

**STUDIES ON WHOLE-BODY NITROGEN TURNOVER,
PROTEIN SYNTHESIS AND BREAKDOWN IN MAN
USING ¹⁵N GLYCINE**

by

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ACKNOWLEDGEMENTS

As a house-surgeon in 1966 I had an interest in fluid and electrolyte balance and the metabolic and endocrine responses to surgery and injury. I asked one of my chiefs, Ian McNeil, who I knew had a similar interest, to recommend a book on the subject. "There is only one book", was the exact reply; he was referring to "The Metabolic Care of the Surgical Patient" by Dr Francis D Moore and Margaret Ball, published in 1958. Two years later, in 1968, the same Dr F D Moore was a visiting professor to the University of Newcastle upon Tyne, and gave the Jacobson Lecture on the subject of Transplantation, which at that time was still in its infancy.

The Vice-Chancellor, the late Dr Henry Miller, introduced him as the world's greatest living surgical scientist, and in his lecture, which described the background leading to the world's first kidney transplant performed in his department at the Peter Bent Brigham Hospital in Boston, Dr Moore demonstrated one of his many qualities - the ability to enthuse others. I nursed an ambition to work for him at some stage, but unfortunately, such an opportunity did not arise for another ten years, by which time a consultant post in surgery beckoned. In addition, Dr Moore had

retired as Mosely Professor of Surgery and Surgeon in Chief at the PBBH, but was given an Emeritus Chair and was still active in surgical research, albeit in a smaller department. It was not the ideal stage in my career to take more time out to do research, but nevertheless I shall always be grateful to Dr Moore for allowing me to work as a Research Fellow in Surgery in his department for one year, and for his help and encouragement during the course of the studies described herein. It was an honour and a privilege to be associated with someone who has made so many contributions to knowledge in the field of surgical metabolism.

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PREFACE

The experimental work described in this thesis was conducted in the surgical research laboratories of Dr Francis D. Moore in the Peter Bent Brigham Hospital (now the Brigham & Women's Hospital) Boston, Massachusetts, USA, between 1978-1979. It formed part of an ongoing programme of research into protein metabolism in man; specifically to measure total body nitrogen turnover and hence protein synthesis and breakdown, initially in normal volunteers receiving various intravenous feeding regimens, and subsequently in patients. The previous year, 1977, had seen the publication of 'Substrate Interaction in Intravenous Feeding' by Bruce Wolfe et al., from the same laboratories. This was an extensive piece of work incorporating many studies and compared nitrogen balance data together with biochemical, hormonal and plasma amino acid data in normal men fed intravenously with a variety of regimens.

Shortly afterwards a series of protein turnover studies was embarked upon, using the ^{15}N glycine method, and in collaboration with Dr Vernon Young of the Massachusetts Institute of Technology. The first experiments were essentially a repeat of the studies described by Wolfe et al. (vide supra) but in addition nitrogen turnover, protein synthesis and breakdown were estimated using a continuous 60 hour infusion of ^{15}N glycine and measuring enrichment of urinary urea with ^{15}N when a plateau was reached. Normal volunteers were studied firstly on normal oral diet and then on a

succession of intravenous regimens:- amino acids alone (AA), amino acids plus 'high dose' glucose (AA+HDG), amino acids plus fat emulsion (AA+FE), amino acids plus 'low dose' glucose (AA+LDG), amino acids, fat emulsion and low dose glucose (AA+LDG+FE), and finally 'low dose' glucose alone (LDG).

The studies on normal diet, AA and AA+HDG were conducted by Andrew Sim (a Glasgow/Harvard exchange fellow) and Bruce Wolfe. The author took no practical role in these experiments, but was responsible for analysis of the data and the protein metabolism calculations, and was a co-author when the work was published in 1979 (Sim et al., *Glucose Promotes Whole-Body Protein Synthesis from Infused Aminoacids in Fasting Man*, *Lancet* i, 68-71).

Subsequently, the author did the experiments using AA+LDG+FE, AA+FE, and AA+LDG and LDG. The results on these four regimens were incorporated in a paper presented in 1979 at the Tripartite Meeting of the Surgical Research Society at Oxford under the title 'Isotope Studies of substrate interaction in parenteral nutrition', and also at the 2nd European Congress on Parenteral and Enteral Nutrition at Newcastle upon Tyne in 1980, and later published as 'The Effect of Fat Infusion on Protein Metabolism' (*Acta. Chir. Scand.*, Suppl. 507, 475-484, 1981). When the studies on the various intravenous feeding regimens were completed, attention was turned to the possible distorting effects of variables such as exercise and diet

on the behaviour of the isotope equilibrium curve and plateau. Such effects, if present, might have significance when studies were carried out on patients at a later stage in the research programme. Because each study lasted 48-60 hours, changes might occur either unintentionally or as a result of the needs of clinical management, and if they affected the plateau would alter the resultant calculations of turnover, synthesis and breakdown. Such a potential source of error clearly needed investigation.

A pilot study was done in two subjects, later repeated on each, to observe any effects on the curve and plateau of both doubling protein intake and bicycle exercise. Subsequently, more extensive studies were done varying the protein and energy intakes, both orally and intravenously, allowing a detailed analysis of curve perturbation, and introducing the concept of basal catabolic rate.

Finally, protein turnover, synthesis and breakdown were estimated seven times in four seriously ill patients.

All of the studies mentioned above form the basis of the thesis. Unfortunately, the gestation period of this thesis has been long. There are two main reasons for this. Firstly, the work done was part of a five-year programme of research, with the intention of publishing a paper in a scientific journal at the completion of each stage. This was done

with the first three regimens (normal diet, AA and AA+HDG) but not with the last four (AA+FE, AA+LDG+FE, AA+LDG, LDG), although the results were presented at two scientific meetings. Shortly after returning to the United Kingdom the author was appointed a consultant surgeon and this career move assumed priority. Secondly, although it was intended to publish the perturbation studies, it proved impossible to reduce the size of the text to a manageable level suitable for publication in the form of a scientific paper.

However, despite the long interval since the experiments were done, no similar work has been published. In particular, virtually no attention has been paid to intentional perturbation. Also, whereas there was a spate of interest in protein turnover studies in the late 1970s and early 1980s, virtually no publications have appeared since 1985. It seems that the potential applications of the method are considered exhausted, and interest has been lost, rather as it was in the 1950s following a short flurry of activity exploring the first cumbersome technique. Hence, it seemed all the more pertinent, even at this late stage, to publish the work in the form of a thesis which could describe in chronological order the continuum of studies as briefly mentioned above. In order to preserve such a progression, the following Introduction contains, with few exceptions, only references up to the time that the experimental studies were commenced, 1978, but the subsequent Discussion(s) in the various sections will attempt to include the relevant literature up to the present time.

Summary of Overall Results

1. Compared to normal oral diet, in which nitrogen equilibrium was observed, there was a negative nitrogen balance when normal subjects were infused with a variety of intravenous substrates. This negative nitrogen balance decreased progressively on LDG, AA, AA+LDG and AA+FE, AA+HDG. Using AA+LDG+FE nitrogen equilibrium was achieved, as with normal oral diet.
2. Nitrogen turnover on normal oral diet was significantly higher than on intravenous AA alone and AA+ADG.
3. Addition of glucose to amino acids improved nitrogen balance compared to amino acids alone, and this was due to increased protein synthesis, breakdown remaining unchanged.
4. Protein synthesis and breakdown were highest in the low dose glucose group. Turnover did not fall in this group, indicating maximal re-utilisation of amino acids for synthesis. In this situation of zero nitrogen intake the model may not be valid.

5. There were insignificant changes in blood glucose and serum insulin levels between groups. In all groups receiving amino acids there were insignificant changes in plasma glucagon levels, but in the LDG group there was a significant fall. Insulin: glucagon molar ratios remained low (2.2-7.5) except in the LDG group (26.2) which was due to suppression of glucagon.

6. Plasma ketones rose in the AA + FE group. Free fatty acids doubled in those groups receiving fat emulsion.

7. Total plasma amino acid and branched chain amino acid levels rose in those groups receiving amino acid infusions. The alanine:TAA ratio fell in those groups receiving fat emulsion. The glycine:TAA ratio rose in all groups, especially those receiving amino acid infusions.

8. In the same five subjects who were studied on normal oral diet, intravenous amino acids, and amino acids plus glucose, a diurnal rhythm existed in urinary excretion of urea and nitrogen. Excretion was lowest in the early hours of the morning and at its highest at

midday and the early hours of the afternoon. Such a rhythm might be explained on normal oral diet by intermittent meals, but this explanation would not suffice for the same observations on continuous supply of intravenous amino acids. This phenomenon has not been described before, and might have implications for cyclical parenteral nutrition.

9. Experiments were conducted to intentionally perturb the build-up curve and plateau enrichment of ^{15}N urinary urea enrichment using acute changes in dietary intake by doubling of protein intake, total cessation, and manipulation of calorie:nitrogen intake.

10. Typically, doubling of protein intake caused synthesis to remain constant, or nearly so, with a marked decrease in breakdown. With cessation of protein intake, synthesis fell sharply, while breakdown remained the same or increased slightly.

11. When nitrogen intake is zero, nitrogen turnover equals total breakdown of body protein. This introduces the concept of the Basal Catabolic Rate, the mean value being about 325 mg/Kg/d.

12. Calorie:nitrogen manipulation produced bizarre alterations. The most consistent was that with cessation of protein intake but maintenance of calories, normal protein synthesis can be quickly resumed upon restoration of normal diet. By contrast, protein intake unsupported by exogenous calories is accompanied, when calories are added, by continuation of net breakdown.

13. In burned and septic patients, protein turnover was markedly elevated. Both synthesis and breakdown were increased, net catabolism being caused by a greater increase in breakdown.

INTRODUCTION

PREAMBLE

The overall long-term purpose of the research, of which the work embodied in this thesis forms a part, was to maximise the effectiveness of supportive nutritional therapy by a better understanding of the metabolism of body fuels as they are influenced by starvation, trauma and sepsis. At the time the work was conducted, intravenous feeding in the form known as "hyperalimentation" was widespread, especially in the USA. When clearly indicated and given appropriately, such treatment appeared to accelerate surgical convalescence and support recovery. However, it was expensive, potentially hazardous, and often given incorrectly. More importantly, it was often used unnecessarily, prompted largely by the industry manufacturing the products and the vested interests of certain clinicians. It was clearly important that such a precious resource was used properly. Hence, a specific objective of the research was to determine the most appropriate mix of intravenously infused substrates as based on the metabolism of the nutrients and the endocrine status of normal man, and patients in situations of chronic starvation, acute trauma and sepsis.

For many years the basis of such metabolic research has been the use of nitrogen balance studies. However, whereas the nitrogen balance gives the difference between protein synthesis and breakdown, it does not give the value of either. A negative nitrogen balance could occur as a result of increased breakdown (the usual assumption), decreased synthesis, or a combination of the two. Knowledge of changes in protein synthesis and breakdown in various clinical settings would not only be of academic interest, but might lead to attempts to influence such changes as part of treatment.

Thus, the work described in this thesis was undertaken with the purpose of investigating changes in protein metabolism in man in terms of nitrogen turnover, protein synthesis and breakdown. Nitrogen turnover was measured from enrichment of urinary urea with the stable isotope ^{15}N at an equilibrium plateau level achieved after constant infusion of ^{15}N labelled glycine. Protein synthesis and breakdown were estimated using this measurement in combination with nitrogen balance data.

Studies were first conducted on normal volunteers; baseline values were obtained on a normal oral diet, and subsequently comparisons made with subjects on six different intravenous feeding regimes. In all subjects analyses were also made of plasma biochemical parameters, endocrine and amino acid profiles.

For future reference it was considered essential to explore the ^{15}N glycine method to its limits. The establishment of the 'correct' plateau is essential for accurate measurement of nitrogen flux, and hence protein turnover, synthesis and breakdown. Perturbation of the curve rising to a plateau, and of the plateau itself, could theoretically be created by acute changes in the diet and perhaps by exercise. Such perturbations have been largely ignored by other workers. An extensive series of experiments was therefore devised to observe the effects of intentional perturbation of the enrichment curve.

Finally, a small number of patients was studied and comparisons made with the results in normal man. This Introduction will now describe the background to the work in a logical sequence, starting with the nature and basic chemistry and importance of proteins, the concept of Protein Turnover, Nitrogen Balance, Protein Metabolism in Health, Starvation, the Metabolic Response to Trauma, Total Intravenous Nutrition, and Measurements of Protein Turnover.

THE NATURE AND IMPORTANCE OF PROTEINS

Whereas almost every individual knows that oxygen is essential to life few probably know why. Similarly the average person probably has a vague concept of what a protein is; if not chemically, at least the common dietary sources, but cannot be more precise. It is likely that even a qualified medical practitioner, certainly one who has long left medical school, would have difficulty in explaining precisely why oxygen is essential to life - respiration and oxygen transport by haemoglobin might easily spring to mind, but the complex cascade of reactions culminating in the electron transport system in the mitochondria will not surprisingly have almost certainly left the memory. Likewise, he would probably struggle to define the word protein to a layman, even though he could illustrate his answer with examples. It is taken for granted that both oxygen transport and proteins are essential for life without fully understanding why. The nature of proteins will therefore be described in some detail from basic principles.

i) Historical background

Early in their study of living matter, chemists found a group of substances which, when heated, turned from liquid to solid rather than the other way round. The white of egg, a substance in milk, and a component of the blood all showed this property.

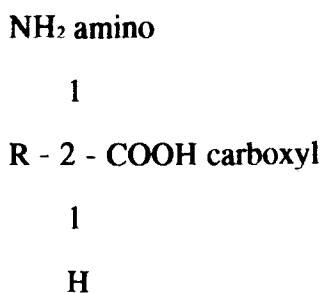
In 1777 the French chemist Pierre Joseph Macquer classified all substances that coagulate on heating as albuminous, after albumen, the word given to egg white by the Roman encyclopaedist Pliny the Elder. However it took the genius of Magendie (1816) to understand the difference between the nitrogenous and non-nitrogenous constituents of food. He described experiments on dogs which received only carbohydrate or fat until death supervened. The experiments were later extended to isolated proteins by the Commission on Gelatine of which Magendie was a member. About the same time as Magendie's later work was reported, urea was recognised as the principal end product of protein metabolism (Dumas and Cahours, 1842) and the first nitrogen balance studies were described (Boussingault, 1839). In 1839 the Dutch chemist Mulder worked out a basic formula, $C_{40} H_{62} O_{12} N_{10}$, which he thought was common to the albuminous substances. At the suggestion of the Swedish chemist Berzelius (who was an early publisher of a table of atomic weights), Mulder named his root formula, protein; from the Greek Proteios, primary, ie. of prime importance. In fact, of course, Mulder's attempts to work out empirical formulae for proteins were doomed to failure, but within a decade of his work, Justus von Liebig had shown that the substances themselves (rather than the root formula) which became known as proteins, were more essential to life than carbohydrates or fats.

A dictionary definition of a protein is "any of a group of complex nitrogenous organic compounds of high molecular weight that contain amino acids as their basic structural units and that occur in all living matter and are essential for the growth and repair of animal tissue" (Collins English Dictionary, 1979). As indicated the amino acids are the basic structural units of proteins, and their properties and sequence control the structure and function of the whole protein. Protein molecules may contain between 200 - 20,000 amino acid molecules, consisting of the 20 naturally occurring amino acids in varying proportions, and the sequence in which they occur is specific to the particular protein. If a protein is hydrolysed with acid, it is broken down into its constituent amino acids which can be separated and identified (except for tryptophan which is destroyed by this process).

Indeed, the first amino acid isolated was discovered by this method when Henri Braconet, in 1820, heated gelatin with acid and obtained a sweet-tasting crystalline substance. This turned out not to be a sugar but a nitrogen-containing compound, because ammonia could be obtained from it, and the compound was christened glycine from the Greek word for "sweet". Shortly afterwards Braconet obtained a white crystalline substance by heating muscle tissue with acid. This was named leucine, from the Greek word for "white". Actually, the amino acid cystine had been isolated as early as 1810 by the English chemist, Wollaston, but had been obtained

pure from a bladder stone rather than being separated from a protein. The last of the important amino acids was identified in 1935.

The general formula for a naturally occurring amino acid is:-

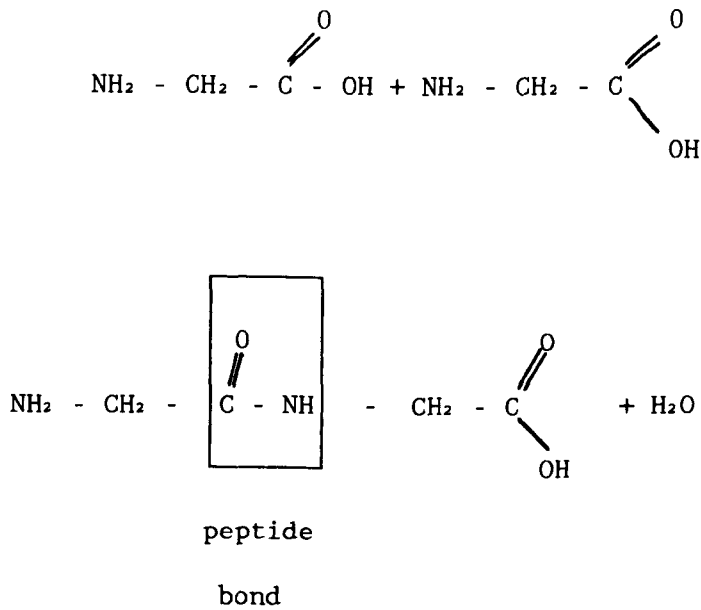


where R represents any side chain.

Amino acids may be grouped together into classes of similar structures, under the general headings of aliphatic, aromatic and heterocyclic amino acids.

By the end of the last century it was known that proteins were giant molecules built up of amino acids but it was not known how the acids were joined together.

In 1901 Emil Fischer achieved the first condensation of one molecule of glycine to another with the elimination of a molecule of water.



This is the simplest condensation possible.

By 1907, Fischer had synthesized a chain made up of 18 amino acids, 15 of them glycine and 3 of leucine. This molecule did not show any of the obvious properties of proteins, but Fischer felt that this was only because the chain was not long enough. He called his synthetic chains peptides, from the Greek meaning "digest", because he correctly believed that proteins broke down into such groups when they were digested. Up to 1946 the longest synthetic peptide chain was only one longer than Fischer's, of 19 amino acids.

However, as stated earlier, protein molecules may contain 200 - 20,000 amino acids. The subdivisions between polypeptides and proteins is arbitrary, and a polypeptide molecule is taken to be one which contains less than 100 amino acid molecules. This gives polypeptide molecular weights up to about 10,000. Proteins have a whole range of molecular weights up to several millions, corresponding to several thousand amino acid molecules.

As stated earlier the dominant role of proteins in nutrition has been recognised since the work of Magendie but it was not until earlier this century that Abderhalden (1912) showed that the fundamental nitrogenous unit required was a mixture of amino acids (derived from completely hydrolysed casein). The same 20 amino acids are found in proteins from all species of micro organisms, plants and animals.

These are:-

<u>Amino acid</u>	<u>Essential</u>
alanine	no
arginine	no
asperagine	no
aspartic acid	no
cystine	no
glycine	no
glutanic acid	no
glutamine	no
histidine	semi
isoleucine	yes
leucine	yes
lysine	yes
methionine	yes
phenylalanine	yes
proline	no
serine	no
threonine	yes
tyrptophan	yes
tyrosine	no
valine	yes.

A few proteins contain several additional amino acids (hydroxylysine, 4-hydroxyproline). A number of other amino acids which are not commonly found in proteins have important physiological roles in man eg. tri-iodothyronine and thyroxine, dihydroxyphenyl - alanine (DOPA), ornithine and citrulline (in the urea cycle).

Amino acids enter the body from the gut via the portal circulation almost entirely in the free form. Elwyn (1970) has shown that in dogs fed a large meal of meat 57% of the absorbed amino acids which pass through the liver are converted to urea, while 6% enter into the formation of plasma proteins and only 23% leave the liver as free amino acids; the residual 14% presumably remain in the liver for synthesis of other hepatic proteins.

Free amino acids make up less than 1% of the total amino acid content of the body. More than 50% of the total pool of free amino acids is in skeletal muscle. Those present in greatest concentration are alanine, glutamic acid, glutamine and glycine.

Corticosteroids increase the uptake of amino acids by liver cells. Growth hormone and insulin increase amino acid uptake by muscle. At usual concentrations, 85% or more of circulating amino acids leave the circulation within 5-15 minutes. There is rapid uptake by liver, kidney, pancreas and gut mucosa, and slower uptake by muscle, but since muscle mass makes up such a large proportion of body tissues the muscles serve as a major "reservoir" for amino acids.

Normally less than 1% of total daily amino acid intake is excreted as free amino acids. When they are found in urine in large amounts, the cause is usually defective tubular reabsorption rather than due to overflow (except when amino acids are infused intravenously at a very rapid rate).

ii) Essential and non-essential amino acids

"Essential" amino acids are those in which the carbon skeletons cannot be synthesized from readily available precursors in amounts adequate to provide for optimal growth and maintenance. The essential amino acids for the adult human are listed on page 10. In addition, histidine is considered essential or semi-essential for infants. Although amino acids may be categorised as "essential" or "nonessential", all of the amino acids present in human proteins must be available in suitable amounts in the cell for protein synthesis to take place. Whenever any of these amino acids, essential or nonessential, is present in less than adequate concentration, this amino acid becomes the limiting factor in protein synthesis. When protein synthesis ceases, the other amino acids are catabolised.

Three of the amino acids have carbon chains with branch configurations and are referred to as the "branched - chain amino acids" (BCAA). They are valine, leucine, and isoleucine. They are rate-limiting in muscle-protein synthesis, because they are absolutely required for the

synthesis of muscle-protein and are oxidised or utilised within muscle. When degraded in muscle they donate their NH₂ groups to pyruvate to form alanine. Alanine is then deaminated in the liver, the NH₂ group entering urea and the alanine skeleton returning to muscle either as pyruvate or glucose (Felig, 1973). The branched-chain amino acids are thus of central importance in post-traumatic metabolism, because they are utilised locally in muscle as a fuel. Once degraded, they are irreversibly lost from muscle. At the same time they are integral in the structure of both actin and myosin.

iii) The Concept of Protein Turnover

Until about 1940 it was not known whether proteins which formed part of a living structure were metabolically inert or not. However, in his Dunham lectures subsequently published posthumously in 1942 as a monograph entitled "The Dynamic State of Body Constituents" Schoenheimer described the first metabolic applications of what were then the recently available radioactive and stable isotopes of common elements. Working at Columbia University in New York City, he had demonstrated for the first time the turnover rates of constituents of body tissues. It had long been suspected that tissue such as muscle, bone, liver and brain were not unchanging rocks in an ocean of shifting body fluids, but were instead being constantly worn away and renewed.

Schoenheimer showed that when ^{15}N - labelled amino acids or ammonia were fed to animals, a large part was incorporated into tissue proteins. ^{15}N fed in the form of amino acids was quickly taken up into plasma proteins, gut mucosa, liver, spleen and bone marrow, while bone, skin and muscle retained only small amounts of ^{15}N per unit weight of tissue.

These observations led to the concept that nearly all proteins in the body were in a state of flux, being continually synthesized and broken down and that this flux was one of the main characteristics of living tissue.

Subsequent studies utilising other tracers such as radio-active isotopes of carbon, hydrogen and sulphur confirmed the findings of Schoenheimer. The term "turnover" which evolved implies the replacement of an amount of degraded protein by an equal quantity of the same protein, newly synthesized from its metabolic precursors. Protein turnover may occur by three different processes:-

- 1) the protein may be part of a structure with a fixed lifespan, eg haemoglobin in the red cell which is almost metabolically inert in the mature cell but is degraded to amino acids when the red cell is destroyed. Thus the lifespan of a haemoglobin molecule is a function of the life expectancy of the red cell.

2) some soluble proteins of cells or proteins of various body fluids which are not part of the organised structure and are degraded randomly at a constant rate. Thus, a recently synthesized molecule of serum albumin has as much chance of being degraded as one that has been in circulation for a number of days. The turnover of such proteins is expressed in terms of "half-life".

3) those proteins which are secreted from one cell and broken down in another part of the body eg. pancreatic enzymes.

iv) The Concepts of Protein Anabolism and Catabolism

The concept of protein turnover implies the concepts of protein anabolism and catabolism which are the rates of protein synthesis and breakdown, respectively.

Protein synthesis is extremely complex and most of our knowledge has been acquired during the last thirty years. Whereas the genetic information required for synthesis of protein is contained in the DNA of the cell nucleus, actual synthesis takes place on ribosomes in cytoplasm. Information is transmitted from nucleus to cytoplasm by messenger RNA (mRNA). The process will not be considered further except to add that a rate-limiting step is the availability of high-energy phosphate bonds as well as supply of amino acids.

NITROGEN BALANCE

It seems appropriate at this point in the Introduction to discuss the subject of Nitrogen Balance. Many studies concerning the general state of protein metabolism have relied upon measurement of total nitrogen intake and excretion, including knowledge of protein metabolism in health, protein-energy relationships, starvation etc., which will be described subsequently. Furthermore, nitrogen balance data form an integral part of the experiments described in this thesis. Whereas nitrogen turnover (or flux) can be measured directly, estimation of protein synthesis and breakdown requires the additional values of nitrogen intake and excretion.

Nitrogen Balance = Nitrogen Intake - Nitrogen Excretion,

or N Balance = N in - N out.

N out = N_{urine} + N_{stool} + N_{skin} + N_{wound} + N_{fistula or nasogastric aspirate}

Stool nitrogen rarely exceeds 1-2 gm/day unless diarrhoea is present.

Skin losses range from 0.1 - 0.4 gm/M²/day. (Calloway et al., (1971).

Usually stool plus skin losses do not exceed 2 gm/day.

Urea accounts for 80-90% of urinary nitrogen lost. By adding an additional 20% to urinary urea measurements (urine urea concentration x 24 hour urinary volume), an approximation of urinary nitrogen losses can be made, 2 gm. added for stool and skin losses and nitrogen balance approximated.

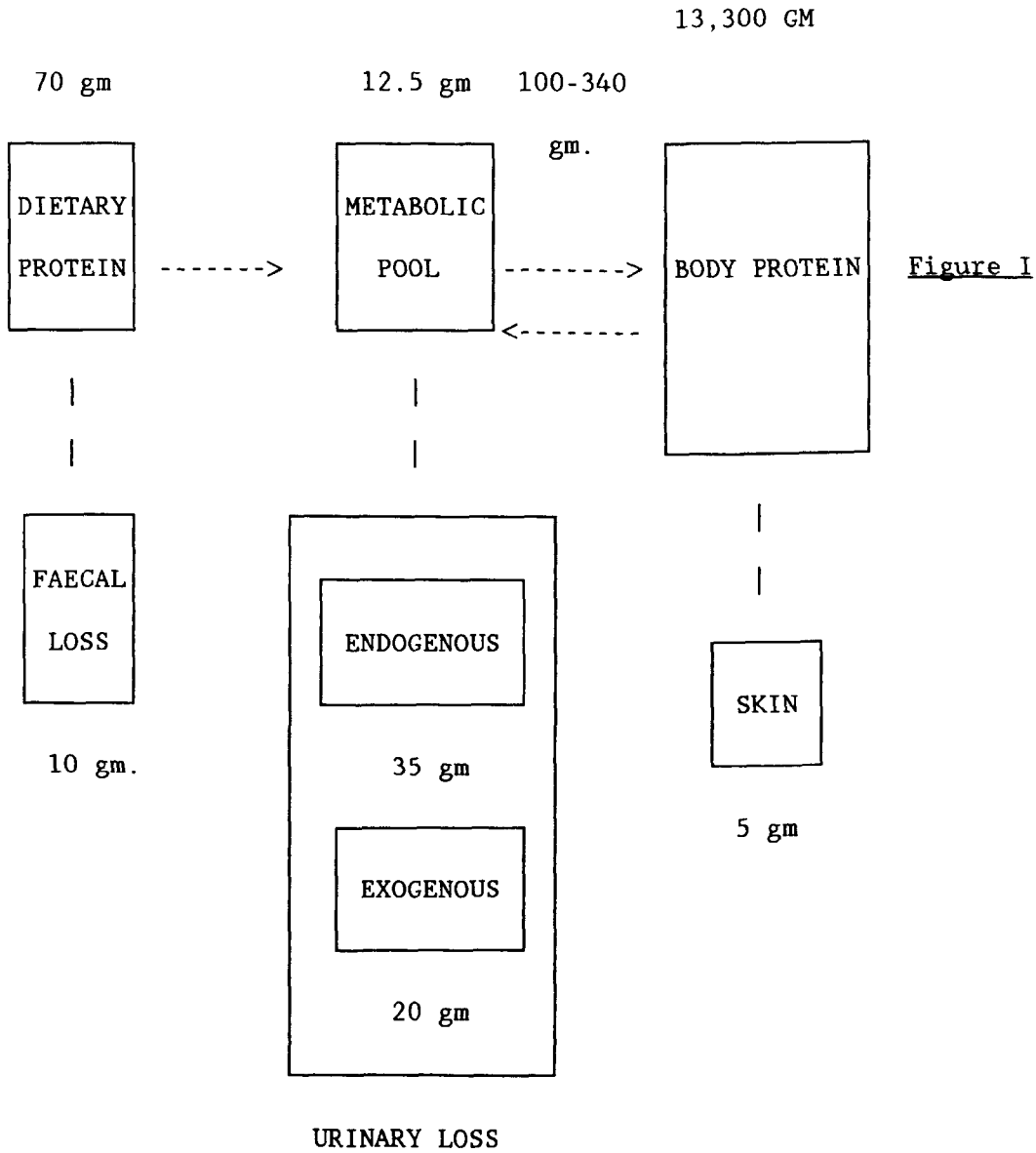
The error of nitrogen balance is always in the positive direction because of a tendency to overestimate nitrogen intake (from diet not consumed or not absorbed) and to underestimate nitrogen output (through incomplete collection of urine) and unexplained or underestimated nitrogen losses from the body. There has been speculation that false positive nitrogen retentions might be explained by reduction and loss of nitrogen as molecular nitrogen gas or ammonia (Hegsted, 1976). Calculated data thus appear more favourable for nitrogen retention and protein synthesis.

