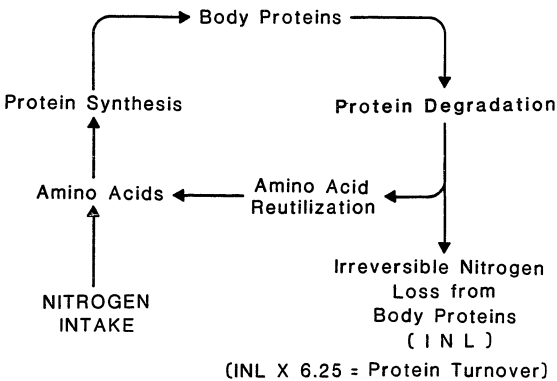

Figure A. Pathways comprising whole body protein turnover.

the pool (Byers, 1982), or by measuring daily excretion rate of 3-methylhistadine (Byers, 1982). 3-Methylhistidine is a non-recyclable metabolite released during myofibrillar protein katabolism. However, Millward et al. (1980) feel that this method does not give an accurate measure of whole body protein turnover because only the degradation of the muscle pool is measured.

Protein turnover has been shown to vary widely between tissue types. For instance, muscle protein is believed to have the slowest turnover while visceral tissues such as kidney, liver and intestine display comparatively high turnover. On the other hand, skeletal muscle is the largest tissue in the body; therefore, whole body protein turnover is mainly a result of protein turnover in muscle (Millward and Garlick, 1972).

Millward et al. (1975) suggest that great care must be taken to correct for errors introduced by recycling of radio-labeled amino acids following their release from degraded proteins. Recycling has been estimated for ^{14}C-lysine at 50 percent in liver, and in muscle at 10 percent to 30 percent (Gan and Jeffay, 1967; Waterlow and Stephen, 1968). However, the degree of recycling may not be the same for all amino acids. Penn et al. (1957) found that seven amino acids yielded seven slightly different half-lifes in serum albumin. It appears that present methods using protein degradation rate to indicate protein

turnover cannot always account for amino acid reutilization. Until recycling can be readily measured, the irreversible loss of a labeled amino acid is most likely the best available measure of protein turnover.

The regulation of protein turnover has yet to be fully understood. It is generally believed that regulation most likely occurs at the sites of protein synthesis, protein degradation or possibly a combination of both (Byers, 1982). Diet may affect whole body protein turnover (Millward and Garlick, 1972; Cahill, 1970; Garlick et al., 1973) as well as stage of maturity or age (Byers, 1982; Reeds et al., 1980) and animal activity (Goldberg, 1969; Laurent and Millward, 1980). The extent to which these factors might influence protein synthesis and degradation rates and ultimately affect whole body protein turnover rate has been the subject of extensive investigation (Goldberg et al., 1980; Reeds and Lobley, 1980; Millward et al., 1976).

The turnover of muscle protein during growth is relatively slow because much of the protein synthesized is structural rather than enzymic and in the growing animal it is incorporated into an increasing muscle protein mass. With increasing age, protein synthesis and muscle growth decline substantially (Waterlow and Stephen, 1968). Whittmore and Fawcett (1976) found that 23 percent of the protein synthesized in early life results in net gain in body protein stores while 77 percent of the protein synthesized

appears to be immediately degraded. As the animal matures, the amount of newly synthesized protein deposited into muscle decreases from nine percent in the 60 percent mature animal to five percent in the 100 percent mature animal.

Protein synthesis rates in muscle of laboratory rats vary in response to protein deprivation and/or starvation (Millward, 1970a). Removal of protein from the diet for 24 hours results in a decline in protein synthesis rate to 50 percent of that found in rats consuming normal protein levels (Millward et al., 1972). In the same study, feeding a protein free ration for 10 days resulted in an even lower protein synthesis rate. During this phase of the study, protein turnover in muscle approached zero. Similarily, Waterlow and Stephen (1967) showed that lysine transfer rate decreased by 30 percent in rats fed protein free rations for extended periods. Cahill (1970) reports that humans starved for prolonged periods undergo a number of metabolic changes, all of which conserve body protein stores. Ultimately, body proteins must be degraded during prolonged starvation to provide essential metabolites. Therefore, increasing protein turnover in starving animals is considered to be an emergency antimortal response.

Changes in diet affect protein turnover; however, the metabolic mechanism is still uncertain. Substantial changes in dietary protein result in small changes in free amino acid concentration at the sites of protein synthesis

(Waterlow and Stephen, 1968). Results show that small changes in free amino acid concentration at the sites of protein synthesis are not highly correlated to dietary levels of the corresponding amino acids (Waterlow and Stephen, 1968). It is unknown if these small changes can trigger observed alterations in protein turnover.

Exercise has been shown to affect whole body protein turnover in various species. Millward et al. (1982) reported that periods of treadmill exercise of three to four hours resulted in a lower whole body protein synthesis rate and increased whole body protein degradation rate. However, protein degradation in muscle actually decreased due to exercise while muscle protein synthesis was maintained at normal levels. Substantial increases in protein degradation and decreased protein synthesis of non-muscle tissues such as liver could explain in part the observed whole body protein turnover. Whole body protein synthesis and degradation rates respond differently in human subjects performing isometric exercise such as weight lifting. Relatively high whole body protein synthesis rates have been measured in weight lifters experiencing muscle hypertrophy while protein degradation rates were lower than sedentary controls (Gudbjaranson et al., 1964).

Protein synthesis rates are primarily limited by the availability of the appropriate amino acids at the sites of transcription in the presence of sufficient energy (ATP)

(Morgan and Wildenthal, 1980). Also the endocrine system is vital for normal regulation of protein synthesis (Millward, 1970b). Recent studies have shown that other factors may influence the rate of protein degradation (Morgan and Wildenthal, 1980). For example, lysosomes contain an array of enzymes and are believed to be instrumental in protein degradation processes. The structure of the proteins themselves together with the availability of enzyme cofactors, most likely influence lysosome function. Hormonal compounds such as insulin, glucagon, corticosteroids and thyroid hormone also appear to be involved in the regulation of protein synthesis and degradation (Morgan and Wildenthal, 1980).

Methodology for measuring protein turnover.

Radio-labeled amino acids such as ^{35}S-methionine and ^{14}C-leucine have been used to label the whole body protein pool in an attempt to measure whole body protein turnover. Use of beta emitters such as ^{35}S or ^{14}C require time series slaughter of animals to determine the whole body irreversible loss rate of the labeled amino acid. Individual differences among animals on the same treatments have been shown to introduce substantial error into time series slaughter experiments (Waterlow and Stephen, 1968). The use of gamma emitting radiolabeled amino acids allows the use of external detection systems to measure whole body burdens of tracer. These systems eliminate the need for slaughter experiments.

Measurement of whole body protein turnover using ^{75}Se-selenomethionine.

Studies conducted twenty-five years ago demonstrated that the seleno analog of methionine could substitute for methionine in protein synthesis of exponentially growing bacterial cells (Cowie and Cohen, 1957). Results from later studies indicated that selenomethionine utilized the same pathways and enzymes as methionine and that the rates of reactions were essentially equal to those of methionine (Hoffman et al., 1970). Other studies have shown that selenomethionine and methionine utilized the same active site of S-adenosyltransferase, the initial enzyme for transmethylation of methionine (Pan and Tarver, 1967). Findings by Ochoa-Solano and Gitler (1968a,b) have indicated that ^{75}Se-selenomethionine (^{75}SeM) is incorporated and subsequently released from tissue proteins at rates similar to the sulfur analog of methionine, ^{35}S-methionine.

There is considerable controversy surrounding the validity of using ^{75}SeM as an indicator of methionine kinetics. Some researchers feel that ^{75}SeM may not always reflect the actual kinetics of body methionine. An unknown amount of ^{75}SeM may be reutilized in the form of selenomethionine, or it is also possible that the ^{75}Se label is reincorporated into de novo seleno-compound synthesis. One study by Millar and Sheppard (1973a) showed that the turnover of an injected dose of ^{75}SeM was altered by the

selenium status of the animal. They compared the distribution and retention of a mixture of ^{75}SeM and ^{35}S-methionine between two groups of laboratory rats. One group was fed a selenium free ration while controls were supplemented with trace amounts of selenium. The selenium deficient group retained ^{75}SeM longer than ^{35}S-methionine. In addition, the distribution of ^{75}SeM in the plasma proteins differed significantly from that of ^{35}S-methionine. In the selenium supplemented rats, no differences between ^{75}SeM and ^{35}S-methionine retention were observed. Similar results were obtained in another study (Holland et al., 1966) using laboratory mice. The whole body turnover of ^{75}SeM was found to be identical in several groups consuming equal amounts of protein and methionine. The supplementation of 0.25 mole of nonisotopic selenomethionine brought about a five-fold increase in turnover, suggesting that selenomethionine and methionine may occupy separate metabolic pools.

Some protein turnover studies have utilized radiolabeled compounds that are not extensively recycled into protein resynthesis. Millward (1970a) compared the protein turnover rates predicted by ^{75}SeM, ^{14}C-arginine and ^{14}C-Na_2CO_3, of which the latter two compounds are thought to be subject to negligible recycling. The results confirmed this for ^{14}C-Na_2CO_3, but slightly higher levels of recycling were observed with ^{14}C-arginine. In contrast, tissues labeled with ^{75}SeM activity did not lose significant

activity in the same time frame. Millward (1970a) concluded that recycling of ^{75}SeM was extensive. Waterlow et al. (1969) compared the turnover rates of injected ^{75}SeM and 15N-glycine in laboratory rats and humans. They concluded that ^{75}SeM was extensively reutilized in protein synthesis and probably was not a valid indicator of whole body protein degradation. However, they did feel that the ^{75}SeM method could yield reliable results concerning changes in whole body protein turnover in response to variations in protein intake.

Despite the potential for reutilzation, several researchers feel that ^{75}SeM can be used to indicate methionine kinetics and provide a good measure for whole body protein turnover (Awwad et al., 1967; Mende and Viamonte, 1965; Yousef and Luick, 1969). ^{75}Se-selenomethionine has been used to determine the relationship between whole body protein turnover and exposure to various environmental, nutritional, physiological and pathological conditions such as: heat and cold exposure (Yousef and Johnson, 1970a,b; Yousef and Chaffee, 1970; Yousef and Luick, 1969); age and rate of growth (Mende and Viamonte, 1965; Yousef and Johnson, 1970a); diet (Yousef and Johnson, 1970b); hormonal effects (Yousef and Johnson, 1970b, 1968); metabolic disorders (Eaton , 1976); trace element status (McConnell and Hsu, 1978; Millar and Sheppard, 1973a); and disease (Penner, 1964a,b).

The present studies dealt with the dynamics of protein metabolism in an arctic herbivore, the reindeer (Rangifer tarandus). All of the ^{75}SeM turnover studies reviewed thus far have dealt with non-ruminant species. Several studies have shown that essentially no pathway exists for non-ruminants for the in vivo synthesis of seleno amino acids from absorbed selenium (Cummins and Martin, 1967; Jenkins, 1968; Scott, 1962). However, Scott (1962) states in his review of selenium metabolism:

> ....there is no doubt that in ruminants a large percentage of the ingested selenium is incorporated by rumen microorganisms into cystine and methionine selenoanalogs which are deposited in their tissues in the form of seleno amino acids.

The bacterial population in the digestive tract of ruminants such as reindeer could potentially introduce error into the results of ^{75}SeM turnover studies.

In a preliminary study investigating the distribution of an injected dose of ^{75}SeM in a single reindeer, six percent of the dose was found in the digestive tract contents five hours post I.V. injection (Blanchard, unpublished observations). ^{75}Selenium activity most likely entered the digestive tract in the form of inorganic ^{75}Se, free ^{75}SeM or as ^{75}SeM reincorporated into new proteins. The saliva could serve as a carrier of ^{75}Se activity to the digestive tract. Although high concentrations of ^{75}Se and/or ^{75}SeM did not appear in the digestive tract contents at a single

point in time, the total amount of ^{75}Se entering the digestive tract was unknown.

Sweeny and Schwarz (1964) found that ^{75}Se ($^{75}SeO_2$) could become non-enzymatically bound to the trichloroacetic acid (TCA) insoluble protein fraction of liver homogenates. In many studies using ^{75}SeM as an indice of protein turnover in tissues, the mere presence of ^{75}Se activity in the protein fraction of these tissues was taken as evidence of incorporation. The latter findings indicate that ^{75}Se activity in protein precipitates may be due to other phenomena.

Ganther (1967) reported that the incorporation of ^{75}Se into organic sulfur compounds can occur by the reaction:

$$4\ RSH + SeO_2 \text{ ----> } RSSeSR + RSSR + 2[H_2O],$$

where the RSSeSR group is known as a selenotrisulfide. Cummins and Martin (1967) demonstrated that the association of sulfur and selenium compounds became stronger with lower pH. Under alkaline conditions (pH> 10), the selenium dissociates from sulfur. Thus, the low pH that would occur in a TCA precipitation would most likely strenghthen the selenium-sulfur association.

The seleno-sulfur association can be disrupted by sulfitolysis. This process involves the incubation of a tissue homogenate with sodiun bisulfite ($NaHSO_3$) prior to TCA precipitation (Awwad et al., 1966a,b; Szentivanyi et al., 1961). Sulfitolysis eliminates that fraction of selenium

non-enzymatically bound to disulfide bonds of protein. Seleno amino acids incorporated into proteins during protein synthesis are precipitated upon treatment with TCA. In a study designed to determine the amount of ^{75}Se activity eliminated by sulfitolysis, Awwad et al. (1966b) found that up to 22 percent of an injected dose of ^{75}SeM was non-enzymatically bound to protein within 10 minutes post injection. After 12 hours, this fraction had been reduced to insignificant levels. Thus an experiment described in Chapter II was designed to test the reliability of using ^{75}SeM as an indicator of methionine turnover. In that experiment the kinetics of simultaneously injected ^{75}SeM and ^{35}S-methionine were compared in the plasma proteins of reindeer. The sulfitolysis method was utilized to insure that only selenomethonine incorporated into the protein structure was measured.

There are several advantages to using ^{75}SeM in protein turnover studies. The use of a gamma emitting radio-label allows the use of whole body gamma counters to measure body burden of the isotope. This eliminates the need for slaughter techniques which are expensive and do not allow the use of an individual experimental animal through a series of measurements. Other advantages of ^{75}SeM are the relatively low cost compared to ^{14}C and ^{35}S labeled amino acids (i.e., one-fifth and the cost) and the short 120-day physical half-life reduces potential health safety problems.

In conclusion, it is believed that ^{75}SeM can provide useful data on the response of protein turnover to changes in nutritional status. For this reason and because of safety and economic considerations, ^{75}SeM was selected as the radio-labeled amino acid most suitable for use in studies of whole body protein turnover in reindeer.

CHAPTER I

Seasonal Changes in Protein Metabolism of Grazing Reindeer

INTRODUCTION

The diet available to indigenous herbivores of circumpolar regions is characterized by a nutrient composition that changes substantially with season (White, 1975). Protein content of the arctic herbivore diet probably fluctuates with season more than any other nutrient. Reindeer (Rangifer tarandus) inhabiting boreal forests and tundra have evolved characteristics enabling them to thrive on a diet that periodically becomes essentially devoid of certain nutrients such as protein. The focus of this study will be on the seasonal changes in protein content of the reindeer diet and how these changes may affect the dynamics of body protein in reindeer. Knowing how the protein metabolism of reindeer responds to changes in protein availability could increase man's understanding of how this specie and possibly other arctic herbivores are especially adapted to northern regions.

In arctic regions, new plant growth begins soon after snow ablation and continues through a very short growing season. During this brief period, an abundance of forage high in nutrients is available to reindeer. The crude protein content of forage during summer ranges from 15

percent in sedges and grasses to as high as 35 percent in mushrooms (Luick, 1977; White et al., 1975; Skogland, 1975). In contrast to summer, the winter diet is somewhat specialized to a variety of lichens that contain only two percent to four percent crude protein (Luick, 1977). Since lichens usually dominate the diet and can account for up to 90 percent of the dry matter intake in winter (Jacobsen and Skjenneberg, 1975), this suggests that reindeer have evolved a minimal protein requirement in winter.

Protein availibility to reindeer in winter is not limited only by the protein content of the diet, but also by feed availability. To obtain feed reindeer may crater in snow that is 0.6 m or more in depth, and this may limit feed intake (Henshaw, 1968). Also during late winter (March-April) snow depths may exceed that which reindeer can successfully excavate for feed (Bergerud, 1974). In addition, encrusted surface snow layers often occur, substantially diminishing access to feed (Stardom, 1975).

The dynamics of whole body protein in reindeer is not well understood. However, variation in the whole body protein turnover in reindeer as a response to changes in nutrition and climate would be consistent with findings for other species. Changes in whole body turnover have been demonstrated in several species exposed to variations in environment or placed on different planes of nutrition (Yousef and Luick, 1969; Yousef and Johnson, 1970b; Millward and

Garlick, 1972; Millward, 1970a,b).

The objectives of the research reported in this chapter were:

1) To determine the feasibility of using external counting methods to measure whole body burdens of ^{75}SeM in reindeer.

2) To determine seasonal variations in methionine transfer rate in free grazing reindeer.

3) To propose how protein metabolism in reindeer may be adapted to improve fitness for life in arctic climates.

METHODS

Climatological Data.

The weather station was located at the McKinley Park, Alaska airstrip (latitude N63° 43', longitude W148° 58'; elevation 631 m), 40 km north of the Cantwell Reindeer Research Station (CRRS). Climatological data from McKinley Park was assumed to be applicable to the field study site located at CRRS. Data was summarized from National Oceanic and Atmospheric Administration records (NOAA, 1978, 1979).

Estimates of snow cover in the grazing paddocks were made by averaging a minimum of six individual depth measurements. These measurements were taken at 15 m intervals along an east-west transect through the middle of each paddock.

Animals and Diets.

Adult female reindeer acquired from a herd on the Seward Peninsula in 1976 were used in five experiments during 1978 and 1979. Table I- 1 shows the sequence of experiments and the reindeer used in each of the five trials.

Two experiments, conducted during early spring of 1978 (early spring, lichens fed - [ES-LKN]; early spring, oats-corn-barley fed - [ES-OCB]), were preliminary in nature

Table I- 1. Time sequence of experiments and reindeer used in preliminary and grazing trials of 1978 and 1979.

DATE	EVENT
Feb 1, 1978	7 reindeer placed in Cantwell paddocks (RD #'s 36, 58, 68, 72, 82, 99, 107)
Feb 1, 1978	Reindeer #'s 77 and 103 placed in feeding pens at Cantwell and fed oats-corn-barley.
Mar 5, 1978	Reindeer #'s 36 and 72 died during early March before beginning of preliminary trials.
Mar 5, 1978	Reindeer #'S 77, 82, 99, 103 transported to Fairbanks. (#'S 82, 99 - fed lichens + Purina Cattle Starter - ES-LKN trial). (#'s 77, 103 - fed oats-corn-barley - ES-OCB trial). All other reindeer remained in Cantwell and fed Quality Texture.
Mar 15, 1978	Beginning of Preliminary Experiments .
Apr 25, 1978	Termination of Preliminary Experiments.
May 30, 1978	Reindeer #'s 58, 68, 99, 107 returned to Cantwell paddocks.
Oct 31, 1978	Began tracer injection of early winter grazing (EW-GRZ) trial with RD #'s 58, 68, 99, 107.
Jan 6, 1979	Terminated EW-GRZ
Jan 20, 1979	RD #107 found dead in paddock
Feb 17, 1979	Began tracer injection of mid winter grazing (MW-GRZ) with RD #'s 58 and 99.
Mar 20, 1979	Terminated MW-GRZ
Jul 30, 1979	Began tracer injection of summer grazing trial (SM-GRZ) with RD #'s 58, 68, and 99.
Sep 1, 1979	Terminated SM-GRZ

designed to determine if ^{75}SeM body burdens could be measured in reindeer using a whole body counting system.

Seven reindeer were placed on alpine pastures on February 1, 1978. This allowed for a 30 day pre-trial period before the beginning of the preliminary trials. Because of heavy snow in the pastures by the beginning of March, 1978, reindeer were removed from the paddocks from March to the end of May, 1978. The preliminary trials were conducted during this time. Two reindeer from this group (RD #99, RD #82) were selected for the ES-LKN preliminary experiment. The diet for these two reindeer consisted of ad libidum levels of hand-picked lichens (2 kg / d) plus a supplement of 1 kg / d of Purina Cattle Starter #1 (percent c.p. = 11.0, Ralston Purina Corp., St. Louis, Mo.). Two reindeer that had been held in feeding pens at the CRRS since February 1, 1978 were selected for the ES-OCB preliminary trial. These reindeer had been consuming ad libidum levels of a diet of 50 percent rolled oats, 25 percent rolled corn and 25 percent barley. The ES-LKN and ES-OCB reindeer were transported to the Institute of Arctic Biology (IAB) and held in outdoor feeding pens for the duration of the tracer trial. The reindeer used in the ES-LKN and ES-OCB were pregnant.