Over a period of several days an individual can adjust his urinary nitrogen excretion to achieve equilibrium over a wide range of nitrogen intakes. When dietary protein is reduced, protein is lost before a new equilibrium is achieved; this protein is thought to arise from organs with high rates of protein turnover or come from free amino acid pools, which are found in intracellular stores in the body. Some sites of deposition of the most labile proteins are in the viscera (liver, pancreas and gut mucosa) - other tissues such as skeletal muscle respond less rapidly (Erik et al., 1972). Brain and connective tissue undergo little change in protein content with starvation. The rapidly fluctuating protein pools have been referred to as "labile protein stores" (reserve protein) and account for the early nitrogen loss which occurs following starvation or trauma.

Studies of nitrogen balance have demonstrated the fundamental importance of the level of energy intake upon nitrogen retention.

Over a considerable range of caloric intake there is a roughly proportional relationship between nitrogen retained and the calories of extra energy added (Plough et al., 1956). A more favourable response is achieved if the caloric source is fed at the same time as the protein. This beneficial effect of an increase in energy intake upon anabolism can be inhibited by inadequate protein intake. The summary of the work of Calloway and Spector (1954) has become an aphorism in this respect :- "on a fixed adequate protein intake, energy level is the deciding factor; on a fixed adequate caloric intake the level of protein intake is the determinant". Despite the known potential errors, nitrogen balance studies remain the mainstay of metabolic studies and meaningful results can be obtained by attention to detail. However, as has been mentioned already in this Introduction, although nitrogen balance estimates the difference between synthesis and breakdown, it does not give a value for either. Such values are obtained from knowledge of nitrogen flux or turnover, measured directly, and nitrogen balance data. Details of turnover measurements will be dealt with extensively later in this Introduction, but at this stage by combining the concept of protein turnover (using results from previous studies) and our knowledge of nitrogen balance studies, one can summarise the daily protein metabolism in health in a standard 70 Kg man.

DAILY PROTEIN METABOLISM IN A 70 Kg MAN



Protein turnover in the whole body is estimated to vary between 100-340 gm. of protein daily for a 70 Kg man, with most values between 285-340 gm. (Wannemacher, 1975). If a normal man eats 70 gm. protein per day but has a turnover of about 300 gm. per day protein synthesis must be several times in excess of protein requirements, confirming that extensive reutilisation of amino acids occurs when they have been liberated by breakdown. Why should this be so? Teleologically, one can argue that the advantage of a fast turnover rate is that the concentration of a specific protein can respond rapidly to changes in hormonal and other signals. Half-lives of individual proteins range from minutes to years. In general, the more important and highly regulated a protein is (usually an enzyme) the faster its turnover rate.

Figure I. shows the kinetic model proposed in the classic paper of Sprinson and Rittenberg (1949). Body protein is in equilibrium with a metabolic pool of amino acids; dietary protein is added to this pool. Amino acids are lost from the pool via nitrogen excreted in urine. It has been estimated that the metabolic pool contains the equivalent of 12.5 gm. protein and is distributed among the various tissues of the body (Tschudy et al., 1959). From analysis of human cadavers it is estimated that the body of a 70 Kg. man contains 19% or 13.3 Kg. of protein (Forbes et al., 1953). Thus, the metabolic pool represents only about 0.1% of total body protein, and only 1 - 2.6% of total body protein is turned over each day in adult man.

Data on turnover of individual proteins in various tissues of man is limited, but animal studies show that there is a great variety in turnover rates. Skeletal muscle in adult man represents about 43% of body weight and 39% of total body protein (Widdowson, 1968) but it is thought that contractile proteins turn over at a relatively slow rate and account for only 13% of total protein synthesis (Song and Swick, 1973). Similarly, whereas collagen accounts for 25% of body protein it is of little significance in the daily protein economy in adults. Efron (1956) calculated a daily turnover of 0.06% collagen representing 0.9% of total body protein synthesis.

The digestive tract is responsible for 10% of body weight and 11% of body proteins (Humphrey, 1961). The intestinal mucosa alone represents only 1% of total body protein but is responsible for about 16% of total daily body protein synthesis (Lipkin, 1965; Nasset, 1968). If one takes into account release of protein into the digestive tract from saliva, gastric juice, pancreas, bile, and small intestine it can be calculated that 120-200 g. per day of protein is lost, accounting for more than 50% of daily protein synthesis in man (Spencer, 1960; Nasset, 1968; Da Costa, 1971; Nasset & Ju, 1961). However, secretion of the various digestive juices is under neural and hormonal control responding to the stimulus of food, and hence the level of secretion and protein synthesis could be a function of the amount and composition of the daily food intake.

The liver accounts for 1.6% of body weight and total body protein in man, and synthesizes 30 gms. of plasma proteins daily (Hoffenberg, 1972). Elwyn (1970) concluded that in dogs plasma protein synthesis accounted for 50% of net protein synthesis in the liver. If the same situation exists in man an organ containing only 1.5% of total body protein is responsible for 20% of total protein synthesis.

In summary, it can be seen from existing data that the structural proteins, which are mainly contractile fibres, collagen and elastin contain 50% of the body protein but only 14% of daily protein turnover. In contrast, cells which are actively dividing, especially those of the gut mucosa (and haematopoietic tissues) contain about 12% of body protein but are responsible for 25% of daily protein turnover. Proteins that are secreted into the digestive tract account for only 1-2% of total body protein but represent 40-45% of the daily amount of protein synthesized.

THE BODY CELL MASS

In the preceding section the figures for protein turnover in various tissues were coupled with knowledge of body composition. Relationships between the two leads to the concept of the Body Cell Mass. Shortly after Schoenheimer's seminal publication, Moore and colleagues started work on measuring body composition, again using the newly available isotopes, and developed the concept of the body cell mass, being defined as the

cellular material in the body separated from the supporting circulation of blood and extracellular fluid, and distinct from the heavy mass of extracellular protein solids, such as bone, tendon, fascia etc. (Moore, 1946; Moore et al. 1963; Moore et al. 1968). The body cell mass is the energy-exchanging portion of the body, and the bulk of it is skeletal muscle. It is most easily measured as the total exchangeable potassium. In a normal adult there is 25-40 Kg. of cellular tissue, of which 15-25 Kg. is muscle cells. The total body cell mass represents 1000 - 1750 gms. of nitrogen, of which 600 - 1000 gm. is in muscle. Analysis of muscle tissue indicates that 1 gm. of nitrogen represents 27-30 gms. of wet muscle tissue, including its extracellular fluid and solids, but excluding fat (1 gm. nitrogen represents 6.25 gm. protein, because the average composition of the body's proteins contains 16% nitrogen, ie $100/6.25$). Hence, a negative nitrogen balance of 1 gm. indicates the loss of 30 gms. of lean wet muscle. The total body fat varies over wide extremes, being as low as 6% in a very lean muscular individual and as high as 60% in an overweight but otherwise healthy woman. In the normal male, body fat is considered to be 15% of body weight; this 10 Kg. provides a caloric reserve of 90,000 (376,200 KJ).

RESTING METABOLIC FLUX (FROM MOORE & BRENNAN, 1975)

The normal adult daily intake ranges around a mean of 10-15 gm. nitrogen (ie 62.5 - 100 gm. protein), potassium 100 mmol., sodium

100 mmol., water 2000 mls. The calorie: nitrogen ratio is 150:1 (630 KJ:1). A typical mixed diet would consist of carbohydrate (starch/sugar) 200 gm = 800 kcal (3340 KJ), protein (meat/cereal) 62.5 gm. = 250 kcal (1045 KJ), fat (dairy/meat) 133 gm = 1250 kcal (5225 KJ), making a total intake of 2300 kcals (8614 KJ). The caloric supply is approximately 70% fat, 15% carbohydrate and 15% protein. Because the experiments to be described were initially carried out on fasting men fed intravenously on a variety of regimens, most of which were hypocaloric in energy requirements, followed by studies on patients, the succeeding sections will cover the metabolic and biochemical responses to firstly starvation and then surgery, trauma and sepsis.

THE METABOLIC AND BIOCHEMICAL RESPONSE TO STARVATION

The first scientific study of starvation in man was carried out by Benedict in 1915 when he persuaded a Mr L., a normal man, to fast for 30 days. In what is now regarded as a classical study, Benedict noted that fat provided more than 75% of the energy utilised after the first few days of starvation. He also observed a progressive decrease in daily urinary nitrogen excretion that suggested an increasing conservation of body protein. Our modern view of starvation owes a great deal to the work of Cahill (1970).

It is obvious that fuel can be accumulated in three forms - carbohydrate, protein or fat. Carbohydrate in animals is stored as glycogen in liver and muscle. Each protein has a non-fuel function, but in the normal state any extra protein in food is metabolised, the nitrogen excreted as urea and excess calories stored as fat. Both glycogen and protein need an aqueous environment and hence are relatively inefficient stores on a weight basis. On a dry weight basis each gram contributes 4 kcal (16.7 KJ). In contrast, one gram of adipose tissue yields close to the theoretical 9.4 kcal (39.3 KJ) per gram of pure triglyceride. In man, muscle and liver glycogen stores amount only to a total of 900 kcals (3762 KJ) and are used as fuel only under conditions of anoxia or vigorous exercise. Man therefore only has two major fuel depots, fat and protein. In a sense, all vertebrates suffer from an inborn error of metabolism, because during starvation they cannot maintain their body cell mass on fat oxidation alone.

There are two reasons for this; firstly, some aspect of the energy requirement for protein resynthesis cannot be met on the basis of endogenous fat oxidation alone, and secondly, some tissues are obligatory users of glucose, which cannot be manufactured from fat. In particular, brain oxidises about 150 gm. glucose daily, certainly in the early stages of starvation; blood cells, bone marrow, renal medulla and peripheral nerves metabolise glucose but convert it primarily to lactate and pyruvate. These are released back into the bloodstream and remade into glucose by liver and kidney - the Cori cycle.

About 20% of glucose utilised daily in starvation is cycled in this way. The energy for conversion of lactate to glucose comes from fat oxidation. By the Cori cycle, peripheral tissues derive their energy indirectly from oxidation of fat, but the net effect is to spare gluconeogenesis from protein by limiting complete oxidation of glucose to carbon dioxide. The remainder of the organism - heart, skeletal muscle, renal cortex etc. - uses either fatty acids released directly into the circulation or fatty acids partially oxidised to acetoacetate or Beta-hydroxybutyrate (the ketone bodies) by liver.

To summarise, fasting man has two major sources of fuel, muscle protein and adipose triglyceride, and three patterns of fuel utilisation: glucose oxidation, mainly in the brain; glycolysis, primarily in red cells; and fatty acids and ketones by the rest of the body.

Forty-five years ago Gamble (1946) showed that small amounts of glucose are capable of decreasing the excretion of nitrogen in the urine. Infusion of 100-150 gm. glucose spared 50-75 gm. protein. Presumably, this small amount of glucose is detected by the pancreatic beta cell, and the resultant higher secretion rate and level of insulin inhibit muscle proteolysis. The need for gluconeogenesis by liver is no longer present, and ketogenesis also ceases parallel to the decrease in gluconeogenesis. Gamble's wartime experiments on the ideal rations for castaways are often misquoted as having demonstrated a "ceiling" on the protein-sparing effect of carbohydrate once the intake had reached 100 gm/day. Actually Gamble's brief was to supply in a small weight and volume some substance which would decrease the rate at which the body cell mass was eroded during starvation. He noted that there was a small further protein-sparing with 200 gm. glucose, but the extra daily weight required in the ration was not worth the benefit. It remained for O'Connell et al. (1974) to demonstrate that nitrogen excretion could be reduced to a minimum, but no further, when the glucose dose was equal to the caloric requirement of the subject, at about 750 gm/day.

If protein depletion continued throughout starvation at the same rate as in the first few days, survival would not be as long as it is, and hence a major reduction in nitrogen metabolism must take place. Urea nitrogen diminishes markedly, and ammonia becomes the predominant nitrogenous produce in the urine. Because urea is normally the major osmotic solute,

there is hence little obligatory water excretion, and urine volume may fall to 200 mls. daily. The most dramatic change is that instead of needing glucose, the brain becomes able to use ketone bodies, thus sparing gluconeogenesis, but the mechanism of this adaptation requires elucidation.

The breakdown of skeletal muscle protein during starvation does not result in release of amino acids in proportion to the amino acid composition of the protein. Alanine is the principal amino acid extracted by liver for gluconeogenesis. Both alanine and glutamine are released by skeletal muscle to an extent that exceeds all other amino acids combined. Plasma concentrations of alanine and glutamine progressively decrease during starvation so that by five weeks there is a marked diminution in the rate of hepatic uptake of these amino acids. Gluconeogenesis is decreased in late starvation primarily as a result of reduced peripheral release leading to decreased precursor substrate.

Where does the alanine come from? It is known that skeletal muscle can oxidise a number of amino acids including valine, leucine, isoleucine, glutamate and aspartate. Oxidation of these amino acids is considered to be via transamination with alpha-oxoglutarate and the resultant keto acid gives rise to intermediates of the citric acid cycle, eg. succinate, oxoglutarate. Eventually these intermediates produce malate and oxaloacetate. Malate is converted to pyruvate and oxaloacetate to

phosphoenol pyruvate. Pyruvate is converted to acetyl-CoA which is then oxidised by the cycle. In addition much of the pyruvate is converted to alanine which leaves muscle in the blood and is converted into glucose by the liver. In addition to alanine, glutamine is released by muscle. Proline, arginine and histidine may give rise to glutamate which forms glutamine when combined with ammonia and ATP. The source of the ammonia is unknown. (Newsholme, 1976).

Alanine is the prime substrate for hepatic gluconeogenesis; glutamine, for renal gluconeogenesis. Early in starvation 90% of gluconeogenesis takes place in liver and 10% in kidney - later, 55% in liver, 45% in kidney (Levenson et al., 1977).

For the first twenty four hours of a fast, about 20% of energy expended comes from protein, equivalent to 100 gms. or about a third of daily turnover. During continuous starvation this contribution is progressively reduced so that by day 8 only 12% (or 50 gm. protein) is supplied and by day 40 5% (or 19 gm. protein). After adaptation to starvation the brain reduces oxidation of glucose to 25 gm. daily enabling the body to reduce loss of protein from 100 gm/day during day 1 to less than 20 gm/day after 30 days of starvation.

The study of starvation is not merely of academic interest to surgeons, for severe starvation without injury or infection is often seen in the practice of surgery. Classical examples are patients with carcinoma of the

oesophagus, stomach or pancreas in which large weight losses can occur irrespective of the specific effects of the malignant process, but due to reduced intake. Indeed, in the busy modern hospital setting, obsessed with throughput and high technology, the inability of ill, weak patients to feed themselves has been largely neglected, such that "starvation in the midst of plenty" is common. Also, virtually every surgical operation will be accompanied by some element of starvation, even though in the majority of cases it will be of short duration. However, there are other biochemical, endocrine and metabolic responses by the body to surgery, trauma and sepsis that can be separated from those of starvation alone, and these require additional consideration.

THE METABOLIC RESPONSE TO TRAUMA

In their studies of typhoid fever victims Coleman and Dubois (1915) showed that febrile patients were in negative nitrogen balance even though they received calories and protein in excess of a normal person's requirements. They therefore defined an increase in amino acid oxidation as a characteristic of the septic state.

In the early 1930s Cuthbertson demonstrated the increased loss of nitrogen and other intracellular constituents following femur fracture in the rat, and subsequently alterations in protein economy became a recognised feature of the post-traumatic metabolic response (Cuthbertson, 1930; 1932). Following moderate to severe trauma (such as uncomplicated surgery) in otherwise healthy adults, there is a marked rise in urinary nitrogen, sulphur, phosphorus, potassium, magnesium, zinc and creatinine. Urea comprises 85-90% of the urinary nitrogen.

When Cuthbertson concluded his earlier studies on protein catabolism following injury, he commented that the nitrogen lost from the body came from systemic stores, rather than from damaged tissue at the site of injury. Most evidence suggests that the main source of catabolised protein is from skeletal muscle - this conclusion is based on the magnitude of nitrogen lost, the clinical evidence of muscle wasting and decreased strength, serial body composition measurements, and muscle biopsies in humans and carcass analysis in animals.

Until recently it was always considered that the negative nitrogen balance, and enhanced protein "catabolism" was due to increased breakdown of muscle protein, and it seems that the possibility of decreased synthesis (producing the same effect) was never considered.

Increased gluconeogenesis follows trauma, but to a much greater extent than in starvation, resulting in formation of large amounts of urea. Indeed, such gluconeogenesis cannot easily be reversed by infusion of glucose as in starvation. The explanation remains unclear and is probably complex (Macfie, 1986). Until recently it was regarded as adaptive by which increased energy requirements are met; in particular it was felt that increased breakdown occurred to provide amino acids as the main substrate for gluconeogenesis. This explanation is no longer accepted because 1) apart from major trauma and burns only a moderate increase in energy expenditure occurs and this can easily be met by endogenous sources other than protein, and 2) fat oxidation remains the most important energy source with protein oxidation amounting to no more than 20-30% of total energy requirements. One possible explanation is that amino acid oxidation occurs to ensure more than adequate glucose available for metabolism of repairing tissues which is totally dependent on glucose as its energy source. Hepatic gluconeogenesis is directed by an interaction of hormones; insulin favours hepatic glucose storage, and catecholamines, augmented by glucagon and cortisol, signal hepatic glucose production.

Hyperglycaemia commonly follows trauma or sepsis and the elevation of fasting blood sugar is generally related to the severity of injury or infection (the so-called "traumatic diabetes" described by Claude Bernard in 1877). This is despite an appropriate response by the pancreas by increased insulin secretion, and this "insulin resistance" is associated with and probably due to the rise in the counter-regulatory hormones glucagon, cortisol and catecholamines. In the early stages of severe trauma, the so-called "ebb phase" (Cuthbertson, 1942) catecholamines may inhibit insulin secretion directly. The subsequent "flow phase" response to injury may last anything from a few days after minor trauma, to a matter of weeks or even months, particularly after major burns. The two main characteristics of this phase, hypermetabolism and increased urinary nitrogen excretion, are also seen in chronic sepsis, and point to the similarity between the two conditions.

Both turnover and oxidation of free fatty acids are also elevated during the flow phase. Thus, the mobilisation and utilisation of endogenous fuel substrates after trauma or in the presence of severe infection differs significantly from the economic pattern of fat utilisation and protein sparing observed in simple starvation.

It has been argued on a teleological basis that the changes in intermediary metabolism, electrolyte physiology and endocrine secretions constitute an integrated response by the body which is advantageous to survival and

which restores the internal environment to the situation which existed before the injury. This response is, however, non-specific in that it occurs irrespective of whether the patient suffers an acute infection, a myocardial infarction, a burn or a surgical operation. Generally speaking, the size of the response is proportional to the magnitude of the trauma; for example, the operation of partial gastrectomy evokes a greater one than does repair of an inguinal hernia, and any complication such as a wound infection or secondary haemorrhage either prolongs the response or causes a further peak.

The widespread endocrine changes prompted Cuthbertson and Tilstone (1969) to coin the phrase "a blanket stimulation of endocrine secretions", but as they stated, apart from the increase in catecholamine secretion, it is not obvious what role increased endocrine activity would have in homeostasis after injury, especially as some of the hormones exhibiting increased secretion have opposing actions. For example, catecholamines, cortisol and glucagon cause hyperglycaemia whereas insulin reduces blood sugar level. Cortisol stimulates protein breakdown and gluconeogenesis, whereas insulin enhances transport of amino acids into cells and their incorporation into protein. Furthermore, catecholamines directly inhibit secretion of insulin.

Increased catecholamine secretion might be considered the primary endocrine response to trauma, because of their manifold and widespread activities both on circulation and metabolism, their effect on activity of other hormones, and their stimulation by almost all of the components of trauma. Whereas the endocrine and metabolic disturbances described above appear obligatory following trauma, they are self-limiting following uncomplicated clean surgery and produce little harm or delay in recovery if not prolonged beyond the usual 2-5 days. However, in massive surgery such as oesophagogastrectomy, especially when there has been pre-existing starvation due to the underlying disease, or when there are post-operative complications, the draught on the body cell mass can be so serious as to lead to severe muscle (and hence respiratory) weakness, loss of immunocompetence, hypoproteinaemia and failure to heal wounds. If the process is not arrested by elimination of the stimulus or by adequate nutrition the end result can be uncontrollable sepsis, multiple organ failure, and death.

Whereas there is no evidence that nutritional support can reverse the negative nitrogen balance in so-called "deep surgical sepsis", it can obtund the loss. In most such cases the crucial step towards recovery is appropriate surgery to eliminate sepsis and necrotic tissue, but adequate nutrition is an important adjunct to treatment. Ideally, nutritional support should be given via the gastro-intestinal tract, if necessary by

tube-feeding or jejunostomy, if the patient cannot manage by mouth. However, in many patients, and almost universally in the Intensive Therapy Unit where most patients require ventilation, the gastrointestinal tract is not functioning, and intravenous feeding is required. The background to intravenous nutrition will therefore be discussed.

TOTAL INTRAVENOUS NUTRITION

In 1938 Elman began using protein hydrolysates (Amigen) for intravenous feeding. He also speculated on the relative value of carbohydrate and fat in supporting protein synthesis, and carried out some experiments along this line (Elman 1940,1953).

In 1943 Albright described observations on two patients, one dying of cancer, and one after spinal fusion, who received "100% intravenous feeding" as he called it, consisting of Amigen and glucose (Albright and Reifenstein, 1944). The results were reported at the Macy Conferences on Bone and Wound Healing, held during World War II in an effort to improve care of the wounded. Albright's principal conclusion was that the method of intravenous feeding was practical, but his main problem was that by giving the material by peripheral vein the infusion site had to be changed frequently because of thrombophlebitis. Shortly afterwards, Gamble reported his work on ideal rations for castaways describing the protein-sparing effect of carbohydrate (Gamble, 1946). As in the case with Albright, this work was done under the stimulus of war. Gamble's work is applied thousands of times every day throughout the world in the form of post-operative 5% dextrose infusions, almost universally in ignorance of the name Gamble, and usually in the belief that it is merely an isosmotic way of providing water. Most housemen are unaware that the 150g. glucose provided, equivalent to 600 Kcals (2508 KJ), is sufficient for the daily requirements of the brain and spinal cord, and reduces the post-operative

nitrogen loss in patients undergoing surgery of moderate severity. Subsequently, Geyer et al. (1948) perfected for the first time a fat emulsion, "Lipmul", based on cottonseed which could be used clinically. By isotopic carbon studies they showed that the intravenously infused fat was promptly metabolised, and early studies were carried out on its protein-sparing effect (Van Itallie et al., 1954). The study on the relative value of fat versus carbohydrate to support protein synthesis was inconclusive (Van Itallie et al., 1953). In 1957, however, severe reactions were reported and the emulsion was withdrawn from clinical use.

Scientific proof that intravenous feeding could completely supplant oral intake and support normal growth and development had to await the work of Dudrick et al.,(1967) who showed that simultaneous provision of a protein substrate and an adequate energy source could provide the basis for normal growth in puppies. This finding was rapidly extended to adult man, to infants, and then taken up worldwide with enthusiasm. Intravenous feeding made it possible to address in a quantitative fashion the question of energy support for the maintenance of body cell mass, and the proper use of such feeding required this knowledge for its most effective use. For example, it might be possible to answer the enigma posed by Calloway and Spector (1954) that:".....on a fixed, adequate protein intake, energy level is the deciding factor in nitrogen balance and that with a fixed adequate caloric intake, protein level is the determinant". To this might be added

a corollary; that is, "at each fixed, inadequate protein intake, there is an individual, limiting energy level beyond which increasing calories without protein or protein without calories is without benefit".

This demonstration by the "Philadelphia group" (Dudrick, Wilmore et al., in the surgical department of Jonathan Rhoads) of the practical nature of this mode of treatment prompted an explosion in research into nutritional biochemistry, the development of new nutritional material, new academic societies and a whole new branch of the pharmaceutical industry.

One unfortunate aspect of the stimulus coming from the USA was the fact that because of the previous problems with a fat emulsion, the energy source used by the leaders in the field was universally hypertonic glucose (usually 25-50%). Such solutions have, of course, to be given by central vein catheter. On the one hand this route makes the method a practicable one, but on the other it leads to its main complications ie. infection, and complications of catheter insertion. The practicalities of intravenous feeding are not relevant to, and outwith the scope of this thesis, but will be referred to where it is considered appropriate. Other problems with the use of glucose as the sole energy source have been the recognition of essential fatty acid deficiency, and glucose overload in what are now recognised to have been misguided attempts at "hyperialimentation" (Carpentier et al., 1981).

However, the modified soy-bean-oil emulsion Intralipid (Kabi Vitrum) was developed by Wretling (Hallberg, Schuberth and Wretling, 1966) and introduced into Sweden as early as 1961, and in most of Europe the following year, and on this Continent parenteral nutrition has commonly taken the form of amino acids, glucose and fat emulsion (as Intralipid). Intralipid was not freely available in Canada until 1973, and in the USA until about 1975, and meanwhile, as stated above, glucose continued to be the main energy source in North America. The safety of Intralipid rapidly became established, and several studies showed at an early stage that it was utilised (Hallberg, 1965; Eckart et al., 1973; Wilmore et al., 1973). Furthermore, the use of a fat emulsion is attractive because of its high energy density of 1.1 Kcal (4.6 KJ) per ml. in the 10% preparation, and because it is isotonic there is the possibility of using peripheral veins for intravenous feeding. Also, the entity of essential fatty acid deficiency is easily prevented or reversed. Nevertheless, although widely used, the precise role which fat plays in the provision of energy for synthesis of new protein remained inadequately defined. This item of substrate interaction will be discussed in the next section.

ENERGY SUPPORT FOR PROTEIN SYNTHESIS IN INTRAVENOUS FEEDING

The purpose of intravenous feeding is to support, restore and maintain the body cell mass. Provision of energy to meet the cost of protein synthesis is a vital aspect of such feeding. Most systems of total parenteral nutrition have provided energy in excess of requirements and until 1975 in the USA hypertonic glucose was the non-protein energy source. Because of concerns about the potential hazards of central vein feeding, and until 1975 the unavailability of a fat emulsion, an attempt was made to devise a satisfactory method of peripheral vein feeding, and this led to a controversy in the field of nutrition. Giving hypocaloric glucose (ie calories insufficient to meet energy requirements) will reduce nitrogen loss in starvation (Gamble, 1946) and this is the basis for the standard post-operative regime of 2-3 litres of 5% dextrose (100-150 gm.). However, Blackburn et al. (1973) suggested that when the total energy infused was insufficient to meet the needs of the patient, infusion of amino acids was preferable to that of glucose. This use of isotonic amino acids alone, tolerated by peripheral veins, was called "protein-sparing therapy". Flatt and Blackburn (1974) subsequently formulated a "metabolic fuel regulatory system" stating that during periods of energy deprivation the anabolic effect of insulin (stimulated by glucose infusion) may increase protein catabolism by curtailing the mobilisation of endogenous fat stores and by preventing starvation ketosis. Postoperatively, and in patients with a variety of illnesses, they found that administration of 90 gm. of

an amino acid mixture resulted in 50-100% less nitrogen loss than was observed in patients infused with 5% dextrose. The omission of glucose with its antiketogenic effects was emphasized as a "crucial factor" in the success of the regimen. Subsequently a leading manufacturer of parenteral solutions in the USA extrapolated this hypothesis as providing a rationale for eliminating dextrose containing solutions during operation (1975). The hypothesis of Blackburn et al. caused controversy because it seemed to challenge the long-held view of insulin as an anabolic hormone, and of glucose as a protein-sparing nutrient. The views of Blackburn et al. were opposed by Greenberg et al. (1976) who argued that if the theory was correct, addition of hypocaloric glucose to the amino acid infusion should be detrimental to nitrogen balance, through the mechanism of increased insulin secretion. Although Greenberg et al. found that amino acids infused at the equivalent of 1g. protein/Kg/d. improved nitrogen balance in postoperative patients (mainly cholecystectomy and vagotomy with pyloroplasty) compared with the usual regime of 150g/d. of glucose, in agreement with Blackburn et al., there was no difference in nitrogen balance between three groups receiving respectively amino acids alone, amino acids with hypocaloric (550 Kcals, 1200 KJ) glucose, and amino acids with hypocaloric (550 Kcals, 1200 KJ) fat emulsion. In other words, addition of glucose did not increase nitrogen excretion although it was associated with increased insulin secretion.

Greenberg et al. concluded that in the absence of total caloric replacement, the major determinant of protein-sparing was protein itself, ie. utilisation of protein was not dependent upon specific substrate or hormonal levels. In support of this Freeman et al. (1971), using amino acids alone, reported that in the postoperative period positive nitrogen balance was achieved only with a protein intake of 1.7g/Kg/d. This balance was associated with no significant change in plasma free fatty acids or ketone bodies as compared with regimens in which amino acid intakes were only 1g/Kg/d.

On reviewing the data of Blackburn et al. it was noticed that although nitrogen balance was less favourable by 2g/d. when glucose was added to amino acids, this group received 20 g/d. less protein than the group receiving amino acids alone (70 g. versus 90 g.). Freeman et al. obtained the same results as Greenberg et al. with amino acids and hypocaloric glucose. They argued that the protein-sparing effects of amino acid solutions obviously depend upon lipolysis, since they provide only 20-25% of energy requirements in the doses used, but are not totally due to lipolysis, and the importance of low insulin levels had been over emphasized. Nevertheless, the mechanism by which amino acids result in protein-sparing remains obscure. Presumably they are available for gluconeogenesis for obligatory glucose requiring tissues such as brain and spinal cord. Alternatively, the branched chain amino acids (BCAA), in the mixture, inhibit flux of amino acids from muscle (Odessey et al., 1974).

Elwyn et al. (1978) found that glucose added in hypocaloric amounts to amino acids reduced nitrogen loss by almost 3g/d. compared with amino acids alone following abdominal surgery, and also showed that glucose decreased total energy expenditure.

McDougall et al. (1977) studied more seriously ill patients who were being treated for burns, both with and without infection, using four different feeding regimens:- amino acids alone, amino acids plus 60g. glucose, amino acids plus 120 g. glucose, amino acids plus fat emulsion. Nitrogen balance improved with administration of amino acids in direct proportion to the dose over a range of 0.20 g/sq.m. body surface area. Addition of glucose also improved nitrogen balance. 60 g. glucose added to the infusion was equivalent to an isocaloric dose of amino acids. In other words, amino acids did not demonstrate a superior effect to glucose at any level of isocaloric infusion.

Some of the differences between the results of Blackburn et al., Greenberg et al., Elwyn et al., and McDougall et al. may be explained, at least in part, by differences in methodology, experimental design and patient population. The patients of Blackburn et al. had for the most part not undergone surgery and many were suffering from serious nutritional depletion. In all the studies cited except that of Blackburn the dose of amino acids was the same given either alone or in combination with glucose. As stated earlier, Blackburn et al. gave 90g. amino acids when given alone, but only 70g. when given with glucose.

In fact, in a later study (O'Keefe et al., 1981) Blackburn's group found that the greatest improvement in nitrogen balance was in patients receiving amino acids (108g/d), glucose (216 g/d.) and insulin (22 units), compared with amino acids alone or glucose and insulin. This was in contrast to their earlier study (Blackburn et al., 1973) and that of Greenberg et al. (1976). The 1981 study is not strictly comparable to the 1973 one, because it was conducted with different doses of amino acids and glucose, although still hypocaloric.

Against this background of controversy about the respective values of amino acids and glucose in the mid 1970s, at least as far as hypocaloric "protein-sparing" therapy was concerned, came further problems with the introduction of the fat emulsion, Intralipid. Up to then there had been little argument about the prescription of total parenteral nutrition or hyperalimentation, because the only non-protein energy source had been glucose, but now once again the old problem arose as to whether a carbohydrate calorie was as good as a fat calorie. Early studies had shown that fat and carbohydrate calories exert equal effects on nitrogen retention when given orally in normal man (Munro, 1951) but it was not known whether the same applied when they were given intravenously to the stressed patient.

Lee has stated ".... I cannot see the relevance of making comparisons between the metabolic requirements of normal volunteers and the types

of patients we are dealing with. A sick patient is an entirely different proposition" (1977). It is true that the metabolic and hormonal milieu is different between normals and stressed patients, but the literature on surgical nutrition is full of studies on critically ill, complicated patients who cannot realistically be compared with others because of so many variables. However, one can say that if a form of intravenous feeding will not maintain or replete nitrogen balance in a fasted but otherwise normal man, it is highly unlikely that it will do so in a sick patient. It was this philosophy that prompted Moore and his colleagues to undertake a series of metabolic studies on normal fasting human volunteers. These studies are the basis on which subsequent studies were carried out on nitrogen turnover, protein synthesis and breakdown which form much of the work described in this thesis, and hence they will be described in some detail.

COMPARATIVE EFFECTS OF VARIOUS SUBSTRATES GIVEN INTRAVENOUSLY ON NITROGEN BALANCE IN NORMAL FASTING MAN

Apart from the baseline data in total starvation (Cahill et al., 1966) all of the results to be described were obtained during studies carried out in the laboratories of Dr Francis D Moore at the Peter Bent Brigham Hospital (now the Brigham and Women's Hospital), Boston, Massachusetts, USA. All the results were combined in a paper by Wolfe et al., (1977), and in addition to the experiments conducted by Wolfe himself, the paper incorporated previous work by Brennan et al., (1975), O'Connell et al. (1974), and Tweedle et al. (1971). Studies were conducted, each over 6-8 days, on healthy male volunteer subjects whose mean age was approximately twenty-five. In starvation alone nitrogen loss stabilises at about 6 gN/M²/d. during the first week in the adult male.

When Brennan et al. (1975) infused fat emulsion (Intralipid) as a single substrate there was a reduction in nitrogen excretion compared with total starvation, but this nitrogen-sparing could be reproduced totally by infusing glycerol in a dose equivalent to that available in the fat emulsion. This finding is consistent with the oral studies of Munro (1951) demonstrating that in starvation fat alone has no impact on nitrogen economy. Presumably under these conditions lipolysis of endogenous fat is maximal and the only contribution which exogenous fat can make is to preserve fat stores. In fact, in Brennan's study the glycerol group lost more weight than the group receiving fat emulsion indicating that this was

the case. In this study plasma free fatty acids reached $2000\mu\text{m/l.}$, a value slightly higher than that seen in total starvation ($1250\text{-}1900\ \mu\text{m/l.}$). Infusion of "low dose glucose" (LDG) at 150g/day , reduces nitrogen excretion by about half what it is in total starvation, to about $3\text{g/m}^2\text{/d}$. This effect of glucose has been mentioned earlier in connection with the life-raft experiments of Gamble (1946) and in the controversy regarding the hypocaloric protein-sparing studies of Blackburn et al. (1973) and Greenberg et al. (1976). It is equivalent to the standard postoperative fluid regime of 5% dextrose given eight hourly.

When amino acids are infused alone, without any other form of energy substrate, in a dose equivalent to a high protein diet (ie $80\text{-}120\ \text{g/day} = 13 - 19\text{gN/day}$) there results an improvement in nitrogen balance compared with total starvation, roughly at the same level as that obtained with LDG, but at the expense of a massive increase in urinary urea and total nitrogen. What is equivalent to an intravenous lean meat meal produces brisk gluconeogenesis and ureagenesis associated with a rise in plasma glucagon and a rise in plasma urea: plasma glucose remains at normal or slightly raised levels. Insulin remains normal or is slightly elevated. The infused amino acids are the substrate for gluconeogenesis but obviously including continued catabolism of endogenous protein because the nitrogen balance remains negative. The reduction in endogenous nitrogen excretion ("protein sparing") is small in relation to the total amount of nitrogen infused.

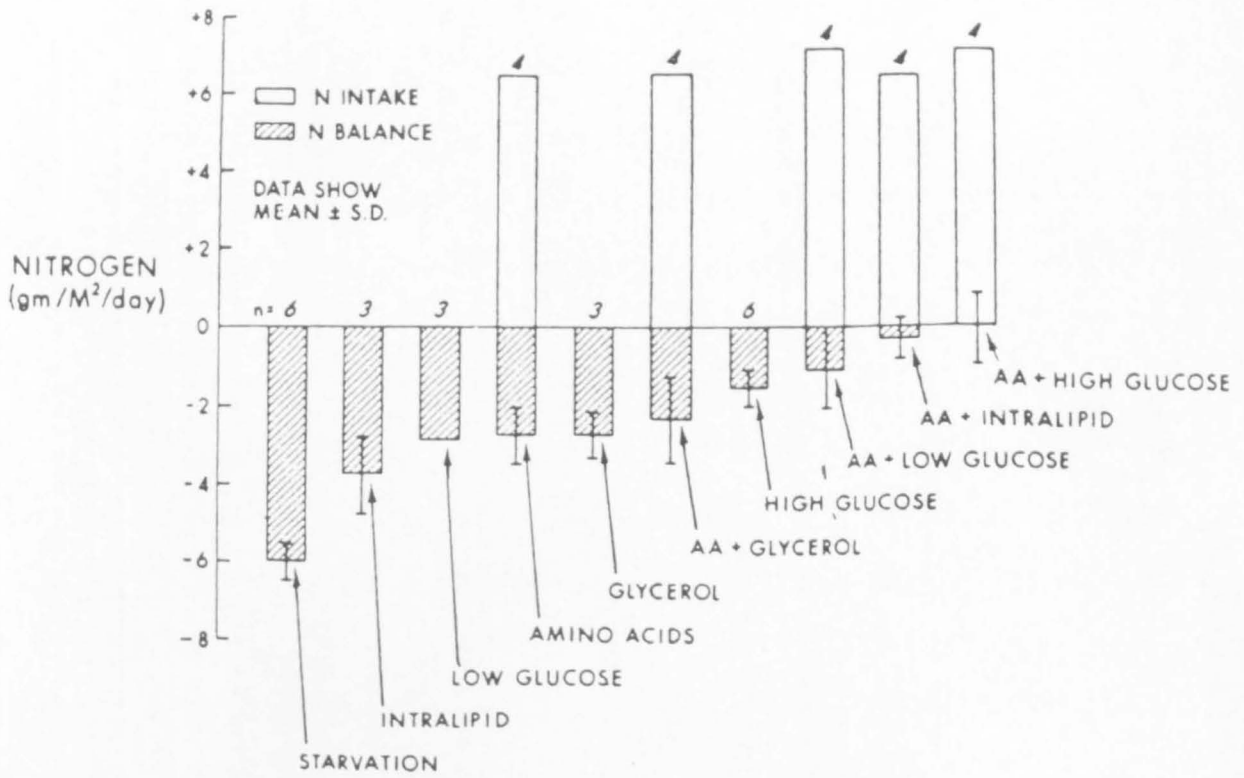
Free fatty acids are elevated and there is progressive ketonaemia but at a lower level than seen in total starvation. In terms of efficiency, both metabolic and financial, the prescription is not a success. However, in chronically starved individuals provision of amino acids alone results in more efficient utilisation than in the normal fasting subject. When the normal fasting subject is given so-called high-dose glucose (HDG), which is an isocaloric dose of between 600-740 g/day, and has to be given by central vein, a nitrogen "floor" is achieved of about 1.8 gM/M₂/day of negative balance, (O'Connell, 1974). Sufficient calories are being provided for energy requirements and yet nitrogen loss still occurs. Presumably this is at least in part because protein resynthesis following breakdown is never 100% efficient. Insulin levels in this group reached 40 μ u/ml. whereas with all other experiments insulin values were within a normal range of 8-15 μ u/ml. Contrariwise, with HDG glucagon secretion was inhibited to the lowest levels seen, with an insulin: glucagon molar ratio of nearly 100.

When amino acids are infused together with glucose the nitrogen balance is improved further, and with addition of glucose at high dose (AA+HDG) nitrogen equilibrium is attained in normal fasting man. Glucose at low dose (AA+LDG) is not as effective, but it does have a statistically significant effect on the utilisation of the amino acids, favouring anabolism and lowering ketones. It is interesting that the low dose glucose does not produce a change in the level of plasma insulin or glucagon. This suggests that it is the energy substrate, rather than

the endocrine setting alone which shuts off ketone production and improves nitrogen economy.

Finally, when amino acids are infused with fat emulsion, (AA+FE), the impact on nitrogen balance is almost the same as with high dose glucose. This result is in marked contrast to when fat emulsion is infused alone. Thus, in the fasting state, the oxidation of endogenous or exogenous fat, alone, has little effect in sparing protein loss; by sharp contrast, when a nitrogen source is also presented, the simultaneously administered long chain fatty acids are effective in promoting protein anabolism. In summary, in comparison with total starvation, there was a progressive reduction in urine nitrogen with the administration of fat emulsion, glycerol (not mentioned), low dose glucose, reaching its lowest point with high dose glucose. When amino acids were provided there was a virtual doubling of total nitrogen excretion with a negative nitrogen balance of about 3gN/M₂/day. There was then analogous improvement in nitrogen economy when amino acids were given with glycerol (not mentioned), amino acids with low dose glucose, amino acids with fat emulsion, and amino acids with high dose glucose. In the latter instance, zero balance was obtained. These results are summarised in Figure II. However, none of these study groups was fed a combination of amino acids, glucose and fat emulsion, and yet in the non-clinical situation a balanced oral diet consists of protein, carbohydrate and fat. In fact, a study using this combination was done by the author, and forms part of this thesis.

FIGURE II



COMPARATIVE EFFECTS OF VARIOUS SUBSTRATES GIVEN INTRAVENOUSLY ON NITROGEN BALANCE POSTOPERATIVELY AND IN TRAUMA AND SEPSIS

The bulk of the experimental work described in this thesis consists of measurements of nitrogen turnover, protein synthesis and breakdown in normal fasting men fed intravenously with the same combinations of substrates as described in the preceding sections, and hence detailed consideration of the studies of Wolfe et al. (1977) are pertinent.

Studies in patients are confined to four case reports at the end of the thesis, but for completeness and because the intention of the work was for it to be used as a baseline for later comparison with studies in patients, a short summary follows outlining present knowledge with respect to comparative effects of substrates on nitrogen balance in postoperative patients, trauma and sepsis. For practical purposes, this amounts to a comparison of glucose and fat as energy sources, and the amounts required.

The main problem with clinical research in the field of metabolic changes in trauma and sepsis is the inhomogeneity of the groups and of necessity often short study periods. From study of such patients three main facts emerge:-

- 1 There is a loss of nitrogen at a rate up to twice normal, depending on the severity of the injury.
- 2 Patients are relatively refractory to attempts to support protein anabolism.

3 Abatement of the stressors is essential to reversal of the sequence (eg drainage of abscesses).

Whereas in normal man endocrine activation will follow and be appropriate to the substrate provided, in trauma and sepsis there are already widespread endocrine changes which may influence the type of substrate preferentially metabolised. In addition, in the presence of tissue necrosis or sepsis, there may be additional effects caused by toxic metabolites (see Final Discussion).

Jeejeebhoy (1976) fed 24 patients intravenously with casein hydrolysate and 40 Kcal (167 KJ)/Kg/d. as either 50% glucose (glucose system) or glucose together with fat emulsion, the latter providing 83% of the non-protein calories (lipid system). Each system was infused for a week at a time and the order of infusion randomised. Nitrogen balance was more positive over a seven day period with glucose than with lipid, but this was only seen during the first four days; no significant difference was seen when the last three days of each system were compared. All patients had experienced a period of protein-calorie malnutrition prior to study. Those with obvious systemic infections were excluded, but several had localised inflammatory masses due to Crohn's disease. The results are similar to those obtained by Munro (1951) on oral over-feeding, when the effect of fat was slightly delayed beyond that of carbohydrate, due probably to the

specific effect of carbohydrate on stimulating insulin, but the longer term effects were equal. Jeejeebhoy et al. concluded that despite very different substrate-hormone profiles and insulin/glucagon ratios the nitrogen balance after equilibrium had been established was insignificantly different in the two groups, and hence the source of non-protein calories does not influence nitrogen balance. The patient uses the substrate that is given and secretes the appropriate hormone to utilise this substrate.

A similar study was reported by Gazzaniga et al. (1975) in which 18 pre- and postoperative patients suffering from such conditions as ulcerative colitis, fistula, carcinoma and malabsorption were alternately assigned to a group given 75% fat and 25% glucose, or a 100% glucose group in addition to amino acids for a mean of 15 days. Weight gain and positive nitrogen balance or equilibrium was achieved with no difference between groups.

Bark et al. (1976) obtained nitrogen equilibrium in nine patients during the first six days after gastrectomy using amino acids plus 50 Kcal (209 KJ)/Kg/d. as either fat emulsion and fructose, or hypertonic glucose and fructose. Fat emulsion contributed 70% of non-protein calories.

Macfie et al. (1980) allocated 42 patients on a gastroenterological surgical service to three TPN groups given amino acids and glucose, glucose and fat emulsion (60% non-protein calories) or glucose and insulin. Changes in body composition over a two week period were measured by the method of Hill et al (1978). Significant weight gain was noted in all groups but only in the glucose plus fat emulsion group was this in the form

of protein, whereas in the other groups weight gain took the form of water and fat.

Although all these publications show that nitrogen equilibrium can be achieved in the postoperative situation using fat emulsion to partially replace glucose as a source of non-protein calories, many of these patients suffered preoperative malnutrition and were not overtly septic. Could similar results be obtained in so-called "hypermetabolic" patients? Willmore et al. (1973) fed ten young adults suffering from burns together with a variety of complications related to burns (mean total burn 44% surface area). Average daily intake was 2100 Kals (8778 KJ)/M₂/day and 15 g. N/n₂/day, an estimate comparable to calorie and nitrogen requirements previously determined at their institution, and 10% Intralipid was included to contribute an average 38% of non-protein calories. Prior to wound coverage these 'hypermetabolic' burn patients demonstrated increased clearance of fat emulsion from the blood, and nitrogen equilibrium was achieved. A subsequent paper from the same institution by Long et al. (1977) described 29 three day studies on five patients, all stable after injury or operation. Three patients had major burns, one an enterocutaneous fistula, and one had had a total gastrectomy. Amino acids were given together with four different doses of glucose and three doses of fat emulsion, and each patient received at least three different diets in a random sequence. Metabolic rates of the patients ranged from 1066-2325 Kcal/d (4456-9718 KJ/d).

Nitrogen excretion decreased as glucose dose increased, reaching a plateau as intake approached resting metabolic rate. Addition of fat did not influence nitrogen excretion at any level of glucose intake. Increasing glucose intake above the resting metabolic rate did not effect any further decrease in nitrogen excretion in patients not receiving insulin. The authors concluded that in these patients the first priority should be to provide glucose calories in dosages approximating the resting metabolic rate, and advised the use of fat only as a source of essential fatty acids and of additional calories to meet extra energy requirements. However, although the studies were extensive, each one only lasted three days, which does not seem adequate to draw any meaningful conclusions. Nevertheless, other workers have similarly demonstrated a dominant effect of carbohydrate over fat in critically ill patients.(Heller 1972) found that nitrogen excretion in patients undergoing radiotherapy for malignancy increased when 30% of total calories was given as fat but equilibrium was established at 15-20%. Similarly, Halmagyi (1974) found that when he gave ITU patients enough calories without fat to cover their basal metabolic rates by 125%, nitrogen balances became increasingly positive with increasing presentation of nitrogen, but this was not the case when 30% of calories was present as fat.

Why should fat emulsion appear to be effective as a major source of energy in some patients but not in others? The balance between glucose and fat oxidation is determined by both the endocrine milieu and relative substrate

levels. Thus, in malnourished patients, normal fasting subjects and to a lesser extent following surgery of moderate severity the endocrine milieu responds appropriately to meet the substrate provided. This is not the case however in hypermetabolic patients typified by severe burns and sepsis. Here there is persistent stimulation of hyperglycaemic hormones together with higher than expected plasma insulin levels. Hepatic glucose production continues and is not suppressed by glucose infusion. Glucose oxidation is increased in absolute terms (Wolfe, 1979).

In addition, both turnover and oxidation of free fatty acids are elevated and this is minimally suppressed by TPN (Askanazi et al. 1980) indicating that lipolysis is not sensitive to the elevated glucose (and hence, insulin) and also that utilisation of fatty acids is accelerated at some stage after their release from adipose tissue. Insulin would normally restrain fat mobilisation, although the counter-regulatory hormones, catecholamines, are also high and have the opposite effects. Indeed, Askanazi et al. argue that endogenous fat may be a preferential substrate in muscle following injury and that attempts to suppress fat oxidation by stimulating insulin release via an excess glucose load causes a compensatory rise in catecholamines which tends to restore fat oxidation. There appears to be a maximal rate of glucose infusion, beyond which physiologically significant increases in protein synthesis and direct oxidation of glucose cannot be expected. In addition, there is a physiological cost of exceeding the optimal glucose infusion rate, as

indicated by increased rates of CO₂ production during infusion as well as large fat deposits in the liver at autopsy in patients infused with large amounts of glucose (Burke et al. 1979). This increased CO₂ production may cause increased respiratory demands in critically ill patients, and in difficulty in weaning patients of ventilators. This is one reason why there has gradually been a swing away from so-called "hyperialimentation" with infusion of large amounts of nitrogen and energy to more modest levels.

This concludes the section describing the background to intravenous feeding.

MEASUREMENTS OF RATES OF PROTEIN TURNOVER

This final part of the Introduction reviews the principles and history of methods of measurement of total body nitrogen (and hence protein) turnover.

The concept of protein turnover was outlined on page 13, and derives from the pioneering work of Schoenheimer (1942). This section considers the methods, assumptions, drawbacks etc. of the various methods which can be used to measure turnover.

Total body nitrogen turnover can be defined as the amount of amino nitrogen, derived from food and from breakdown of tissue protein, that enters a metabolic pool in unit time, or leaves it through synthesis of protein or excretion in urine. It represents the sum of all the turnovers in all the individual tissues and of all the proteins in those tissues, and is hence analogous to total oxygen uptake which is the sum of the uptake of individual cells differing widely in their metabolic rates.

The basis of most methods of estimating total nitrogen turnover is to measure what is termed the flux of amino acid or nitrogen. The flux is the rate of amino acid flow through the free amino acid pool into protein and other metabolic pathways. In the steady state the basic flux equation can be written:-

$$Q = I + B + N = E + S + M.$$

where Q = flux

I = intake from food

B = amino acid from protein breakdown

N = de novo synthesis

E = amino acid oxidised and excreted

S = amino acid synthesized to protein

M = amino acid metabolised through other pathways.

In most cases M is assumed to be negligible. N is zero if an essential amino acid is used. Hence, if M and N were eliminated -

$$Q = I + B = E + S$$

It can be seen that whereas Q is measured directly by the methods to be described, protein synthesis and breakdown can only be estimated by the added knowledge of the nitrogen balance.

i) Historical background

Sprinson and Rittenberg (1949) were the first to measure total nitrogen turnover in man. They gave a single dose of ^{15}N glycine and measured excretion of isotope in urine for 72 hours. They proposed a simple two-part model consisting of a metabolic pool and a protein pool, but probably because they used complicated mathematical reasoning, their method was not followed with any degree of enthusiasm. Indeed, only a few papers

seem to have been published by others using the method. Sharp et al (1957) studied two young and two elderly subjects and found that turnover was lower in the elderly. It is interesting that they used ^{15}N labelled yeast and found that the average value for synthesis in the young subjects was the same as that obtained by Sprinson and Rittenberg using ^{15}N glycine, suggesting that glycine is a satisfactory tracer for total amino nitrogen.

Bartlett and Gaebler (1952) used the method in dogs which were either in nitrogen balance or given growth hormone, and found that synthesis was 50% higher in the latter. Levenson and Watkin (1959) applied the technique to experimental animals following injury and showed that there was an increase in the labelling by ^{15}N glycine of visceral tissue and plasma proteins, but unfortunately they did not appreciate the significance of muscle protein in the metabolic response to injury and did not study muscle.

The original method of Sprinson and Rittenberg was superceded by that of San Pietro and Rittenberg (1953) requiring estimation of the time of maximum urinary urea enrichment with ^{15}N after a single dose of ^{15}N glycine, but once again the mathematical treatment was complex.

Tschudy et al (1959) made a detailed analysis of the San Pietro-Rittenberg method and concluded that it had serious drawbacks. Among other problems, it seemed unsatisfactory to base estimates of turnover rate on a measurement made at one point in time, when ^{15}N abundance is likely to be changing rapidly. Tschudy et al. (1959) used the method to study protein

turnover under conditions of low and high intakes of protein and energy. The same method was used as recently as 1977 by Long et al. to study normals and septic patients. Estimates of turnover rate even in normal subjects showed a wide range of variation. The method is also quite complex, requiring a large number of urine samples at frequent intervals. It is perhaps not surprising that few clinical applications were made. In retrospect, it is possible that the unnecessarily complex mathematical treatment deterred clinicians.

Wu et al. (1959) analysed in detail the pattern of ^{15}N excretion in the first few hours after giving various labelled compounds - glycine, phenylalanine, aspartic acid and ammonium nitrate. The data obtained in the first few hours reflected mainly the metabolism of the particular amino acid given. By making urine collections at very frequent intervals they showed that the shape of the excretion curve is complex, and must represent the sum of several exponentials which cannot be separated. It seems that it is impossible to interpret data on isotope excretion obtained during the first twenty four hours after the dose.

The most complete and sophisticated of these early studies was that of Olesen et al. (1954). Urine was collected for 15 days after giving ^{15}N glycine and three exponential slopes were derived from the semilog plot of ^{15}N abundance in urine against time. A four-pool model was postulated - a slow and fast protein pool, a metabolic pool and a urea pool. The same workers (Shonheyder et al., 1954) used the method to

measure synthesis rate in subjects who had been immobilised for several days in a plaster cast. Immobilisation produced a negative nitrogen balance, due partly to a fall in protein synthesis rather than an increase in breakdown. The method did allow for recycling, which is an advantage, but the main practical disadvantage is that it takes such a long time. Apart from the inconvenience, in the clinical situation it is unlikely that patients will remain in a steady state for two weeks.

Following the mid-to late 1950s there was then a loss of interest in the subject, possibly because of the obscurantist effect of the papers by the Rittenberg group.

ii) The method of Picou and Taylor-Roberts

Interest in measurement of nitrogen turnover was revived about 10 years later by Waterlow's department at the MRC Tropical Metabolism Research Unit in Jamaica. Picou and Taylor-Roberts (1969) used the constant infusion of ^{15}N glycine to estimate protein turnover from the enrichment of urinary urea with ^{15}N after establishing a plateau of excretion. This method avoided many of the difficulties previously encountered, allowing for a simpler mathematical treatment. Constant infusion has the advantage that since a plateau is a straight line it can be defined by fewer points than are necessary for the specific activity-time curve after a single dose.

The fact that a plateau is reached means that the amount of tracer entering the metabolic pool in unit time is equal to the net amount leaving. Hence the infusion rate of isotope (d) at steady state is equal to the disposal or turnover rate (Q) multiplied by the specific activity at plateau (S_a). (see later for detailed treatment of the calculations).