The remaining reindeer (RD's 58, 68, 107) not used in the preliminary trials were held in outdoor feeding pens at the CRRS until they were returned to the grazing paddocks

in late May, 1978. This group of reindeer was maintained on a complete livestock ration, Quality Texture (QTX) (Fisher Mills, Seattle, Wash.). On May 30, 1978, reindeer #'s 58, 68, 99 and 107 were returned to the grazing paddocks and used for the early winter (EW-GRZ), mid winter (MW-GRZ) and summer (SM-GRZ) grazing trials. The reindeer remained in the grazing paddocks until the end of the grazing trials in September, 1979. The diet for these reindeer consisted solely of natural browse from the pastures. During the grazing trials none of the reindeer were pregnant.

Experimental protocol.

For the preliminary experiments, all tracer injections and whole body counting were performed at the IAB. The three grazing experiments were conducted at the CRRS.

On the day prior to tracer injection, the reindeer were herded into holding pens and polyethylene indwelling catheters (Becton, Dickinson and Co., Parsippany, N.J., #PE-190, 1.19 mm i.d.) were placed in each jugular vein. The reindeer were held overnight and fed hand-picked lichens. Reindeer were weighed immediately prior to the tracer injection and reweighed before each whole body count taken during the experiment. Reindeer were returned to the pastures, or the feeding pens as appropriate immediately following whole body counting and blood sampling.

Injections and sampling.

Sterile injectable doses containing 275 μCi (10.2 MBq) of ^{75}Se (as ^{75}SeM) were obtained from Amersham Searle Corp, Arlington Heights, Ill. The 275 μCi doses (2.75 ml) were transferred into syringes and diluted to 10 ml with sterile physiological saline.

Tritiated water, TOH) was obtained from New England Nuclear Corp., Boston, Mass., and diluted with physiological saline to a specific activity of one mCi per ml. The use of TOH for determination of body water space and body water turnover rate has been discussed by Holleman et al. (1982). One mCi of the TOH was transferred to syringes and diluted with physiological saline to a total injection volume of five ml.

Immediately prior to injection of the tracers, pre-injection blood samples were drawn into heparinized tubes from the left catheter. The injection syringes were connected to the right catheter by a three-way valve. The ^{75}SeM and the TOH doses were injected through the same catheter. The right catheter was removed following the injection of the tracers and all subsequent blood samples were drawn from the left catheter.

Sixty to ninety minutes following injection of the tracers, the reindeer were whole body counted for ^{75}Se activity using a lead shielded NaI (TI) crystal detector (6.5 cm diameter) and a portable single channel analyzer/ scaler

(Ludlum Measurements Inc., Sweetwater, Tx., Model #20-A). Reindeer were positioned next to the detector in a stanchion (Figure I- 1), using a method similar to that described by Holleman et al. (1975). The ^{75}SeM body burden for a particular post injection time was estimated from the mean of five consecutive one-minute counts. Reindeer were whole body counted twice on day zero, once daily for the next three days, and then approximately once each week until one-half of the initial body burden remained. A 150 μCi (5.6 MBq) ^{75}SeM reference source was attached to the stanchion and counted prior to all whole body counts. Whole body counts were expressed as a fraction of those of the reference source, thus correcting for instrument and geometrical variability between different counts as well as correcting for the physical decay of ^{75}Se. Background counts were taken immediately prior to whole body counting and again after reindeer had been removed from the room.

Blood samples, taken immediately after each whole body count, were divided into two five ml aliquots. One was used for analysis of ^{75}SeM specific activity in whole blood and the other was analyzed for ^{75}SeM and TOH in plasma. Haematocrit was determined from whole blood. Blood and plasma samples were stored in glass vials at -10 ^{o}C until analyzed.

Analytical methods.

Two one ml aliquots of each whole blood and plasma

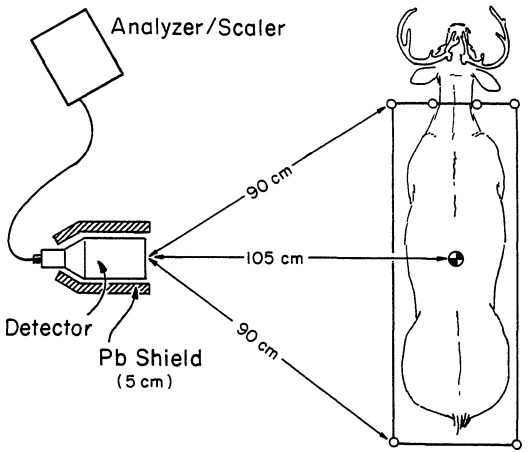

Figure I- 1. Geometry of whole body counting system.

sample were pipetted into glass gamma counting tubes. ^{75}Se-selenomethionine specific activity was measured with an automatic gamma well counter (Searle Analytic Inc., Des Plaines, Ill.). Samples were counted with ^{75}SeM standards, which consisted of one ml of a 1:5000 dilution of the ^{75}SeM stock solution.

Plasma aliquoted for TOH analysis was lypholized and the resulting water from plasma was then analyzed for tritium specific radioactivity using a Beckman LS 7500 liquid scintillation system (Beckman Instruments, Inc., Irving, Ca.). One ml of plasma water was placed in a scintillation vial with five ml of an aqueous scintillation cocktail [9/4 triton-X100 (Amersham, trademark of Rohn and Haas, Inc., Philadelphia, Pa.) / toluene; 5 g/l PPO (2,5-diphenly- oxazole); 0.1 g/l POPOP {1,4-bis(2-{5-phenyl-oxazoyl}) -benzenel}]. Variability in quenching was automatically indexed with an "H" number system. The "H" number was obtained by external standardization with a Cs137 external source (Beckman Operators Manual, 1979).

Calculations - ^{75}SeM body burdens.

^{75}Se-selenomethionine body burden with respect to time following a single tracer injection was characterized by a multiexponential relationship, namely:

$$\ln A = \sum_{i=1}^{n} \ln A_i + k_i t$$

where i is the number of components in the exponential relationship, A is the body burden at time t after injec-

tion, A_o is the intercept of the i^{th} component, and k_i is the slope or the fractional turnover rate.

The biological half-life ($T_{1/2}$) is the time in days to eliminate 50 percent of the injected dose of tracer. The $T_{1/2}$ is a function of the fractional rate constant and is calculated by the equation:

$$T_{1/2} \text{ (days)} = \ln 2 / k = 0.693 / k.$$

Blood volume (BV) was assumed to be eight percent of body weight. Red blood cell volume (RCV) was calculated by the equation:

$$RCV = (BV)\ (\text{Fractional Haematocrit})\ (0.97)$$

where 0.97 is a correction factor for trapped plasma in the red cell pack (Hodgetts et al. 1959).

Calculations - Total body water volume and transfer rate.

The equation

$$\ln A = A_o + kt$$

describes the first order exponential decline found in plasma TOH activity. The biological half-life for injected TOH was calculated using the formula described above.

Total body water (TBW) was calculated from the equation:

$$TBW \text{ (liters)} = I / A_o$$

where I is the equilibrium injected dose in dpm and A_o is the equilibrium specific activity in dpm/ml. The equilibrium specific activity was equal to the extrapolated t_o specific activity.

Water transfer rate (WTR) is that amount of body water entering or leaving the total body water pool per unit time (Holleman et al., 1982). The WTR is derived by the equation:

$$WTR = K\ (TBW).$$

Simulation Modeling.

A computer program, Simulation Analysis and Modeling (SAAM27) was used to solve a three compartment model which was proposed to describe the kinetics of methionine metabolism for grazing reindeer (Berman and Weiss, 1978). The ^{75}SeM retention data from whole body counting as well as the ^{75}SeM concentration in plasma and red blood cells were used as input data for the model. The SAAM 27 program calculated fractional turnover rate for all flow vectors between the compartments and out of the system.

Statistical Methods.

The least squares linear regression method was used to describe tracer retention as a function of time following a single injection. Significant differences between means were determined by the Students t test. Significance among fractional turnover rates were determined by covariance analysis. Regression lines for each component of multiexponential elimination relationships were formulated by the method of least squares.

RESULTS

Climatological data.

Climatological data from the McKinley Park airstrip weather station is summarized in Table I- 2 . Mean maximum temperatures during the EW-GRZ and MW-GRZ trials ranged between $-3^{o}C$ and $-10^{o}C$. The mean minimum temperature ranged between $-14^{o}C$ and $-23^{o}C$. During the SM-GRZ trial, the mean minimum temperature was $+6^{o}C$ and the average maximum was $+21^{o}C$.

Table I- 3 shows the mean snow depth in the CRRS grazing paddocks on specific dates during the EW-GRZ and MW-GRZ trials. Snow cover increased uniformly up to 0.25m by late November. High winds during the first week of December created snow-drifts which remained until the end of the winter experiments.

Body weights, body water pool size and body composition.

Table I- 4 shows the changes in body weight of grazing reindeer during the winter and summer. All reindeer lost body weight during the winter grazing trials. The daily rate of loss for the reindeer in the MW-GRZ trial (January-March) was more than three times that for those in the EW-GRZ trial (November-January). This is most likely a

Table I- 2 . Climatological data(2) occuring during the grazing trials of 1978 and 1979.

EXPERIMENT (1)	AVE.MAX °C	AVE.MIN °C	AVE. °C	HIGHEST °C	LOWEST °C	AVE.PRECIP cm	MX.SNOW DEPTH cm
EW-GRZ	3	-14	- 9	12	-29	5.5	78.1
MW-GRZ	10	-23	-16	6	-42	5.5	179.1
SM-GRZ	21	6	12	27	1	11.0	0.0

(1)
EW-GRZ - Early winter grazing trial (Nov-Jan, 1978-79).
MW-GRZ - Mid-winter grazing trial (Feb-Mar, 1979).
SM-GRZ - Summer grazing trial (Jul-Aug, 1979).
(2) Climatological data obtained from National Oceanic and Atmospheric Administration, Environmental Data and Information Services, Climatic Center, Ashville, N.C. (Climatological Data, Alaska, 1978, 1979).

Table I- 3 . Snow cover in Cantwell Reindeer Research Station grazing paddocks during the winter of 1978-79.

DATE	MEAN SNOW COVER cm (1)	COMMENTS/CONDITIONS
OCT 13,1978	5	Uniform Cover
OCT 31,1978	13	"
NOV 3,1978	15	"
NOV 10,1978	20	"
NOV 18,1978	25	"
NOV 25,1978	25	"
DEC 11,1978	46	Drifts 61-119 cm
JAN 5,1979	46	" w/Crust
JAN 29,1979	56	" w/Crust
FEB 15,1979	71	" w/Crust
FEB 28,1979	53	Drifts 30-71 cm
MAR 7,1979	60	" w/Crust
MAR 20,1979	69	" w/Crust
MAR 29,1979	66	" w/Crust

(1) The mean of 6 measurements taken along an east-west transect at 15 m intervals.

Table I- 4 . Changes in body weight of reindeer during the preliminary and grazing trials.

EXPERIMENT	TIME OF YEAR	DURATION (days)	MEAN INITIAL B.W. (kg)	TOTAL CHANGE IN B.W. (kg)	% OF INITIAL B.W.	DAILY CHANGE IN B.W. (kg)
ES-LKN	MAR-APR	49	81.8	-5	-5	-0.08
ES-OCB	MAR-APR	49	84.4	-2	-2	-0.03
EW-GRZ	NOV-JAN	67	80.0	-3	-4	-0.04
MW-GRZ	FEB-MAR	40	83.5	-6	-7	-0.14
SM-GRZ	JUL-AUG	30	91.4	+6	+7	+0.21

ES-LKN - Early spring pen-feeding trial, lichens fed.
ES-OCB - Early spring pen-feeding trail, oats-corn-barley fed.
EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.

result of lower feed availability because of adverse snow conditions. Reindeer grazing on new green growth during the SM-GRZ trial gained weight at an mean rate of 0.2 kg / d.

Changes in total body water (TBW) as measured by TOH dilution are shown in Table I- 5 . Mean TBW volume remained a constant 66 percent of body weight in the EW-GRZ reindeer; however, there was a 2.6 percent decrease in the absolute volume of TBW. During the MW-GRZ trial, TBW declined slightly from an initial 64 percent to 61 percent of body weight. The decrease in actual volume amounted to 4.3 percent. During summer, the TBW of grazing reindeer declined from 70 percent to 64 percent of body weight by the end of the trial.

Regression equations for predicting body composition from in vivo TOH pool size have not been reported for reindeer. Searle (1970) derived equations from slaughter and TOH measurements in sheep. Cameron (1972) obtained reasonable results for reindeer when he applied Searle's equations to his TOH data. Table I- 6 shows the initial and final fat, protein, ash and total body solids content for grazing reindeer. In the EW-GRZ reindeer, loss of body solids accounted for 71 percent of the total body weight loss. Of the decline in body solids for this group, fat accounted for 75 percent while protein remained essentially constant. In contrast, TBW loss accounted for 74 percent of the body weight decline in the MW-GRZ reindeer.

Table I- 5 . Seasonal changes in total body water pool of grazing reindeer.

EXP	RD #	TIME OF YEAR	# OF DAYS	INITIAL TBW (l)	% OF INITIAL B.W.	FINAL TBW (l)	% OF FINAL B.W.	CHANGE IN TBW (l)
EW-GRZ	58	NOV-JAN	67	53	63	63	74	+10
"	99	" "	"	59	68	55	64	- 4
"	107	" "	"	41	67	43	66	+ 2
"	68	" "	"	49	64	37	59	-13
"	MEAN	" "	"		66		66	
MW-GRZ	58	FEB-MAR	40	53	62	52	59	- 1
"	99	" "	"	53	65	48	62	- 5
"	MEAN	" "	"		64		61	
SM-GRZ	58	JUL-AUG	30	69	72	61	60	- 8
"	68	" "	"	65	75	60	60	- 5
"	99	" "	"	59	64	64	67	+ 7
"	MEAN	" "	"		70		64	

EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.

Table I- 6 . Initial and final body composition of grazing reindeer.

EXP	FAT INIT	FAT FINAL	FAT DIFF	PROTEIN INIT	PROTEIN FINAL	PROTEIN DIFF	ASH INIT	ASH FINAL	ASH DIFF	TOTAL SOLIDS INIT	TOTAL SOLIDS FINAL	TOTAL SOLIDS DIFF
EW-GRZ												
(kg)	17.0	15.2	-1.7	10.9	10.6	-0.3	2.8	2.5	-0.3	30.7	28.4	-2.3
(%b.w.)	23.3	20.4	-2.9	15.0	14.2	-0.8	2.9	3.5	+0.6	41.2	38.1	-3.1
MW-GRZ												
(kg)	19.4	17.9	-1.5	11.7	12.1	+0.4	2.9	2.7	-0.2	34.0	32.7	-1.3
(%b.w.)	23.3	22.9	-0.4	14.0	15.4	+1.4	3.5	3.5	0.0	40.8	41.8	+1.0
SM-GRZ												
(kg)	15.0	22.8	+7.8	12.6	13.6	+1.0	3.4	3.4	0.0	31.0	39.8	+8.8
(%b.w.)	16.3	23.2	+6.9	13.8	13.8	0.0	3.7	3.5	-0.2	33.8	40.5	+6.7

Data calculated from TOH pool size using equations from Searle (1970).
FAT (kg) = 0.01 - 1.05 (TOH SPACE) + 0.90 (BOD.WT.).
PROTEIN (kg) = 0.007 + 0.139 (TOH SPACE) + 0.90 (BOD.WT.).
ASH (kg) = -0.08 + 0.04 (TOH SPACE) + 0.01 (BOD.WT.).
EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.

Again body protein content remained constant while body fat decreased by 1.5 kg. In the SM-GRZ trial, increasing body fat stores accounted for 90 percent of the gain in total body solids while body protein accounted for 10 percent. In general, there appeared to be little change in the protein and ash content of grazing reindeer. Body protein was maintained at approximately 14.5 percent of body weight and ash was constant at 3.5 percent. Therefore, in these experiments, substantial net accumulation or loss of tissue protein did not occur. Fat was the most variable constituent, ranging between 16.3 percent and 23.3 percent of body weight.

Retention of ^{75}Se-selenomethionine - whole body.

Whole body counting of reindeer provided estimates of ^{75}SeM body burdens over time. The whole body ^{75}SeM counts for reindeer in the preliminary and grazing trials are shown in Appendix one. With the exception of the EW-GRZ reindeer, the ^{75}SeM retention data indicated that a first order exponential relationship existed between ^{75}SeM body burden and time post injection. For the EW-GRZ reindeer, the whole body retention of ^{75}SeM over time was characterized by a two component exponential relationship. Figure I- 2 (a,b), shows the whole body retention of ^{75}SeM as a percent of dose remaining in reindeer for the preliminary and grazing trials. The k values , Y intercepts, correlation coefficients and calculated biological half-lives ($T_{1/2}$) of the

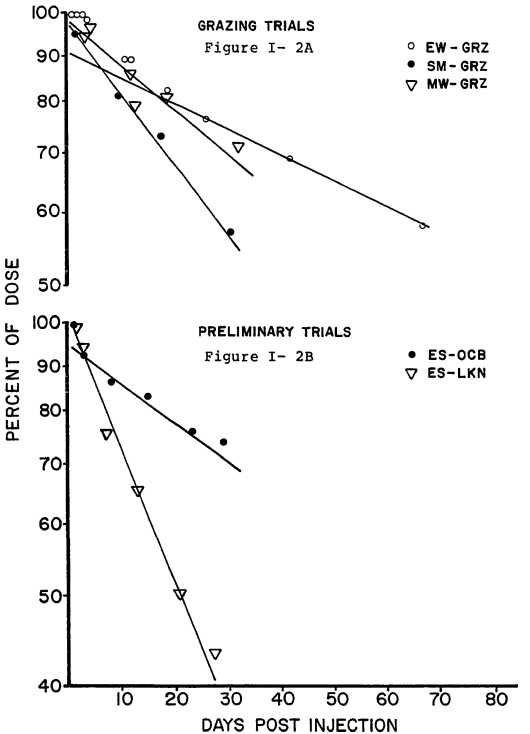

Figure I- 2. Whole body 75Se-selenomethionine retention in reindeer from (A) grazing and (B) preliminary trials.

terminal regression line of whole body ^{75}SeM retention are shown in Table I- 7.

The $T_{1/2}$ was used to compare the retention time of injected ^{75}SeM. Biological half-life varied with season from a high of 79 days for the EW-GRZ reindeer to a low of 36 days for reindeer grazing summer pastures. For the two preliminary trials, $T_{1/2}$ of injected ^{75}SeM was 21 days in lichen fed reindeer (ES-LKN), compared to 69 days for grain fed reindeer (ES-OCB). Covariance analysis indicates that the $T_{1/2}$ for the ES-OCB, EW-GRZ and MW-GRZ trials did not significantly differ, nor did the $T_{1/2}$ for the ES-LKN and SM-GRZ trials. However, the $T_{1/2}$ of the ES-OCB, EW-GRZ and MW-GRZ reindeer were significantly different from those of the ES-LKN and SM-GRZ reindeer.

Retention of ^{75}Se-selenomethionine - Red blood cells and plasma.

Whole blood and plasma drawn after each whole body count were analyzed for ^{75}SeM specific activity. The ^{75}SeM specific activity in red blood cells (RBC) was calculated as the difference between the specific activities of whole blood and plasma. These values are shown in Appendix two. Changes in ^{75}SeM specific radioactivity in RBC'S and plasma are shown in Figure I- 3 (a,b,c) for each of the three grazing trials as a percent of dose remaining.

The decline in ^{75}SeM activity with time in plasma was characterized by a multiexponential relationship.

Table I- 7 . Pooled regression line data of terminal exponential components resolved from whole body 75Se-selenomethionine retention curves of grazing reindeer.

EXPERIMENT	POOLED FRACTIONAL TURNOVER RATE (k)	N	Y INTERCEPT (cpm)	STD ERROR OF MEAN	CORREL. COEF. (r)	BIOLOGICAL HALF-LIFE (d)
ES-LKN	-0.0326	6	52445	89.3	-.98	21.3
ES-OCB	-0.0102	6	47704	65.4	-.91	67.8
EW-GRZ	-0.0088	12	44996	15.0	-.95	78.7
MW-GRZ	-0.0116	10	29671	16.2	-.95	59.6
SM-GRZ	-0.0191	6	56683	37.1	-.99	36.3

ES-LKN - Early spring preliminary trial, lichens fed.
ES-OCB - Early spring preliminary trial, oats-corn-barley fed.
EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.
n = number of observations.

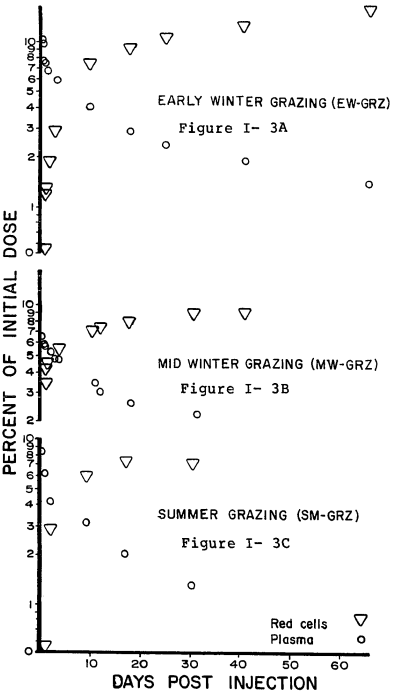

Figure I- 3. 75Se-selenomethionine retention in red blood cells and plasma in reindeer of (A) EW-GRZ, (B) MW-GRZ and (C) SM-GRZ trials.