Such end-product methods are called stochastic methods. The word stochastic comes from the field of statistical analysis and means random, or characterised by conjecture (derived from Greek Stokhos, an aim or target; whence, stokhastikos = capable of aiming, or conjectural).

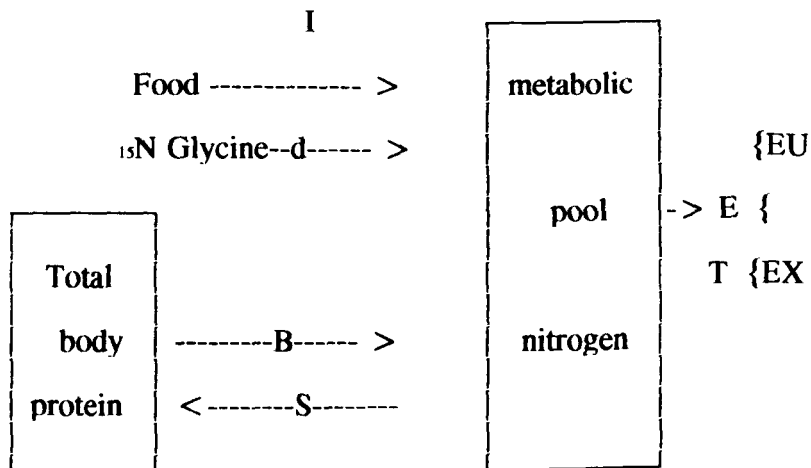
The beauty of a stochastic method is that it does not matter how many protein pools communicate with the metabolic pool. At any given rate of total nitrogen uptake into protein the rate of isotope excretion at plateau (constant infusion) or the cumulative excretion (if a single dose is used) will not be affected by the number of protein pools, their relative sizes and turnover rates. In theory these factors will affect the shape of the curve by which the plateau is reached in a constant infusion, but not its final level. They will affect the slope of the excretion curve of isotope in the single dose method, but not the total excretion. In practice, the shape of the curve in both cases is determined to a large extent by the turnover rate of the excretory pool. The stochastic method greatly simplifies the measurement of protein turnover both practically and mathematically.

The simplicity of the arithmetic is an advantage whereas the complexity of the compartmental analysis used in earlier studies is unsatisfactory because of the relative imprecision of the data.

a) Theoretical Considerations & Calculations

The model is a simple one consisting of single metabolic and protein pools, with the addition of an excretory pool which acts in the main as a "delay" pool, as well as being the pool which is sampled.

Figure III



Amino acids from the diet and from breakdown of body protein enter a metabolic pool and are either synthesized into protein, or degraded and excreted in the urine, mainly as urea. I, B, S and E_T denote respectively the rates of entry of amino nitrogen from the diet and from breakdown, synthesis and excretion of total nitrogen in the urine.

ET is the sum of EU , the rate of excretion of urinary urea nitrogen and Ex , the rate of excretion of non-urea nitrogen. Isotope is infused at the rate of d (usually mg/Kg. body wt./day).

At plateau (ie a steady state) the rate at which unlabelled amino-nitrogen enters the metabolic pool must be the same as the rate at which it leaves the pool.

Therefore, $I + B = S + ET = Q$,

where Q is the total turnover rate or flux of amino-nitrogen.

At plateau, the proportion of the infused isotope which is excreted as urea (eu/d) should be the same as the proportion of total amino-nitrogen entering the pool which is excreted as urea (EU/Q).

i.e. $eu/d = Eu/Q$ (1) (eu = excretion rate of ^{15}N in urea).

If Sa is the measured ^{15}N enrichment in urinary urea (measured by mass spectrometry), then

$$eu = Sa \cdot Eu \quad (2)$$

Substituting $Sa \cdot Eu$ for eu in equation (1)

$$Sa \cdot Eu/d = Eu/Q, \text{ and re-arranging the equation, } Q = d/Sa.$$

Thus, the flux or turnover rate is measured directly from knowledge of the infusion rate of ^{15}N and the enrichment of ^{15}N in urinary urea at plateau.

Protein synthesis and breakdown are calculated from the measured

nitrogen intake (I) and the total nitrogen excretion (Et) as follows:-

$Q = I + B = S + Et$, all expressed as grams nitrogen per day. Then, $B = Q - I$ and $S = Q - Et$, and when multiplied by 6.25 is expressed in grams of protein per day.

b) Assumptions and limitations of the method.

Such a simple model relies upon certain assumptions. These are:-

- 1) the metabolism of the tracer reflects that of the total amino acid mixture.
- 2) protein synthesis occurs from a homogeneous precursor pool.
- 3) the synthesis rate can be calculated as flux minus excretion, other metabolic pathways being regarded as quantitatively unimportant.
- 4) recycling of isotope derived from labelled protein can be ignored.

Assumption 2) is by far the most important. It is self-evident that this assumption is a gross over-simplification of a complex situation. The acceptance of the concept of a single pool stems mainly from an absence of workable alternative models and from a lack of studies which have examined the validity of the assumptions. If a single pool of metabolic nitrogen existed, three theoretical expectations should follow -

- i) the rate of protein turnover as measured with ^{15}N should be the same regardless of the route of tracer administration,
- ii) choice of end product should not influence the measured value for turnover,
- iii) the same rate should be obtained with any ^{15}N - labelled amino acid tracer.

Stein et al. (1976) described a series of experiments to investigate under what conditions the assumption of a single metabolic pool is valid. ^{15}N glycine is frequently used as a tracer because it is readily available and comparatively cheap. It has, however, been suggested that when ^{15}N glycine is used the resulting ^{15}N excretion pattern reflects the particular metabolism of glycine rather than that of amino acids in general, and the rates of various nitrogen interchange reactions may be slow because transamination is not a major pathway for glycine metabolism (Nyhan, 1972). Subjects were given ^{15}N glycine, ^{15}N labelled NH_4Cl or ^{15}N L-aspartate, either orally or intravenously, and ^{15}N enrichment of plasma amino nitrogen, urinary ammonia, hippuric acid and urea were measured. Irrespective of the carrier used or the route of administration, all enrichments showed a plateau at 6 - 10 hours. It was concluded that metabolism of ^{15}N glycine was not atypical and that mixing, rather than nitrogen interchange reactions determines the rate in attaining an approximate plateau.

When a tracer labelled with ^{15}N is used to estimate total body protein turnover, and the results are based on enrichment of urinary urea as an end product, it is assumed that both urea and newly synthesized protein are derived from the same precursor nitrogen pool.

Because urea is synthesized in the liver the enrichment of the precursor nitrogen pool for protein synthesis in the liver may determine the enrichment of urea. Thus the measurement of total body protein turnover

using ^{15}N glycine may be weighted heavily by the rate of hepatic protein turnover. The same problem may arise if urinary ammonia is the end product. The extent of labelling of the precursor nitrogen pool is affected by at least two factors (Golden and Jackson, 1977). Firstly, after infusion of a ^{14}C amino acid, its specific radioactivity at plateau is lower in tissues than in plasma, due to dilution with unlabelled amino acid derived from intracellular protein breakdown. This is termed internal recycling and also applies when ^{15}N glycine is infused. Secondly, when ^{15}N glycine is infused extensive rapid intracellular transamination occurs especially in liver and kidney, so that the ^{15}N is distributed to other amino acids which will have a higher enrichment within the cell than in the plasma. Thus, depending on the balance between internal recycling and transamination, the intracellular enrichment of amino acids may be greater or less than the plasma enrichment. To investigate the problem Golden and Jackson (1977) infused ^{15}N glycine and measured the enrichment of alpha amino nitrogen and ammonia-nitrogen in various tissues. The data obtained showed that none of the tissue pools of alpha amino nitrogen could be identified as the single precursor for urea synthesis. However, the method for measuring total body protein turnover requires only that the nitrogen originating from glycine contributes to both protein and urea in the same ratio as the total alpha amino nitrogen contributes to protein or urea. This assumption is supported by the close agreement between results obtained by ^{14}C and ^{15}N glycine measurements and between ^{15}N yeast and

¹⁵N egg protein measurements.

In conclusion, inhomogeneity of the precursor metabolic pool is unavoidable but will not usually be important in comparative measurements. There may, however, be situations in which it does become significant. For example, in starvation it is possible that increased amounts of amino acids liberated by breakdown become available for synthesis, and hence dilute the precursor pool. The disposal rate could then seriously underestimate the true rate of synthesis (see discussion on the low dose glucose expts).

In addition to the theoretical objection of a single metabolic pool is the objection to a single protein pool. This is true, but measurements are of the entity of whole body protein turnover. As previously mentioned, Oleson (1954) assumed two protein pools, a fast and slow, but a complete model is required in which turnover can only be determined by compartmental analysis, and measurements had to be continued for ten days. It has been suggested that measurements of whole body protein turnover only reflect rapidly turning over proteins. A measurement can give a good estimate of the weighted average turnover rate for a heterogeneous mixture of proteins present in different amounts and turning over at different rates if the time of measurement is short in relation to the average half-life of the mixed proteins. In man a turnover of 3 g. protein/Kg/day (see later) corresponds to a fractional rate of about 2% total body protein/day, or a half-life of 35 days. Thus a measurement made over 2 days probably does fulfil this condition.

It has also been assumed that pathways other than synthesis or breakdown for amino acids can be ignored. However, for many amino acids there are other pathways which may be important eg. glycine uptake into haem and creatine. If the life-span of the haemoglobin molecule is 100 days, the amount of glycine needed for synthesis of haemoglobin is 0.5 mg/Kg/day, which is negligible. On the other hand about 7.5 mg/Kg/d. of glycine is needed for synthesis of creatine (based on the rate of creatinine excretion, 20 mg/Kg/d.) which represents about 5% of glycine flux, and this is not negligible (Waterlow, Garlock, |Millward, 1978). It is therefore pertinent to briefly discuss glycine metabolism.

c) Glycine Metabolism in Man.

Although ^{15}N glycine is a commonly used tracer for measuring human total body protein turnover, the metabolism of glycine nitrogen is not well defined in man. Matthews et al. (1981) measured the transfer of ^{15}N glycine to other plasma amino acids and urea. After giving ^{15}N glycine orally those plasma amino acids showing ^{15}N incorporation significantly greater than zero after 40 hours were serine (54%, relative to glycine), glutamine/glutamate amino - N (15%), alanine (7%), leucine,, isoleucine, valine, ornithine, proline and methionine (3-8%). Urea ^{15}N enrichment was 20%. The remaining amino acids contained much lower degrees of enrichment. The pattern of ^{15}N incorporation was similar to that of rat liver proteins, (Aqvist, 1951). Thus, conversion of glycine to serine must be an important consideration, and the entire glycine molecule, not just the N atom, is

transferred to serine. When actual ^{15}N urea enrichments were compared with predicted ^{15}N urea enrichments calculated from plasma amino acid ^{15}N enrichments, predicted values fell within 10% of observed values. This implies that although glycine transfers a considerable amount of ^{15}N to glutamine/glutamate amino-N, the actual ^{15}N enrichments of glycine and serine are the major determinants of urea ^{15}N , and that the partitioning of glycine between incorporation into protein and breakdown to urea is representative of the other amino acids. Furthermore, as mentioned earlier, Wu et al. (1959) used ^{15}N labelled glycine, phenylalanine and aspartic acid and the three amino acids gave similar estimates of the synthesis rates.

d) Definition of the plateau

The nitrogen flux $Q = d/Sa$

d = infusion rate of tracer ie. ^{15}N glycine.

Sa = ^{15}N enrichment of end-product ie. urea, ammonia, at plateau (ie. a steady state).

Hence, the measurement of Sa is crucial and depends upon how the plateau is defined. It should first of all be pointed out that with constant infusion of labelled amino acids the plateau reached in the infused pool is not a true plateau but a "pseudo"-plateau. The amino acids taken up into protein are not disposed of or irreversibly lost for ever. There is a slow return of tracer, so that the specific activity in the infused pool will gradually rise until it is the same in all the amino acids in the system. It has

been calculated that in the rat this would take about 1000 hours (Waterlow, 1978). Thus, in an infusion of only a few hours the deviation from a plateau can be regarded as negligible.

Constant infusion has the great advantage that since the plateau is a straight line it can be defined by fewer points than are necessary for the specific activity - time curve after a single dose of tracer. Nevertheless, the curve of rise to plateau with a constant infusion is the inverse of the specific activity curve after a single dose. The time when plateau is reached corresponds with the time when zero specific activity is reached after a single dose.

Steffee et al. (1976) defined the plateau in two ways - by simple inspection and non-linear regression analysis. In practice definition of the plateau by inspection is not likely to lead to serious error. It can be made more objective if one takes as the starting point of the plateau the first value which is followed, at some later time, by a lower value. Once the first point has been determined, the plateau can be defined as the least squares line through the first and all subsequent points.

e) The extent of recycling.

If recycling of isotope from labelled protein is significant, the ^{15}N enrichment in urine will not maintain a plateau, but will continuously increase, because the rate of entry of isotope into the metabolic pool will exceed its rate of disposal. As mentioned above, this must occur to some extent on theoretical grounds ("pseudo"-plateau) but the data of Steffee

(1976) indicate that in practice recycling is not significant, the plateau being unlikely to be in error by more than 5%. The effect of this error would be a small under-estimate of the rate of protein synthesis.

f) The nature of the tracer. Up to the present time the tracer substance used in most constant infusion studies has been ^{15}N glycine. In theory, the ideal tracer for overall measurements of protein turnover should be a labelled protein. In fact, results similar to those with glycine have been obtained with ^{15}N labelled yeast protein (Golden, Waterlow & Picou, 1981), ^{15}N labelled egg protein (Picon & Taylor-Roberts, 1969) and ^{15}N labelled wheat protein (Fern & Garlick, 1983).

g) Choice of end-product.

With ^{15}N glycine as tracer the usual end-product is either urea or ammonia. Simplistically, it might be supposed that the labelling of urea reflects that of its precursors in liver and the labelling of ammonia that of the free amino acid pool in muscle, since the greater part of urinary ammonia is derived from the amide-N of glutamine synthetase, which is largely confined to muscle.

Urea is synthesized in the liver, and the precursor pool in the liver may be unrepresentative of that in the body as a whole because of rapid exchange of ^{15}N by transamination, a rapid inflow of amino-N from the gut, and extensive dilution with unlabelled amino acids derived from protein breakdown. From the practical point of view, the disadvantage of urea is

that it is a large pool which turns over slowly, so that in an adult on a normal diet it may take 48 hours for plateau, using continuous infusion.

With ammonia the time required for the measurement is only about 12 hours. The tracer can be given by intermittent dosage instead of by continuous infusion. With urea as end-product it can be given at intervals of 3-4 hours, and fluctuations in labelling are smoothed out because of slow turnover rate of the urea pool. With ammonia the tracer must be given at least every hour.

It is likely that neither end-product is "better" than the other. There are objections to the validity of results obtained with any end-product. The basic assumption is being made that protein in the whole body and urea in the liver or ammonia in the kidney are formed from the same precursor pool. It is clear that this assumption can never be more than an approximation, but it is not possible to test the error resulting from this approximation. The only test of validity is a practical one by comparing results obtained by different methods and tracers, and by reproducibility of results. Golden and Jackson (1977) found that in rats infused with ^{15}N glycine the ^{15}N abundance in urea in the liver and in ammonia in the kidney was the same and only half that of free amino N in the tissues. The free amino acid pool is therefore not the same thing as the precursor pool. The amino acids from which urea N is derived are known (Krebs, Hems and Lund, 1973), as are the precursors of urinary ammonia (Owen and Robinson,

1963; Pitts and Pilkington, 1966). These precursors are not the same; it is therefore understandable that the results of the two end products do not agree. In fact, the values obtained with ammonia were about two-thirds of those obtained with urea, probably because glycine contributes preferentially to formation of urinary ammonia. The important practical question is whether either measurement gives reliable comparative results, not whether it is absolutely correct.

To quote Stein (1982) "there has been a divergence of approaches between the biochemists on the one hand and the clinical scientists on the other.

The former are interested in obtaining the "correct" values; the latter with being able to obtain good approximations that are compatible with clinical realities and internally consistent. Thus, the aim of the medical scientist has been to develop a "simple" assay even at the expense of sacrificing some scientific rigor".

SUMMARY

Several methods have been devised to measure rates of whole body protein turnover in man. These methods use isotopically labelled amino acids to trace the rates of exchange between body proteins, the free amino acid pool, and the catabolic end-products of protein metabolism. Because nitrogen (N) is the common element in these processes, amino acid tracers labelled with ^{15}N have found the widest application. Furthermore, radioactive amino acids such as ^{14}C leucine cannot ethically be given to children or adults of child-bearing age. Since the original measurements by Sprinson and Rittenberg (1949) a number of methods have been developed that make use of ^{15}N . All except one depend upon measurements of isotope excreted in an end product in urine. Typically, a dose of ^{15}N -labelled amino acid is administered and the fraction of the ^{15}N dose appearing in the end products, urinary urea and ammonia, is measured. From analysis of a constructed model for protein metabolism, the rates of whole body protein turnover, synthesis and breakdown can be solved. Two approaches have been taken: 1) to administer a single dose of tracer and to interpret rates of protein turnover from the end-product decay kinetics, or 2) to administer a ^{15}N labelled amino acid tracer as a continuous infusion until isotopic steady state in the end-products is reached.

Long et al. (1977) have greatly improved the first method (based on that of San Pietro and Rittenberg, 1953) by applying sophisticated

modelling and computer-assisted kinetic analysis techniques. In contrast, the second method as approached by Picon and Taylor-Roberts (1969) is a stochastic one using a simple formula not requiring complicated computer algorithms.

Either approach depends upon the partitioning of the ^{15}N between incorporation into protein, into end-product formation (urea and ammonia), and into amino acids other than the tracer amino acid.

Comparison of results obtained by other whole body protein metabolism methods (eg infusion of L-(1- ^{14}C leucine) with those from the N end-product methods have given similar figures. The importance of this comparison is that the two methods rely on independent assumptions (Golden and Waterlow, 1977).

PROTEIN TURNOVER STUDIES IN MAN CONDUCTED PRIOR TO THOSE DESCRIBED IN THIS THESIS.

As a conclusion to this Introduction, and before describing the studies on which this thesis is based, it is pertinent to review those studies on protein turnover in man which preceded the present work. For practical purposes, this subtitle encompasses those studies published over the decade 1969-78.

Following the pioneering studies of Spinson and Rittenberg (1949), San Pietro and Rittenberg (1953), Olesen et al. (1954), Schönheyder et al. (1954), Tschudy et al. (1959) and Wu et al. (1959), the next major advance in the measurement of whole-body protein metabolism was by Picou and Taylor-Roberts (1969). This stochastic end-product method has become a benchmark method and has since been used to study the effects of dietary protein intake, malnutrition and fasting, age, and burn-injury or surgical trauma on protein metabolism in man. It was the method used to conduct the work described in this thesis.

In their paper, Picou and Taylor-Roberts described measurement of total nitrogen turnover, protein synthesis and breakdown in infants using constant intragastric infusion of ^{15}N glycine. 9 infants (10-20 months) were studied. 5 infants were suffering from malnutrition, and 4 were studied after complete recovery from malnutrition. Each infusion lasted 28-31 hours. The average time taken to reach a plateau was 19 hours, except for those on a low protein diet in which it was 24 hours.

Nitrogen turnover, protein synthesis and breakdown were all significantly higher in the malnourished compared to the recovered infants. Protein synthesis in recovered infants was about 6g/Kg/d. and was the same in infants on a low or high protein intake. However, protein breakdown was significantly increased and there was greater utilisation of nitrogen for protein synthesis in infants on a low protein diet.

Golden, Waterlow and Picou (1977) used the same method to study 17 children (ages unspecified) who were recovering and had recovered from severe malnutrition. Protein metabolism did not change when dietary protein intake was reduced from 5.1 to 1.2 to 0.6 g/Kg/d. However, there was a highly significant correlation between protein turnover and synthesis and energy intake. Synthesis increased by about 1g/Kg/d. for each increment of 25 kcal (105 kJ) in the diet over the range 60-270 kcal/Kg/d. (252-1134 kJ). Over this range there was nearly a five-fold increase in protein synthesis. However, large changes in dietary intake resulted in only small changes in protein breakdown, breakdown being least on an inadequate intake. The results led to the conclusion that synthesis, unlike breakdown, is extremely labile and that variations in synthesis are the most important determinants of a change in body protein and nitrogen balance.

The same authors (Golden, Waterlow and Picou, 1977) studied five children before and after recovering from severe malnutrition (study diet 0.6 g/K/d.

and 95 kcal (397 kJ)/Kg/d.). Malnourished children had significantly lower turnover, synthesis and breakdown before, than after they recovered. During recovery from malnutrition two children on a diet of 1.2 g/Kg/d. and 605 kJ/Kg/d. had rates of protein kinetics twice those found on admission and higher than after recovery. On the study diet the malnourished children maintained their weight whereas the recovered children lost weight. In recovered children, synthesis was unchanged over a wide range of protein intake, whereas breakdown rose with reduction in intake. These findings differ from the first paper by these authors, cited above. This study and the one by Picou and Taylor-Roberts produced different results for malnourished children, but are not strictly comparable. In the present study measurements were made within two days of admission, whereas in that of Picou and Taylor-Roberts the children were studied during rapid weight gain.

It can be concluded from these studies that the malnourished child adapts by decreasing synthesis and breakdown, hence reducing metabolic expenditure. A diet which is adequate for a malnourished child to maintain body weight is inadequate for the recovered child. In recovered children, synthesis remains constant over a ninefold range of protein intake - provided sufficient protein is given, synthesis is not directly related to the level of protein intake. However, breakdown increases with decreased intake.

Young et al. (1975) used the method of Picou and Taylor-Roberts to compare the rate of total body protein synthesis in humans of various age groups.

	<u>body wt. Kg</u>	<u>Total body synthesis, g/Kg.</u>
Newborn	1.94	17.4
Infant	9.0	6.9
Young adult	71	3.0
Elderly	56	1.9

These differences in the intensity of protein metabolism between various age groups are eliminated when synthesis is related to the energy expenditure for the different age groups. The data indicate that 1g. dietary protein is required to support synthesis of 4.5 g. total body protein. This suggests that the efficiency of total dietary nitrogen utilisation is similar in the newborn, adult and elderly, and that the differences in protein needs, expressed per Kg. body weight for the various age groups are related to differences in the amount of protein synthesized per unit time.

The same group (Steffee et al., 1976) studied six healthy adults, using the method of Picou and Taylor-Roberts, after adaptation to dietary protein intakes of 1.5 and 0.38 g/Kg/d. and energy of 45 kcal (189 kJ)/Kg/d. At 1.5 g/Kg/d. mean synthesis was 3.0 g/Kg/d. and breakdown 2.7 g/Kg/d. Reducing intake to 0.38 g/Kg/d. caused a fall in turnover of 8%

(just significant) with a 27% increase in breakdown and a 15% increase in synthesis, indicating that endogenous amino acids are re-utilised more efficiently under such conditions.

Clinicians interested in surgical metabolism were then stimulated by two papers about studies on surgical patients. It had long been assumed that the increased "catabolism" of protein following surgery was due to increased breakdown of skeletal muscle. Whereas nitrogen balance gives the difference between synthesis and breakdown it does not provide a value for either. O'Keefe, Sender and James (1974) used ^{14}C leucine infusion to measure protein turnover, synthesis and breakdown before and after abdominal surgery. Postoperative negative nitrogen balance was due to a fall in synthesis of about 12% representing 34g. protein. Breakdown also fell but to a much lesser extent. However, in this study no protein was provided postoperatively.

Crane et al. (1977) studied eleven patients before and after elective orthopaedic surgery using ^{15}N glycine given orally. Patients were maintained on a constant protein intake. Synthesis fell from 3.83 g/Kg/d. preoperatively to 2.94 g/Kg/d. postoperatively, with no significant change in breakdown.

Kien et al. (1978) used ^{15}N glycine to study burned children aged 4-15 years both before and after five days after reconstructive surgery,

postoperatively, and the authors came to the same conclusion as O'Keefe, Sender and James; that is, minor surgery caused a small decrease in synthesis. However, a similar criticism can be made of this study as that of O'Keefe, Sender and James in that protein intake fell postoperatively (calories by 9%, protein by 22%). These differences did not achieve statistical significance, although the fall in synthesis did ($p < 0.05$). In fact, in the two out of six cases in which postoperatively energy and protein did not change there was no difference in synthesis. In a similar study by the same authors (Kien et al., 1978b) 24 studies were done in 11 acutely burned children. Synthesis and breakdown were significantly positively correlated with percentage area total burned surface, third degree burn, and open wound. Mean synthesis of 7.1 g/Kg/d. and breakdown of 6.3 g/Kg/d. were greater in the children with burned surfaces greater than 60% compared to those with burns less than 25%, or to surgical patients. Synthesis was also significantly positively correlated with energy and protein intake, as was breakdown to a lesser extent.

Golden and Waterlow (1977) published an important paper in which protein synthesis was estimated in elderly people. After constant infusion of ^{14}C leucine, turnover and synthesis were calculated in four ways:-

- 1) specific activity of plasma leucine was measured at plateau, and 2) the proportion of the dose excreted as $^{14}\text{C}\text{O}_2$ at plateau. After constant infusion of ^{15}N glycine, turnover and synthesis were measured,

3) from final rates of ^{15}N excretion in urea or total urinary nitrogen, 4) from final or plateau rates of ^{15}N excretion in urinary ammonia. Estimates of synthesis obtained by 2) and 3) agreed closely. This is an important point, because both methods rely upon different assumptions. Results obtained by methods 1) and 4) were lower. Synthesis ranged from 2.34 ± 0.26 to 3.43 ± 0.37 g/Kg/d. In these elderly subjects the synthesis rate given by method 1) of 2.67 g/Kg/d. is much lower than that obtained by James et al. (1976) using ^{14}C tyrosine (4.6 g/Kg/d.) and by O'Keefe et al. (1974) using ^{14}C leucine (3.97 g/Kg/d/) in patients who were middle-aged rather than elderly. The results suggested that the rate of protein turnover is reduced in the elderly, compared with younger subjects.

With ammonia as the end-product, Waterlow, Golden and Garlick (1978) found synthesis rates only 60% of those obtained with urea, but for comparative purposes it may be just as useful. The probable reason for low results with ammonia is that glycine contributes more than most amino acids, other than glutamine, to the formation of ammonia. The rates calculated from specific radioactivity of leucine in plasma agreed well with those based on urinary ammonia. However, it has been suggested that the plasma leucine method gives results which are too low by about 20%. (Golden and Waterlow, 1977). If this is so, it confirms that the combination of ^{15}N glycine as tracer with ammonia as end-product underestimates the synthesis rate, but it does not necessarily reduce the value of the method for comparative measurements.

Using yet another tracer, L-[alpha - ¹⁵N] lysine given by continuous infusion, Halliday and McKeran (1975) found mean total body protein turnover to be 3.5 g/Kg/d. (range 2.5 - 5.0) in five normal adults on a standard diet of 40g. protein daily. Long et al. (1977) studied two normal subjects and three septic patients using a pulse injection of ¹⁵N alanine (and ¹⁵N urea). The best-curve fits and acceptable confidence limits were obtained with a 4-pool model containing two metabolic pools and two urea pools. Synthesis and breakdown were calculated for a fast and slow turnover pool. Normal synthesis was 3.6 g/Kg/d. compared with 4.48 g/Kg/d. in sepsis. Breakdown was 4.38 g/Kg/d. in normals and 5.30 g/Kg/d. in sepsis. All patients were in negative nitrogen balance because they were given low-dose glucose for two days before the study, with no nitrogen intake.

Waterlow, Golden and Picou (1977) summarised rates of overall protein synthesis in man from various studies up to that date. These are tabulated over the page.

Rates of overall protein synthesis in man

<u>Subjects</u>		<u>Tracer</u>	<u>Synthesis g/Kg/d.</u>	<u>Reference</u>
Age	No		Mean \pm S. D.	
1 - 46 days	10	^{15}N glycine	18.4 ± 7.9	Young et al 1975
6 - 19 mths	10	^{15}N glycine	6.4 ± 1.3	Golden, Waterlow and Picon, 1977.
10 - 20 mths	6	^{15}N glycine	6.1 ± 1.1	Picon & Taylor-Roberts, 1969.
20 - 23 yrs	4	^{15}N glycine	3.0 ± 0.2	Young et al. 1975.
20 yrs	2	^{15}N glycine	3.7	Crane et al. 1977.
			3.9	
30 - 65 yrs	6	$\text{U-}^{14}\text{C}$ tyrosine	3.9 ± 0.8	James et al. 1976.
47 - 65 yrs	9	^{15}N glycine	3.8 ± 0.7	Crane et al. 1977.
52 - 75 yrs	4	$1\text{-}^{14}\text{C}$ leucine	4.0 ± 0.5	O'Keefe et al 1974
66 - 91 yrs	6	$1\text{-}^{14}\text{C}$ leucine	2.7 ± 0.7	Golden & Waterlow 1977.
68 - 91	5	^{15}N glycine	3.2 ± 0.4	Golden Y Waterlow 1977.
69 - 91 yrs	4	^{15}N glycine	1.9 ± 0.2	Young et al 1975.

It can be seen that for similar age groups there is reasonable agreement for the values for protein synthesis obtained by constant infusion of

¹⁴C labelled amino acids and those obtained by constant infusion of ¹⁵N glycine. This close agreement provides some validation for the basic assumption in the ¹⁵N glycine method that urea and protein are synthesized from the same amino acid precursor pool.

The aforementioned studies are referred to in the Discussion following Study I and hence will not be analysed further at this stage.

The Introduction to this thesis is now concluded. The following experimental studies will now be described as they were conducted in chronological sequence.

EXPERIMENTAL STUDIES

STUDY 1 - Whole Body Nitrogen Turnover, Protein Synthesis and Breakdown on a Variety of Intravenously Infused Substrates in Normal Man.

The experiments to be described were the first of this nature to be conducted in the laboratory of Dr F D Moore. As mentioned in the Preface and Introduction data contained in the paper "Substrate Interaction in Intravenous Feeding", (Wolfe et al., 1977), provided the foundation for these further studies, which involved repetition of the various intravenous feeding regimens in fasting normal men, but in addition measurements of total body nitrogen turnover, protein synthesis and breakdown were made.

Because prolonged starvation alters the receptivity of the body to protein precursors while trauma, surgery and sepsis produce transient but severe dislocation of the energy-protein synthesis relationship, it was felt that examination of the effects of various substrates fed intravenously on nitrogen turnover, protein synthesis and breakdown should be undertaken firstly under highly controlled conditions in normal fasting man.

i) Subjects and Methods

a) Subjects

Sixteen healthy male volunteers (aged 19-34: mean 27 years) were interviewed, examined clinically and by biochemical screening, blood count and glucose tolerance test to ensure normality. Procedures and risks were explained and informed consent obtained in accordance with the regulations of the Peter Bent Brigham Hospital and the Harvard Medical School. Studies were conducted in the Clinical Centre of the Peter Bent Brigham Hospital. This hospital ward, fully cubicalised, was originally devised for metabolic studies by the pioneering endocrinologist George Thorn. The continued main purpose of the ward was for metabolic and endocrine research, and the nursing staff were practised in and vigilant to the problems of nitrogen balance studies.

Subjects were allowed to be ambulant with the intravenous infusion running. Only water was allowed ad libitum by mouth. Subjects were weighed daily, naked.

b) Infusion Protocols

Seven different feeding regimens were used; firstly, normal oral diet, followed by six different intravenous regimens. The same five subjects (JF, JG, WW, DC, HK) received in succession, the following three intakes, for six days each, with a minimum of two weeks between study periods.

1. Normal mixed oral diet

Four approximately equal meals were given at regular times, to provide a protein intake of 1g/Kg/day and 30 Kcal/Kg/d. (125 KJ). Comparability of total fluid intake with the subsequent intravenous regimens was achieved by adding to the oral fluid intake an intravenous infusion of 0.9% saline at a constant rate of 62.5 mls/hr.

2. Amino acids alone (AA alone)

Crystalline amino acids (3.4% Fre Amine II), manufactured by McGaw Laboratories, Irvine, California, were packaged individually for each subject to achieve a total nitrogen intake identical with that of the normal oral diet, and given intravenously at a rate of 125 mls/hr.

3. Amino acids plus high dose glucose (AA + HDG)

Glucose was added to the same amino acid infusions to provide the equivalent of 30 Kcal/Kg/d. (125 kJ). This amounted to about 500 gms, and required the insertion of a central venous catheter via an antecubital vein.

4. Amino acids, low dose glucose and fat emulsion. (AA + LDG + FE).

Four subjects (RN, CT, PJ, DM) were studied for ten days each. Subjects received 3 litres daily of 3.4% Fre Amine II (13.5 grams nitrogen daily), with 180 gms. of glucose, and 1.5 litres 10% Intralipid (150 gm. fat emulsion) - manufactured by Cutter Laboratories, Berkeley, California. (Calorie: Nitrogen ratio 156 ± 12.1).

5. Amino acids and fat emulsion (AA + FE)

Four subjects (JR, MC, JS, CM) were studied for five days each. Amino acids as above with 2 litres of 10% Intralipid (200 gm. fat emulsion). (Calorie: nitrogen ratio $167 \pm 11:1$).

6. Amino acids and low dose glucose (AA + LDG)

Four subjects (MC, JS, GS, JR) were studied for six days each. Amino acids as above plus 150 gm. glucose. (Calorie: nitrogen ratio 38:1).

7. Low dose glucose alone (LDG)

Four subjects (CT, PB, RT, JS) were studied for six days each. 150 gms. glucose given as 3 litres 5% solution. In the intravenous regimens the electrolyte intake was standardised (mmol/day): sodium 90, potassium 60, phosphate 48, magnesium 15; vitamins were added.

Apart from the AA + HDG group all infusions were given by peripheral vein. Infusions were regulated by an infusion pump (Imed Corp., San Diego, California).

All solutions used were prepared in the hospital pharmacy under sterile laminar flow conditions. The bags for infusion were weighed before and after infusion to provide an accurate measure of the volume infused as based on the weight and specific gravity of the solution. Residual infusate was analysed for total nitrogen content. Fat emulsions were infused using a Y connection with the amino acid bag, and Intralipid bottles were weighed before and after infusion.

c) Collection and Analysis of Samples

Each study day commenced at 8am. Twenty-four hour urine collections were made throughout the studies. Precise 3-hourly urine collections were made during the period of ^{15}N glycine infusion for measurement of ^{15}N enrichment. This was the last 60 hours of each study. 50% aliquots from each sample were pooled to calculate 24 - hour urinary nitrogen output. All urine was stored at - 20 C. until analysed.

Urine nitrogen determinations were carried out by the standard macro Kjeldahl technique. Analyses for urinary urea, creatinine and uric acid were carried out by the Auto Analyser (Technicon, Tarrytown, New York) utilising a modification of the carbamido diacetyl reaction, the alkaline picrate (Jaffe) reaction and the phosphotungstic acid reactions, respectively. Urinary ammonia was analysed using an ammonia electrode (Orion Research Inc., Model 95-10). Urinary creatinine data were used as a check on completeness of collections.

Venous blood samples were taken at 8 am. Plasma glucose was determined by the Hoffman reaction and blood urea nitrogen on the Auto Analyser. Serum free fatty acids (FFA) were measured by a single extraction method. (Dole, 1956). Serum triglycerides and ketones (acetoacetate and beta-hydroxybutyrate) were analysed by enzymatic methods (Bergmeyer, 1974). Serum immunoreactive insulin was measured by combined radio-immunoassay (Juan & AuRuskin, 1971). Plasma immunoreactive glucagon was measured by radio-immunoassay using a 30K antibody (Faloona & Unger, 1974).

Amino acid analyses in the plasma were done at Bio-Science Laboratories, Santa Monica, California using the Durrum D-500 Amino Acid Analyzer.

d) Isotope Procedure and Calculations

Nitrogen turnover was measured, and protein synthesis and breakdown were estimated by the method of Picou and Taylor-Roberts (1969).

During the final sixty hours of each study period, ^{15}N glycine was added in equal amounts to infusion bags to give a daily dose of 0.5 mg ^{15}N per kilogram body weight.

^{15}N glycine powder was purchased from either Stohler Laboratories, Waltham, Mass. or Prochem, Summit, New Jersey - usually 5 grams at a time. ^{15}N glycine solution was prepared in the hospital pharmacy in a concentration of 10 mg. glycine per ml. by dissolving the ^{15}N glycine powder in sterile isotonic saline, passed through a 0.22 μ millipore filter, autoclaved and stored in sealed vials. Randomly selected vials were submitted to pyrogen testing using a limulus lysate endotoxin assay, and bacteriological culture before use. Total nitrogen content of the solution was checked by the Kjeldahl method.

Precise 3-hourly urine collections were made during the final 60 hours of the study. This obviously involved waking the subjects in the night.

Enrichment of urinary urea with ^{15}N was determined on the 3-hourly urine samples as follows. Urinary urea nitrogen was isolated as ammonia by the Conway diffusion method (Hawk et al., 1954) after pretreatment with Permutit (Folin and Bell, 1917). The ammonia was then reacted with

hypobromite to produce nitrogen gas (Sprinson and Rittenberg, 1949) and the $^{29}\text{N}/^{28}\text{N}$ ratio determined using a double-collector, isotope-ratio mass spectrometer (Model 3-60 RMS, Nuclide Corporation, State College, Pennsylvania). These measurements were carried out by Dr Denis Rohrbaugh under the auspices of Dr Vernon Young of the division of Food Sciences, Massachusetts Institute of Technology, Cambridge, Mass.

In the method of Picou and Taylor-Roberts, calculation of nitrogen turnover (Q) depends upon proportionality between the dose rate of ^{15}N in glycine (d) and the achieved enrichment in urinary urea at plateau (S_a), or $Q = d/S_a$.

The calculation clearly depends upon the validity of the plateau. In all the curves involved in the study plateau was achieved at about 42 hours. The mean of the last five values from 48-60 hours was taken to represent the plateau of urinary urea enrichment, or S_a .

The intake was based on analysis of the oral diet and the infusions. Urinary nitrogen was measured as previously mentioned. Faecal losses were measured on the oral diet but were negligible after the first day on the intravenous regimens and were not analysed. 5mg/Kg/day were allowed in the determinations for nitrogen loss through the skin (Calloway, Odell, Morgan, 1971).

The calculation of protein synthesis and breakdown was based on the assumption of a single metabolic and a single protein pool.

Nitrogen values were converted to protein by multiplying by 6.25.

Then, intake (I) and breakdown (B) are related to synthesis (S) and excretion (E) by the equation:- $Q = I + B = s + E$.

e) Instrumentation and ^{15}N measurement.

Although measurements of ^{15}N urinary urea enrichment were carried out at M.I.T. outlined above, a brief explanation of the principles involved is necessary.

Stable isotopes such as ^{15}N , by their very nature, are already present in living systems and must be administered and measured "in excess" of their natural abundance backgrounds. Whereas in radioactive tracer studies, the standard unit of measurement is specific activity, for stable isotope tracers, with no "activity" the equivalent unit of measurement is "atom % excess" which refers to the isotopic enrichment in excess of the naturally occurring isotopic component. The naturally occurring abundance or atom % of ^{15}N is 0.366 leaving 99.634% ^{14}N .

To appreciate the instrumentation required to measure stable isotope enrichment one has to consider the degree of dilution involved. The infused tracer will be diluted in the blood with unlabelled amino acids entering the free amino acid pool via the diet and body protein breakdown and conversely leave the system via urea and protein synthesis. The possibilities of stable isotope label dilution range from as little as 20-fold for the amino acid infused in the free amino acid pool to greater than 10,000 fold in the end products of protein metabolism. For example

the degree of enrichment of urinary urea with ^{15}N at plateau in the experiments to be described was about 0.1%, whereas the enrichment of ^{15}N glycine as stated by the manufacturer was 96-99%. In fact, solutions were consistently found to be only 85% enriched with ^{15}N when measured at M.I.T. by Dr Rohrbaugh on the 3-60 RMS Nuclide Magnetic Mass Spectrometer. This degree of enrichment was well below the 96-99% enrichment as stated by the manufacturer. However, as long as the urinary urea nitrogen enrichment is measured by the identical chemical and isotopic technique employed for the study of the standard, the precise enrichment level becomes unimportant in the calculation, since it appears in both numerator and denominator of the final equation.

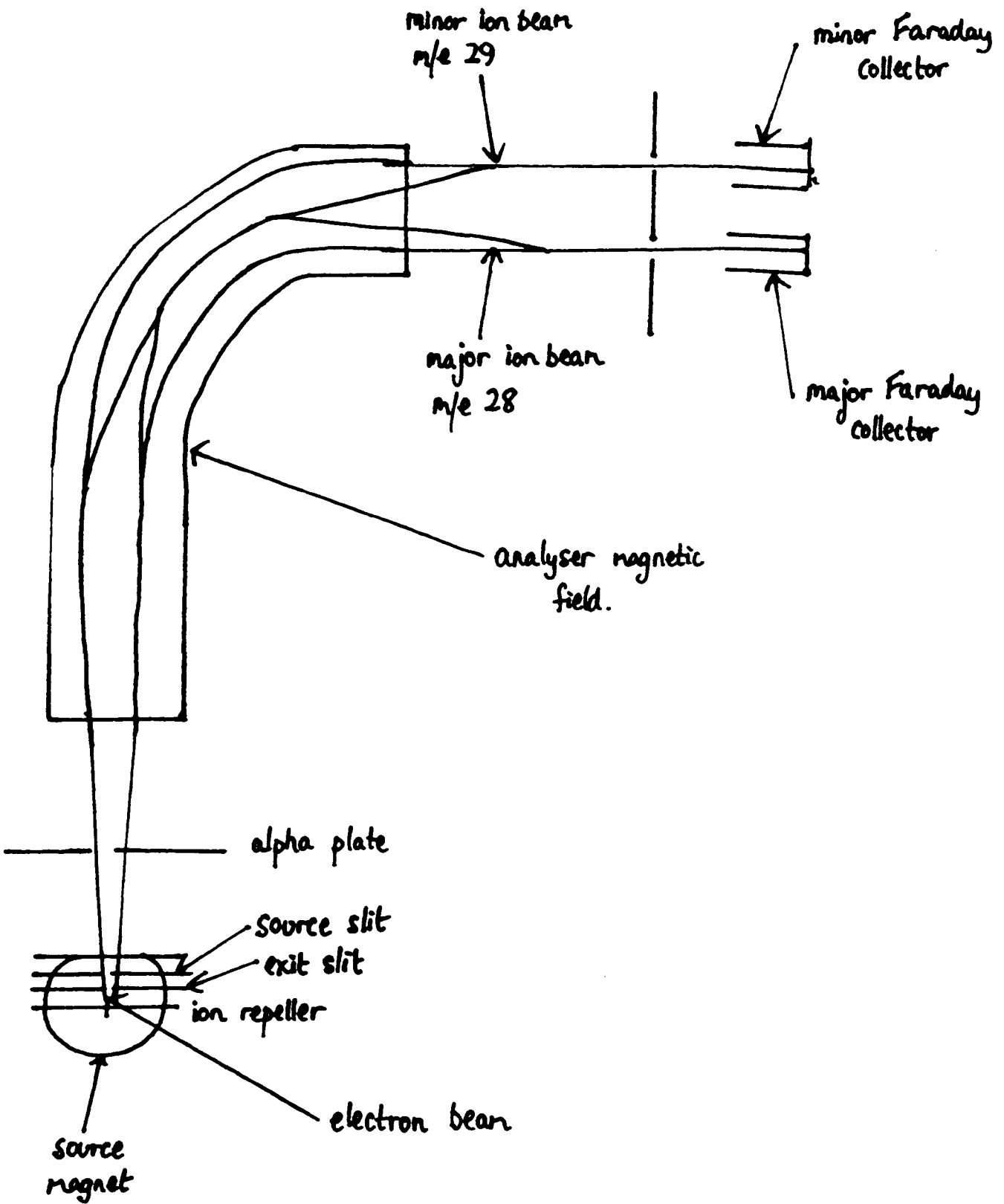
The principle of mass spectrometry is that isotopes of different mass, in gaseous form, are diverted to differing degrees by an applied magnetic field. The classical dual-inlet dual-collector isotope ratio mass spectrometer can determine very small differences in enrichment (c. 0.0001 atom % excess) by measuring only small gas molecules (N_2 , H_2 or CO_2), by simultaneously measuring both major and minor isotope-produced ions with a set of ion-collectors, and by always measuring the isotope ratio of the sample gas against the isotope ratio of a reference gas of known isotopic content via periodic shifting with a dual-gas-inlet system. Such instruments have been in use since the 1930s but were tedious to use until recently when the application of microcomputers allowed automatic

operation. At present, the limitation of the technique is not the instrument, but the sample preparation procedures.

Taking nitrogen as an example (see Figure IV), the objective of isotopic analysis is to separate the m/e 28 (major) and 29 (minor) ion species (N_2 retains its diatomic molecular structure) such that they can be collected and measured separately and a ratio of their intensities obtained. On entering the ion source gas molecules are ionised in a stream of electrons, extracted from the source under the influence of an accelerating voltage as a mixed ion beam, which is segregated on passage through the magnetic field into component ion beams of fixed mass: charge value.

The segregated ion beams are focused into individual deep Faraday buckets, having passed through major and minor beam resolving slits and suppressor electrodes which act both to minimise ion drift between the collectors and to reject any secondary electrons formed by ion collisions. The impact of the major and minor ion beams in their respective collector buckets produces a current. This original signal is amplified at two stages, transformed into a usable voltage and thence fed into voltage to frequency convertors whose output pulse rate is linearly proportional to the inlet voltage. These pulses are fed into separate major and minor beam counters and the desired ratio obtained. Under optimal conditions on isotope ratio mass spectrometer can detect one ^{15}N atom in 10^6 unlabelled atoms difference in the isotopic content of two gases.

FIGURE IV



ii) Results

a) Clinical

The infusions were well tolerated. Of the four subjects on AA+LDG+FE for ten days, three needed to have the IV cannula re-sited because of local discomfort, although only one had overlying redness. Of the remainder, one subject discontinued his LDG study because of discomfort at the cannula site, but returned at a later date and repeated the study. Two others had slight redness at the infusion site by the time of completion.

There were no significant complaints of hunger after the first day, except when fast-food advertisements were on the television!

All subjects lost weight, ranging from 0.1 - 3.8 Kg. The mean weight loss on normal diet was 1.2 Kg, on AA 3.1 Kg. and on AA+HDG 1.8 Kg. This weight loss on AA alone was significantly greater than that observed in the other two study periods ($p < 0.05$, paired t-test).

Mean weight losses for the other groups were: AA+LDG+FE, 1.15 ± 1.2 Kg; AA+FE, 2.3 ± 0.7 KG; AA+LDG, 1.9 ± 0.6 Kg; LDG, 2.8 ± 0.8 Kg. None of these differences between groups is statistically significant.

b) Intake Constancy Comparability (Table 1)

There was no significant difference in nitrogen intake per square metre body surface area in those groups receiving amino acids. The normal diet, AA, and AA + HDG groups were all the same individuals and received

INTAKE SUMMARY (MEAN \pm S.D. DAILY)

GROUP	NO	STUDY DAYS	N g/M ²	GLUCOSE g	GLUCOSE Kcals	F.E. m/s	F.E. Kcals	NON-PROTEIN CALORIES	CALORIE:N Kcals:g
AA+LDG+FE	4	10	7.4 \pm 0.3	179 \pm 6	609 \pm 20	1407 \pm 125	1548 \pm 137	2157 \pm 135	156 \pm 12:1
AA+FE	4	5	7.2 \pm 0.4			1915 \pm 50	2106 \pm 56	2106 \pm 56	167 \pm 11:1
AA+LDG	4	6	7.6 \pm 0.3	150 \pm 4	512 \pm 14			512 \pm 14	38 \pm 2:1
LDG	4	6		148 \pm 2	503 \pm 7			503 \pm 7	

TABLE 1

1g/Kg/d. of protein or amino acids, but in the other groups there were differences in surface area between individuals, range 1.73 - 2.05 M₂, together with small differences in nitrogen content and volumes of infusion bags, and residual volumes after infusion. Similarly, in groups AA+LDG+FE and AA+FE, non-protein calories were insignificantly different at 30 ± 2 and 32 ± 2 Kcals/Kg/d. (125 and 134 KJ) respectively, and calorie:nitrogen ratios of $156 \pm 12: 1$ and $167 \pm 11: 1$.

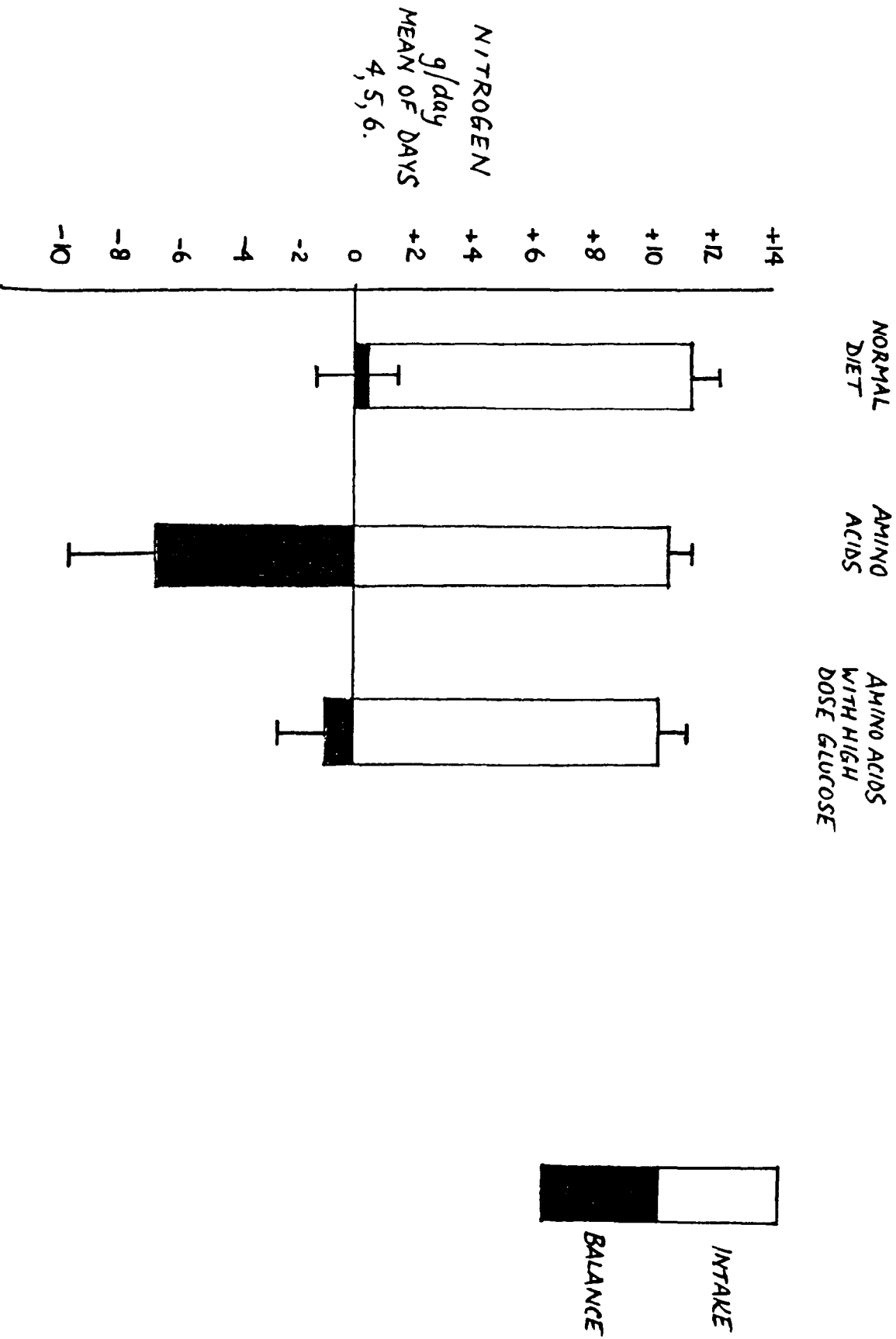
In groups AA + LDG and LDG, non-protein calorie intakes were 8 ± 0.6 and 7 ± 8 Kcals/Kg/d. (33 and 29 KJ) respectively; insignificantly different.

c) Nitrogen Balance

It is appropriate to divide the nitrogen balance results into two groups. The first group consisting of the same five subjects on normal oral diet, amino acids and amino acids plus high dose glucose are considered separately from the remaining four groups which involved eleven subjects. Because the two groups were also separated chronologically, the nitrogen balance results were expressed slightly differently, the first group in terms of grams/Kg/d, the second group in terms of grams/M₂/d.

Figure V shows histographically the nitrogen balance data on normal diet, amino acids, and amino acids plus high dose glucose, showing the mean results of the last three days of study. The mean 24-hour urinary nitrogen excretion on normal diet was 10.1 ± 1.3 g.N/day, increasing to 17.5 ± 2.7 g/d. on amino acids alone, which was statistically significantly higher

FIGURE V



than that on normal diet and on amino acids plus high dose glucose (11.5 ± 2.1 g/d.), $p < 0.001$. The mean daily loss of nitrogen in the faeces on normal diet was 0.7 ± 0.5 g/d. Skin-loss mean for all periods based on 5mg/Kg/d. was 0.4 ± 0.1 g/d.

The nitrogen balance findings show a net balance on normal diet of 0.3 ± 1.2 g/d., i.e. no significant change. On amino acid infusion the net nitrogen balance was -6.7 ± 2.8 g/d., a significant loss when compared with the normal diet ($p < 0.001$). When glucose was added to the amino acid infusion the balance was -1.0 ± 1.6 g/d., significantly less than that observed on amino acids ($p < 0.001$) but not significantly different from that observed on normal diet.