Table I-8 shows the k-values, Y-intercepts and $T_{1/2}$ of each exponential component. For all three grazing trials, the exponential decline in plasma ^{75}SeM activity consisted of three distinct components. The $T_{1/2}$ of the initial and second components were relatively short, ranging between 0.3 and 0.5 days for the initial and 5.6 and 7.9 days for the second component. The $T_{1/2}$ of the terminal component was considerably longer, ranging from 20 days in the EW-GRZ reindeer to 99 days in the MW-GRZ group.

Changes in RBC ^{75}SeM activity for the grazing trials were characterized by a 20 to 30 day build-up followed by a plateau which persisted until the termination of the trials 30 to 60 days post injection. The increase in ^{75}SeM activity in RBC's was described as a two component exponential relationship (Table I- 9). The fractional turnover rate of the initial component ranged between 13.2 percent (SM-GRZ) and 58.4 percent (MW-GRZ) of the injected dose per day. The fractional turnover rate of the terminal component ranged from 1.6 percent (EW-GRZ) to 14.4 percent (MW-GRZ) of the injected dose per day.

Body water transfer rate.

The results of the TOH turnover measurements for the three grazing trials are shown in Table I-10 . The retention of TOH over time was described as a single component exponential relationship. Mean body water transfer rate (WTR) during the SM-GRZ trial (9.9 l / d) was nearly double

Table I- 8 . Exponential components of the mean 75Se-selenomethionine retention curves in plasma of grazing reindeer.

EXP	INITIAL COMPONENT			SECOND COMPONENT			TERMINAL COMPONENT		
	k	$T_{1/2}$ (d)	Y-INT (dpm)	k	$T_{1/2}$ (d)	Y-INT (dpm)	k	$T_{1/2}$ (d)	Y-INT (dpm)
EW-GRZ	2.165	0.3	9432	0.125	5.6	9476	0.012	57.8	6277
MW-GRZ	2.688	0.3	8219	0.117	5.9	7027	0.007	99.0	4890
SM-GRZ	1.397	0.5	6594	0.095	7.3	1504	0.035	19.8	5375

EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.
$T_{1/2}$ - Biological half-life in days.

Table I- 9 . Exponential components resolved from 75Se-selenomethionine activity build-up curves in red blood cells of grazing reindeer.

EXPERIMENT	INITIAL COMPONENT			TERMINAL COMPONENT		
	FRACTIONAL TURNOVER RATE (k)	Y-INTERCEPT (dpm)	$T_{1/2}$ (days)	FRACTIONAL TURNOVER RATE (k)	Y-INTERCEPT (dpm)	$T_{1/2}$ (days)
EW-GRZ	0.190	6562	3.7	0.016	19227	43.3
MW-GRZ	0.584	5362	1.2	0.144	6670	4.8
SM-GRZ	0.132	5617	5.3	0.054	4987	12.8

Tl/2 - Biological half-life.
EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.

Table I-10 . Total body water turnover in grazing reindeer.

EXP	R D #	TIME OF YEAR	FRACTIONAL TURNOVER RATE (k)	BODY H2O TRANSFER (WTR) (l / d)	BIOLOGICAL HALF-LIFE (d)
EW-GRZ	58	NOV-JAN	0.092	4.8	7.5
	99	" "	0.084	5.0	8.2
	107	" "	0.075	3.0	9.2
	68	" "	0.084	4.2	8.2
	MEAN	" "	0.084	4.3	8.3
MW-GRZ	58	FEB-MAR	0.099	5.3	7.0
	99	" "	0.083	4.4	8.4
	MEAN	" "	0.091	4.9	7.7
SM-GRZ	58	JUL-AUG	0.160	11.0	4.4
	68	" "	0.147	9.5	4.7
	99	" "	0.157	9.3	4.4
	MEAN	" "	0.155	9.9	4.5

EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.

that observed during the winter grazing trials (4.3-4.9 l / d). These WTR observations are comparable to those found for grazing reindeer by Cameron (1972).

DISCUSSION

Protein metabolism in grazing reindeer.

Reindeer apparently protect themselves from the extremes of the arctic winter predominantly by physical means, such as reducing peripheral blood flow and growing a highly insulating haircoat (Hart, et al., 1961). Increasing metabolic rate as a means of thermogenesis occurs in reindeer only at very low termperatures. In fact, lower critical temperature for reindeer has been reported to be approximately -50°C (White, 1979). During winter, the metabolic rate has been found to be approximately 30 percent lower than during summer (McEwan and Whitehead, 1970; Druri, 1970). A depressed metabolic rate during winter when feed is often less available could help to conserve body energy stores. Other results indicate that reindeer have an enhanced ability to conserve other vital nutrients. Wales et al. (1975) found that caribou were able to recycle significantly more urea nitrogen than cattle or sheep when fed low protein feeds during winter. During summer, the proportion of urea recycled was similar for all animals tested.

A reduced protein turnover during winter when feed protein is in short supply could aid reindeer in conserving

body protein. In the present studies, the relatively small ^{75}SeM fractional turnover rate observed during winter (compared to summer) in grazing reindeer may reflect lower protein turnover resulting from depressed protein intake. Protein deprivation has been shown to lower protein turnover in man (Motil et al., 1981). During the SM-GRZ trial, ^{75}SeM turnover rate increased approximately two-fold over winter turnover rates. This is most likely an indicator of increased protein turnover, in part due to higher availability of feed protein.

The highest ^{75}SeM turnover rates were obtained in the preliminary trial where lichens were the predominant feed (ES-LKN). A high protein turnover rate in reindeer consuming a low protein diet, such as lichens would appear to be wasteful of vital body protein. The reindeer of the ES-LKN group were observed to be in very poor body condition due to body weight loss during February. In fact, two reindeer (RD #'s 36 and 72) died of malnutrition in the grazing paddock during February. During this time, increasing snow cover ultimately eliminated access to feed. Protein turnover rates are known to be reduced during the initial periods of starvation (Cahill, 1970). When protein deprivation occurs over prolonged periods, body proteins can be mobolized to meet the immediate need for critical metabolites (Cahill, 1970). Thus, the high ^{75}SeM turnover rate observed in the ES-LKN reindeer may have been a result

of rapid protein katabolism to fulfill essential metabolite requirements.

The reindeer in the grazing trials had whole body ^{75}SeM retention curves that could be characterized by a single exponential (MW-GRZ, SM-GRZ) or a two component multi-exponential (EW-GRZ) relationship. Single exponential relationships between ^{75}SeM whole body burden and time have been reported from other experiments with rats (Thomson et al., 1975a,b) and laboratory mice (Yousef and Luick, 1969). Multi-exponential relationships have been observed in humans (Toohey et al., 1979; Johnson, 1977; Thomson and Stewart, 1973).

Schimke (1970) suggested an explanation for the observed exponential elimination of radio-labeled amino acids from body proteins. He proposed that a major portion of body proteins display first-order (single component) exponential elimination functions. When a single dose of a radio-labeled amino acid is injected into the body, it is rapidly incorporated into newly synthesized proteins. These new proteins then enter a single pool of body proteins that are randomly degraded, yielding a single exponential function.

Single component exponential elimination is observed in the whole body although some proteins, such as those in RBC's, appear to display life-time kinetics. Body proteins characterized by life-time kinetics may as a whole display

exponential elimination of injected radiotracers. Schimke (1970) suggested that newly formed radio-labeled proteins may enter numerous protein pools (e.g., RBC proteins) where each group displays a unique lifespan. The summation of the degradation of these various groups yields an overall elimination function that appears to be exponential.

A possible explanation for the two component exponential elimination observed in the EW-GRZ reindeer may be a variation of the above explanation for single exponential elimination. Instead of one pool of newly labeled proteins, radio-labeled proteins may enter two pools. Waterlow et al. (1969) have suggested that the body may contain two major protein pools, one having a relatively fast rate of turnover and a second characterized by a slow protein turnover rate. If radio-labeled proteins in both of these pools are degraded randomly, a two component exponential elimination function may be observed.

Methionine metabolism in red blood cells and plasma.

The elimination of ^{75}SeM activity in plasma was characterized by a three component exponential function. With the exception of the MW-GRZ trial, the $T_{1/2}$ of the terminal component for plasma ^{75}SeM retention was approximately one-half that from whole body retention. This is most likely a result of plasma proteins being part of a pool of proteins that have relatively rapid turnover rates. The relatively long terminal $T_{1/2}$ displayed in plasma of MW-GRZ reindeer

could be in part due to error introduced by having only three sample points along the terminal component.

Red blood cell (RBC) ^{75}SeM activity over time was characteristic of proteins that display life-span kinetics. Similar results for RBC ^{75}SeM activity have been observed by others (Awwad et al., 1966b; Griffiths et al., 1976; Thomson and Stewart, 1973). In these experiments, RBC ^{75}SeM activity in humans was maintained at a plateau for approximately 100 days, at which time activity declined precipitously. Retention curves such as this are thought to be a result of the life-span kinetics of the red blood cells. The life-span of RBC's has been estimated to be 80 days in antelope, 95 days in mule deer and 235 days in the llama (Berlin, 1964). The results of the present study indicate that the life-span of RBC's in grazing reindeer is greater than 70 days, as the terminal elimination component was not observed before the termination of the grazing trials.

Compartmental simulation model of methionine metabolism.

Simulation modeling can be a useful tool to provide an overall conception of tracee kinetics (Shipley and Clark, 1972). A three compartment model shown in Figure I- 4 was proposed for methionine metabolism.

Simulation models have previously been proposed for whole body protein kinetics based upon results from radiolabeled amino acid tracer studies (Waterlow et al., 1969;

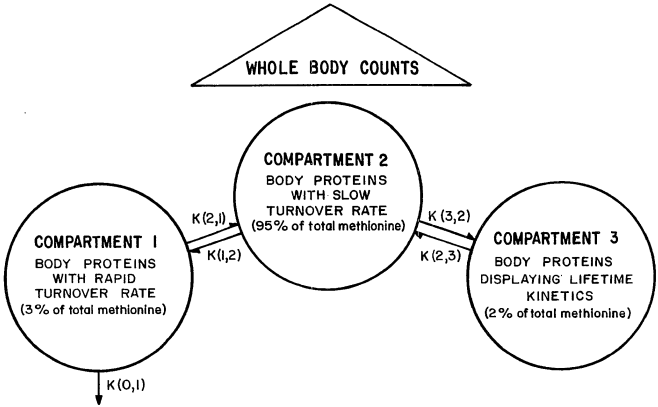

Figure I- 4. Three compartment simulation model for methionine metabolism.

Olesen et al., 1954; San Pietro et al., 1953; Wu et al., 1959). The models proposed in the latter two studies (in order of listing) treated whole body proteins as a single pool. The model proposed by Olesen et al. (1954) was based upon whole body ^{15}N-glycine retention curves, which were resolved into three exponential components. Their four compartment model consisted of an excretory pool, a metabolic pool and two protein pools. The model proposed by Waterlow et al. (1969) was based upon whole body ^{75}SeM retention curves in infants. The retention curves were resolved into two exponential components. Their ^{75}SeM retention data was incorporated into a model consisting of three freely exchanging compartments. All of the above models failed to give realistic solutions for whole body protein kinetics based upon ^{75}SeM whole body retention curves.

The model proposed in the present study was intended to simulate methionine metabolism in reindeer. In this model, three compartments were considered. Compartment one represented methionine in body proteins which display relatively rapid turnover rates. The ^{75}SeM retention data from plasma was incorporated into this compartment. The second compartment contained methionine located in body proteins that display relatively slow turnover such as that observed in muscle proteins. No measured data were taken to represent the kinetics of compartment two. The third compartment represented proteins that display kinetics dictated

by the life-span of the cells they occupy such as red blood cells (RBC). The RBC ^{75}SeM retention curves were taken to represent the kinetics of compartment three. The whole body ^{75}SeM retention data was taken to represent the the total of the three methionine compartments.

The calculated distribution of methionine in the plasma, RBC's and the whole body of reindeer are shown in Table I- 11. The whole body methionine estimates were based upon whole body amino acid analysis in other species which showed that whole body methionine content was 1.75 percent of the whole body protein content (Williams et al., 1954). The whole body protein content was estimated by the equations of Searle (1970) (Table I- 6) and was used to determine whole body methionine pool size.

The methionine content of plasma was derived from amino acid analysis (Table II-12). The methionine content of RBC's was assumed to be 1.75 percent of the protein content 57 percent of RBC dry matter as reported by Davey and Luscher (1967).

In the present model, only one route of excretion of methionine was used, because of the method of tracer administration. Waterlow et al., (1969) found that two major routes, urine and feces, may exist for ^{75}SeM excretion. Human subjects on high protein diets excreted an oral dose of ^{75}SeM predominantly via the feces. In contrast, urinary excretion of ^{75}SeM predominates when the tracer is injected

Table I- 11. Estimated methionine content of the whole body, plasma and red blood cells of grazing reindeer.

EXPERIMENT	WHOLE BODY CRUDE PROTEIN (kg)	WHOLE BODY METHIONINE (g)	TOTAL PLASMA METHIONINE (g)	TOTAL RBC METHIONINE (g)
EW-GRZ	11.4	200.0	0.7	3.4
MW-GRZ	12.5	219.0	0.8	3.7
SM-GRZ	13.8	242.0	0.9	4.1

EW-GRZ - Early winter grazing trial (Nov-Jan, 1978,79).
MW-GRZ - Mid winter grazing trial (Feb-Mar, 1979).
SM-GRZ - Summer grazing trial (Jul-Aug, 1979).

intravenously, regardless of protein intake. In subjects on low protein diets, fecal excretion of an intravenous dose of ^{75}SeM did not exceed five percent of the total over 30 days. Therefore fecal excretion of an intravenous dose of ^{75}SeM in reindeer that are grazing on winter feed appears to be negligible. Analysis of fecal samples collected periodically throughout the winter and summer grazing trials did not contain measurable ^{75}SeM.

Selenium may also be eliminated in expired air as McConnell and Roth (1966) found that significant amounts of ingested selenium (as $^{75}SeO_3$ or ^{75}SeM) were methylated and eliminated via the respiratory gases as dimethylselenide. Respiratory elimination of dimethylselenide is believed to be a detoxification mechanism active in animals suffering from selenium toxicity. The garlic-like odor of dimethylselenide is a symptomatic of animals suffering from selenium toxicity.

McConnell and Roth (1966) also found that the percentage of a dose of ^{75}SeM eliminated via the exhaled gases increased with increasing dose. Up to 36 percent of a dose of ^{75}SeM was eliminated in the first 24 hours following injection. Griffiths et al. (1976) found no detectable ^{75}Se in the exhaled gases of women given 20 μCi of ^{75}SeM.

Appling McConnell and Roth's (1966) data to the present studies with grazing reindeer shows that reindeer receiving 275 μCi of ^{75}SeM, no more than 1.5 percent of the injected

dose should have been exhaled. This is an extremely small error and was concluded that for the present simulation model, a single excretory pathway was appropriate. Methionine leaving the body protein pool could only leave via compartment one. The fractional turnover rate for each flow vector of the three compartment model as solved by the SAAM27 program are shown in Table I-12 . The fractional turnover rate of the excretion vector [k(0,1)] was used along with the methionine pool size of compartment one (three percent of total pool size) to calculate the daily methionine transfer rate from body proteins. The calculated methionine transfer rate was then assumed to be representative of the irreversible loss of all other amino acids. Therefore, a daily irreversible loss of protein nitrogen from body proteins (INL) was calculated for the grazing reindeer.

The daily methionine transfer rate and INL predicted by the three compartment model or the terminal whole body ^{75}SeM fractional turnover rates are shown in Table I-13 . Reindeer would need to have an equal amount of nitrogen and methionine entering their systems through absorption to maintain body protein stores. The body protein content of the grazing reindeer calculated by the equations of Searle (1970) appeared to be relatively constant.

The calculated INL values in Table I-13 were compared to results of nitrogen balance trials conducted on reindeer

Table I-12 . Fractional rate constants of flow vectors in the three compartment model resulting from the best solution to 75Se-selenomethionine retention data.

EXPERIMENT (1)	k(0,1)	k(2,1)	k(1,2) --- (2) ---	k(3,2)	k(2,3)
EW-GRZ	.265	.092	.015	.006	.008
MW-GRZ	.330	.122	.018	.006	.004
SM-GRZ	.603	.060	.018	.001	.000

(1)
EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.
(2)
Flow vectors corresponding to Figure II- 5. Data represent the fraction of methionine in a particular compartment leaving via a particular flow vector (per day).
k(0,1) Flow from compartment 1 to outside of system (excretory).
k(2,1) Flow from compartment 1 to compartment 2.
k(1,2) Flow from compartment 2 to compartment 1.
k(3,2) Frow from compartment 2 to compartment 3.
k(2,3) Flow from compartment 3 to compartment 2.
k(0,2) and k(0,3) were flow vectors leaving the system (excretory) that were also tested in various forms of the 3 compartment model.

Table I-13 . Estimated methionine and nitrogen excretion from the body tissues of grazing reindeer.

	EXCRETION IN GRAMS PER DAY			
	DERIVED FROM: WHOLE BODY COUNTING OF 75SEM		DERIVED FROM: THREE COMPARTMENT MODEL	
EXPERIMENT (1)	METHIONINE	PROTEIN N	METHIONINE	PROTEIN N
EW-GRZ	1.3	11.9	0.2	1.8
MW-GRZ	2.6	23.8	0.3	2.7
SM-GRZ	4.6	42.1	0.5	4.6

(1)
EW-GRZ - Early winter grazing trial (Nov-Jan, 1978-79).
MW-GRZ - Mid-winter grazing trial (Feb-Mar, 1979).
SM-GRZ - Summer grazing trial (Jul-Aug, 1979).

by Jacobsen and Skjenneberg (1975). From their data, the regression equation

$$Y = 1.37 X - 4.23$$

was formulated, which predicts daily nitrogen intake (Y, g) from known daily nitrogen excretion (X, g/d).

Although INL is only a portion of total nitrogen excretion, applying the INL values shown in Table I-13 could yield the amount of nitrogen needed to maintain body nitrogen stores. The predicted daily nitrogen intake for reindeer to maintain body stores based upon the INL values as predicted by whole body counting and the model are shown in Table I-14 .

The estimated daily nitrogen intake derived from daily INL (0.7 - 1.8 g N / kg $^{0.75}$ b.w.) is comparable to nitrogen intake values reported by others. Daily nitrogen intake for reindeer feeding on lichens has been estimated to range from 0.2 - 1.3 g N / kg $^{0.75}$ b.w. (Hansen et al., 1975; Holleman et al., 1979).

The predicted daily nitrogen intake of reindeer grazing on summer pastures was 1.8 g N / kg $^{0.75}$ b.w. This is comparable to values reported for captive caribou (1.5 g N / kg $^{0.75}$ b.w. (White, 1976).

The daily nitrogen intake needed to maintain body N stores, based on INL values from the model are unrealistic. For example, based upon these intake predictions, reindeer in the EW-GRZ trial did not require any nitrogen input

Table I-14 . Predicted daily nitrogen intake of grazing reindeer based upon whole body counting of 75SeM and a three compartment model.

	PREDICTED NITROGEN INTAKE BASED UPON: WHOLE BODY COUNTING		BASED UPON: THREE COMPARTMENT MODEL	
EXPERIMENT(1)	(g)	(g / kg $^{0.75}$ b.w.)	(g)	(g / kg $^{0.75}$ b.w.)
EW-GRZ	18.2	0.7	0.0	0.0
MW-GRZ	27.8	1.1	0.1	<0.1
SM-GRZ	51.9	1.8	0.5	<0.1

(1)
EW-GRZ - Early winter grazing trial (Nov-Jan, 1978-79).
MW-GRZ - Mid-winter grazing trail (Feb-Mar, 1979).
SM-GRZ - Summer grazing trial (Jul-Aug, 1979).

to maintain a constant body protein content. Therefore, this three compartment model was rejected because a realistic solution to methionine and protein nitrogen kinetics based upon ^{75}SeM turnover was not found.

Several combinations of the three compartment model were tested. When excretion pathways were added (in addition to k(0,1)) from compartment three [k(0,3)], or from the slow turnover compartment [k(0,2)], the solution (i.e., best data fit) resulted in the same predicted methionine transfer rate.

Characterizing body methionine pools as rapid turnover, slow turnover or life-span kinetics may be incorrect. Methionine most likely occupies numerous metabolic pools, which exhibit an array of turnover rates ranging from very rapid to life-span. Also, separate pools of different size, distribution and kinetics for methionine and its seleno analog could introduce gross errors into a methionine model based upon selenomethionine turnover. The subject of selenomethionine and methionine occupying separate metabolic pools will be discussed in Chapter II.

Adaptive characteristics of reindeer nitrogen metabolism.

The INL rate of grazing reindeer predicted by whole body ^{75}SeM turnover appears to change with season. Winter INL rates in grazing reindeer are substantially lower than those during summer. The seasonal change in feed nitrogen intake could be the predominating factor affecting INL rate.