The nitrogen balance data for the remaining four groups are shown in Figures VI to IX inclusive. Figure X is a summarising chart showing mean nitrogen intakes and balances during the last three days of each study. The group receiving LDG alone exhibited the most negative nitrogen balance (-3.1 ± 0.1 N/M₂/d.) (NB the change of units to gN/M₂/d.). Provision of amino acids in addition to low dose glucose (AA + LDG) lessened the net nitrogen loss significantly (-1.4 ± 0.6 g/M₂/d., $p < 0.01$, unpaired t-test), when compared with LDG alone. There was no further reduction in net nitrogen loss when low dose glucose was replaced by fat emulsion in a higher caloric dose (AA+FE, -1.3 ± 0.4 g/M₂/d.). Additional reduction in nitrogen loss was achieved, however, when an isocaloric combination of low dose glucose and fat emulsion was given with amino acids (AA+LDG+FE, $+0.7$

FIGURE VI

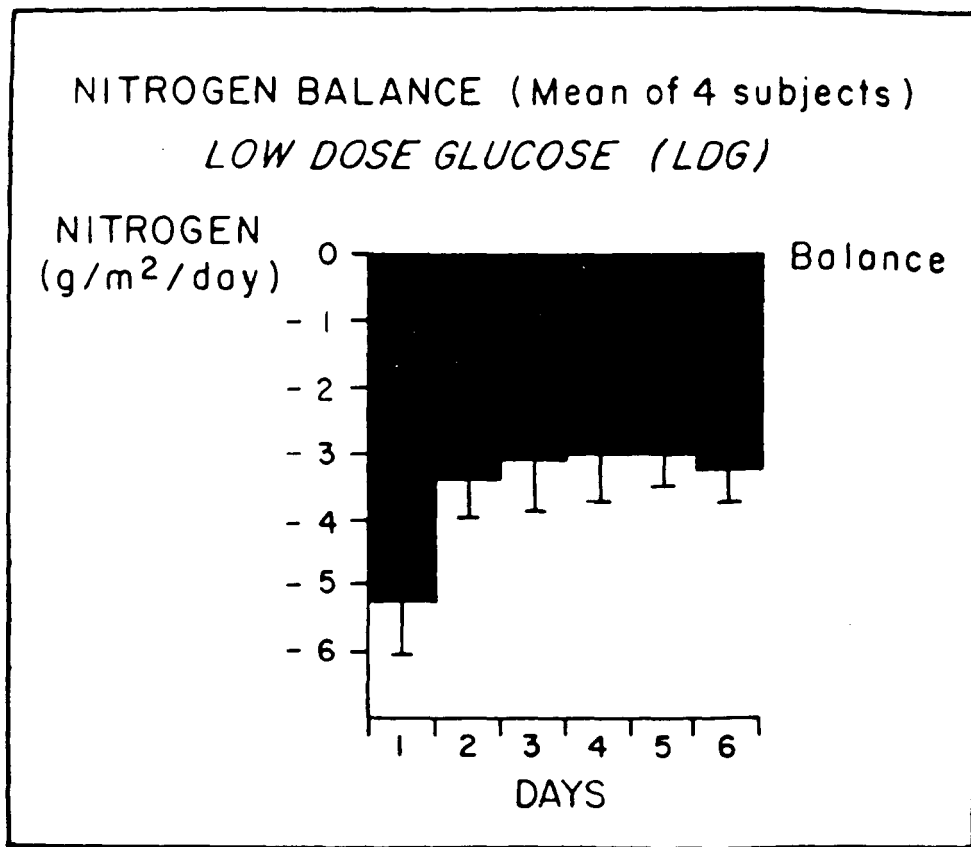


FIGURE VII

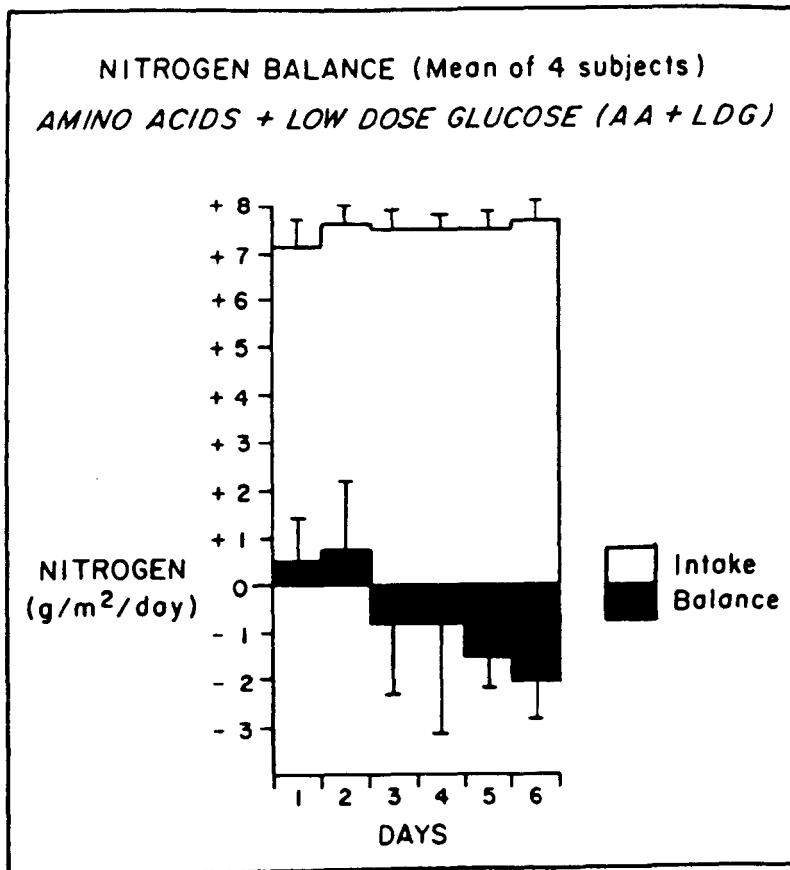


FIGURE VIII

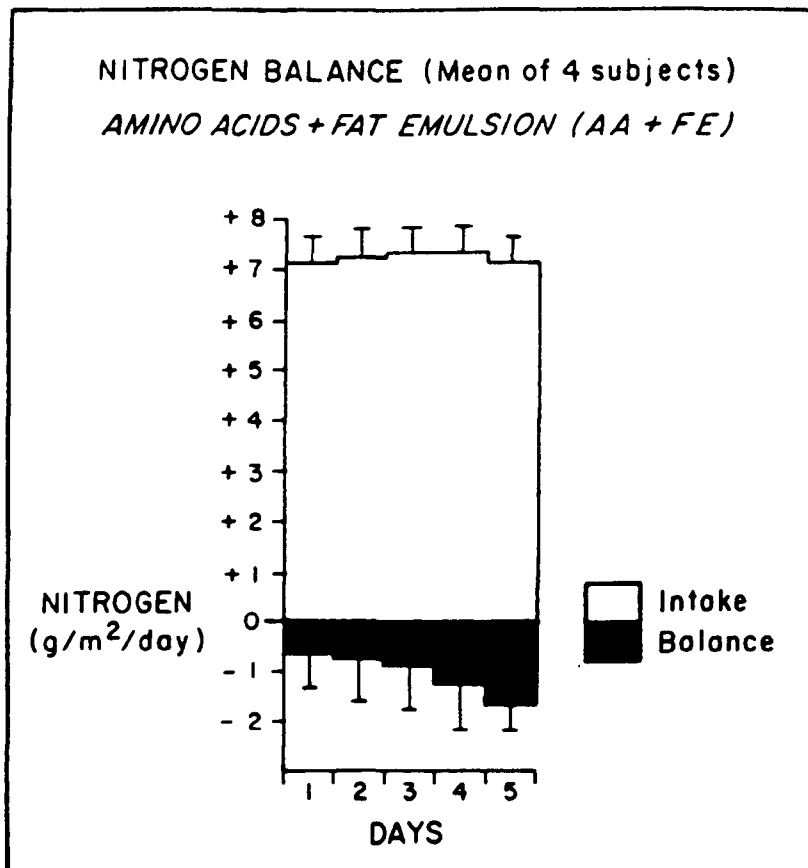


FIGURE IX

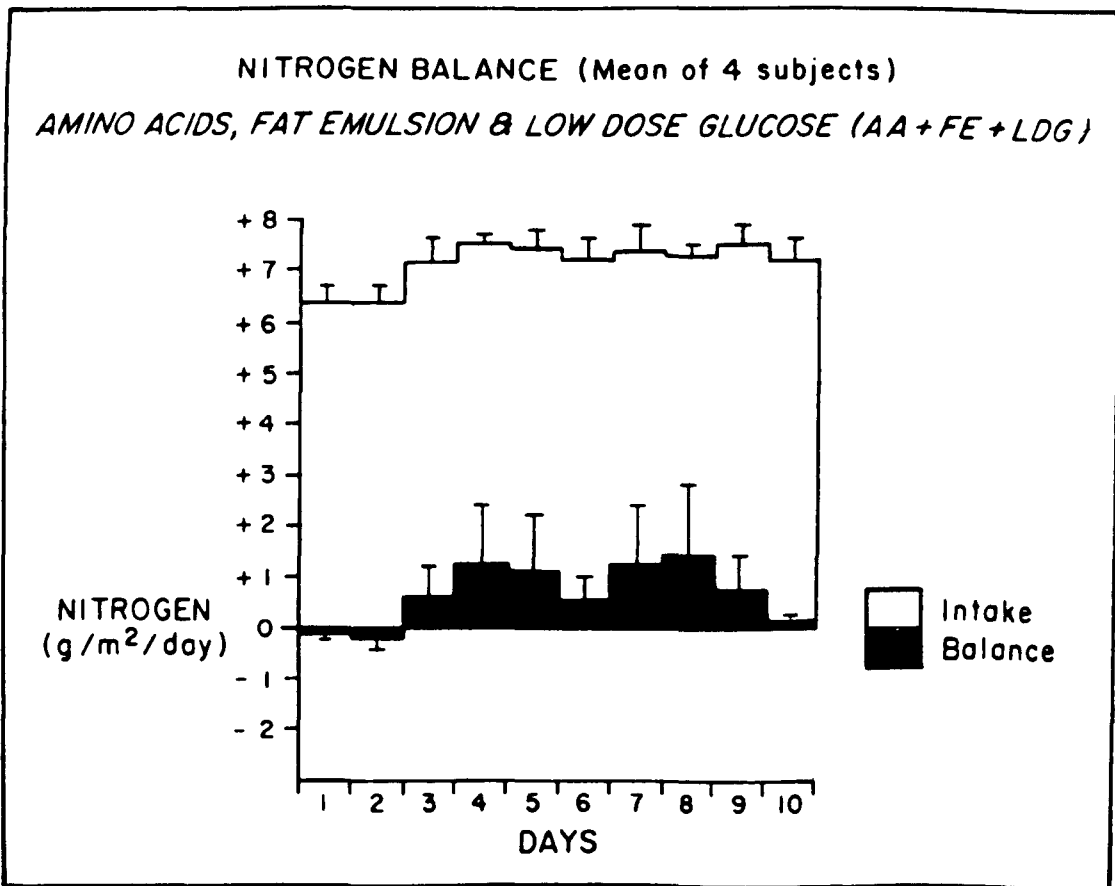
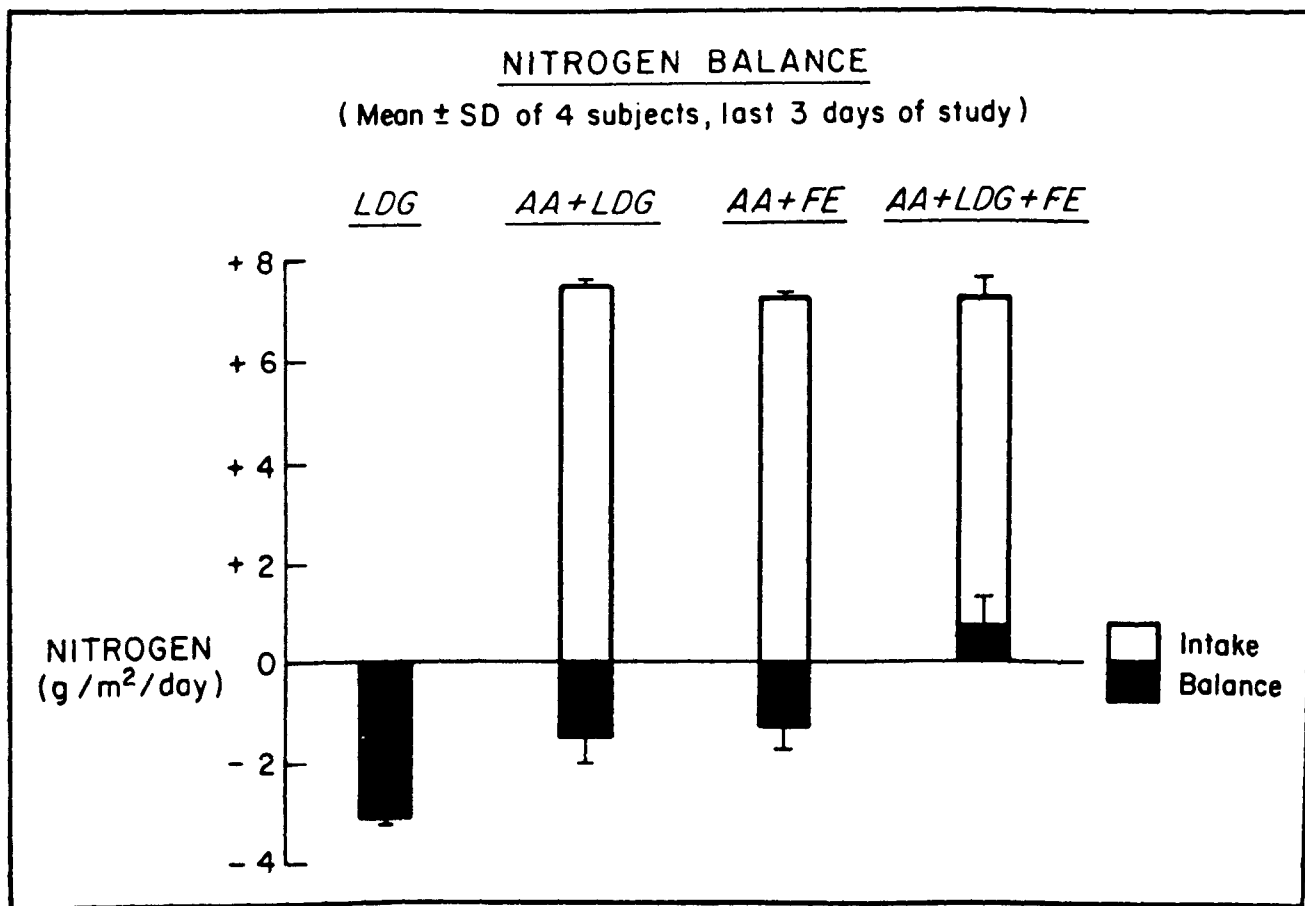


FIGURE X



± 0.6 g/M²/d., $p < 0.01$) instead of AA+FE.

d) Whole Body Nitrogen Flux, Protein Synthesis and Breakdown.

The raw data for the subjects on normal diet, amino acids and amino acids with high dose glucose are shown in Tables 2,3,4. The Tables show the isotope doses, plateau enrichment of urinary urea with ¹⁵N intake, nitrogen excretion and calculated Q, S and B. The nitrogen flux on normal diet was 543 ± 47 mg N/Kg. per day. This was significantly higher than with AA alone (442 ± 37 mg N/Kg/d.) and with AA + HDG (434 ± 39 mg N/Kg/d) ($p < 0.01$).

Figure XI shows diagrammatically the calculated protein synthesis and breakdown rates. Whole-body protein synthesis rate was 2.4 ± 0.3 g/Kg/d. on normal diet. This was significantly higher than that of 1.0 ± 0.13 g/Kg/d. observed on AA alone ($p < 0.001$). With AA + HDG the synthesis rate was 1.6 ± 0.13 g/Kg/d., which was significantly higher than that observed on AA alone ($p < 0.001$) but still significantly lower than that observed on normal diet ($p < 0.005$).

On normal diet protein breakdown rate was equal to synthesis rate, at 2.4 ± 0.3 g/Kg/d., significantly higher than that observed on AA alone of 1.8 ± 0.25 g/Kg/d. ($p < 0.02$). With AA + HDG the rate was also 1.8 ± 0.2 g/Kg/d. and significantly lower than that observed on normal diet ($p < 0.005$).

ISOTOPE DATA, NITROGEN FLUX, PROTEIN SYNTHESIS AND BREAKDOWN - NORMAL ORAL DIET

SUBJECT	MEAN WT KG	d	Sa	Q	I Mean of days 4, 5, 6	E	B	S
JF	60.80	0.42	0.0732	579	10.5	10.01	2.54	2.59
JG	61.80	0.46	0.0816	562	11.1	12.14	2.39	2.28
WW	81.20	0.39	0.0822	474	12.7	12.14	1.99	2.03
DC	71.10	0.44	0.0845	518	11.8	11.93	2.20	2.19
HK	73.90	0.44	0.0754	584	11.4	10.12	2.69	2.79
MEAN	69.76			543	11.5	11.27	2.36	2.38
S.D.	±8.56			±47	±0.82	±1.10	±0.27	±0.3

d isotope dose mg ¹⁵N/Kg/day

Sa Urinary Urea ¹⁵N enrichment (atoms % excess)

$$\frac{S}{Q} = \frac{2.38}{3.39} = 70\%$$

Q whole body nitrogen turnover rate (mgN/Kg/day)

TABLE 2

ISOTOPE DATA, NITROGEN FLUX, PROTEIN SYNTHESIS AND BREAKDOWN - AMINO ACIDS

SUBJECT	MEAN WT KG	d	Sa	Q	I Mean of days 4, 5, 6	E	B	S
JF	56.90	0.43	0.0927	464	11.24	18.10	1.67	0.91
JG	62.40	0.45	0.0904	496	9.43	18.50	2.16	1.25
WW	79.00	0.43	0.1039	411	10.56	20.80	1.73	0.92
DC	68.00	0.54	0.1294	414	10.43	17.20	1.63	1.01
HK	69.90	0.51	0.1211	425	11.33	17.80	1.64	1.06
MEAN	67.24			442	10.60	18.48	1.76	1.02
S.D.	±8.31			±37	±0.76	±1.38	±0.25	±0.13

$$\frac{S}{Q} = \frac{1.02}{2.76} = 37\%$$

TABLE 3

ISOTOPE DATA, NITROGEN FLUX, PROTEIN SYNTHESIS AND BREAKDOWN - AMINO ACIDS PLUS HIGH DOSE GLUCOSE

SUBJECT	MEAN WT KG	d	Sa	Q	I Mean of days	E 4, 5, 6	B	S
JF	58.70	0.51	0.1094	462	9.17	9.80	1.910	1.84
JG	63.80	0.43	0.0919	473	10.12	13.90	1.970	1.59
WW	80.80	0.44	0.1105	395	11.60	13.00	1.570	1.46
DC	70.00	0.47	0.1153	407	10.51	11.00	1.610	1.57
HK	—	—	—	—	—	—	—	—
MEAN	68.32			434	10.35	11.92	1.775	1.62
S.D.	±9.51			±39	±1.01	±1.86	±0.2	±0.13

$$\frac{S}{Q} = \frac{1.62}{2.71} = 60\%$$

TABLE 4

Tables 5.6.7 and 8 show the isotope doses, plateau enrichment of urinary urea with ^{15}N , and calculated nitrogen flux, protein synthesis and breakdown in the remaining four intravenous regimens, and are shown diagrammatically for comparison with normal diet, AA, and AA + HDG in Figure XI.

Nitrogen flux on AA + LDG (534 ± 31 mg N/Kg/d.) was significantly higher than that on AA + FE (470 ± 32 MG N/KG/D), $P < 0.05$, but there were no other significant differences between groups in terms of flux.

Protein synthesis was significantly greater on AA + LDG + FE (2.0 ± 0.2 g/Kg/D.) and on LDG (2.4 ± 0.4 g/Kg/d.) than on AA + FE (1.6 ± 0.2 g/Kg/D.), $P < 0.02$, but differences between these groups and AA + LDG (1.8 ± 0.2 g/Kg/d.) were insignificant.

Protein breakdown on LDG (2.9 ± 0.5 g/Kg/d) was the highest of all, being significantly higher than that on AA + LDG + FE (2.0 ± 0.25 g/Kg/d.), AA + FE (1.75 ± 0.1 g/Kg/d.) and AA + LDG (2.1 ± 0.1 g/Kg/d.), $p < 0.01$ in all cases.

Protein breakdown on AA + FE was significantly higher than that on AA + LDG, $p < 0.05$. Protein synthesis and breakdown were equal on AA + LDG + FE in accordance with the nitrogen balance data.

In the final four intravenous regimens (ie. AA + LDG + FE, AA + FE, AA + LDG and LDG alone) blood glucose, insulin, glucagon, urea nitrogen, fatty intermediates, and amino acid profiles were measured.

ISOTOPE DATA, NITROGEN FLUX, PROTEIN SYNTHESIS AND BREAKDOWN - AA + FE + LDG

SUBJECT	MEAN WT KG	d	Sa	Q	I	E	B	S
RN	67.95	0.488	0.0895	545	13.98	12.30	2.12	2.27
CT	72.46	0.494	0.1076	459	14.10	11.45	1.65	1.88
PJ	82.68	0.501	0.1068	470	14.30	13.75	1.86	1.89
DM	70.32	0.505	0.0933	541	13.30	14.80	2.20	2.10
MEAN	73.35			504	13.92	13.07	1.96	2.03
S.D.	±6.48			±49	±0.43	±1.49	±0.25	±0.19

$$\frac{S}{Q} = \frac{2.03}{3.15} = 64\%$$

TABLE 5

ISOTOPE DATA, NITROGEN FLUX, PROTEIN SYNTHESIS AND BREAKDOWN - AA + FE

SUBJECT	MEAN WT KG	d	Sa	Q	I	E	B	S
JR	73.60	0.461	0.0979	471	13.13	15.4	1.83	1.72
MC	61.60	0.489	0.1150	425	11.40	14.2	1.54	1.25
JS	64.50	0.508	0.1020	498	13.63	14.7	1.79	1.69
CM	67.70	0.476	0.0996	486	12.90	15.3	1.85	1.63
MEAN	66.85			470	12.76	14.9	1.75	1.57
S.D.	±5.14			±32	±0.96	±0.56	±0.14	±0.22

$$\frac{S}{Q} = \frac{1.57}{2.94} = 53\%$$

TABLE 6

ISOTOPE DATA, NITROGEN FLUX, PROTEIN SYNTHESIS AND BREAKDOWN - AA + LDG

SUBJECT	MEAN WT KG	d	Sa	Q	I	E	B	S
MC	60.80	0.524	0.0970	540	12.60	14.60	2.08	1.87
JS	64.40	0.504	0.0881	572	14.20	15.10	2.20	2.11
GS	66.30	0.485	0.0978	496	13.00	15.70	1.87	1.61
JR	71.60	0.500	0.0947	528	14.00	18.80	2.07	1.65
MEAN	65.77			534	13.45	16.05	2.06	1.81
S.D.	±4.50			±31	±0.77	±1.89	±0.14	±0.23

$$\frac{S}{Q} = \frac{1.81}{3.34} = 54\%$$

TABLE 7

ISOTOPE DATA, NITROGEN FLUX, PROTEIN SYNTHESIS AND BREAKDOWN - LDG

SUBJECT	MEAN WT KG	d	Sa	Q	I	E	B	S
CT	67.30	0.484	0.0954 *	506	—	4.2	3.16	2.80
PB	83.50	0.473	0.1125	420	—	7.1	2.63	2.10
RT	74.70	0.491	0.1200	409	—	6.2	2.56	2.03
JS	64.35	0.501	0.0973 *	515	—	5.4	3.22	2.69
MEAN	72.46			462	0	5.7	2.89	2.40
S.D.	±8.50			±56		±1.2	±0.34	±0.40

* Curve still rising

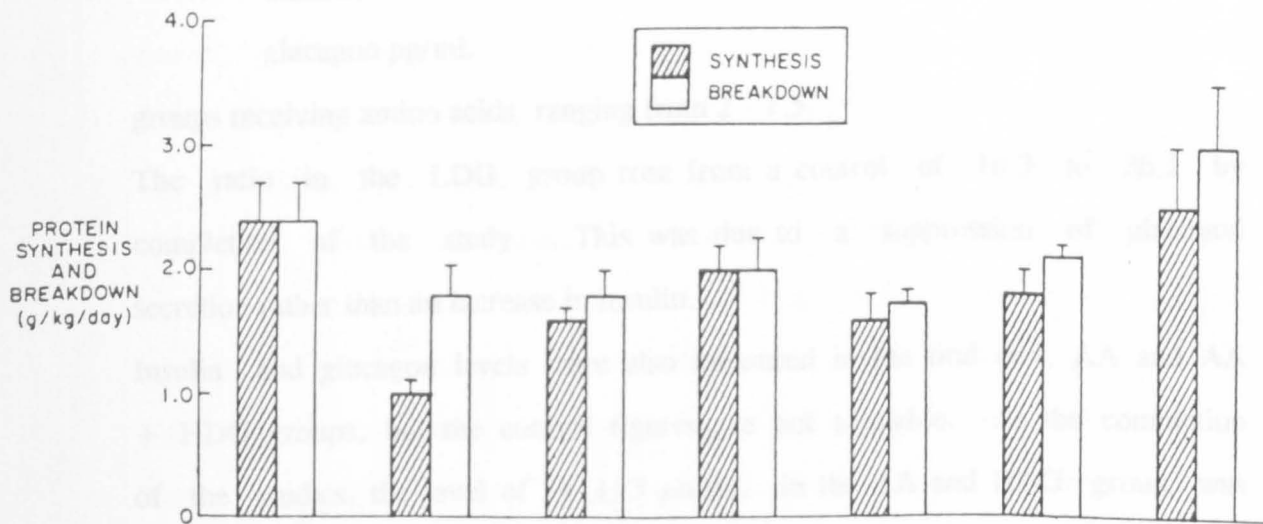
$$\frac{S}{Q} = \frac{2.40}{2.89} = 83 \%$$

TABLE 8

FIGURE XI

NITROGEN TURNOVER, PROTEIN SYNTHESIS AND BREAKDOWN ON VARIOUS SUBSTRATES

	<u>NORMAL DIET</u>	<u>AMINO ACIDS (AA)</u>	<u>AA + HIGH DOSE GLUCOSE</u>	<u>AA + FAT EMULSION & LOW DOSE GLUCOSE</u>	<u>AA + FAT EMULSION</u>	<u>AA + LOW DOSE GLUCOSE</u>	<u>LOW DOSE GLUCOSE</u>
NITROGEN TURNOVER Q (mg/kg/day)	543 ± 47	442 ± 37	434 ± 39	504 ± 46	470 ± 32	534 ± 31	462 ± 56



e) Blood glucose; insulin, glucagon and their ratios, blood urea nitrogen (Tables 9 and 10).

Blood glucose levels were supported at the control level throughout each study and there were insignificant differences between groups.

The serum insulin concentrations gave analogous results. Although the mean control level of insulin in the LDG group was 39 $\mu\text{u/ml}$. the remaining concentrations all fell between means of 11-24 $\mu\text{u/ml}$. Similarly, in all three groups receiving amino acids there were insignificant changes in plasma glucagon levels, but in the LDG group there was a significant fall from a control of 88 ± 21 pg/ml . to 16 ± 13 pg/ml . ($p = 0.05$).

Insulin: glucagon ratios were expressed as molar ratios

insulin $\mu\text{u/ml}$ x 23.33 and were low in all
glucagon pg/ml .

groups receiving amino acids, ranging from 2 - 7.5.

The ratio in the LDG group rose from a control of 10.3 to 26.2 by completion of the study. This was due to a suppression of glucagon secretion rather than an increase in insulin.

Insulin and glucagon levels were also measured in the oral diet, AA and AA + HDG groups, but the control figures are not available. At the completion of the studies, the level of 34 ± 3 $\mu\text{u/ml}$. in the AA and HDG group was significantly higher than that on AA alone (11 ± 3 $\mu\text{u/ml}$., $p < 0.001$) and normal diet (17 ± 6 $\mu\text{u/ml}$., $p < 0.001$). Contrariwise, the highest level of

glucagon on AA alone (212 ± 67 mg/ml) was significantly higher than on normal diet (62 ± 54 pg/ml., $p < 0.001$) and AA + LDG (80 ± 45 pg/ml., $p < 0.02$). Blood urea nitrogen levels remained within the normal range in all groups, but there was a significant fall from the control level by the end of the study in the AA + LDG + FE and LDG groups ($p = 0.05$ in both cases).

f) Fatty Intermediates - Ketones, free fatty acids (FFA), Triglycerides and Cholesterol (Table 10).

Plasma ketone levels rose significantly in the AA + FE group ($p < 0.02$) but remained unchanged in the remaining groups. FFA approximately doubled in AA + LDG + FE and AA + FE ($p < 0.02$ in both cases). No significant changes occurred in AA + LDG and LDG.

Serum triglycerides did not alter in any group, and the results are not shown. Cholesterol and glycerol levels rose in both groups receiving fat emulsion but not in the others, and the results are not shown.

g) Plasma Amino Acid Levels (Table 11)

The mean plasma total amino acid concentration (TAA) and branched chain amino acid concentration (BCAA) rose in those groups receiving amino acid infusions, but these rises were statistically significant only for TAA in AA + LDG and BCAA in AA+FE and AA + LDG. No change in BCAA occurred in the LDG group, but there was an insignificant rise in TAA. No change occurred in the BCAA: TAA ratio in AA + LDG + FE or AA + LDG, but the ratio rose significantly in AA + FE and fell in LDG.

GLUCOSE (MEAN PLASMA CONC. mg/dl \pm S.D.)

GROUP	CONTROL	FINAL DAY OF STUDY
AA+LDG+FE	82 \pm 11	88 \pm 7
AA+FE	84 \pm 4	72 \pm 6
AA + LDG	90 \pm 4	80 \pm 8
LDG	97 \pm 21	93 \pm 7

INSULIN (MEAN SERUM CONC. μ u/ml)

GROUP	CONTROL	FINAL DAY OF STUDY
AA+LDG+FE	11 \pm 6	24 \pm 9
AA+FE	16 \pm 16	20 \pm 18
AA+LDG	18 \pm 8	20 \pm 16
LDG	39 \pm 32	18 \pm 17

GLUCAGON (MEAN PLASMA CONC. pg/ml)

GROUP	CONTROL	FINAL DAY OF STUDY
AA+LDG+FE	122 \pm 88	126 \pm 39
AA+FE	63 \pm 22	92 \pm 39
AA + LDG	56 \pm 17	90 \pm 27
LDG	88 \pm 21	16 \pm 12

p < 0.05

TABLE 9

BLOOD UREA NITROGEN (mg/dl \pm S.D.)

GROUP	CONTROL	FINAL DAY OF STUDY	
AA+LDG+FE	19 \pm 5	13 \pm 2	p < 0.05
AA+FE	15 \pm 3	12 \pm 1	
AA+LDG	15 \pm 1	17 \pm 2	
LDG	15 \pm 3	7 \pm 3	p < 0.05

FREE FATTY ACIDS (μ Eq/l)

GROUP	CONTROL	FINAL DAY OF STUDY	
AA+LDG+FE	545 \pm 138	1182 \pm 249	p < 0.02
AA+FE	1259 \pm 374	2732 \pm 333	p < 0.02
AA+LDG	980 \pm 67	794 \pm 132	
LDG	967 \pm 364	1240 \pm 638	

KETONES μ m/l

GROUP	CONTROL	FINAL DAY OF STUDY	
AA+LDG+FE	60 \pm 50	30 \pm 17	
AA+FE	250 \pm 280	990 \pm 560	p < 0.02
AA+LDG	150 \pm 260	80 \pm 80	
LDG	140 \pm 170	170 \pm 70	

TABLE 10

BRANCHED CHAIN AND TOTAL AMINO ACIDS AND THEIR RATIOS

GROUP	BRANCHED CHAIN (BCAA) mm/l \pm S.D.		TOTAL (TAA) mm/l \pm S.D.		BCAA / TAA	
	CONTROL	FINAL	CONTROL	FINAL	CONTROL	FINAL
AA+LDG+FE	43 \pm 11	55 \pm 11	296 \pm 26	371 \pm 49	0.14	0.15
AA+FE	50 \pm 7	110 \pm 34	323 \pm 44	437 \pm 102	0.15	0.25
AA+LDG	36 \pm 5	75 \pm 14	276 \pm 55	457 \pm 64	0.13	0.16
LDG	37 \pm 7	39 \pm 5	251 \pm 23	312 \pm 38	0.15	0.12

ALANINE AND GLYCINE : RATIOS TO TOTAL AMINO ACIDS

GROUP	ALA / TAA		GLY / TAA	
	CONTROL	FINAL	CONTROL	FINAL
AA+LDG+FE	0.11	0.09	0.08	0.16
AA+FE	0.09	0.06	0.08	0.14
AA+LDG	0.10	0.08	0.09	0.16
LDG	0.10	0.085	0.09	0.10

TABLE 11

The alanine: TAA ratio fell significantly in AA + LDG + FE and AA + FE but there was no change in AA + LDG or LDG.

The glycine: TAA ratio rose in all groups, especially in those receiving amino acid infusions in which glycine is in high concentration.

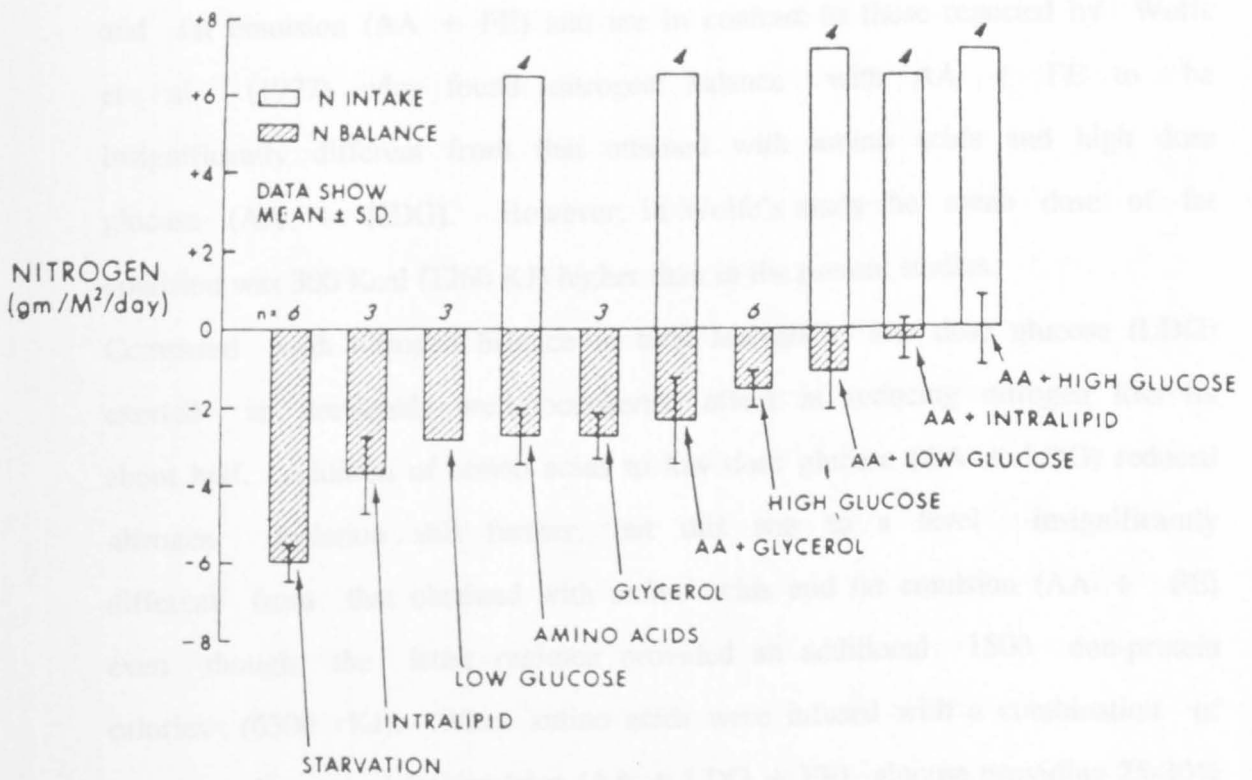
iii) Discussion

Discussion of the results must cover the main purpose of the studies, that is, measurement of nitrogen turnover, whole body protein synthesis and breakdown, but also the nitrogen balance data on the various regimens, the endocrine and biochemical changes, and the plasma amino acid results.

a) Nitrogen Balance Data

Figure XII summarises the data of Wolfe et al. (1977) referred to earlier, in which it can be seen that in normal fasting man there is a progressive reduction in negative nitrogen balance with the administration of fat emulsion, low dose glucose, high dose glucose, amino acids with low dose glucose, amino acids with fat emulsion and finally nitrogen equilibrium was obtained using amino acids with high dose glucose. These measurements were all done by conventional nitrogen balance methods. Whether the improved nitrogen balance was due to an effect on protein synthesis or on protein breakdown or a combination of the two is unclear.

FIGURE XII



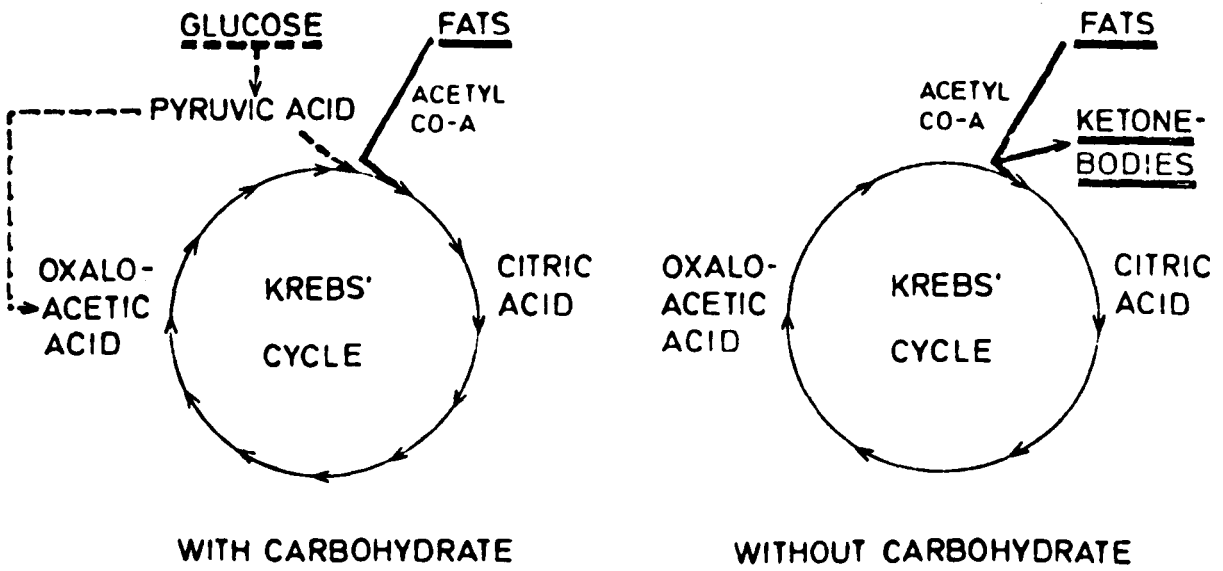
In the present studies a mixed oral diet resulted in nitrogen equilibrium. When exactly the same amount of protein equivalent was infused as amino acids with no added energy, nitrogen balance became negative. The nitrogen economy improved when glucose equivalent to the energy intake of the oral diet was added, but it was still slightly negative and evidently not as efficient as that observed on the oral diet, although statistically not significantly different.

These results show a relative inefficient nitrogen economy with amino acids and fat emulsion (AA + FE) and are in contrast to those reported by Wolfe et al. (1977) who found nitrogen balance with AA + FE to be insignificantly different from that attained with amino acids and high dose glucose (AA + HDG). However, in Wolfe's study the mean dose of fat emulsion was 300 Kcal (1260 KJ) higher than in the present studies.

Compared with nitrogen balance in total starvation, low dose glucose (LDG) exerted its previously well-documented effect in reducing nitrogen loss by about half. Addition of amino acids to low dose glucose (AA + LDG) reduced nitrogen depletion still further, but this was to a level insignificantly different from that obtained with amino acids and fat emulsion (AA + FE) even though the latter regimen provided an additional 1500 non-protein calories (6300 KJ). When amino acids were infused with a combination of low dose glucose and fat emulsion (AA + LDG + FE), glucose providing 25-30% of the non-protein energy, nitrogen equilibrium was obtained, the optimal result in this setting.

In the early stages of starvation the effect of glucose in reducing nitrogen loss is presumed to be due to its effect in reducing the obligatory need for gluconeogenesis from either body protein or infused amino acids (Cahill, 1970). Additional energy requirements are met through mobilisation of endogenous fat stores. It seems that when fat emulsion alone is given with amino acids, the available glycerol is used for gluconeogenesis, but the additional energy appears to be of lesser significance for protein-sparing, perhaps only contributing to preservation of fat stores. This may be because in the presence of a high fat and negligible carbohydrate intake, there is insufficient oxaloacetic acid derived from pyruvate available to initiate the Krebs cycle for the oxidation of acetyl-CoA. Thus, the accumulating acetyl-CoA forms a surplus of ketone bodies and ketosis develops (see Figure XIII). This would produce a biochemical explanation for the old adage that "fats burn in the fire of carbohydrate". Plasma ketones were certainly significantly higher at the end of the infusion in the AA+FE group, whereas there was no change in any of the other groups. Presumably sufficient glucose is available in the combination AA+LDG+FE to limit gluconeogenesis to a small fraction of the infused amino acids and sufficient energy is oxidised from fat for protein synthesis. Nevertheless, it is difficult to explain why, in terms of nitrogen balance (as opposed to weight change) this combination is more effective than AA+LDG. For example, Greenberg et al. (1976) concluded that in hypocaloric regimens endogenous fat mobilisation is

FIGURE XIII



adjusted appropriately to meet the deficit of the exogenous energy input, whether of glucose or fat. Furthermore, serum insulin levels were insignificantly different (at about 20 μ u/ml. in all groups (except AA and AA + HDG), a level which should permit endogenous fat mobilisation. Whatever the mechanism, these results in normal man agree with those of Jeejeebhoy et al. (1976) and Hansen et al. (1976) that glucose can be substituted by fat emulsion as 60-80% of the total energy provided, with no consequent deterioration in nitrogen balance, in patients requiring total parenteral nutrition. However, the patients described by Jeejeebhoy et al. were all protein-energy depleted before study (mean weight 47 ± 1 Kg; mean percent of ideal body weight 79 ± 10). Those of Hansen et al. included patients following operation and trauma as well as some with gastrointestinal disorders. In contrast, Long et al. (1977) found that addition of fat (up to 1100 Kcal, 4620 KJ per M²/day) to a combination of amino acids and glucose in five patients who were stable following burns or surgery did not affect nitrogen balance at any level of glucose intake. These patients were "hypermetabolic", following severe injury. Long suggested that the results of Jeejeebhoy were dependent upon adaptation to semi-starvation (because insulin levels fell from 53 ± 2 μ u/ml on the glucose system to 13 ± 2 μ u/ml. on the lipid system), a situation which severely stressed surgical patients are allegedly unable to achieve. The problem, once again, is not comparing like with like.

In summary, these data on normal fasting man suggest that the combination of amino acids with fat emulsion favours the nitrogen economy no more than the provision of amino acids with low dose glucose. In contrast, when amino acids were infused with an isocaloric combination of low dose glucose and fat emulsion (rather than fat emulsion alone), nitrogen equilibrium was attained. This mixture was well tolerated by peripheral veins. The only other combination which achieved this effect was amino acids and high dose glucose at a much higher osmolality which can only be infused via a central vein catheter.

b) Nitrogen Turnover, Protein Synthesis and Breakdown.

This constant infusion method for equilibration of intake and output using a stable-isotope tagged amino acid, as developed by Picou and Taylor-Roberts with glycine, has many theoretical and practical advantages. The stability of the plateau equilibrium attained can be established by variance limits for S_a ; the whole organism integrates the different equilibrium rates to a single value; the dose is small and the glycine load trivial. Constancy of infusion can be maintained for as long as the investigator wishes, for standardisation in the particular experimental setting under consideration.

Using this method, the whole body nitrogen turnover value, Q , is ~~the~~ the unique datum provided. It represents a plateau relationship between the infusion rate and the degree of urinary urea enrichment. The degree of enrichment

is found to rise slowly towards that of the infusate, which it can never exceed. At about 48 hours a slow steady rise occurs, termed a plateau. This can be arbitrarily defined as a slope showing no change greater than 5% over a 12-hour period. The early rapidly rising limb of the curve represents the integrated sum of a series of short half-life proteins, each newly made molecule taking up the isotope and if of short half-life, discharging it again, where it is taken up by others. The end-product (urea) reflects the net output of this mixture of enrichment and re-entry. The "plateau" is not flat (example shown in Figure XIV). The small continuing rise at equilibrium represents continuous representation of slow half-life turnover species of proteins in the curve. This curve, if it could be continued by constant injection of the isotope for weeks, months, or even years (biological infinity) would finally come into complete equilibrium with the degree of enrichment of the total infusate (ie the $^{15}\text{N} : ^{14}\text{N}$ ratio). At that time the entire body would be equally enriched as the intake.

In practice, during an experiment, what is accomplished is an equilibrium of the total body proteins whose synthetic half-time is encompassed within the duration of the infusion, a duration which should insure equilibrium for the majority of body proteins turning over at their normal rates. Almost certainly some extracellular proteins such as collagen and bone matrix, skin and hair, have not reached equilibrium.

The calculation of whole body nitrogen turnover, Q , is independent of the

BUILD-UP CURVE & PLATEAU ENRICHMENT OF URINARY UREA WITH ^{15}N
SUBJECT D.C. (NORMAL DIET) (EXAMPLE OF A PLATEAU)

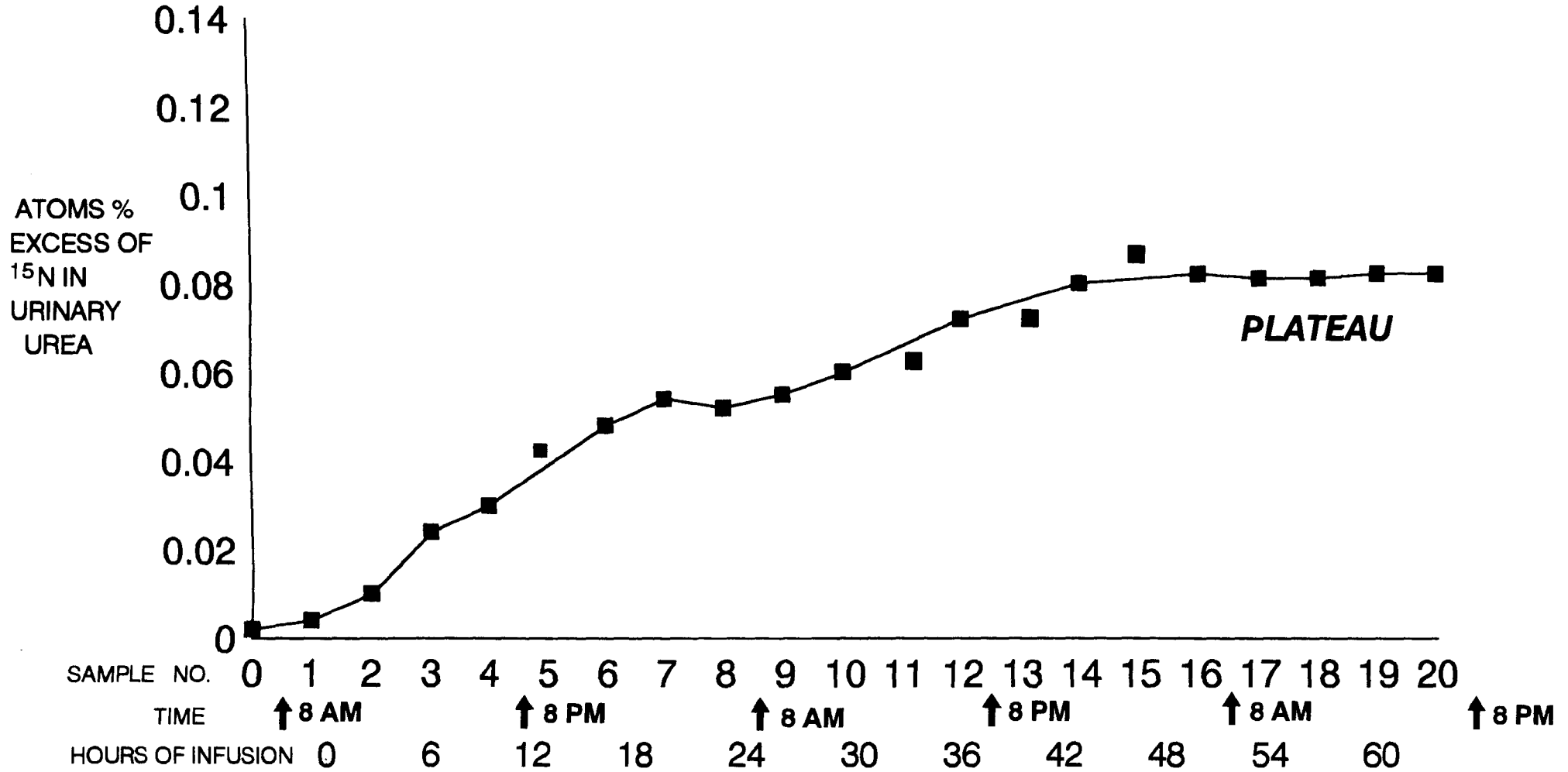


FIGURE XIV

analytic results for either total nitrogen intake or excretion, and hence it is customary to express it in terms of milligrams of nitrogen per kilogram body weight per day. Some workers express it in mg N/Kg/hour, or convert it into grams of protein per Kg. per day by multiplying by 6.25.

In these 30 studies in 16 subjects the range of Q was 395 - 584 mg.N/Kg/day. However, the range for those on normal diet was 474-584 mg N/Kg/d. Sim and Sugden (unpublished) found a range of 550 - 623 mg N/Kg/day in four subjects taking 1g. protein/Kg/day by mouth, and repeat studies on the same subjects under identical conditions at a later date revealed differences of only 7 - 21 mg/Kg/d. (about 1-3%) which were statistically insignificant. There is little other information about reproducibility in the same individual, but in one subject eight measurements made with ¹⁵N glycine over a period of 4 years gave coefficients of variation of 3.5% for turnover and 6.3% for synthesis (Fern et al., 1984). The results from the present studies seem to be slightly lower than the unpublished series of Sim and Sugden. They are significantly lower than those of Steffee et al. (1976) who reported a range of 581 - 766 mg N/Kg/d. in six subjects on a diet of 1.5 g. protein/Kg/d.

When converted into grams of protein there is a turnover of 3.4 g/Kg/d. or an average of 236 grams per person for those on a normal diet. Hence, whereas 236 grams of new protein are synthesized and broken down every day, only about 70 grams are excreted (excretion of 11.27g N x 6.25) indicating

that most of the amino acids originating from protein breakdown are reincorporated into protein. In the situation of normal diet, synthesis amounts to 70% of turnover, or, in other words, the body makes an average of 166 grams of protein despite an intake of only 72 gms. Stated in graphic fashion these numbers correspond to the synthesis of more than 4 tons of protein during an average lifetime but with a protein intake of less than 2 tons. Nevertheless, the mean synthesis rate of 2.4 g. protein per Kg/d. on normal oral diet is lower than that of most other workers (see page 86) using ^{15}N glycine on subjects of the same age group. Coefficients of variation in the studies cited were 8-30%. Norton, Stein and Brennan (1981) found a similar level of protein synthesis of 2.5 gm./Kg/d. in three normal controls using ^{15}N glycine, but they used ^{15}N enrichment of ammonia as end-product, which tends to give lower results than urea (see page 84). Similar results to those presented were also obtained by Jeevanandam, Lowry and Brennan (1987) studying normal volunteers during a ten-day fast (turnover 2.43, synthesis 1.77 g./Kg/d) and depleted patients with benign gastrointestinal disease (turnover 2.49, synthesis 1.96) similarly fasted. One possibility for the low values may be the relatively low protein and energy intakes in the present studies (1g. protein/Kg/d. and 30-35 Kcal, 126-147 KJ/Kg/d.) when compared with those of Steffee et al. (1976) of 1.5 g. protein/Kg/d. and 45 Kcals (189 KJ) per Kg/d. Although the difference in protein intake may not make much difference at this level (Steffee et al. found a barely significant fall

in Q of 8% when protein intake fell from 1.56 to 0.38 g/Kg/d.), Golden, Waterlow and Picou (1977) found that in children who were recovering from or had recovered from protein-energy malnutrition, the level of protein intake did not affect protein synthesis but over the range of energy intake 60 - 270 Kcal (252 - 1134 KJ) per Kg/d., synthesis rate increased by about 1g. protein/Kg/d. for every 25 Kcal/Kg/d. There is no doubt that the effects of protein and energy intake on protein turnover are quite complex because account has to be taken of three separate factors: the immediate food intake during the measurement; the prevailing or previous diet; the nutritional state of the subject. One of the most striking findings in the present studies is that the nitrogen turnover in the subjects on normal oral diet (543 ± 47 mgN/Kg/d.) is statistically significantly higher than that on intravenous amino acids (442 ± 37) and amino acids with high dose glucose (434 ± 39). The finding is especially convincing because the two intravenous regimens provided exactly the same nitrogen and energy intake as the oral diet, and all subjects acted as their own controls in these three studies. This 20% reduction in protein kinetics in normal adults compares with the subsequent demonstration by Duffy and Pencharz (1986) of a 40% reduction in rapidly growing postsurgical neonates when enteral feeding was changed to the intravenous route. Similarly, Jeevanandan, Lowry and Brennan (1987) showed that during refeeding of previously starved normal volunteers, and depleted patients either with or without cancer, the kinetic parameters of protein metabolism were generally increased during

oral feeding compared to intravenous feeding in all groups. Their results suggested that oral feeding preferentially stimulates whole body protein synthesis and that intravenous feeding retards protein breakdown.

A possible explanation for our findings proposed by us was that there may be a sharp reduction in gastrointestinal enzyme secretion and intestinal mucosal cell turnover when oral intake ceases. It was mentioned earlier that the intestinal mucosa alone represents about 16% of daily protein synthesis (Lipkin, 1965; Nasset, 1968) and total release of protein into the digestive tract from saliva, gastric juice, bile, pancreas and small bowel can be calculated to account for 50% of daily protein synthesis (Spencer, 1960; Nasset, 1968; Da Costa, 1971; Nasset and Ju, 1961). Certainly it is known that in the absence of oral feeding the gastrointestinal tract does not fully recover from the insult of prolonged diarrhoea (Greene et al., 1975) nor does the bowel adapt fully after partial resection (Feldman et al., 1976).

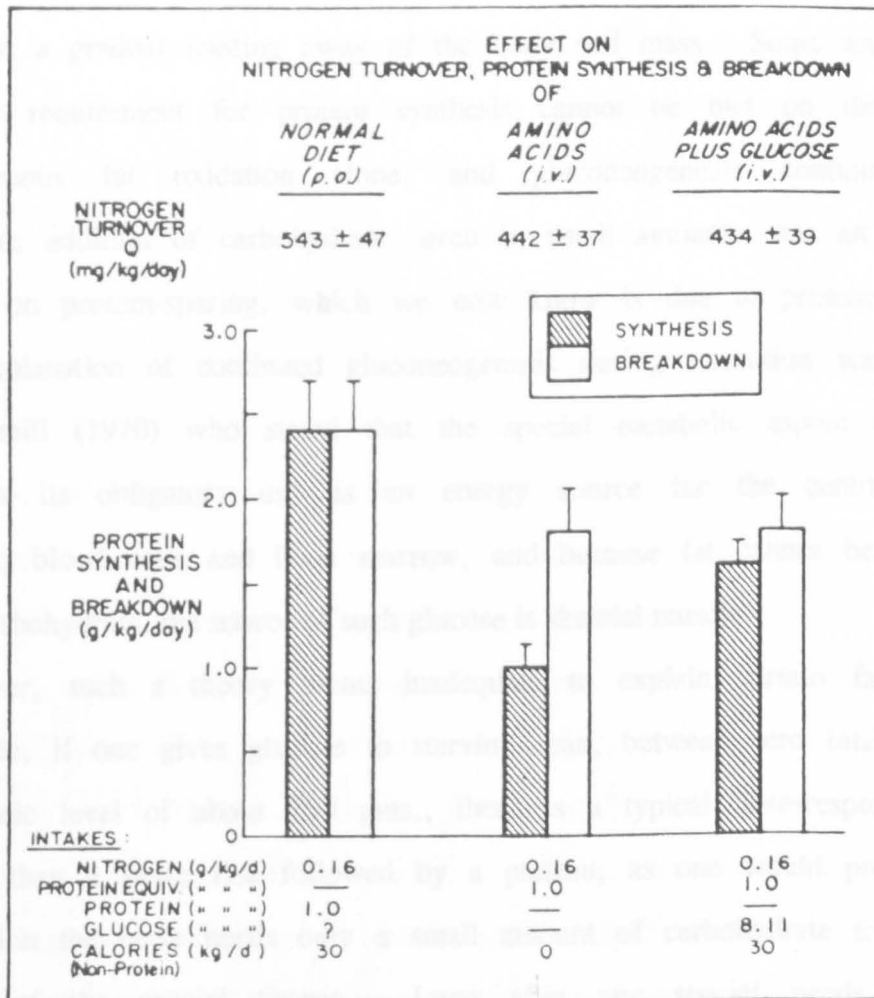
An alternative explanation is given by Fern et al. (1981). They estimated ^{15}N in both urea and ammonia after a single dose of ^{15}N glycine given orally or intravenously and obtained different results with the two end-products depending upon which route the isotope was given and whether the subjects were in the postabsorptive or absorptive states. Their results do not need to be discussed here, other than to state that Fern et al. believed that differences in the postabsorptive and absorptive states were due to incomplete mixing of unlabelled nitrogen in the food with the

metabolic nitrogen pool of the body. ^{15}N in the small bowel and the liver will be diluted more by dietary nitrogen than the isotope present in other tissues. As urea is formed in the liver its ^{15}N -enrichment will consequently be lowered by the absorption of dietary protein, which in turn will produce higher estimates for nitrogen flux. Extending this alternative hypothesis proposed by Fern et al. to our findings would indicate that the apparent decrease in nitrogen flux on the intravenous regimens was an artefact due to the switch from oral to intravenous feeding serving to increase the isotopic enrichment in the hepatic nitrogen pool relative to the peripheral tissue. The true explanation still awaits elucidation.

The other striking finding in the comparisons between the five subjects on normal oral diet, intravenous amino acids, and amino acids with a high dose glucose, is that the addition of glucose to an infusion of amino acids in healthy fasting man increases the whole-body protein synthesis rate, without affecting either nitrogen turnover (when compared with AA alone) or protein breakdown rates, see [Figure XV](#). This was the first time that such an effect of glucose had been demonstrated. The effect of glucose, particularly at high dose, in reducing negative nitrogen balance during starvation, is well-known (as described in the Introduction) but it was not clear until now whether this effect was by increasing protein synthesis, decreasing breakdown, or a combination of both.