If this relationship is true, then it would appear that reindeer have a highly developed ability to conserve body protein when intake of this nutrient is low.

Urea recycling is a well known nitrogen conservation mechanism active in ruminants. Results from studies indicate that reindeer are able to recycle urea very efficiently. Hove and Jacobsen (1975) observed that reindeer calves consuming a lichen ration reabsorbed 93 percent of the urea filtered by the kidneys. In contrast, reindeer on a high protein diet reabsorbed only 50 percent of the filtered urea. Wales et al. (1975) found that caribou consuming a low protein ration recycled more urea per unit metabolic size than did sheep or cattle. On summer feeds, caribou, sheep and cattle recycle approximately the same amount of urea per unit metabolic size. Hove and Jacobsen (1975) estimated that urinary nitrogen losses in reindeer on low protein diets may be reduced by up to 50 percent due to urea recycling.

Conservation of body nitrogen would seem beneficial to reindeer grazing on winter pastures. Reduced protein degradation rates are usually observed during periods of protein deprivation (Millward et al., 1972). Reutilization of amino acids in protein synthesis is thought to be an important conservation mechanism, active during periods of protein deprivation (Waterlow et al., 1969). Although protein synthesis and degradation rates are most likely

reduced in reindeer grazing on winter browse, amino acid reutilization may be important in reducing protein turnover. The relatively high availability of feed protein nitrogen in the summer diet of reindeer may eliminate the need for amino acid reutilization. Therefore, the high protein turnover observed in reindeer during summer could be the result of increased synthesis and degradation rates and substantially reduced amino acid reutilization.

Relationship between water turnover and methionine turnover.

Previous results indicate that in reindeer there is a high positive correlation between water transfer rate and nitrogen intake (Cameron, 1972). In the present studies, a high correlation exists ($r=.96$; $p<0.01$) between water transfer rate and the fractional turnover rate of ^{75}SeM (Figure I-5). This may indicate the existance of a relationship between water and protein turnover. It has been suggested that the metabolism of water and nitrogen may be interrelated (Utley et al., 1970). This proposal was based upon the finding that as nitrogen intake increased in cattle, urine volume also increased. The increase in urine volume was most likely required to eliminate the increased amount of nitrogen. In the present study, the low water transfer rates observed in reindeer grazing on winter browse may have been due to low protein intake.

Another explanation for the high correlation between water and methionine (as ^{75}SeM) turnover in grazing reindeer

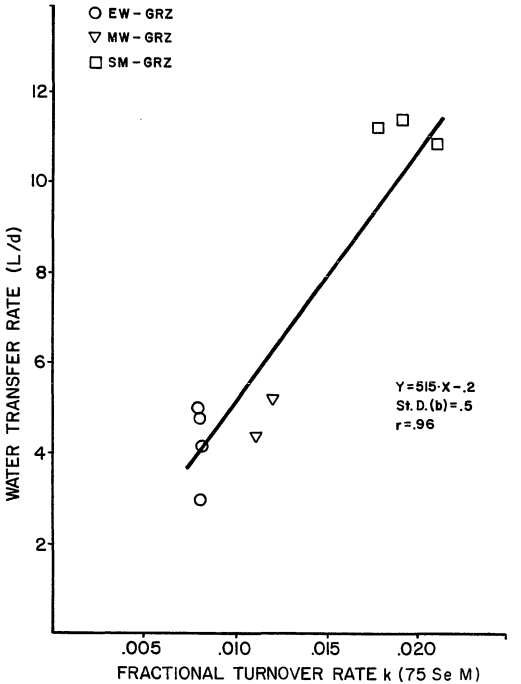

Figure I- 5. Relationship between water transfer rate and fractional turnover rate of 75Se-selenomethionine.

could be in part a result of the intake patterns of these two nutrients. In reindeer grazing on winter browse, water (snow) and crude protein intakes are both at an annual low. In contrast, water and protein are more abundant in the diet in the summer. The high correlation could be due to the relatively parallel rates of intake of these two nutrients.

The relationship between the intake of various nutrients and whole body protein turnover as indicated by ^{75}SeM turnover will be the focus of the experiments discussed in Chapter II.

Summary and conclusions.

The results of ^{75}SeM turnover studies in grazing reindeer indicate the following:

1) ^{75}SeM body burdens in reindeer can be easily estimated by external gamma counting methods.

2) Significant seasonal changes in the turnover rate of ^{75}SeM occur in grazing reindeer. The turnover rate of ^{75}SeM is lowest during the winter, when protein intake is low. This probably reflects decreased protein synthesis and degradation and increased amino acid reutilization in protein synthesis. Higher ^{75}SeM turnover is observed in summer when protein intake is high. This may be due to higher protein synthesis and degradation resulting from a higher protein intake. There may be little need for amino acid reutilzation when protein intake is high.

3) A very high ^{75}SeM turnover is observed in starved

reindeer. This is most likely a result of increased body protein mobilization to provide highly essential metabolites and probably occurs only as an antimortal response.

4) A three compartment model for methionine metabolism utilizing ^{75}SeM retention data failed to produce a reliable solution to the data. Methionine and selenomethionine may occupy separate metabolic pools of different size, distribution and kinetics. ^{75}SeM retention data may only be applicable to the distribution and kinetics of selenomethionine.

5) A high correlation between body water turnover and ^{75}SeM turnover exists.

The turnover of ^{75}SeM may be affected by the intake of certain nutrients. Protein and ash intake is known to vary to extremes with season. The relationship between protein turnover and the intake of various nutrients will be investigated in Chapter II.

CHAPTER II

The Relationship Between Nutrient Intake and Protein Metabolism in Pen-Fed Reindeer

INTRODUCTION

In the previous chapter, a marked seasonal difference in the turnover of injected ^{75}SeM was demonstrated in grazing reindeer. It was proposed that this was an indication of substantial changes in whole body protein turnover. Variables that could alter whole body protein turnover were discussed. Of these, nutritional variables probably have the greatest influence on protein turnover. The seasonal variation in protein availability to reindeer is well known. However, the relationship between protein turnover and protein intake is essentially unknown. The primary objective of the experiments described in this chapter will be to determine the extent of this relationship in reindeer. The relationship between the intake of other nutrients and protein turnover was also investigated. Knowing how protein turnover responds to variation in nutrient intake could provide a better understanding of some of the unique metabolic characteristics of arctic herbivores.

Some of the experiments in this chapter test the validity of using ^{75}SeM as an indicator of whole body protein turnover in reindeer. Simultaneous injection of ^{75}SeM and ^{35}S-methionine in addition to a sulfitolysis

procedure (bisulfite incubation) were methods utilized to evaluate the reliability of using ^{75}SeM to measure protein turnover rates.

METHODS

Animals and diets.

Five rations ranging from three percent to 18 percent crude protein were offered to reindeer in feeding trials conducted during the winters of 1980-81 and 1981-82. Three feeding groups of two reindeer each (see Table II- 1) were formed during the winter (November-April) of 1980-81. Each group received ad libidum levels of one of the following:

Hand picked lichens (LKN8081) consisting of mixed species harvested from the Coal Creek area 15 k south of the CRRS.

Quality Texture (QTX8081), a pelleted livestock ration from Fisher Mills, Seattle, Washington.

Whole grain barley (Hordeum vulgare) (BAR8081), harvested Fall 1979 from the Delta Agricultural Project (DAP), Delta, Alaska.

During the winter of 1981-82, two feeding groups of two reindeer each were formed. Both groups were fed whole grain barley, ad libidum, that had been harvested Fall 1981 from the DAP. One group (B+M8182) received a supplement of 15 g / d of trace mineral salt (American Salt Corp., Kansas City, Mo.) while the other group (BAR8182) received only

Table II- 1. Isotope injection data and experimental protocol for pen-feeding experiments.

EXPERIMENT	DURATION OF EXP.	RD#	AMOUNT 75SeM µCi	DATE mo/day	AMOUNT TOH mCi	DATE mo/day	AMOUNT 35S-M mCi	DATE mo/day
LIC-8081	12/29/80- 2/15/81	82	269	12/29	2.0 1.0	12/29 2/15	----	----
	12/29/80- 2/15/81	56	269	12/29 2/15	2.0 1.0	12/29 2/15	----	----
QTX-8081	12/29/80- 3/16/81	99	269	12/29	2.0 1.0	12/29 3/16	----	----
	12/29/80- 3/16/81	58	269	12/29	2.0 1.0	12/29 3/16	----	----
BAR-8081	12/29/80- 4/21/81	7	269	12/29	2.0 1.0	112/29 4/21	----	----
	12/29/80- 4/21/81	68	269	12/29	2.0 1.0	12/29 4/21	----	----

Table II- 1. Continued.

EXPERIMENT	DURATION OF EXP.	RD#	AMOUNT 75SeM µCi	DATE mo/day	AMOUNT TOH mCi	DATE mo/day	AMOUNT 35S-M mCi	DATE mo/day
BAR-8182	1/12/82-4/08/82	99	80	1/12	---	----	1.05	1/12
	1/12/82-4/08/82	58	80	1/12	---	----	1.07	1/12
B+M-8182	1/12/82-4/08/82	7	80	1/12	---	----	1.15	1/12
	1/12/82-4/08/82	105	80	1/12	---	----	1.04	1/12

LKN-8081 - Lichen fed reindeer, 1980-81.
QTX-8081 - Quality Texture fed reindeer, 1980-81.
BAR-8081 - Whole grain barley fed reindeer, 1980-81.
BAR-8182 - Whole grain barley fed reindeer, 1981-82.
B+M-8182 - Whole grain barley plus trace mineral salt, 1981-82.

whole barley. The level of salt supplementation in the B+M8182 group provided a level of sodium approximately equal to that of the QTX8081 group from the previous winter.

All reindeer had been fed whole grain barley for 280 days prior to the first three experiments in November 1980. During this period, the reindeer increased in body weight as a group by 35 percent. This resulted in an apparent excellent body condition.

Commencing November 1, 1980, six reindeer were separated into the LKN8081 (RD #'s 56,82), QTX8081 (RD #'s 58, 99) and BAR8081 (RD #'s 7, 82) feeding groups. All reindeer were held in adjacent individual outdoor feeding pens of equal size. Therefore, the only obvious difference between groups was diet. The BAR8081 reindeer were continued on the same barley ration that had been fed for the previous 10 months. The QTX8081 and LKN8081 groups were gradually adapted to their respective diets over a 30-day period. These reindeer were then maintained on these diets for an additional 30 days of pretrial.

On November 5, 1981, two barley feeding groups, BAR8182 (RD #'s 58, 99) and B+M8182 (RD #'s 7, 105) were formed. Trace mineral supplementation of the B+M8182 reindeer commenced at this time. The reindeer had been consuming ad libidum levels of a barley ration for the previous 60 days. As in the feeding trials of the previous winter, reindeer were placed in adjacent outdoor feeding pens so diet would

be the only apparent difference between groups.

For all five feeding trials during 1980-81 and 1981-82, snow was provided as the only source of water.

Body weights, daily feed intake and climatological data.

For all five feeding groups, daily feed intake was measured. Feed refused at the end of each 24 hours was weighed and remixed with the feed offered for the next 24 hour period. The amount of feed offered each day was continuously adjusted to minimize weigh-back. Body weights were taken approximately once each week during the pretrial. During the tracer trial, body weight was measured immediately prior to each whole body count. Climatological data from the University of Alaska Agricultural Experiment Station, in Fairbanks, Alaska, was summarized from National Oceanic Atmospheric Administration records (NOAA, 1980, 1981, 1982). Temperature at this weather station (1 k west of feeding pens) was assumed to be the same as that at the feeding pens.

Injections and sampling.

Tracers were injected according to the experimental protocol shown in Table II- 1 . In 1980-81 (LKN8081, QTX8081, BAR8081), reindeer received a simultaneous injection of 269 μCi of ^{75}SeM and two mCi of TOH at the beginning of the experiment. A second one mCi injection of TOH was given at the termination of the experiment. The initial TOH injection provided data for total body water volume (TBW)

and total body water transfer rate. Only TBW was measured from the second TOH injection. Doubling the initial TOH dose compared to the grazing trials facilitated the TOH analysis in plasma samples taken towards the end of the experiments.

The tracer injections in 1981-82 (BAR8182, B+M8182) consisted of a simultaneous injection of approximately (see Table II- 1) one mCi of ^{35}S-methionine (^{35}SM) and 80 uCi of ^{75}SeM. This resulted in an average ratio (^{35}SM:^{75}SeM) of radio-activity in the injected doses of 13.5 : 1. The relatively high level of ^{35}SM was used to insure good counting efficiency of ^{35}S.

The whole body counting and blood sampling protocol was essentially the same as that used in the preliminary and grazing experiments. In the present experiments, only plasma was collected from blood samples. Plasma samples were placed in glass vials and stored at -10^{o}C until analyzed.

Analytical procedures and calculations.

For all experiments, ^{75}SeM body burdens were measured using the same NaI crystal detector and scaler/analyzer described in Chapter I. The analytical methods for ^{75}SeM and TOH in plasma samples as well as calculations for ^{75}SeM and TOH turnover were all the same as those used for the grazing experiments.

Analysis of ^{75}SeM and ^{35}SM in plasma and plasma proteins. For the 1981-82 experiments, frozen plasma was

thawed, aliquoted and analyzed according to the flow chart shown in Figure II- 1 . Measurement of ^{75}Se specific radio-activity in plasma and plasma proteins was performed using a Searle model 1195 gamma counter. Plasma samples were aliquoted as shown in Figure II- 1 . Three ml of each plasma sample were divided equally into glass gamma counting tubes. One tube was used for ^{75}Se analysis in whole plasma while the other two were used for ^{75}Se analysis in plasma proteins. Plasma proteins were precipitated in both aliquots using 10 percent cold trichloroacetic acid (TCA). One of the two aliquots had been incubated at 37°C for 30 minutes with 0.1 ml of a 0.05 molar solution of sodium bisulfite ($NaHSO_3$) prior to TCA precipitation. Plasma proteins were then washed two times with five percent TCA, two times with distilled water, freeze-dried and weighed prior to ^{75}Se analysis.

$^{35}Sulfur$ specific radio-activity was measured in both plasma and plasma proteins using a Beckman liquid scintillation counter, model LS7500. Analysis of samples containing both gamma emitting (i.e., ^{75}Se) and beta emitting (i.e.,^{35}S) tracers require that special procedures be used to correct for gamma interference during analysis of the beta emitting tracer. A series of standards made up of plasma spiked with variable amounts of ^{75}SeM were counted in both the gamma and liquid scintillation counters (LSC). The resulting measurements indicated that 56 percent of the

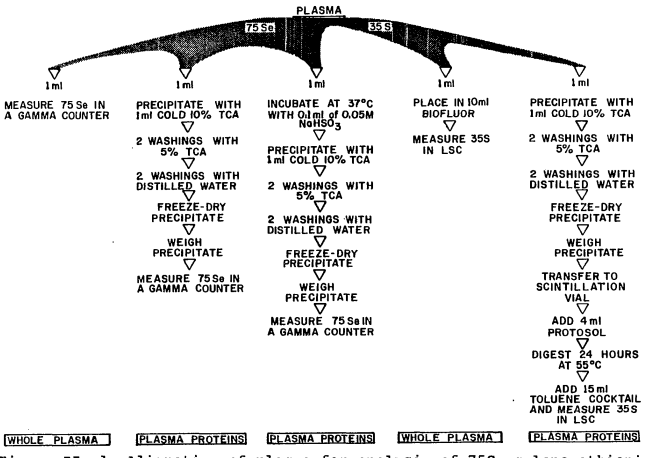

Figure II- 1. Aliquoting of plasma for analysis of 75Se-selenomethionine and 35S-methionine in whole plasma and plasma proteins of pen-fed reindeer.

^{75}Se activity measured in the gamma counter was detected in the LSC. The 0.56 correction factor was assumed to also be applicable to plasma protein samples.

All plasma and plasma protein samples were counted in both the gamma and LSC counters. The ^{35}S activities measured by the LSC were corrected for ^{75}Se interference using the general formula:

$$S\ (cpm) = [\ Y - s_y\ ] - 0.56\ [\ X - s_x\ ]$$

where S is the corrected ^{35}S activity per unit volume (ml) or dry weight (g) of plasma or plasma proteins respectively, Y is the total activity (cpm) per unit weight or volume measured in the LSC , s_y is the background activity of the LSC, X is the total activity (cpm) per unit weight or volume measured in the gamma counter and s_x is the background activity of the gamma counter.

For the analysis of ^{35}S in whole plasma, one ml of plasma was placed into a glass scintillation vial along with 10 ml of Biofluor (trademark of New England Nuclear Corp). The vials were allowed to equilibrate for 24 hours at counting temperature ($+2^{o}C$) to minimize interference from chemoluminesence.

For ^{35}S analysis in plasma proteins, the entire protein precipitate (range 100-130 mg) was transferred to a glass scintillation vial. Transfer was accomplished by placing the precipitation tube (2.2 ml) inside of the counting vial. No additional quenching resulted from the presence of a

precipitation vial inside of a counting vial. The precipitate was redissolved in the counting vial with four ml Protosol (trademark of New England Nuclear Corp.), a quartenary ammonium hydroxide solubilizer. The precipitate was digested in the counting vials, which were tightly capped, and placed in a 55°C water bath for 24 hours. The vials were removed from the bath and allowed to cool before adding 15 ml of a toluene based scintillation cocktail. The cocktail consisted of four g PPO per liter and 50 mg POPOP per liter of toluene. Samples were allowed to equilibrate at counting temperature (+2°C) for 24 hours. Sample ^{35}S activity was corrected for ^{75}Se interference as described above.

Counting efficiency was indexed by external standardization with a ^{137}Cs source using the Beckman H number indexing system. The efficiency curve (Appendix three) for ^{35}S was derived by counting a series of internally spiked (each standard contained 8404 dpm of ^{35}S) plasma standards variably quenched with lysed whole reindeer blood. Plasma was obtained from an uncontaminated reindeer. Counting efficiency ranged between 80 percent and 82 percent in plasma.

Amino acid analysis of plasma and feed. Feeds and pooled plasma from each reindeer were analyzed for amino acid content. Plasma was analyzed for both protein bound and free amino acids. Plasma samples analyzed for free

amino acid content were prepared by oxidizing with performic acid. This procedure was performed prior to hydrolysis to protect methionine from destruction. For the analysis of protein bound amino acids in plasma, proteins and peptides were first precipitated. The dried precipitate was then oxidized with performic acid prior to hydrolysis and analysis.

Amino acid content (total) of the feed was determined after an oxidation and hydrolysis procedure similar to that used for plasma. All sample preparation and amino acid analysis were performed at the Department of Biochemistry, University of California, Davis, California. A Durrum model D-500 amino acid analyzer was used for all analyses.

Statistical analysis.

Methods of statistical analysis were the same as those used in the preliminary and grazing trials described in Chapter I.

RESULTS

Feed analysis.

The composition of the feeds offered in the pen-feeding trials is summarized in Table II- 2 . The crude protein content (percent N times 6.25) ranged from a low of 3.0 percent in hand-picked lichens to a high of 18.1 percent in Quality Texture. Of the three different feeds offered, nutrient content in lichens was lowest with the exception of crude fiber, calcium, manganese, molybdenum, and iron, for which whole grain barley was lowest.

The results of the barley analysis were comparable to the values reported for thresher run barley (National Academy of Sciences, 1971). The major discrepancies were between the selenium (0.050 ppm present vs. 0.415 ppm thresher run) and sodium (0.05 percent present vs. 0.01 percent thresher run) contents.

Analysis of mixed lichens harvested from the same locality as those used in the LKN8081 group have been previously reported by Luick et al. (1971). In general, the results of their analysis were similar to those of the present feed analysis. However, their lichens contained 34.1 percent crude fiber compared to 49.2 percent for those

Table II- 2 . Feed analysis of the diets offered to pen-fed reindeer (dry matter basis).

	BARLEY 8081	BARLEY 8182	BAR+MIN 8182	QTX	LICHENS
Dry Matter %	91.5	89.5	89.5	93.3	65.7
Nitrogen %	1.8	2.0	2.0	2.9	0.5
Crude Prot.%	11.3	12.5	12.4	18.1	3.0
Crude Fat %	1.7	1.4	1.4	3.4	1.0
Crude Fibr.%	7.8	8.0	7.9	7.5	49.2
Ash %	3.2	3.3	4.8	8.8	2.3
Calcium %	0.12	0.13	0.10	1.04	0.17
Phos. %	0.34	0.34	0.33	0.78	0.04
Magnesium %	0.14	0.09	0.09	0.96	0.06
Potassium %	0.52	0.58	0.57	1.07	0.19
Sodium %	0.05	0.03	0.62	0.42	0.02
Sulfur %	0.08	0.08	0.08	0.24	0.08
Manganese PPM		29.3	40.8	115.0	68.3
Copper PPM		4.6	6.4	6.9	0.6
Colbalt PPM		0.2	0.3	1.8	0.2
Zinc PPM		38.0	52.9	107.8	7.6
Iron PPM		71.5	99.6	789.0	190.7
Molybden. PPM		0.7	1.0	2.9	0.3
Selenium PPM	0.05	0.06	0.06	0.57	0.01
Vit.E mg/kg (total atocopherols)	3.0	2.3	2.3	6.5	1.4

Proximate Analysis performed at U of A Palmer Research Station, Palmer, Ak.
Trace element analysis performed at Triple S Labs, Loveland, Colorado.
Vit E analysis performed at Hoffman-LaRoche Animal Research Labs, Nutley, NJ.
QTX - Quality texture, Fisher Mills, Seattle, Wash.

used in the LKN8081 group. The trace mineral content of their lichens was similar to those fed in the present trial with the exception of Mg (0.20 percent present vs. 0.04 percent), Na (0.08 percent present vs. 0.02 percent) and S (800 ppm present vs. 200 ppm). Their lichens were not analyzed for selenium.

The results of the amino acid analysis of feed are shown in Appendix Four. When amino acid composition is expressed as a percent of crude protein (Table II- 3), barley and Quality Texture (QTX) are very similar. Barley is a major ingredient of QTX, which most likely accounts for this similarity in the amino acid composition of protein. Lichen protein contained significantly less ($p<0.01$) GLU, TYR and PHE and significantly more ($p<0.01$) ALA.

Feed and nutrient intake.

Daily feed consumption and temperatures are shown in Appendix Five for all five feeding trials during 1980-81 and 1981-82. The mean temperatures of 1980-81 and 1981-82 are not significantly different ($p<0.01$). The temperature and feed intake summary is shown in Table II- 4. The results indicate that with the exception of the BAR8182 group, feed intake at or below temperatures of $-20^{o}C$ was higher than at temperatures above $-20^{o}C$. The increase in feed intake at temperatures below $-20^{o}C$ was significant ($p<0.01$) for the QTX and lichen-fed reindeer.

Feed intake and temperature data show that consumption

Table II- 3. Amino acid composition of various feeds (% of c.p. w/w) offered to pen-fed reindeer.

AMINO ACID	BAR-8081	BAR-8182	QTX-8081	LKN-8081
ASP	6.27	6.18	7.97	6.93
THR	3.39	2.96	3.34	4.11
SER	4.39	3.53	4.68	4.55
GLU	19.73	17.08	19.88	7.62
PRO	8.90	7.65	5.94	3.51
GLY	4.13	3.49	4.99	4.24
VAL	4.36	3.75	4.38	3.55
MET	1.19	0.95	1.31	0.65
ILE	2.92	2.63	2.95	2.81
LEU	6.17	5.25	6.27	4.55
TYR	3.06	2.47	3.08	1.56
PHE	4.11	3.57	4.27	2.86
HIS	1.93	1.74	2.81	1.34
LYS	3.76	3.28	4.01	3.16
ARG	4.95	4.32	8.32	3.03
ALA	4.43	3.78	4.69	5.41

BAR-8081 - Barley-fed reindeer, 1980-81.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
LKN-8081 - Lichen-fed reindeer, 1980-81.
Bar-8182 - Barley-fed reindeer, 1981-82.

Table II- 4. Mean ambient temperature and mean daily feed intake (dry matter) of pen-fed reindeer.

DIET	O V E R A L L			B E L O W (<) -20°C			A B O V E (>) -20°C		
	TOTAL FEED DAYS	MEAN TEMP °C	MEAN INTAKE (kg)	TOTAL FEED DAYS	MEAN TEMP °C	MEAN INTAKE (kg)	TOTAL FEED DAYS	MEAN TEMP °C	MEAN INTAKE (kg)
BAR8081	121	-15.3	1.14	34	-33.5	1.26	87	-8.2	1.09
± SD			.26			.19			
BAR8182	115	-17.4	1.31	49	-28.0	1.26	66	-9.7	1.34
± SD			.26			.30			
B+M8182	115	-17.4	1.20	49	-28.0	1.22	66	-9.7	1.19
± SD			.34			.36			
QTX8081	120	-15.3	1.39	34	-33.5	1.54	86	-8.2	1.31
± SD			.32			.30			
LKN8081	82	-17.7	1.36	26	-35.1	1.58	56	-9.7	1.26
± SD			.40			.50			

BAR8081 - Barley-fed reindeer, 1980-81.
BAR8182 - Barley-fed reindeer, 1981-82.
B+M8182 - Barley-fed + trace mineral salt, 1981-82.
QTX8081 - Quality Texture-fed reindeer, 1980-81.
LKN8081 - Lichen-fed reindeer, 1980-81.
Change in intake in relation to ambient temperature is significant ($p<0.01$) for QTX8081 and LKN8081 reindeer.

increased for some reindeer as ambient temperature declined. Figure II- 2 shows the regression lines for the relationship between ambient temperature and daily feed dry matter intake for the QTX8081 and LKN8081 reindeer. The LKN8081 reindeer (especially #82) were frequently observed to be shivering at lower temperatures. This may indicate that these reindeer were not in a thermo-neutral status even though ambient temperature did not fall below -42^{o}C. This is contrary to previous findings indicating that the lower critical temperature in reindeer appears to be at or below -50^{o}C (White, 1979). The LKN8081 reindeer may have increased feed intake in response to increased energy demands at lower temperatures. Shivering was not observed in the QTX8081 reindeer. The increased feed intake observed in these reindeer at lower temperatures may have been due to temperature induced changes in appetite.

The daily consumption of individual nutrients excluding amino acids is shown in Table II- 5. The daily individual amino acid consumption is summarized in Table II- 6 . When expressed on a / kg $^{0.75}$ body weight basis, the LKN8081 reindeer had the highest daily dry matter consumption rate. The daily lichen dry matter intake of 1.35 kg / animal is slightly higher than that observed in reindeer (0.8 to 0.9 kg / head) by Jacobsen and Skjenneberg (1975).

The daily intake of all trace minerals ranged from two to 10 times lower for BAR8081, BAR8182 and LKN8081 reindeer

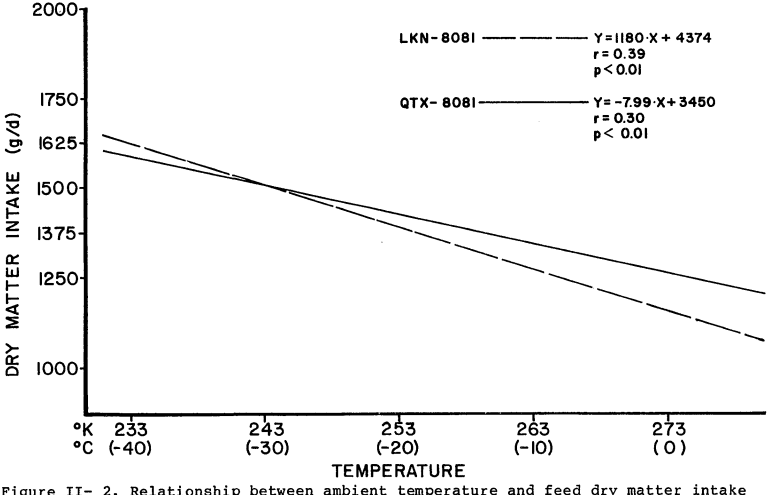

Figure II- 2. Relationship between ambient temperature and feed dry matter intake of lichen- and Quality Texture-fed reindeer.

Table II- 5 . Mean daily intake of nutrients by pen-fed reindeer. (in unit weight / reindeer and unit weight / kg $^{0.75}$ b.w.).

	BAR-8081	BAR-8182	B+M-8182	QTX-8081	LKN-8081
DRY MATTER (kg)	1.12	1.33	1.39	1.45	1.35
(g/kg $^{0.75}$ b.w.)	35	44	47	45	55
NITROGEN (g)	20.1	26.7	27.8	42.0	6.4
(g/kg $^{0.75}$ b.w.)	0.6	0.9	0.9	1.3	0.3
TOT.C.PROT. (g)	125.7	166.6	173.8	262.2	40.2
(g/kg $^{0.75}$ b.w.)	4.0	5.5	5.8	8.2	1.6
CRUDE FAT (g)	19.4	18.4	18.1	49.8	14.1
(g/kg $^{0.75}$ b.w.)	0.6	0.6	0.6	1.6	0.6
CRUDE FIBER (g)		107.1	111.7	108.1	665.4
(g/kg $^{0.75}$ b.w.)		3.5	3.7	3.4	26.8
ASH (g)	35.8	44.2	46.2	127.0	31.5
(g/kg $^{0.75}$ b.w.)	1.2	1.5	2.3	4.0	1.3
CALCUIM (g)	1.3	1.7	1.9	14.9	2.3
(g/kg $^{0.75}$ b.w.)	0.04	0.06	0.05	0.47	0.09
PHOSPHOROUS (g)	3.4	4.5	4.7	11.2	0.6
(g/kg $^{0.75}$ b.w.)	0.11	0.15	0.16	0.35	0.02
MAGNESIUM (g)	1.6	1.3	1.3	8.1	0.8
(g/kg $^{0.75}$ b.w.)	0.05	0.04	0.04	0.25	0.03
POTASSIUM (g)	5.8	7.8	8.1	15.4	2.6
(g/kg $^{0.75}$ b.w.)	0.19	0.26	0.27	0.48	0.10
SODIUM (g)	0.6	0.4	8.7	6.0	0.2
(g/kg $^{0.75}$ b.w.)	0.02	0.01	0.29	0.19	0.01
SULFUR (g)		1.0	1.1	3.5	1.1
(g/kg $^{0.75}$ b.w.)		0.03	0.04	0.11	0.04

Table II- 5. Continued.

	BAR-8081	BAR-8182	B+M-8182	QTX-8081	LKN-8081
MANGANESE (mg)		39.1	91.9	166.4	92.5
(mg/kg $^{0.75}$bw)		1.29	3.08	5.22	3.73
COPPER (mg)		6.1	10.7	9.9	0.8
(mg/kg $^{0.75}$bw)		0.20	0.36	0.31	0.03
COLBALT (mg)		0.3	2.4	2.6	0.3
(mg/kg $^{0.75}$bw)		0.01	0.08	0.08	0.01
ZINC (mg)		50.7	57.2	156.0	10.3
(mg/kg $^{0.75}$bw)		1.67	1.92	4.89	0.41
IRON (mg)		95.4	133.7	1141.7	258.2
(mg/kg $^{0.75}$bw)		3.15	4.48	35.82	10.40
MOLYBDENUM (mg)		0.9	1.0	4.2	0.4
(mg/kg $^{0.75}$bw)		0.03	0.03	0.13	0.02
SELENIUM (mg)		0.1	0.1	0.8	<0.1
(mg/kg $^{0.75}$bw)		<0.01	<0.01	0.03	0.00
VITAMIN E (mg)	3.4	3.1	3.2	9.4	1.9
(mg/kg $^{0.75}$bw)	.11	.10	.11	.30	.08
(total tocoperol)					

Vitamin E was analyzed as total tocopherols.
BAR-8081 - Barley-fed reindeer, 1980-81.
BAR-8182 - Barley-fed reindeer, 1981-82.
B+M-8182 - Barley + trace mineral salt-fed reindeer, 1981-82.
QTX-8081 - Quality Texture-fed reindeer.
LKN-8081 - Lichen-fed reindeer.

Table II- 6. Daily intake of amino acids by pen-fed reindeer.
(in g / reindeer and in g / kg $^{0.75}$ b.w.).

A.A.	BAR-8081 g/da	BAR-8081 g/kg $^{0.75}$	BAR-8182 g/da	BAR-8182 g/kg $^{0.75}$	B+M-8182 g/da	B+M-8182 g/kg $^{0.75}$	QTX-8081 g/da	QTX-8081 g/kg $^{0.75}$	LKN-8081 g/da	LKN-8081 g/kg $^{0.75}$
ASP	8.56	0.27	11.45	0.38	11.97	0.40	22.49	0.71	2.33	0.09
THR	4.63	0.15	5.48	0.18	5.73	0.19	9.42	0.30	1.38	0.06
SER	6.01	0.19	6.56	0.22	6.85	0.23	13.20	0.41	1.53	0.06
GLU	26.98	0.86	31.68	1.05	33.11	1.09	56.09	1.76	2.56	0.10
PRO	12.17	0.39	14.19	0.47	14.83	0.49	16.77	0.53	1.18	0.05
GLY	5.56	0.18	6.46	0.21	6.76	0.22	14.08	0.44	1.42	0.06
VAL	5.97	0.19	6.96	0.23	7.27	0.24	12.34	0.39	1.19	0.05
MET	1.62	0.05	1.77	0.06	1.85	0.06	3.69	0.12	0.22	0.01
ILE	3.98	0.13	4.87	0.16	5.09	0.17	8.34	0.26	0.95	0.04
LEU	8.43	0.27	9.74	0.32	10.18	0.34	17.68	0.56	1.53	0.06
TYR	4.18	0.13	4.58	0.15	4.76	0.16	8.70	0.27	0.53	0.02
PHE	5.62	0.18	6.62	0.22	6.92	0.23	12.04	0.38	0.96	0.04
HIS	2.63	0.08	3.22	0.11	3.36	0.11	7.94	0.25	0.45	0.02
LYS	5.14	0.16	6.08	0.20	6.35	0.21	11.32	0.36	1.06	0.04
ARG	6.77	0.22	8.61	0.27	8.37	0.28	23.49	0.74	1.02	0.04
ALA	6.05	0.19	7.01	0.23	7.33	0.24	13.24	0.42	0.46	0.02

BAR-8081 - Barley-fed reindeer, 1980-81.
BAR-8182 - Barley-fed reindeer, 1981-82.
B+M-8182 - Barley + Trace mineral salt, 1981-82.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
LKN-8081 - Lichen-fed reindeer, 1980-81.

than for QTX8081 reindeer. The supplementation of the B+M8182 reindeer with trace mineral salt increased the daily intake of Co, Cu, Mn, K and Na to levels comparable to those of the QTX8081 reindeer.

The daily intake of the amino acid methionine on a / kg $^{0.75}$ body weight basis ranged 12-fold over the various feeding groups. The methionine intake of the LKN8081 reindeer was lowest at 0.01 g / d. Methionine intake for the barley-fed reindeer (0.06 g / d) was approximately 50 percent of that for the QTX8081 reindeer.

Body weight, TBW and body composition changes.

Changes in body weight over the duration of the five pen-feeding experiments are shown in Table II- 7 . The LKN8081 reindeer lost an average of 15.4 percent of their initial body weight over 46 days, equivalent to a daily loss of 0.27 kg. Losses of up to 20 percent of initial body weight during the winter are not unusual in reindeer (Jacobsen and Skjenneberg, 1975; Skjenneberg and Slagsvold, 1968). The average daily change in body weight for all other feeding groups ranged between +0.01 kg / day (BAR8081) and -0.04 kg / day (BAR8182).

Changes in the TBW over the duration of the feeding trials conducted during 1980-81 are shown in Table II- 8 . The TBW of the BAR8081 reindeer increased slightly by an average of 2.7 percent. The TBW of the QTX8081 reindeer remained essentially unchanged. In contrast, the LKN8081

Table II- 7. Body weight changes in pen-fed reindeer on various diets.

EXPERIMENT	TIME OF YEAR	RD#	# OF DAYS	INIT. B.W. (kg)	TOTAL CHANGE IN B.W. (kg)	% OF INIT. B.W.	DAILY CHANGE IN B.W. (kg)
BAR-8081	DEC-MAR	7	83	92.8	+ 1.4	+ 1.4	+0.02
		68	83	104.2	0.0	0.0	0.00
		MEAN	83	98.5	+ 0.7	+ 0.6	+0.01
BAR-8182	JAN-APR	58	86	98.0	- 2.7	- 2.7	-0.03
		99	86	90.7	- 4.5	- 4.9	-0.05
		MEAN	86	94.4	- 3.6	- 3.8	-0.04
B+M-8182	JAN-APR	7	86	87.4	- 5.0	- 5.5	-0.06
		105	86	97.8	+ 2.3	+ 2.3	+0.03
		MEAN	86	92.7	+ 0.9	+ 1.0	+0.01
QTX-8081	DEC-MAR	58	83	102.4	- 5.0	- 4.9	-0.06
		99	83	98.7	+ 2.3	+ 2.3	+0.03
		MEAN	83	101.0	- 1.4	- 1.4	-0.02
LKN-8081	DEC-FEB	56	46	75.4	-12.7	-15.3	-0.28
		82	46	69.5	-11.8	-15.5	-0.26
		MEAN	46	72.5	-12.3	-15.4	-0.27

LKN-8081 - Lichen-fed reindeer, 1980-81.
BAR-8081 - Barley-fed reindeer, 1980-81.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
B+M-8182 - Barley + Trace mineral salt-fed reindeer, 1981-82.
BAR-8182 - Barley-fed reindeer, 1981-82.

Table II- 8. Changes in total body water (TBW) in pen-fed reindeer.

EXPERIMENT	RD #	DATE (d/mo)	INITIAL TBW (l)	% OF INITIAL B.W.	FINAL TBW (l)	% OF FINAL B.W.	CHANGE IN TBW (l)
BAR-8081	7	29/12	54.6	58.1			
	"	21/03			57.3	60.3	+ 2.7
	68	29/12	63.5	60.4			
	"	21/03			64.1	60.9	+ 0.6
	MEAN	29/12		59.3			
	"	21/03				60.6	
QTX-8081	58	29/12	65.1	63.2			
	"	06/04			63.6	64.9	- 1.5
	99	29/12	65.5	66.2			
	"	06/04			65.7	65.0	+ 0.2
	MEAN	29/12		64.7			
	"	06/04				65.0	
LKN-8081	56	29/12	56.7	68.3			
	"	13/02			48.5	69.0	- 8.2
	82	29/12	60.1	79.0			
	"	13/02			49.4	76.6	-10.7
	MEAN	29/12		73.7			
	"	13/02				72.8	

BAR-8081 - Barley-fed reindeer, 1980-81.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
LKN-8081 - Lichen-fed reindeer, 1980-81.
B.W. = Body weight.

reindeer lost an average 16.2 percent of their initial TBW during the 46 day experiment.

The fat, protein, ash and total body solid content in the reindeer fed during 1980-81 estimated by the equations of Searle (1970) are shown in Table II- 9 . The BAR8081 reindeer had the highest initial percentage of body fat at 27.2 percent of body weight. Body fat content remained essentially constant for both the BAR8081 and QTX8081 reindeer.

The LKN8081 reindeer had the lowest initial body fat content, ranging between 7.2 percent and 18.3 percent of body weight. These reindeer had been feeding on lichens for 30 days prior to the initial TOH injection. This group lost an average 5.6 percent of their initial pre-trial body weight during this 30 day period.

The body protein content of the BAR8081 and QTX8081 reindeer remained essentially constant during the tracer experiments. In contrast, reindeer in the LKN8081 group lost between 15 percent and 17 percent of their initial protein content.

The slight increase in body weight of the BAR8081 reindeer was due to a small increase in TBW. Body solids accounted for 51 percent of the slight loss in body weight of the QTX8081 reindeer. Fat accounted for 71 percent of the body solids loss.

Table II- 9. *Initial and final body composition of pen-fed reindeer.* (in total kg and as a percent of body weight).

EXP/RD#	FAT INIT	FAT FINAL	FAT DIFF	PROTEIN INIT	PROTEIN FINAL	PROTEIN DIFF	ASH INIT	ASH FINAL	ASH DIFF	TOTAL SOLIDS INIT	TOTAL SOLIDS FINAL	TOTAL SOLIDS DIFF
BAR-8081												
# 7 (kg)	27.2	25.6	-1.6	12.3	12.7	+0.4	3.2	3.3	+0.1	42.7	41.6	-1.1
"(%BW)	28.9	26.8	-2.1	13.1	13.4	+0.3	3.4	3.5	+0.1	45.2	43.7	-1.5
#68 (kg)	28.0	27.4	-0.6	14.2	14.2	+0.1	3.7	3.7	0.0	45.8	45.1	-0.7
"(%BW)	26.6	26.1	-0.5	13.4	13.5	+0.1	3.5	3.5	0.0	43.5	43.1	-0.4
MEAN(kg)	27.6	26.6	-0.9	13.2	13.5	+0.3	3.4	3.5	+0.1	44.2	43.6	-0.6
"(%BW)	27.2	26.5	-1.2	13.3	13.4	+0.1	3.5	3.5	0.0	44.5	43.4	-1.1
QTX-8081												
#58 (kg)	24.3	21.4	-2.9	14.2	13.8	-0.5	3.7	3.6	-0.1	42.2	38.8	-3.4
"(%BW)	23.6	21.9	-1.7	13.8	14.0	+0.2	3.6	3.7	+0.1	41.0	39.6	-0.4
#99 (kg)	20.2	22.1	+1.8	14.1	14.2	+0.1	3.7	3.7	0.0	37.9	40.0	+2.1
"(%BW)	20.5	21.8	+1.3	14.2	14.0	-0.2	3.7	3.7	0.0	38.4	39.8	+1.4
MEAN(kg)	22.3	21.7	-0.6	14.1	14.0	-0.1	3.7	3.7	0.0	40.1	39.4	-0.7
"(%BW)	22.1	21.9	-0.2	14.0	14.0	0.0	3.7	3.7	0.0	39.8	39.6	-0.2
LKN-8081												
#56 (kg)	15.2	12.6	-2.6	12.0	10.3	-1.7	3.2	2.7	-0.5	31.4	25.6	-4.8
"(%BW)	18.3	17.6	-0.7	14.5	14.6	+0.1	3.8	3.9	+0.1	36.6	36.1	-0.5
#82 (kg)	5.5	6.1	+0.6	12.2	10.1	-2.1	3.3	2.7	-0.6	20.9	18.9	-2.0
"(%BW)	7.2	9.5	+2.3	16.0	15.7	-0.3	4.3	4.2	-0.1	27.5	29.4	+1.9
MEAN(kg)	10.2	9.2	-1.0	12.1	10.2	-1.9	3.2	2.7	-0.5	25.6	22.1	-3.5
"(%BW)	12.9	13.6	+0.7	15.2	15.1	-0.1	4.0	4.0	0.0	32.1	32.7	+0.6

BAR-8081 - Barley-fed reindeer, 1980-81.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
LKN-8081 - Lichen-fed reindeer, 1980-81.

Thirty-eight percent of the body weight loss in reindeer #56 (LKN8081) was due to a loss in total body solids. Fat accounted for 53 percent and protein for 37 percent of this loss. In reindeer #82 (LKN8081) body solids accounted for only 17 percent of the loss in body weight, of which almost 100 percent was due to loss in body protein content. Body fat in this reindeer had apparently approached a critical lower limit by the beginning of the isotope trial.

Whole body burden of ^{75}Se-selenomethionine.

The whole body counts of injected ^{75}SeM measured over time in pen-fed reindeer are shown in Appendix One. The whole body ^{75}SeM retention curves are shown in Figure I- 3. All retention curves are the mean of two reindeer, with the exception of the LKN8081 curves. Reindeer #56 and #82 of the LKN8081 group had vastly different ^{75}SeM turnover rates. Therefore, the retention curves of these two reindeer were plotted separately. Table II-10 shows the fractional turnover rate (k), the Y-intercepts and the biological half-lifes ($T_{1/2}$) of the initial and terminal components of the exponential ^{75}SeM whole body retention curves. The BAR8081 and B+M8182 reindeer eliminated a single dose of ^{75}SeM by a single component exponential function. All other reindeer eliminated ^{75}SeM by a two component exponential rate function. The $T_{1/2}$ of the initial component ranged between 0.6 and 1.2 days for the BAR8182

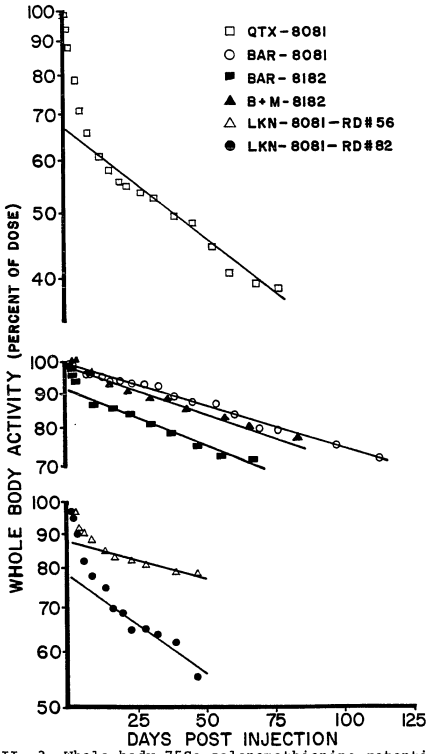

Figure II- 3. Whole body 75Se-selenomethionine retention in pen-fed reindeer.

Table II-10. Regression line data of exponential components resolved from whole body 75Se-selenomethionine retention curves of pen-fed reindeer.

EXPERIMENT	RD #	INITIAL COMPONENT k	INITIAL COMPONENT Y-INT (t=0)	INITIAL COMPONENT $T_{1/2}$ (d)	TERMINAL COMPONENT k	TERMINAL COMPONENT Y-INT (t=0)	TERMINAL COMPONENT $T_{1/2}$ (d)
BAR-8081	7				-0.003	49204	233
	68				-0.003	49534	237
	MEAN				-0.003	49371	235
BAR-8182	99	-1.124	6960	0.6	-0.003	52471	277
	58	-0.596	3750	1.2	-0.004	55460	178
	MEAN	-0.833	5691	0.8	-0.003	53601	231
B+M-8182	7				-0.003	61495	284
	105				-0.004	56733	179
	MEAN				-0.003	59109	233
QTX-8081	99	-0.206	17374	3.4	-0.006	29570	112
	58	-0.217	15103	3.2	-0.008	33926	86
	MEAN	-0.209	16028	3.3	-0.008	32064	92
LKN-8081	56	-0.167	6890	4.1	-0.002	46526	289
	82	-0.137	10288	5.1	-0.007	37198	103

k = Fractional turnover rate (/ d).
Y-INT (t=0) - Y intercept at time zero.
$T_{1/2}$ - The biological half-life (days).
BAR-8081 - Barley-fed reindeer, 1980-81.
BAR-8182 - Barley-fed reindeer, 1981-82.
B+M-8182 - Barley + trace mineral salt, 1981-82.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
LKN-8081 - Lichen-fed reindeer, 1980-81.

reindeer, and between 3.2 and 5.1 for the QTX8081 and LKN8081 reindeer.

The $T_{1/2}$ of the terminal component of whole body ^{75}SeM retention curves ranged from a high of 289 days in LKN8081 reindeer #56 to a mean low of 92 days in the QTX8081 reindeer. The mean $T_{1/2}$ of the barley-fed reindeer ranged between 231 and 235 days. The $T_{1/2}$ of reindeer #56 and those of the barley-fed reindeer were not significantly different ($p<0.01$). However, reindeer #82 of the LKN8081 group had a whole body $T_{1/2}$ approximately one-third of the other lichen-fed reindeer (RD #56). Reindeer #82 died of malnutrition two days after the termination of the lichen feeding trial. The rapid ^{75}SeM turnover was most likely due to increased tissue protein mobilization to provide essential metabolites.

^{75}Se-selenomethionine retention in plasma and plasma proteins.

The ^{75}SeM retention curves of plasma from pen-fed reindeer is shown in Figure II- 4 . The fractional turnover rate (k), Y-intercepts and $T_{1/2}$ of each component of the exponential elimination rate functions for ^{75}SeM in plasma are shown in Table II-11. Plasma ^{75}SeM retention curves could be resolved into three exponential components with the exceptions of the BAR8182 reindeer and LKN8081 reindeer #82. The plasma ^{75}SeM retention curves for the BAR8182 reindeer could be resolved into two exponential components.

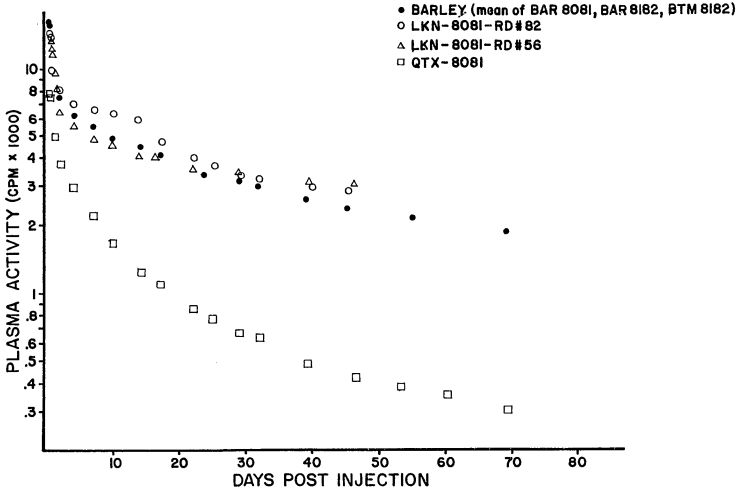

Figure II- 4. 75Se-selenomethionine retention in plasma of pen-fed reindeer.

Table II-11. Regression line data of exponential components resolved from 75Se-selenomethionine plasma retention curves in pen-fed reindeer.

EXPERIMENT	FRACTIONAL TURNOVER RATE (k)	Y-INTERCEPT (CPM)	BIOLOGICAL HALF-LIFE (DAYS)	CORRELATION COEFFICIENT (r)
BAR-8081				
Initial Comp.	-1.298	10305	0.5	-0.99
Second Comp.	-0.066	3870	10.5	-0.99
Termin. Comp.	-0.007	3086	99.1	-0.97
BAR-8182				
Initial Comp.	-0.125	4785	5.5	-0.99
Termin. Comp.	-0.012	3740	58.1	-0.99
B+M-8182				
Initial Comp.	-1.549	3978	0.5	-0.93
Second Comp.	-0.073	3375	9.5	-0.99
Termin. Comp.	-0.008	3495	85.1	-0.98

Table II-11. Continued.

EXPERIMENT	FRACTIONAL TURNOVER RATE (k)	Y-INTERCEPT (CPM)	BIOLOGICAL HALF-LIFE (DAYS)	CORRELATION COEFFICIENT (r)
QTX-8081				
Initial Comp.	-1.214	4670	0.5	-0.99
Second Comp.	-0.119	2949	5.8	-0.99
Termin. Comp.	-0.016	873	43.5	-0.99
LKN-8081				
REINDEER # 56				
Initial Comp.	-1.918	5898	0.4	
Second Comp.	-0.232	3717	3.0	
Termin. Comp.	-0.009	4330	80.6	
REINDEER # 82				
Initial Comp.	-1.220	8545	0.6	
Second Comp.	-0.016	7404	43.3	
Third Comp.	-0.174	20695	4.0	
Termin. Comp.	-0.009	4330	75.3	

BAR-8081 - Barley-fed reindeer, 1980-81.
BAR-8182 - Barley-fed reindeer, 1981-82.
B+M-8182 - Barley + trace mineral salt-fed reindeer, 1981-82.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
LKN-8081 - Lichen-fed reindeer, 1980-81.

Reindeer #82 exhibited a step-wise retention curve, possibly representing four rate functions. The $T_{1/2}$ of the terminal components of the ^{75}SeM retention curves ranged between 44 and 99 days. Thus, ^{75}SeM turnover rate in plasma was two to four times higher than whole body ^{75}SeM turnover rates. These results are comparable to those observed in grazing reindeer (Chapter I).

Amino acid content in plasma.

The amino acid content in the plasma of pen-fed reindeer is shown in Table II-12 . In general, QTX reindeer exhibited slightly elevated total (for each a.a.) amino acid levels. This was a result of the relatively high level of bound amino acid in the plasma of this group. In contrast, the plasma of LKN8081 reindeer generally contained elevated levels of free amino acids. Similar results have been observed in laboratory rats. Small increases in free amino acid concentration in serum and plasma have been observed in rats when they are given low-protein and protein- free rations (Waterlow and Stephen, 1968). In reindeer, an elevated bound plasma fraction may result from high-protein diets such as Quality Texture.

Validation experiments of the ^{75}Se-selenomethionine method.

Table II-13 summarizes the results of the bisulfite treatment of plasma proteins in four barley-fed reindeer (BAR8182, B+M8182). Sulfitolysis released only a slight amount of ^{75}Se from plasma proteins. The amount of ^{75}SeM

Table II-12. Amino acid content in plasma of pen-fed reindeer.

A.A.	BAR-8081		BAR-8182		B+M-8182		QTX-8081		LKN-8081	
	FREE	TOTAL	FREE	TOTAL	FREE	TOTAL	FREE	TOTAL	FREE	TOTAL
	(µg / ml)		(µg / ml)		(µg / ml)		(µg / ml)		(µg / ml)	
ASP	3	3734	1	2793	1	3100	1	4464	2	3074
THR	13	1536	6	1266	13	1413	9	2141	21	1497
SER	9	2186	7	1213	10	1457	7	2173	18	1546
GLU	200	4597	175	3501	192	3945	212	5678	212	3909
PRO	18	1600	11	1260	14	1414	12	2071	28	1438
GLY	40	950	28	754	41	820	30	1211	62	894
VAL	22	1121	13	850	24	1063	19	1758	46	1282
MET	4	256	3	206	4	209	2	343	6	241
ILE	18	388	9	329	16	353	14	581	23	401
LEU	16	2683	12	2104	18	2371	13	3458	32	2386
TYR	11	1433	10	1137	17	1165	9	1844	21	1298
PHE	9	1405	9	1089	11	1103	9	1800	14	1236
HIS	15	1229	13	707	15	971	13	981	16	841
LYS	25	2999	16	2367	31	2521	19	3730	45	2069
ARG	27	1539	22	1183	32	1293	28	1994	28	1358
ALA	31	1853	34	1362	47	1625	29	2236	47	1528

BAR-8081 - Barley-fed reindeer, 1980-81.
BAR-8182 - Barley-fed reindeer, 1981-82.
B+M-8182 - Barley + trace mineral salt, 1981-82.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
LKN-8081 - Lichen-fed reindeer, 1980-81.

Table II-13. Mean ^{75}Se activity in plasma protein precipitate (with and without sulfitolysis [Na_2SO_4] treatment) of four barley-fed reindeer.

ELAPSED TIME (days)	75Se ACTIVITY (cpm/100mg ppt)	75Se ACTIVITY AFTER Na_2SO_4 (cpm/100mg ppt)	PERCENT OF 75Se NOT PROTEIN INCORPORATED
0.3	1512	1443	5
0.7	1227	1187	3
2.7	901	892	1
9.8	674	653	3
16.9	561	541	4
23.9	452	447	1
30.8	406	379	7
37.8	343	331	4
44.7	308	300	3
58.8	268	251	7
68.8	238	227	5

non-enzymatically bound to plasma proteins ranged between one percent and seven percent (mean = 3.9, ±2.0 SD). The retention curves in plasma and plasma proteins of simultaneously injected ^{35}S-methionine (^{35}SM) and ^{75}SeM were compared. The ratio of specific activities of ^{35}S to ^{75}Se are shown for plasma and plasma protein samples taken over an 86-day period (Table II-14). The initial ratio values are lower than those of the injection ratio, indicating that relatively more ^{75}SeM was present in samples drawn during the first 20 days. The value of the ^{35}S/^{75}Se ratio in the plasma and plasma proteins approached that of the injection doses (13.5) approximately 20 days post injection. Millar and Sheppard (1973b) reported that for a number of tissues including blood, liver and kidneys, proportionally more ^{75}SeM (than ^{35}SM) was incorporated into protein when measured one to three days after simultaneous injection of the two tracers.

For plasma, the ^{35}S/^{75}Se ratio was maintained at a plateau of approximately 13.8 until the end of the experiment. In plasma protein, a plateau of 13.6 was maintained for only 30 days before a slight decline in the ratio was observed. This may have been a real decline or a result of error introduced by low counting efficiency due to low tracer activity in the final samples.

Body water turnover.

The results of the body water transfer measurements in

Table II-14. Ratio of ^{35}S to ^{75}Se in plasma and plasma protein precipitate (bisulfite treated) of four barley-fed reindeer.

ELAPSED TIME (days)	PLASMA $^{35}S/^{75}Se$	± SD	PLASMA PROTEINS $^{35}S/^{75}Se$	± SD
0.4	4.5	1.1	4.8	2.5
0.3	5.8	1.7	4.0	1.3
0.7	6.2	2.0	6.6	1.3
2.9	7.3	1.8	8.0	1.3
9.9	10.2	1.7	11.1	2.3
16.9	11.2	3.0	11.9	2.1
23.8	13.7	3.4	13.6	4.0
30.8	12.6	1.7	13.2	3.5
37.8	13.6	3.0	13.7	3.7
44.7	13.8	2.7	13.8	3.4
58.8	14.7	2.8	12.3	2.4
68.8	14.0	2.0	11.2	1.2
85.9	14.0	2.0	10.3	1.8

the three pen-feeding experiments conducted during 1980-81 are shown in Table II-15 . the QTX8081 reindeer displayed the highest body water transfer rate at 4.8 l / d. This was over two times that of the BAR8081 reindeer (2.2 l / d). Reindeer #56 of the LKN8081 group had the lowest body water transfer rate at 1.7 l / d and reindeer #82 lost 2.8 l / d.

Table II-15. Total body water turnover in pen-fed reindeer.

EXPERIMENT	RD #	TIME OF YEAR	FRACTIONAL TURNOVER RATE (k)	BODY H_2O TRANSFER (l / d)	$T_{1/2}$ (d)
BAR-8081	7	DEC-APR	-0.042	2.3	16.6
	68	" "	-0.033	2.1	20.8
	MEAN	" "	-0.038	2.2	18.7
QTX-8081	58	DEC-APR	-0.061	3.9	11.3
	99	" "	-0.087	5.7	8.0
	MEAN	" "	-0.074	4.8	9.7
LKN-8081	56	DEC-FEB	-0.032	1.7	21.4
	82	" "	-0.050	2.8	13.8
	MEAN	" "	-0.041	2.2	17.6

BAR-8081 - Barley-fed reindeer, 1980-81.
QTX-8081 - Quality Texture-fed reindeer, 1980-81.
LKN-8081 - Lichen-fed reindeer, 1980-81.
$T_{1/2}$ = Biological half-life.

DISCUSSION

Nitrogen metabolism in pen-fed reindeer.

Jacobsen and Skjenneberg (1975) conducted a series of nitrogen balance trials with reindeer. During these trials they measured nitrogen balance resulting from nitrogen intake that ranged from three to 20 g / d. A regression line (Figure II- 5) was constructed from their data relating daily nitrogen intake with nitrogen balance. The regression equation:

$$Y = 0.24 X - 2.69$$

was used to predict nitrogen balance (Y) from daily nitrogen intake (X) in reindeer used in the present study. The relationship between nitrogen intake and nitrogen balance was assumed to remain linear at relatively high nitrogen intake levels, such as those of the QTX8081 reindeer (40 to 43 g N/day). Therefore, the regression line in Figure II-5 was extended.

The whole body methionine and protein nitrogen content of the pen-fed reindeer (Table II-16) were calculated using the assumptions stated in Chapter I. The terminal whole body ^{75}SeM fractional rate constants were used to calculate methionine transfer rate and irreversible nitrogen loss

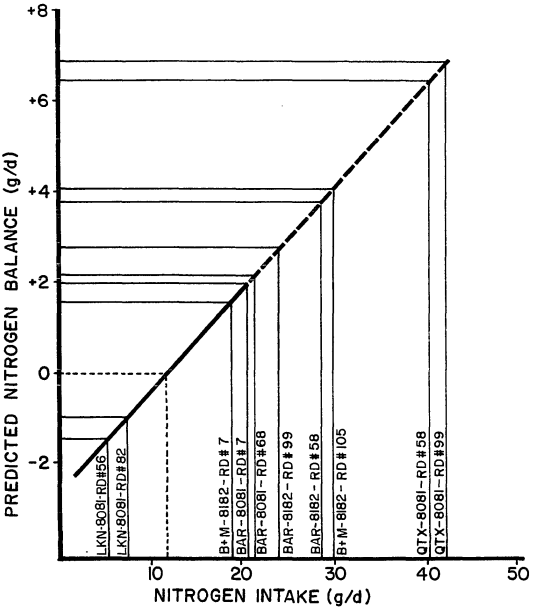

Figure II- 5. Predicted nitrogen balance from measured nitrogen intake.

Table II-16. Whole body methionine and protein content and irreversible nitrogen loss (INL) in pen-fed reindeer.

EXPERIMENT	WHOLE BODY CONTENT METHIONINE (g)	PROTEIN N (g)	METHIONINE TRANSFER RATE (g/d)	I N L (g/d)
LKN-8081				
RD # 56	195	1792	0.4	3.6
RD # 82	194	1775	1.4	12.4
QTX-8081				
RD # 58	245	2240	2.0	17.9
RD # 99	247	2256	1.5	13.5
BAR-8081				
RD # 7	219	2000	0.7	6.0
RD # 68	249	2272	0.7	6.8
BAR-8182				
RD # 58	240	2195	1.0	8.9
RD # 99	222	2032	0.7	6.1
B+M-8182				
RD # 7	214	1958	0.6	5.9
RD #105	240	2191	1.0	8.8

LKN-8081 - Lichen fed reindeer.
QTX-8081 - Quality Texture fed reindeer.
BAR-8081 - Barley fed reindeer.
BAR-8182 - Barley fed reindeer, 1981-82.
B+M-8182 - Barley + trace mineral salt fed reindeer, 1981-82.

rate from body proteins (INL). These values are also shown in Table II-16 .

Table II-17 shows the measured daily nitrogen intake, the estimated nitrogen balance from Figure II- 5, total nitrogen excretion (intake minus balance), INL, and INL, as a percentage of total nitrogen excretion for the pen-fed reindeer. Based upon nitrogen intake, the LKN8081 reindeer had negative nitrogen balances ranging between 0.8 to 1.4 g N / day. In contrast, barley- and Quality Texture-fed reindeer had positive nitrogen balances ranging between 2 to 8 g N / d. A negative nitrogen balance is usually observed when reindeer are fed lichens exclusively (Jacobsen and Skjenneberg, 1975; Cameron, 1972). During winter (simulated in a $-20^{o}C$ environmental chamber) negative nitrogen balance has been observed in reindeer even though nitrogen intake was 25 g / d (Cameron, 1972). According to Figure II- 5 , a daily nitrogen intake of 25 g should correspond to a nitrogen balance of approximately +3 g N / d. Nitrogen balance values based solely upon nitrogen intake may not be reliable. Factors such as temperature and animal response to high protein intake during winter most likely influence nitrogen balance. Therefore direct measurement of nitrogen balance may be the only reliable method to determine nitrogen balance.

The INL ranged from a low of 3.6 g N / d in reindeer #56 of the LKN8081 group to an average high of 15.7 g N/d

Table II-17. Nitrogen balance and irreversible nitrogen loss from body proteins (INL) of pen-fed reindeer.

EXPERIMENT	RD#	DAILY N INTAKE (g N/d) (1)	BALANCE (g N/d) (2)	TOTAL N EXCRETION (g N/d) (3)	I N L (4) (g N/d)	% OF TOTAL N EXCRETION
LKN-8081	56	5.4	- 1.4	6.8	3.6	52.9
	82	7.7	- 0.8	8.5	12.5	147.1
QTX-8081	58	40.1	+ 7.1	33.0	17.9	54.2
	99	43.6	+ 7.9	35.7	13.5	37.8
BAR-8081	7	20.5	+ 2.3	18.2	6.0	33.0
	68	20.6	+ 2.3	18.3	6.8	37.2
BAR-8182	99	23.7	+ 3.1	20.6	6.1	29.6
	58	28.6	+ 4.3	24.3	8.9	36.6
B+M-8182	7	18.7	+ 1.9	16.8	5.9	35.1
	105	29.4	+ 4.5	24.9	8.8	35.3

(1) Measured nitrogen intake.
(2) From Figure II- 5.
(3) Intake minus balance.
(4) Irreversible nitrogen loss from body proteins, derived from 75SeM whole body counting.

LKN-8081 - Lichen fed reindeer, 1980-81.
QTX-8081 - Quality Texture fed reindeer, 1980-81.
BAR-8081 - Barley fed reindeer, 1980-81.
BAR-8182 - Barley fed reindeer, 1981-82.
B+M-8182 - Barley + trace mineral salt fed reindeer, 1981-82.

in the QTX8081 reindeer, indicating that INL is positively related to nitrogen intake. The effect of the intake level of various nutrients on whole body protein turnover (INL times 6.25) is discussed in the following section.

The INL of lichen-fed reindeer #56 (LKN8081) was only 16 percent of that observed in reindeer grazing on winter forage (EW-GRZ and MW-GRZ of Chapter I). The large discrepancy in INL between reindeer on diets that are assumed to be similar could be a result of confinement. Rehbinder et al. (1982) have documented the effects of manual handling and restraint on reindeer. Extensive human contact with untamed reindeer resulted in lesions of the digestive tract and skeletal and myocardial muscles. Reindeer were also more susceptible to parasites. However, the reindeer used in the present study were tamed, and daily handling of reindeer produced minimal visible stress.

Confined reindeer are certainly less active than those that are free roaming. Activity (i.e. exercise) is known to increase whole body protein turnover rate (Millward et al., 1982). Higher activity levels in the grazing reindeer (EW-GRZ, MW-GRZ) may have contributed to the larger INL indicated by ^{75}SeM fractional turnover rate.

Relationship between nutrient intake and methionine transfer rate.

Cameron et al. (1976) confirmed in reindeer that water intake was directly related to body water transfer rate.

For the present experiments, it was not surprising that of the daily intake of all feed nutrients, the highest correlation found was between methionine intake and methionine transfer rate ($r=.93$, $p<0.01$).

The relationships between methionine and nitrogen intake and methionine transfer rate are shown in Figure II-6 (a,b). The relationship between nitrogen intake and methionine transfer rate is less highly correlated ($r=.90$, $p<0.01$) than that between methionine intake and transfer rate. A highly correlated relationship ($Y=0.92X + 3.12$, $r=.98$, $p<0.01$) exists between daily nitrogen intake (X) and INL (Y). It would appear that as has been observed for water and methionine, the INL is for the large part a result of nitrogen intake. The methionine intake expressed as a percentage of protein nitrogen intake was 3.4 percent for the LKN8081 reindeer, 6.6 percent to 8.1 percent for the barley-fed reindeer and 8.8 percent for the QTX8081 reindeer. As a result, methionine intake was not a constant fraction of protein nitrogen intake. This divergence between the relative intake of methionine and nitrogen may explain for the lower correlation found between nitrogen intake (compared to methionine intake) and methionine transfer rate.

The daily intake of ash and methionine for reindeer in the pen-feeding trials was not significantly correlated. However, the relationship between daily ash intake

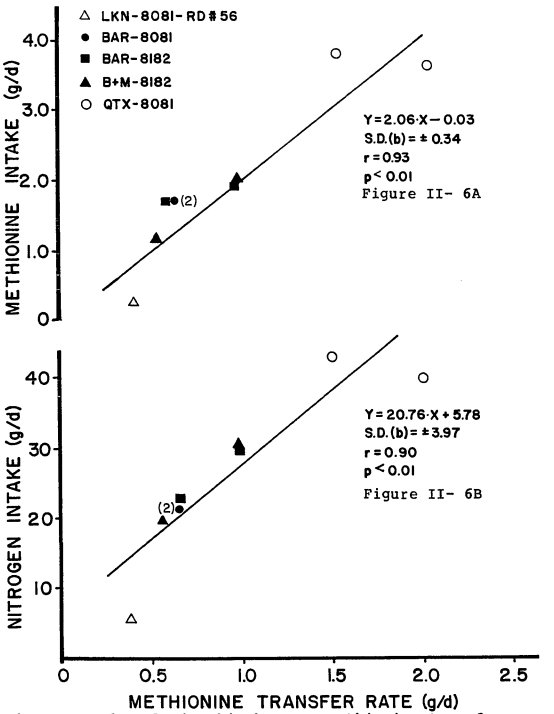

Figure II- 6. Relationship between methionine transfer rate and methionine intake (A); nitrogen intake (B); in pen-fed reindeer.

and methionine transfer rate (Figure II- 7 b) was found to be highly correlated. In fact, methionine intake verses methionine transfer was the only relationship with a higher coefficient of correlation. The body's need for electrolytes to maintain normal tissue function is well known. A close interrelationship between body protein and mineral (ash) metabolism could explain the high correlation between ash intake and methionine transfer rate.

The intake of several constituents in the ash fraction were correlated to the methionine transfer rate. The correlation obtained between selenium intake and methionine transfer rate (Figure II- 7 a) was highest. The high correlation between selenium intake and methionine tranfer rate could be a result of an interrelationship in their metabolism rather than any similarities in their intake patterns. In fact, the relationship between selenium intake and methionine intake was not significant.

Selenomethionine has been shown to be the predominant form of selenium in forage (Hidiroglou et al., 1974). Selenium in the organic form as selenomethionine may be the most effective treatment of selenium deficiency disease (Griffiths et al., 1976). In fact, selenomethionine may be the major form of animal tissue selenium, essential as a trace amino acid to the normal function and structure of certain body proteins and enzymes (Holland et al., 1966; Stadtman, 1974).

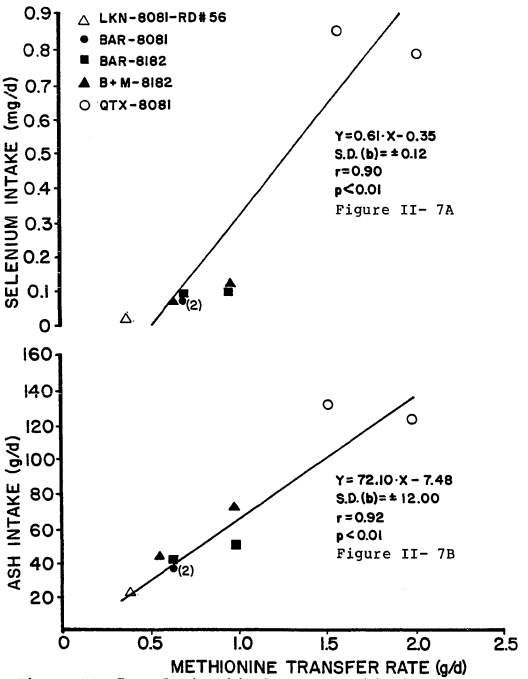

Figure II- 7. Relationship between methionine transfer rate and selenium intake (A); ash intake (B); in pen-fed reindeer.

It has been assumed by some researchers that selenomethionine and methionine occupy a common metabolic pool (Ochoa-Solano and Gitler, 1968a,b; Yousef and Luick, 1969). The seleno- and sulfur forms of methionine have been shown to be biochemically similar (see Literature Review). Selenium and sulfur are very similar chemically. Both elements have valencies of two, four and six, and are incorporated into similar compounds (-2 - H_2Se and hydrogen sulfide; +2 - H_2Se2O_3 and hydrogen thiosulfate; +4 - H_2SeO_3 and hydrogen sulfite; +6 - H_2SeO_4 and hydrogen sulfate). However, results from other studies indicate that the seleno- and sulfur forms of methionine may occupy separate metabolic pools (Millar and Sheppard, 1973a; Millar et al., 1972).

Evidence supporting the existence of a single selenium metabolic pool has been reported (Thomson and Stewart, 1973). The turnover rates of three chemical forms of ^{75}Selenium such as ^{75}SeM, ^{75}Se-selenocystine and ^{75}Se-sodium selenite, were found in rats to be indistinguishable. The authors concluded that regardless of chemical form, all selenium absorbed into the body occupied a single metabolic pool. Selenomethionine may be the predominant form of selenium in this pool.

If the intake of selenium by the pen-fed reindeer was predominantly in the form of selenomethionine or its metabolic precursors, it would not be surprising that

methionine transfer rate predicted by ^{75}SeM turnover is highly correlated with selenium intake. The turnover of injected ^{75}SeM may actually be a result of the turnover of a separate selenomethionine pool in the body. Therefore, another interpretation of the relationship between selenium intake verses methionine transfer rate (Figure II- 7 a) could be: selenium intake (as selenomethionine) is highly correlated to selenomethionine transfer rate. The effect of changes in the level of selenium intake on the turnover of injected ^{75}SeM has been demonstrated (Millar and Sheppard, 1973a; Millar et al., 1972). The results from these studies indicate that selenium supplementation in the diet results in an apparent increase in methionine transfer rate when ^{75}SeM is used as an indice of methionine transfer rate. However, changes in selenium intake level do not appear to affect methionine transfer rate when ^{35}S-methionine (^{35}SM) is used as an indice methionine transfer rate. Unfortunately, the reindeer (BAR8182, B+M8182) that received simultaneous injections of ^{75}SeM and ^{35}SM consumed essentially equal levels of selenium (0.06 to 0.09 mg / d). Consequently, the effect of variable selenium intake could not be examined.

Selenium intake is the single constituent of ash most highly correlated with methionine transfer rate. The biochemical role of selenium in the body is unclear. However, selenomethionine may be the major active form of

selenium in the body. The size and distribution of this possible selenomethionine pool has not been defined. The elimination of ^{75}SeM may be a direct result of the turnover of a selenomethionine pool rather than the methionine pool. Evaluation of the ^{75}Se-selenomethionine technique as an index of protein metabolism.

The results of the simultaneous injection of ^{75}SeM and ^{35}SM in reindeer indicate that, initially, sulfur methionine incorporation into plasma proteins is less than that of the seleno-analog (Table II-14). The sulfitolysis of plasma eliminated only a minor portion of ^{75}Se activity in plasma proteins (Table II-13). After 20 days post injection, the ^{35}S/^{75}Se ratio in plasma proteins approached that of the injection solution, Indicating that ^{35}SM and ^{75}SeM had similar kinetics. The presence of separate metabolic pools for the two forms of methionine could explain the divergence in the initial rates of incorporation into plasma proteins. Simultaneously injected ^{35}SM and ^{75}SeM may enter separate metabolic pools, where they equilibrate at rates dependent upon the size and distribution of these pools.

The chemical similarities between selenium and sulfur could have allowed for the evolution in reindeer as well as other species of similar metabolic reactions for the seleno- and sulfur forms of methionine. Trace amounts of selenomethionine may be utilized in protein synthesis as an

essential amino acid component of certain proteins (Stadtman, 1974). The involvement of selenomethionine in protein synthesis reactions may be in addition to that of methionine. The method of measuring whole body protein turnover with a single radio-labeled amino acid is dependent upon the assumption that most amino acids have similar irreversible loss rates. Based upon this assumption, selenomethionine and methionine should have similar whole body fractional turnover rates.

Results indicate that the fractional turnover rates of simultaneously injected ^{35}SM and ^{75}SeM can be similar when a constant intake ratio of selenium to methionine is maintained between treatments (Ochoa-Solano and Gitler, 1968a,b; Millar et al., 1972). The selenium/methionine ratio that produces similar turnover rates for ^{35}SM and ^{75}SeM is unknown and may vary with each situation. However, the intake ratio of one mg selenium / 17.5 grams ($\pm$ 1.2 g SD) methionine in barley-fed reindeer resulted in similar terminal fractional turnover rates for ^{35}S and ^{75}SeM in plasma proteins.

In conclusion, results support the possibility of separate metabolic pools for methionine and selenomethionine. The possibility that selenomethionine occupies a metabolic pool separate from that of methionine could have contributed to the failure of the three compartment model in Chapter I. A more appropriate model may be

one constructed around selenomethionine content within each of the three compartments.

A particular intake ratio of selenium to methionine may produce similar fractional elimination rate constants for ^{75}SeM and ^{35}SM. However, the results obtained indicate that initially, selenomethionine turnover in some proteins may differ from that of methionine. Results from other studies indicate that when selenium is added to the diet, the ^{75}SeM fractional turnover rate will increase while the ^{35}SM fractional turnover rate remains unchanged. When this occurs, the methionine transfer rate calculated from ^{75}SeM turnover rate will be overestimated. Selenomethionine may be inappropriate as an indicator of protein turnover when selenium is added to the diet.

Relationship between water and nitrogen metabolism in pen-fed reindeer.

Water transfer rate and water input rates (i.e., intake + metabolic water) have been shown to be essentially equal in reindeer (Cameron et al., 1976). In the present experiments, a correlation between water transfer rate and nitrogen intake rate of pen-fed reindeer was found (Figure II- 8 a) Similar results in reindeer have been previously reported (Cameron et al., 1982; Syrjala et al., 1980). Utley et al. (1970) proposed that the relationship between water transfer rate and nitrogen intake may be the result of an increased need for water to eliminate the increased

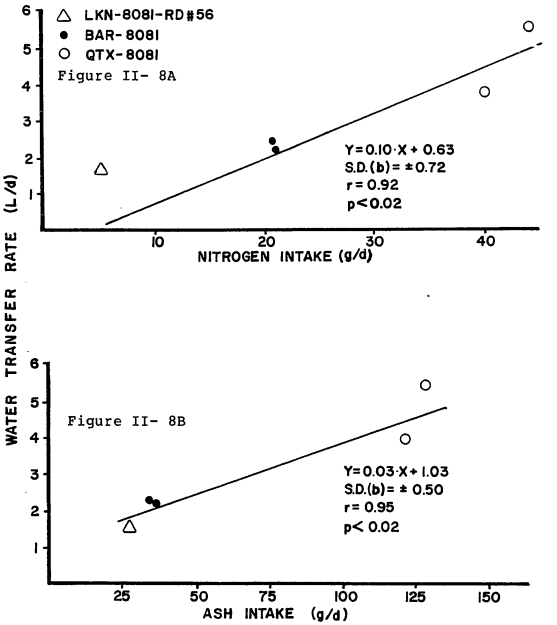

Figure II- 8. Relationship between water transfer rate and nitrogen intake (A); ash intake (B); in pen-fed reindeer.

amounts of nitrogen resulting from high protein diets. Valtonen (1980) has demonstrated that the reindeer kidney is incapable of producing urine containing a high concentration of nitrogen. This indicates that for reindeer, an increased urine volume is the only means of eliminating large amounts of nitrogen.

In the present study, water transfer rate and the rate of irreversible nitrogen loss from body proteins (INL) are not significantly correlated. Forbes (1968) proposed that an increased body water transfer rate may result from higher metabolic rate. He suggested that the higher dry matter intake observed in the animals of his study may be related to higher metabolic rate. The LKN8081 and QTX8081 reindeer consumed similar amounts of dry matter (Table II- 4), however; these animals demonstrated a three-fold difference in their water transfer rates. Unfortunately metabolic rate was not measured in these reindeer so the relationship between water turnover and metabolic rate could not be determined.

Figure II- 8 b shows the relationship between water transfer rate and the rate of ash intake in pen-fed reindeer. No other nutrient intake was more highly correlated with water transfer rate than ash intake. Cameron et al. (1982) also reported a high correlation for the relationship between water transfer rate and ash intake. The relationship between water and ash metabolism has been thoroughly

discussed by Cameron (1972). It appears that water metabolism may be closely related to the status of various electrolytes in the body. Unfortunately, water turnover was not measured in the trace mineral salt supplemented (B+M8182) and unsupplemented (BAR8182) barley-fed reindeer. The relationship between water turnover and variable ash intake in reindeer on otherwise equal diets could not be observed.

Summary and conclusions.

The following can be concluded from the pen-feeding experiments:

1) Initially, the dissimilar metabolism of simultaneously injected ^{35}S- methionine and ^{75}Se-selenomethionine in plasma proteins indicates that separate metabolic pools with different equilibrium kinetics exist. After an initial equilibration period, the metabolism of seleno and sulfur methionine become similar, indicating that after equilibration the turnover kinetics of the two metabolic pools are essentially the same.

2) There was no evidence in the present experiments showing that the turnover of methionine and selenomethionine were substantially different. This indicates that ^{75}SeM may be a useful indicator of methionine and possibly protein turnover. When selenium is supplemented in the diet or injected directly into the animal, turnover may be overestimated. Unsupplemented controls should be used under

these conditions.

3) Diet appears to be the major factor influencing whole body protein turnover as indicated by ^{75}Se-selenomethionine turnover in pen-fed reindeer.

4) Methionine transfer rate was highly correlated with methionine intake rate. The turnover of most amino acids may be a direct result of their respective intake levels.

5) Body water transfer does not appear to be closely related to protein turnover in reindeer.

SUMMARY OF CHARACTERISTICS OF PROTEIN METABOLISM IN REINDEER

Environmental factors such as temperature and wind most likely affect protein turnover rates in reindeer. However, diet appears to be the major factor affecting protein turnover. The intake rate of methionine appears to be highly correlated to its transfer rate from the body. The same relationship may be true for most amino acids and protein in general.

Low methionine transfer rates as indicated by ^{75}SeM turnover were observed in lichen-fed reindeer (reindeer #56, LKN8081). This was a result of a low methionine intake and since the intake of all amino acids was low it undoubtably reflects reduced protein synthesis and degradation rates (Millward, 1970b; Motil et al., 1981). Conservation of nitrogen may be maximally expressed on these low protein diets by reutilizing during protein synthesis those amino acids released upon degradation. In reindeer which must consume low protein feeds for six to eight months each year, conservation of amino acids could be an effective survival mechanism.

Reindeer grazing on winter feeds have considerably

lower protein turnover than those grazing on green summer vegetation. The relatively rapid protein turnover rates observed in reindeer during summer most likely result from increasing protein intake and an abundant supply of amino acids in the diet. Based on estimates of the conservation of nitrogen by means of recycling, Wales et al. (1975) concluded that urea recycling in caribou (Rangifer tarandus) is substantially reduced during summer. Therefore higher protein synthesis and degradation rates plus minimal amino acid reutilization during protein synthesis are probable characteristics of reindeer metabolism during summer.

During winter when feed is variably available to reindeer, body weight losses are usual but excessive losses can be fatal. If starvation conditions persist for an extended time, reindeer mobilize body proteins to provide essential metabolites. The present studies on one reindeer near death shows that protein turnover in starved reindeer may increase four times that for reindeer in less critical condition but consuming the same feed. This antimortal rise phenomena in methionine transfer rate takes place without an increase in methionine intake, suggesting that the ^{75}SeM technique measures turnover rather than intake of methionine. The extent to which it reflects whole body protein turnover will depend on the extent to which methionine is a good marker amino acid.

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APPENDIX ONE

Whole Body 75Se-Selenomethionine Counts

Table Al- 1 . Whole body 75Se-selenomethionine counts in Reindeer (Preliminary trials).

EXPERIMENT	ELAPSED TIME (DAYS)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)
		RD # 77	RD # 103	MEAN
ES-OCB	0.91	54166	48407	51217
	2.05	45616	44629	45123
	7.15	42652	42027	42339
	14.11	41930	40050	40990
	21.09	39212	35485	37348
	28.16	37131	-----	37131
		RD # 99	RD # 82	MEAN
ES-LKN	1.07	50889	56055	53471
	1.99	49085	54081	51583
	6.01	38378	40410	39394
	11.75	34070	33618	34844
	18.98	28092	25867	26979
	26.00	25193	22605	23899

ES-OCB - Early spring preliminary trial, oats-corn-barley fed.
ES-LKN - Early spring preliminary trial, lichens fed.

Table A1- 2 . Whole body 75Se-selenomethionine counts in grazing reindeer.

EXPERIMENT	ELAPSED TIME (DAYS)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)
EW-GRZ		RD # 68	RD # 58	RD # 107	RD # 68	MEAN
	0.05	45464	45934	46494	45712	45902
	0.29	44640	48139	45680	48939	46850
	0.94	43028	44207	47653	47802	45673
	1.16	42857	45240	46086	43939	44531
	1.96	42842	43375	46628	44539	44346
	2.90	44233	44477	45341	45009	44766
	10.11	38322	40057	42815	39069	40066
	10.94	37937	40054	43296	39324	40153
	18.09	34608	36964	38831	36398	36700
	25.07	32576	33090	36117	34887	34169
	41.04	29652	30737	31034	32616	31010
	66.15	24941	25994	27857	26372	26291

Table A1- 2 . Continued.

EXPERIMENT	ELAPSED TIME (DAYS)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)
MW-GRZ		RD # 99	RD # 68			MEAN
	0.23	30622	28865			29744
	0.94	29365	31916			30641
	1.24	28239	30500			29370
	2.03	28016	31197			29607
	3.00	26755	29288			28021
	4.02	27092	30608			28850
	11.11	24332	26474			25403
	11.98	23757	23405			23581
	18.08	24230	24500			24365
	31.11	20757	21978			21368
SM-GRZ		RD # 99	RD # 58	RD # 68		MEAN
	0.07	61541	56885	57686		58698
	0.62	57430	54946	54567		55646
	2.72	55404	52838	53070		53745
	8.85	46116	43179	47837		45706
	16.84	43363	38794	42185		41443
	29.74	35341	30006	31830		32389

EW-GRZ - Early winter grazing trial
MW-GRZ - Mid-winter grazing trial
SM-GRZ - Summer grazing trial

Table Al- 3 . Whole body 75Se-selenomethionine counts in reindeer (pen-feeding trials).

EXPERIMENT	ELAPSED TIME (DAYS)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	% OF INITIAL DOSE REMAINING
QTX-8081		RD # 99	RD # 58	MEAN	MEAN
	0.1	46224	49089	47657	99
	0.8	44499	46136	45318	94
	1.8	40901	43611	42256	88
	3.9	36777	37951	38184	79
	6.4	33309	35296	34302	71
	9.8	30069	33210	31639	66
	13.4	29057	31029	29543	61
	16.8	26208	29506	27857	58
	21.8	25743	28396	25656	56
	24.8	25547	27435	26521	55
	28.9	25284	26769	26026	54
	32.0	24329	25353	25491	53
	39.0	22923	24103	23513	49
	46.0	21835	23154	22494	47
	53.0	20943	22431	21687	45
	60.0	19516	20514	20015	42
	69.4	18726	19200	18963	39
	77.0	17955	18647	18301	38

Table Al- 3 . Continued.

EXPERIMENT	ELAPSED TIME (DAYS)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	% OF INITIAL DOSE REMAINING
BAR-8081		RD # 7	RD # 68	MEAN	MEAN
	0.1	48703	50284	49494	100
	0.8	49167	50493	49830	101
	1.8	49113	49513	49313	100
	3.9	49397	50289	49843	101
	6.8	47051	48151	47601	96
	9.8	47236	47221	47229	96
	13.8	46896	46995	46945	95
	16.8	45635	46507	46071	93
	21.4	46494	46094	46294	94
	24.9	46555	45606	46081	93
	28.8	45811	46222	46017	93
	32.1	45462	46073	45768	93
	39.0	44818	43425	44122	89
	46.0	42665	43049	42857	87
	53.0	42455	42820	42638	86
	60.0	41731	40801	41086	83
	69.9	39237	39205	39221	79
	76.4	38797	39202	38999	79
	97.9	36716	37814	37265	75
	113.0	35125	36355	35740	72

Table Al- 3 . Continued.

EXPERIMENT	ELAPSED TIME (DAYS)	WHOLE BODY COUNT(cpm)	% OF INITIAL DOSE REMAINING	WHOLE BODY COUNT(cpm)	% OF INITIAL DOSE REMAINING
LKN-8081		R E I N D E E R # 82		R E I N D E E R # 56	
	0.1	47886	101	51880	97
	0.8	45840	97	54171	101
	1.8	45288	95	51825	97
	3.9	42901	90	49233	92
	6.8	38974	82	48192	90
	9.8	37134	78	46744	88
	13.8	35763	75	45519	85
	16.8	33090	70	44513	83
	21.8	32815	69	-----	--
	24.8	31039	65	43816	82
	28.8	30861	65	43062	81
	32.0	30496	64	-----	--
	39.0	29329	62	42352	79
	46.0	26748	55	42052	79

Table A1- 3 . Continued.

EXPERIMENT	ELAPSED TIME (DAYS)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	% OF INITIAL DOSE REMAINING
BAR-8182		RD # 58	RD # 99	MEAN	MEAN
	0.1	59279	59100	59190	100
	0.3	58293	57407	57850	98
	0.7	57814	55441	56628	96
	2.7	57259	54072	55666	94
	9.9	52055	51431	51743	87
	17.0	51886	49672	50779	86
	23.9	49462	49641	49552	84
	30.8	48945	46983	47964	81
	37.8	47517	47048	47283	79
	44.7	47038	46749	46893	75
	58.8	44452	44693	44572	72
	68.8	42738	-----	42738	73

Table A1- 3 . Continued.

EXPERIMENT	ELAPSED TIME (DAYS)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	WHOLE BODY COUNT(cpm)	% OF INITIAL DOSE REMAINING
B+M-8182		RD # 7	RD # 105	MEAN	MEAN
	0.1	61348	56183	58766	99
	0.7	61648	56031	59624	101
	2.7	61669	57579	59624	101
	9.9	60148	54459	57303	97
	16.9	58431	52017	55224	93
	23.9	56993	50207	53600	91
	30.8	56583	49210	52897	89
	37.8	55707	49221	52464	89
	44.7	54462	47124	50793	86
	58.8	53190	45693	49441	84
	68.8	52652	43414	48033	81
	85.9	50379	41466	45922	78

APPENDIX TWO

75Se-Selenomethionine Retention in Blood

Table A2- 1. 75SeM Retention in Plasma and RBC's (Grazing Trials).

Table A2- 1 . 75Se-selenomethionine activity in plasma and red blood cells of grazing reindeer.

EXPERIMENT	ELAPSED TIME (DAYS)	PLASMA ACTIVITY dpm/ml	% OF DOSE	RBC ACTIVITY dpm/ml	% OF DOSE
EW-GRZ	0.11	21027	10.1	659	0.3
	0.23	20765	10.0	1210	0.6
	0.30	20550	9.9	1518	0.7
	0.87	15970	7.7	1128	0.5
	1.10	15432	7.4	1542	0.7
	1.90	13917	6.7	2292	1.1
	2.85	12538	6.0	3567	1.7
	10.04	8486	4.1	8945	4.3
	10.87	7896	3.8	9230	4.5
	18.03	6118	2.9	11263	5.4
	25.01	4993	2.4	13314	6.4
	40.99	4048	1.9	15659	7.6
	66.05	2906	1.4	19443	9.4

Table A2- 1 . Continued.

EXPERIMENT	ELAPSED TIME (DAYS)	PLASMA ACTIVITY dpm/ml	PLASMA ACTIVITY % OF DOSE	RBC ACTIVITY dpm/ml	RBC ACTIVITY % OF DOSE
MW-GRZ	0.21	16453	8.6	6977	3.6
	0.93	11900	6.2	5290	4.4
	1.23	11132	5.8	8879	4.6
	2.02	10397	5.4	7989	4.2
	3.00	9538	5.0	10265	5.4
	4.03	9396	4.9	10652	5.6
	11.10	6657	3.5	13739	7.2
	11.97	6088	3.2	14302	7.5
	18.08	5068	2.6	15572	8.1
	31.13	4174	2.2	17006	8.9
	40.16	3709	1.9	17388	9.1
SM-GRZ	0.08	12618	8.2	0	0.0
	0.64	9614	6.2	797	0.5
	1.73	6754	5.4	4319	2.8
	8.86	4812	3.1	9426	6.1
	16.86	3145	2.0	11208	7.3
	29.77	2034	1.3	10691	6.9

EW-GRZ - Early winter grazing trial.
MW-GRZ - Mid-winter grazing trial.
SM-GRZ - Summer grazing trial.

APPENDIX THREE

Efficiency Curve for 35S

Figure A3- 1. Efficiency verses "H" Number.

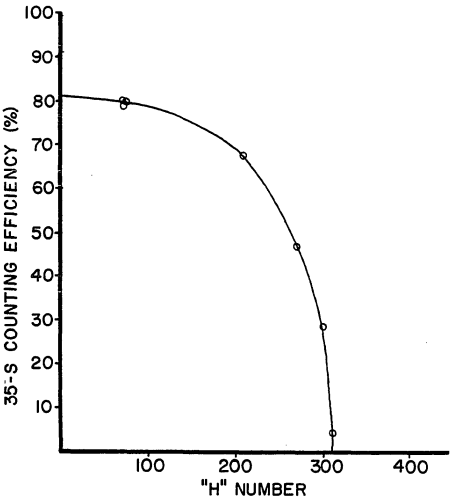

Figure A3- 1. Efficiency curve for 35Sulfur.

APPENDIX FOUR

Amino Acid Analysis of Feeds

Table A4- 1. Amino Acid Analysis of Barley, Quality Texture and Lichens.

Table A4- 1. Amino acid analysis of feeds offered to pen-fed reindeer. (mg/kg dry matter).

AMINO ACID	BARLEY 8081	BARLEY 8182	QTX	LICHENS
ASP	7.70	8.01	15.54	1.72
THR	4.16	4.12	6.51	1.02
SER	5.40	4.93	9.12	1.13
GLU	24.26	23.82	38.76	1.89
PRO	10.94	10.67	11.59	0.87
GLY	5.08	4.86	9.23	1.08
VAL	5.37	5.23	8.53	0.88
MET	1.46	1.33	2.55	0.16
ILE	3.58	3.66	5.76	0.70
LEU	7.58	7.32	12.22	1.13
TYR	3.76	3.44	6.01	0.39
PHE	5.05	4.98	8.32	0.71
HIS	2.37	2.42	5.49	0.33
LYS	4.62	4.52	7.82	0.78
ARG	6.09	6.02	16.23	0.75
ALA	5.44	5.27	9.15	0.34

QTX - Quality Texture, Fisher Mills, Seattle, Washington.

APPENDIX FIVE

Feed Intake and Temperature of Pen-Feeding Trials

Table A5- 1 .Daily temperature and fe
experiments of 1980-81.

DAY	TEMP °C	I N T A K E (kg) BAR8081	QTX8081	LKN8081
NOVEMBER 1980				
1	-13			
2	-16			
3	-14			
4	-10			
5	- 9			
6	- 6			
7	- 8			
8	-11			
9	-16			
10	-17			
11	-11			
12	-14			
13	-11			
14	-14			
15	-16			
16	- 9			
17	-13			
18	-18			
19	-14			
20	-12			
21	-12	1.87	0.98	1.91
22	-14	0.57	0.08	1.96
23	-15	1.06	0.22	1.81
24	-11	1.18	0.68	1.29
25	-13	1.20	1.05	1.12
26	-13	0.58	0.78	1.38
27	- 9	1.27	1.19	2.30
28	-10	1.39	0.68	1.55
29	-13	1.02	1.29	1.69
30	-14	1.53	1.18	2.14
--	--	----	----	----

ed intake for pen-feeding

DAY	TEMP °C	I N T A K E (kg) BAR8081	QTX8081	LKN8081
DECEMBER 1980				
1	-17	1.46	1.29	2.71
2	-20	1.39	1.25	1.30
3	-18	1.32	1.22	1 25
4	-16	1.33	1.39	1.42
5	-17	0.79	1.57	1.49
6	-26	1.42	1.70	1.29
7	-29	1.35	1.40	1.29
8	-33	1.09	1.47	1.16
9	-35	1.49	1.77	1.90
10	-38	1.32	1.53	1.38
11	-39	1.09	1.79	2.04
12	-40	1.50	2.69	2.28
13	-42	1.30	1.74	1.90
14	-39	1.42	2.01	1.86
15	-39	1.20	1.70	2.37
16	-40	1.53	1.70	2.40
17	-36	1.63	1.30	3.09
18	-35	1.22	1.69	2.84
19	-35	1.53	0.94	2.93
20	-30	1.28	1.89	2.51
21	-31	1.67	2.00	3.30
22	-33	1.47	1.90	3.25
23	-36	1.52	1.62	2.76
24	-31	1.64	1.72	2.89
25	-34	1.52	2.03	3.66
26	-41	1.60	2.06	3.32
27	-41	1.29	1.49	2.01
28	-38	1.13	1.52	2.99
29	-38	1.18	1.57	3.50
30	-34	0.84	1.11	2.58
31	-16	1.35	1.26	3.54

Table A5- 1. Continued.

DAY	TEMP °C	INTAKE (kg) BAR8081	QTX8081	LKN8081	DAY	TEMP °C	INTAKE (kg) BAR8081	QTX8081	LKN8081
JANUARY 1981					FEBRUARY 1981				
1		0.79	0.92	2.95	1	- 5	0.81	1.40	1.52
2	- 3	0.85	1.05	2.89	2	0	1.54	1.63	2.08
3	- 9	0.98	1.83	1.89	3	- 2	1.35	1.57	2.03
4	-19	1.35	1.80	2.62	4	- 6	1.28	1.81	1.64
5	-18	0.96	0.98	1.93	5	-10	1.46	1.90	2.01
6	-12	0.99	1.56	2.14	6	- 7	0.79	1.84	1.74
7	-10	0.71	1.53	2.25	7	-12	1.49	1.90	1.80
8	- 8	0.78	1.66	2.40	8	-13	0.84	1.53	1.70
9	-12	0.81	1.64	1.63	9	- 9	0.83	1.23	1.73
10	-15	1.22	1.83	1.90	10	-12	1.29	1.84	1.77
11	-17	0.96	1.72	2.52	11	-15	1.56	1.79	1.91
12	-14	1.22	1.49	1.77	12	-18	1.37	1.90	
13	-10	1.11	1.96	1.55	13	-25	1.11	1.80	
14	- 8	0.88	1.35	2.17	14	-30	1.69	1.56	
15	+ 2	0.85	1.43	1.98	15	-31	1.50	1.57	
16	- 1	0.75	2.01	3.02	16	-27	1.26	1.84	
17	- 1	2.11	1.78	1.75	17	-33	1.56	1.22	
18	- 3	0.68	1.07	1.74	18	-33	1.55	1.70	
19	- 3	1.36	1.45	1.73	19	-26	1.19	1.63	
20	- 5	0.96	1.42	1.56	20	-23	1.22	1.33	
21	-12	1.01	1.39	1.64	21	-18	1.35	1.26	
22	-14	1.30	1.64	2.07	22	-16	1.31	1.50	
23	-11	0.82	1.28	1.84	23	-19	1.03	1.46	
24	- 3	1.66	1.25	1.74	24	-15	0.91	1.56	
25	- 3	1.15	2.01	1.97	25	-13	1.49	1.65	
26	- 5	1.08	1.62	1.62	26	-11	1.12	1.22	
27	- 6	1.01	1.38	1.73	27	- 9	1.36	0.81	
28	- 9	1.25	1.73	1.98	28	- 7	1.25	0.91	
29	-10	1.42	1.42	1.69					
30	- 3	1.20	1.29	2.21					
31	- 4	1.15	1.50	1.87					

Table A5- 1. Continued.

DAY	TEMP °C	INTAKE (kg) BAR8081	QTX8081	LKN8081
MARCH 1981				
1	- 6	1.20	1.38	
2	- 5	1.35	1.20	
3	- 7	1.33	1.42	
4	- 6	1.37	1.46	
5	- 6	1.37	1.46	
6	- 6	1.12	1.25	
7	- 5	1.46	1.62	
8	- 8	1.67	1.79	
9	+ 1	0.98	1.53	
10	- 4	1.59	1.70	
11	- 1	1.11	0.84	
12	+ 3	1.01	1.17	
13	- 1	1.35	1.57	
14	- 2	1.73	1.55	
15	0	1.22	1.59	
16	- 1	1.59	1.72	
17	+ 3	1.11	1.67	
18	+ 4	0.89	1.08	
19	+ 2	1.30	0.65	
20	+ 2	1.73	0.61	
21	+ 2	1.86	1.45	
22	- 6	1.60	1.36	

DAY	TEMP °C	I N T A K E (kg) BAR8081	QTX8081	LKN8081

Table A5- 2 . Daily temperature and feed intake for pen-feeding experiments of 1981-82.

DAY	TEMP °C	INTAKE (kg) BAR-8182	B+M-8182	DAY	TEMP °C	INTAKE (kg) BAR-8182	B+M-8182
DECEMBER 1981				JANUARY 1982			
1	-15			1	-25	1.32	1.07
2	-15			1	-28	2.17	2.11
3	-15			3	-27	1.36	1.41
4	-20			4	-21	1.36	1.41
5	-21			5	-23	1.42	2.20
6	-20			6	-30	1.38	1.80
7	-24			7	-30	1.25	1.80
8	-27			8	-38	1.18	1.83
9	-13			9	-31	1.40	1.56
10	-16			10	-26	1.34	0.67
11	-18			11	-21	1.34	0.67
12	-18			12	-22	1.67	1.69
13	-16			13	-29	0.78	1.43
14	-19			14	-29	0.78	0.86
15	-21	1.66	0.96	15	-24	0.78	0.85
16	-15	1.22	0.61	16	-30	1.29	1.81
17	- 6	1.22	0.61	17	-33	0.95	1.18
18	- 1	1.78	1.52	18	-29	0.95	1.18
19	- 7	2.06	1.15	19	-26	1.60	2.24
20	-11	1.60	1.15	20	-26	1.30	1.32
21	-16	1.61	1.15	21	-21	1.26	0.98
22	-21	1.09	1.62	22	-18	1.28	1.46
23	-19	0.96	1.43	23	-19	1.20	1.73
25	-19	1.43	1.30	25	-28	1.04	0.94
26	-24	1.33	1.30	26	-33	1.79	1.91
27	-30	1.74	1.56	27	-32	1.49	1.91
28	-33	1.49	1.45	28	-22	1.16	1.20
29	-37	1.48	1.60	29	-17	1.27	0.71
30	-37	1.67	1.80	30	-18	1.62	1.32
31	-29	1.33	1.07	31	-19	1.29	1.62

Table A5- 2 . Continued.

DAY	TEMP °C	INTAKE (kg) BAR-8182	INTAKE (kg) B+M-8182	DAY	TEMP °C	INTAKE (kg) BAR-8182	INTAKE (kg) B+M-8182
FEBRUARY 1982				MARCH 1982			
1	-17	1.30	0.56	1	-18	1.45	1.18
2	-11	1.45	0.56	2	-18	1.66	1.90
3	- 4	1.66	0.96	3	-14	1.08	1.16
4	- 2	1.67	1.22	4	-17	1.38	1.47
5	- 2	1.59	1.56	5	-10	1.57	1.84
6	- 3	1.56	0.93	6	-10	1.57	1.28
7	- 1	1.76	0.67	7	- 8	1.57	1.28
8	- 3	1.66	0.99	8	-10	1.18	0.78
9	- 1	1.77	0.94	9	- 6	1.18	0.78
10	- 9	1.57	1.06	10	- 6	1.98	1.36
11	-14	1.87	1.28	11	- 8	1.50	1.59
12	-21	1.26	1.15	12	- 8	1.40	1.13
13	-19	1.29	0.99	13	-16	1.59	1.45
14	-21	1.29	1.00	14	-16	1.59	1.45
15	-25	1.08	0.78	15	-18	2.01	1.77
16	-29	1.08	0.78	16	-15	1.12	1.74
17	-30	2.10	1.97	17	- 2	1.94	1.60
18	-33	1.94	1.46	18	0	1.72	1.36
19	-31	1.60	1.39	19	+ 1	1.31	1.28
20	-36	1.23	1.42	20	+ 3	1.12	1.74
21	-35	1.78	1.19	21	+ 1	1.29	1.64
22	-34	1.78	1.18	22	+ 2	1.34	1.20
23	-32	2.14	1.30	23	- 1	1.56	1.33
24	-24	1.57	1.10	24	- 3	1.55	0.96
25	-26	1.94	1.69	25	- 7	1.53	1.49
26	-20	1.67	1.52	26	-12	1.53	1.26
27	-19	1.72	0.94	27	-13	1.44	1.34
28	-20	1.45	1.18	28	-13	1.45	1.43
				29	-13	1.49	1.55
				30	-14	1.63	1.81
				31	-17	1.72	1.93

Table A5- 2 . Continued.

DAY	TEMP °C	I N T A K E (kg) BAR-8182	B+M-8182	DAY	TEMP °C	I N T A K E (kg) BAR-8182	B+M-8182
APRIL 1982							
1		1.18	1.60				
2	-16	1.59	1.45				
3	-15	1.60	1.43				
4	-10	1.58	1.43				
5	- 2	1.59	1.43				
6	+ 5	1.56	1.96				
7	+ 4	1.57	1.87				
8	- 1	1.36	1.69				