This demonstration, which is clear-cut, adds to our knowledge of the

FIGURE XV



relationship between protein metabolism and energy requirements, but there remain unanswered questions. For example, what role exactly does glucose play? In starvation the oxidation of body fat can meet all of the measurable external requirements of the body, at least for a time, and yet there is a gradual wasting away of the body cell mass. Some aspect of the energy requirement for protein synthesis cannot be met on the basis of endogenous fat oxidation alone, and gluconeogenesis continues. By contrast, addition of carbohydrate, even in small amounts, has an immediate effect on protein-sparing, which we now know is due to protein synthesis. An explanation of continued gluconeogenesis during starvation was provided by Cahill (1970) who stated that the special metabolic aspect of glucose lies in its obligatory use as an energy source for the central nervous system, blood cells and bone marrow, and because fat cannot be converted into carbohydrate, the source of such glucose is skeletal muscle.

However, such a theory seems inadequate to explain certain facts. For example, if one gives glucose to a starving man, between zero intake and an isocaloric level of about 750 gms., there is a typical dose-response curve, rather than a sharp rise followed by a plateau, as one would predict if in starvation the body needs only a small amount of carbohydrate to meet the needs of the special tissues. Long after any special needs are met, glucose continues its effect. This suggests a unique or special role of glucose in meeting the energy cost of protein synthesis. Also, when the maximum amount of glucose is given, ie 750 gm., there is still a basal

gluconeogenesis - the so-called "nitrogen floor" of O'Connell et al. (1974). Gluconeogenesis should have been shut off if the only purpose of glucose was to satisfy special tissues. Giving additional insulin will not push the nitrogen floor any lower (Moore F.D - unpublished observations), perhaps not surprisingly because such a large amount of carbohydrate produces insulin concentrations of 40-80 μ u/ml.

It seems likely that glucose does indeed have a special role in protein synthesis. Lehniger (1975) has calculated that it costs 29.2 Kcal (123 KJ) per mole of peptide bond synthesized. By contrast, the hydrolysis of protein yields only 5 Kcal (21 KJ) per mole, making a net cost for protein synthesis of 24.2 Kcal (102 KJ). However, if it is a matter of ATP regeneration, alone, to provide the energy to support protein synthesis, why does endogenous fat oxidation fail to support nitrogen equilibrium?

Estimates from higher vertebrates, including man, suggest that for the organism as a whole, 15-20% of resting energy expenditure is for protein synthesis. Is glucose simply a general utility carbon source, yielding energy for oxidative phosphorylation in all the tissues of the body, and stimulating the production of insulin which itself may provide a protein synthetic signal (it does increase uptake of amino acids by cells) or does it have some other effects, traceable more to its chemical configuration, rather than simply as an energy source? In bacteria the gene transcription of the lac operon is specifically inhibited by a glucose catabolite (an

operon consists of a group of structural genes, responsible for enzyme induction or repression together with an operator gene which can switch activity of the group in replicating M-RNA on or off). This specific catabolite interferes with gene transcription, favouring the oxidation of glucose over that of alternatives such as lactose. The system is important in bacteria and always favours glucose as the energy source (Majors 1975; Tubay, Schwartz and Beckwith, 1970). The lac operon is less important in mammalian metabolism, but the point is that in describing the effect of glucose as a fuel it might be a mistake to refer to its effect as entirely due to the energy provided. It is also evident that glucose can operate as a specific chemical signal at the gene transcription level, a step involved in all protein synthesis, but whether or not such an effect exists in man is purely speculative.

Apart from acting as a nonspecific energy source, or specifically because of the obligatory glucose requirements of the special tissues, glucose may also have a specific protein-sparing effect because of its capacity to stimulate the output of insulin. Insulin is the anabolic hormone par excellence favouring ingress of amino acids into muscle cells, maintenance of liver function, ingress of glucose into peripheral cells for oxidative metabolism and deposition of excess carbohydrate as fat and inhibiting mobilisation of fat as a body fuel. Studies of 3-methylhistidine excretion indicate that insulin produces increased synthesis and decreased breakdown in muscle protein (Wassner, Orloff and Halliday, 1976).

Returning to the results of nitrogen turnover, the range in the remaining four groups (AA + LDG + FE, AA + FE, AA + LDG, and LDG) of 409 - 545 mg N/Kg/d. is similar to that obtained on normal oral diet, AA, and AA + HDG. Turnover on AA + LDG (534 ± 31 mg N/Kg/d.) was significantly higher than on AA + FE (470 ± 32 mg N/Kg/d.), $p < 0.05$. It is difficult to know why this should be so, but three subjects out of four were in both studies, acting as their own controls. No significant differences were observed between other groups, possibly because it was necessary to use eleven subjects for sixteen studies. Although there seems to be close reproducibility in the same individuals, inter-individual variation among similar subjects is of the order of 10%.

Relationships between synthesis and breakdown were not clear-cut in AA + LDG + FE, AA + FE and AA + LDG as in normal oral diet, AA, and AA + HDG. Differences between the various regimens were not dependent solely on changes in either synthesis or breakdown alone.

However, the results obtained with low dose glucose (LDG) are of outstanding interest. Although as would be expected, the difference between protein synthesis and breakdown is the largest of these last four groups, the actual values for both are also higher than in any other. These results suggest that with a zero protein intake (as in LDG) turnover is unchanged, but that a much larger proportion of the total turnover is re-utilised for protein synthesis (83%, compared with 37-70% with the other six regimens; that on normal oral diet being 70%). Similar results have

been obtained in rats and infants on deficient diets (Waterlow, 1968; Golden, Waterlow and Picou, 1977).

Since the only source of nitrogen entering the metabolic pool on zero nitrogen intake is from protein breakdown, it follows that there is a greater re-utilisation of endogenous amino acids for protein synthesis when even small amounts of glucose are present. Adaptive enzyme changes are thought to play a major role in the economic use of amino acids in this situation. For example, in rats on a low protein diet there is increased activity of amino acid activating enzymes in the liver (Mariani et al., 1963; Gaetari et al., 1964) and a decrease in activity of urea-cycle enzymes (Schimke, 1962). However, it is difficult to conceive that these changes can be responsible for such rapid adaptation witnessed in short-term studies involving acute alterations in dietary intake in man.

Steffee et al. (1976) found that with adaptation to a low protein diet of 0.38 g/Kg/d. turnover fell just significantly by 8%, but breakdown increased by 27% and synthesis by 15%. Endogenous amino acids were reutilised more efficiently under these conditions, but it is still difficult to know why synthesis should be greater than when dietary intake was 1.5 g. protein/Kg/d. Steffee et al. felt that the significant increase in synthesis on reduced intake might be more apparent than real. They state that reducing protein intake to 0.38 g./Kg/d. causes about a 50% fall in turnover rate of the body urea pool, and their ¹⁵N urea data are in line with this and indicate that plateau was achieved after 36 - 39 hours on a

higher protein diet, but not until the 47th hour on the lower protein diet. Thus, the plateau enrichment might be underestimated and values for Q overestimated at the lower protein intake. In our studies, turnover Q was not larger than in the other groups, and they were continued for 60 hours, but in two cases the curve was still rising.

Long et al., (1977) similarly found what seems to be high results when they used a pulse injection of ^{15}N alanine to compare normal with septic subjects. As in our studies all subjects received 150 g. glucose only daily for two days beforehand. Synthesis was 3.69 in normals and 4.48 in sepsis. Birkhahn et al. (1980) compared normals with trauma patients and found that trauma produced a 50% increase in synthesis and a 79% increase in breakdown, but the point is that in the normals synthesis of 2.33 and breakdown of 2.67 were similar to ours, and once again these subjects were receiving low dose glucose for 72 hours prior to study. However, measurements were made using ^{14}C leucine.

Halliday and McKeran (1975) believe that the ^{15}N enrichment in urinary urea is more a reflection of hepatic than whole-body nitrogen turnover, and that with a low protein intake hepatic protein breakdown continues with relative segregation of the hepatic amino acid precursor nitrogen pool from the plasma pool of labelled amino acids, giving rise to a reduced enrichment of urinary urea synthesized in the liver, and thus an increase in the calculated total body synthesis.

Stein et al. (1976) studied rats on four diets, equivalent to our studies

of AA + HDG, AA, HDG and LDG and measured rates of protein synthesis in heart, lung, muscles, kidney and liver. Heart and lung protein synthesis were depressed by both nitrogen and energy restriction, but muscle protein synthesis was affected only by omission of glucose. With dietary deficiency liver protein breakdown increased. With the nitrogen-deficient diets there was no agreement between the urea-based and intracellular precursor pool values for liver protein synthesis. With the high dose glucose diet, the urea value suggested that liver protein synthesis was increased threefold and whole-body synthesis sixfold. The authors concluded that it was unlikely that these results were correct. They felt that the 'one-amino acid pool' assumption does not hold under conditions where nitrogen is limiting. When no exogenous nitrogen enters the body intracellular recycling of nitrogen and compartmentalization are maximised. The urinary urea reflects muscle nitrogen of low enrichment that cannot be utilized by the liver and is therefore excreted without mixing with the liver pool.

Thus, in making deductions about reutilisation of amino acids derived from protein breakdown it is assumed that complete mixing of these amino acids takes place with the infused tracer. This assumption may not be correct, and in the situation in which no or low nitrogen intake occurs, the model may not work.

Studies of protein metabolism following trauma and sepsis are discussed in greater detail following the description of the patient studies, but

suffice it to say that some workers have shown a simultaneous increase in both synthesis and breakdown (ie turnover) (Stein et al., 1977; Millward et al., 1976; Birkhahn et al., 1980) while others have shown only a decrease in protein synthesis (O'Keefe et al., 1974; Crane et al., 1977; Kien et al., 1978). One of the reasons for the discrepancy is likely to be because of differences in intake between the studies. For example, there was no nitrogen intake postoperatively in the studies of O'Keefe et al, and Crane et al., and decreased intake in Kien et al., which correlated with the decrease in synthesis.

In summary, the results with low dose glucose show no fall in turnover compared with the other groups, which might be expected with a lack of nitrogen intake. However, they do show a much greater degree of re-utilisation of amino acids following breakdown. This would be expected, and has previously been demonstrated following adaptation to a low-protein diet (Waterlow, 1968), but the mechanism in the acute situation remains obscure. Also in this situation, synthesis and breakdown were higher than in any of the other regimens, including normal oral diet. It is possible that the assumptions inherent in the method are not correct when there is no nitrogen intake. It may be valid to make comparisons between groups of controls and patients given low dose glucose such as Birkhahn et al. (1980), when trauma produced increase in both synthesis and breakdown (the latter to a greater extent), but probably not if subjects

are studied on a certain protein diet and subsequently on a zero intake.

c) Endocrine and Biochemical Changes.

All of our study groups received amino acids except those on normal diet, and those on low dose glucose. Low dose glucose did not produce increase in insulin secretion compared to control levels but did produce a significant reduction in glucagon to the lowest level seen. The insulin: glucagon molar ratio was 26, much higher than the 2 - 7.5 range seen in the remaining groups. In Wolfe et al. (1977) high dose glucose alone formed one of the groups, and here, in addition to suppression of glucagon, a strong insulin response occurred, giving the highest insulin: glucagon molar ratio seen, approaching 100. Whereas high dose glucose reduces negative nitrogen balance in starvation by a combination of exogenous carbohydrate calories and altering the endocrine setting of the body, favouring insulin, glucose at low dose provided fewer calories and did not alter insulin secretion, but the sharp fall in glucagon levels indicated diminished gluconeogenesis. Provision of amino acids with high dose glucose does lead to increased insulin secretion, but the insulin: glucagon ratio is not altered because of maintenance of glucagon secretion produced by the amino acids. Amino acids alone cause the highest levels of glucagon seen indicating increased gluconeogenesis. In Wolfe et al (1977) glucagon concentration was higher with AA + FE than AA alone suggesting stimulation by the fat emulsion, but this finding was not reproduced in the present studies.

One might suppose that because insulin is the prime anabolic hormone, the feeding regimen with the highest insulin: glucagon molar ratio would be the most effective. In fact, this is not the case, because the ratio of 26 in the LDG group is by far the highest, but this was due more to suppression of glucagon than by stimulation of insulin.

Significant rises in free fatty acids occurred in both groups receiving fat emulsion, although the final figure in the AAFE group (2731 ± 333) was double that of AA + LDG + FE (1182 ± 249). Provision of glucose maintained free fatty acids at near control levels. Similarly, provision of glucose prevented ketosis.

d) Amino Acid Concentrations (Tables 12,13,14)

Table 13 shows the plasma amino acid levels which were measured and the normal range of concentration of each, together with the amino acid composition of the 3.4% FreAmine II solution which was used in each study.

It can be seen that the FreAmine II solution contains fifteen amino acids including all eight of the essential amino acids (EAA), plus the semi-essential histidine. Glycine, methionine and isoleucine are present in a greater concentration than their normal levels in plasma.

There are several ways of interpreting the plasma amino acid levels. Nicholson et al. (1975) studied what they termed pattern-recognition in the "plasma aminogram" in infants and found that in normal infants the ratio of total essential amino acids to total amino acids (TEAA/TAA) was

about 0.4. In FreAmine II the ratio is 0.38 (115.2/298.7). Tweedle et al. (1977) found that in fasting adults the ratio was 0.41, which increased on the fourth and eighth days of amino acid infusions with FreAmine II to 0.48 and 0.51 respectively. Culebras et al. (unpublished - quoted by Tweedle) found that when amino acids were accompanied either by low dose or high dose glucose the ratio remained stable. The subjects on the regimens AA + LDG + FE, AA + FE, AA + LDG and LDG, all had plasma amino acids estimated. Seven of the eight essential amino acids were measured, not including histidine. These were:- threonine, valine, methionine, isoleucine, leucine, phenylalanine and lysine. Tryptophan is not measured by the ion exchange chromatographic technique used for the other amino acids.

It can be seen that the control ratios of TEAA/TAA were constant at 0.29 - 0.30. (Table 14). The only groups in which a notable change occurred were in AA+FE (0.30 to 0.40) and AA + LDG (0.295 to 0.34). In general, plasma amino acid levels in starvation conditions show an overall decrease, and a low TEAA/TAA ratio (Adibi, 1971). This was certainly the case with LDG in which the TEAA/TAA ratio remained constant and low at 0.29, and which had the lowest TAA of all the groups, apart from, oddly enough, the control group on a normal oral diet. It is not immediately clear why the ratio should have risen in AA + FE and AA + LDG. However, the essential amino acids contain the three branched chain amino acids (BCAA) - valine,

isoleucine, and leucine. More information may be obtained by studying the ratio BCAA/TAA. The normal ratio is 0.19 - 0.22 (mean 0.2), Wolfe et al., 1977. Interestingly, in FreAmine II the ratio is $60.6/298.7 = 0.20$.

In the control group on normal oral diet, the ratio was 0.22, as expected. With infusion of amino acids alone it rose to 0.37, a situation seen in early starvation, and probably due to a decrease in insulin secretion which reached its lowest level in this group. When glucose alone is given (LDG) the ratio falls to its lowest level. In this group insulin levels were maintained, but glucagon levels were the lowest seen. Taking these two results together, an elevation in the BCAA level might be used as an indicator of gluconeogenesis (Swendseid et al., 1967). Addition of amino acids to glucose (AA + LDG) and in (AA + LDG + FE) resulted in ratios with no significant change from control levels, which were low normal. The ratio rose in AA + FE, a situation in agreement with Wolfe et al (1977), but although this suggests increased gluconeogenesis, insulin and glucagon levels were insignificantly different from the other groups receiving amino acids. Wolfe did find increased glucagon secretion in this group, which would fit with increased gluconeogenesis.

Two other ways of expressing plasma amino acid concentrations are the proportions of alanine and glycine to total amino acid levels, ala/TAA and gly/TAA. (Table 12). With ala/TAA, the control level is 0.16. The changes with amino acid infusion alone (AA) provide the background for assessing

the impact on amino acid infusions of concomitant energy substrates. Despite the fact that alanine is present in high concentration in FreAmine II (26.9 mM/l., but within the normal range in plasma of 17-50), the alanine/TAA ratio fell to 0.07, presumably secondary to rapid gluconeogenesis. Thus, low alanine does not indicate restricted gluconeogenesis per se, and coupled with an increased BCAA/TAA ratio, it is a feature of gluconeogenesis and accords with the increased glucagon levels and ureagenesis. With AA + HDG the alanine/TAA ratio is closest to the control ratio, and with AA + LDG, although there is a fall, it is not significant. Both findings suggest reduced necessity for gluconeogenesis. Strangely, there is a statistically significant fall in the ratio in AA + LDG + FE. It is difficult to know why this should be so. This combination gave nitrogen equilibrium and showed no endocrine setting for gluconeogenesis. Perhaps it was in some way related to provision of fat emulsion, because the ratio also fell in AA + FE. Although glucagon did not change significantly in AA + FE, Wolfe et al (1977) found that it rose, and to a greater level than in AA alone or amino acids plus glycerol. Jeejeebhoy et al. (1976) reported a similar stimulation. An insignificant fall occurred with LDG presumably indicating reduced requirement for gluconeogenesis. Glycine is present in large amounts in FreAmine II (90.5 mM/l., much higher than its normal level in plasma of 13 - 49). The control glycine/TAA ratio was 0.12. Presumably because of the high levels in Fre

Amine II the ratio rose in all groups receiving amino acids, and remained unchanged in LDG. The ratio would also be expected to rise in starvation as plasma glycine concentration rises then (Wannemacher, 1977; Lund & Williamson, 1985). The ratio will therefore finally depend upon the rate of infusion of Fre Amine II, the utilisation for protein synthesis and degree of transamination of glycine, and the degree of starvation which will itself depend upon the dose and nature of non-protein energy substrate(s).

Only 2-3% of the total amino acids in the body are present in the free pool, with only about 1% in the plasma since red cells contain amino acids. Diurnal variations in the plasma concentrations are relatively small (Wurtman et al., 1968). This is surprising in view of the intermittent influx of amino acids derived from the diet, short-term changes in rates of protein synthesis and breakdown, and the large inter-tissue fluxes of certain amino acids (eg glutamine, alanine). Moreover, the plasma concentrations bear little relation to the intracellular concentrations in different tissues (each tissue has a different profile), and they do not reflect the composition of dietary protein.

Experiments on the isolated perfused liver suggest that the liver plays the key role in "setting" plasma concentrations (Lund & Williamson, 1985). There is a thirty-fold range in concentration of plasma amino acids between the lowest (aspartate 0.02 mM/l.) and the highest (glutamine, about 60 mM/l.) which seems to be determined by the amount and composition in

the diet, rate of delivery to the tissues, kinetic properties of the systems which transport individual amino acids into and out of tissues, their intracellular fate, and relative rates of synthesis and breakdown and interconversion of amino acids. Tissue-plasma concentration gradients are well known in animals but not in man except for skeletal muscle. The gradient is less than two for the branched chain amino acids and phenylalanine. 30% of dietary protein is glutamine, glutamate, aspartate and alanine (glutamine is 50-60% of the free amino acid pool in skeletal muscle, and alanine 4-5%).

Following dietary ingestion, the fate of incoming amino acids depends upon whether they are essential or non-essential. If essential, the amount allowed to pass through the liver depends upon the needs of the body; intake above requirements induces the degradative liver enzymes. On the other hand, the three branched chain amino acids, valine, leucine, and isoleucine, are exceptions to this and are transferred intact to the systemic circulation and undergo transamination and degradation in peripheral tissues such as muscle, kidney, adipose tissue and brain. Thus, the plasma amino acid profile leaving the liver after a protein meal is enriched in BCAA because excess of the other essential and non-essential amino acids have been selectively degraded by the liver. More than 70% of the increase in free amino-N leaving the liver is accounted for by BCAA compared with a concentration of 20% of BCAA consumed in the meal.

Entry of BCAA into muscle and fat is facilitated by insulin secreted in

response to the carbohydrate and protein components of the meal. Carbohydrate causes a reduction in plasma free amino acids which is most extensive with BCAA. In contrast, in early starvation the BCAA initially rise in plasma due to lack of insulin. This rise lasts 5-7 days and then falls. Alanine falls rapidly in starvation, and glycine progressively increases (Wannemacher, 1977). In man certain bacterial and viral infections lead to a decrease in plasma total amino acids, the largest decrease being in the BCAA (Wannemacher, 1977). In such cases phenylalanine and tryptophan rise in the plasma; they are released due to muscle breakdown and cannot be metabolised within muscle. Possibly a rising phenylalanine in injured or infected patients might be useful as an index of skeletal muscle breakdown as it is catabolised entirely by the liver.

BRANCHED CHAIN AND TOTAL AMINO ACIDS AND THEIR RATIOS

GROUP	BRANCHED CHAIN AMINO ACIDS (BCAA) mm/l \pm S.D.		TOTAL AMINO ACIDS (TAA) mm/l \pm S.D.		BCAA / TAA	
	CONTROL	FINAL	CONTROL	FINAL	CONTROL	FINAL
NORAML DIET		45 \pm 10		206 \pm 29		0.22
AA		133 \pm 23		364 \pm 52		0.37
AA+HDG		62 \pm 6		325 \pm 25		0.19
AA+LDG+FE	43 \pm 11	55 \pm 11	296 \pm 26	371 \pm 49	0.14	0.15
AA+FE	50 \pm 7	110 \pm 34	323 \pm 44	437 \pm 102	0.15	0.25
AA+LDG	36 \pm 5	75 \pm 14	276 \pm 55	457 \pm 64	0.13	0.16
LDG	37 \pm 7	39 \pm 5	251 \pm 23	312 \pm 38	0.15	0.12

ALANINE AND GLYCINE : RATIOS TO TOTAL AMINO ACIDS

GROUP	ALA / TAA		GLY / TAA	
	CONTROL	FINAL	CONTROL	FINAL
NORMAL DIET		0.16		0.12
AA		0.07		0.15
AA+HDG		0.13		0.23
AA+LDG+FE	0.11	0.09	0.08	0.16
AA+FE	0.09	0.06	0.08	0.14
AA+LDG	0.10	0.08	0.09	0.16
LDG	0.10	0.085	0.09	0.10

TABLE 12

NORMAL RANGE OF PLASMA AMINO ACID LEVELS, WITH AMINO ACID COMPOSITION OF 3.4% FREAMINE II (mm/L)

		Freamine	
TAURINE	(3.5 - 14)		
THREONINE	(7.5 - 25)	11.4	essential
SERINE	(6.1 - 19)	16.0	
ASPARAGINE & GLUTAMINE	(45 - 85)		
PROLINE	(8.9 - 44)	33.0	
GLUTAMIC ACID	(0 - 12)		
CITRULLINE	(1.2 - 5.5)		
GLYCINE	(13 - 49)	90.6	
ALANINE	(17 - 50)	26.9	
VALINE	(12 - 33)	19.1	BCAA essential
CYSTINE	(3.1 - 14)		
METHIONINE	(1.3 - 3.9)	12.1	essential
ISOLEUCINE	(3.5 - 10)	18	BCAA essential
LEUCINE	(6.9 - 16)	23.5	BCAA essential
TYROSINE	(3.2 - 8.7)		
PHENYLALANINE	(3.4 - 12)	11.6	essential
ORNITHINE	(3.0 - 13)		
LYSINE	(9 - 26)	17.0	essential
HISTIDINE	(5.6 - 12)	6.2	semi-essential
ARGININE	(4.6 - 15)	7.1	
TRYPTOPHAN	not measured	2.5	essential
CYSTEINE	not measured	< 0.7	
		298.7	

TABLE 13

RATIO OF TOTAL ESSENTIAL AMINO ACIDS TO TOTAL AMINO ACIDS ($\frac{TEAA}{TAA}$)

	CONTROL	END OF STUDY
AA(Tweedle et al)	0.41	0.51
AA+LDG+FE	0.30	0.325
AA+FE	0.30	0.40
AA+LDG	0.295	0.34
LDG	0.29	0.29

TABLE 14

FREAMINE II 0.38

SUMMARY AND CONCLUSIONS

1. Compared to normal oral diet, in which nitrogen equilibrium was observed, there was a negative nitrogen balance when normal subjects were infused with a variety of intravenous substrates. This negative nitrogen balance decreased progressively on LDG, AA, AA + LDG and AA + FE, AA + HDG. Using AA + LDG + FE nitrogen equilibrium was achieved, as with normal oral diet.
2. Nitrogen turnover on normal oral diet was significantly higher than on intravenous AA alone and AA + HDG.
3. Addition of glucose to amino acids improved nitrogen balance compared to amino acids alone, and this was due to increased protein synthesis, breakdown remaining unchanged.
4. Protein synthesis and breakdown were highest in the low dose glucose group. Turnover did not fall in this group, indicating maximal re-utilisation of amino acids for synthesis. In this situation of zero nitrogen intake the model may not be valid.
5. There were insignificant changes in blood glucose and serum insulin levels between groups. In all groups receiving amino acids there were

insignificant changes in plasma glucagon levels, but in the LDG group there was a significant fall. Insulin: glucagon molar ratios remained low (2.2 - 7.5) except in the LDG group (26.2) which was due to suppression of glucagon.

6. Plasma ketones rose in the AA + FE group (they were not measured in AA but ketosis would have occurred here also). Free fatty acids doubled in those groups receiving fat emulsion.

7. Total plasma amino acid and branched chain amino acid levels rose in those groups receiving amino acid infusions. The alanine: TAA ratio fell in those groups receiving fat emulsion. The glycine: TAA ratio rose in all groups, especially those receiving amino acid infusions.

STUDY I A

Circadian Rhythm in Urinary Nitrogen and Urea Excretion

An interesting and serendipitous finding in Study I was provided by the urinary nitrogen and urea results. As was mentioned in the section on Materials and Methods, in each study the volunteers were required to pass urine at three-hourly intervals so that the curve could be plotted and plateau enrichment of urinary urea with ^{15}N could be determined, and the specimens were also pooled to estimate 24 hour nitrogen and urea excretion. Such specimens provided an opportunity to study the pattern of diurnal excretion of nitrogen and urea, and to compare that obtained with normal oral diet consisting of four equally spaced meals with continuous provision of protein and energy in the form of intravenous amino acids and amino acids and glucose. Although such collections were made in all seven studies it is unfortunate that in the last four studies the three-hourly urinary urea and nitrogen levels were pooled to calculate daily balances and the individual figures not kept.

It was found in the five subjects who were studied on normal oral diet, intravenous amino acids, and amino acids plus glucose, that a diurnal rhythm existed in urinary excretion of urea and nitrogen. This is easily discernible to the eye in Figures XVI to XXV inclusive. As far as normal diet is concerned, it is perhaps not surprising to see a diurnal rhythm in view of the daytime intake of four equally-spaced meals, with no intake

at night. The rhythm seemed to be a twenty-four hour one, and apart from one exception (WW), the lowest rate of urea and nitrogen excretion occurred in the early hours of the morning, roughly from midnight to 0630 hours. In WW the lowest excretion was later in the morning, at 0930 hours. What is surprising is the strikingly similar diurnal rhythm in excretion of nitrogen when the subjects were receiving a constant infusion of intravenous amino acids, thus eliminating the effect of intermittent supply of nutrients in the form of meals. Because in the latter situation subjects were not receiving added energy and were in negative nitrogen balance, the urinary excretion of urea and nitrogen was greater, and thus when shown graphically, the points are placed at a higher level than those of normal diet, and the rhythm is easy to observe in the two curves which only occasionally cross. Once again, because the curves generally follow one another, the excretion of urea is lowest in the night. Again, the exception is WW in which it occurs later in the morning.

In addition, the rhythm appears to have individual patterns despite the generalities just described. For example, JF demonstrates a pattern of smooth undulations with a range of excretion on normal diet of only 0.79 - 1.29 whereas JG and WW in particular show wide swings, and apart from a few aberrant points, the pattern of the graph for amino acids follows that for normal diet. This individuality is destroyed by plotting the means of the five subjects, where a diurnal rhythm persists but is less easy to discern

visually. A similar, but possibly slightly less obvious, diurnal rhythm exists in those four subjects who completed the study using intravenous amino acids and glucose.

Many biochemical, physiological and behavioural processes demonstrate endogenous circadian rhythms which will persist in the absence of environmental variation. These circadian variations appear to represent internally timed adaptations to environmental changes which are predictably correlated with the earth's twenty-four hour day-night cycle. The independence of some circadian rhythms from daytime patterns of dietary intake, activity, and posture has been recognised for a long time, (Moore-Ede et al., 1978).

Garlick et al., (1980) measured protein synthesis and oxidation in seven obese women using continuous infusion of ^{14}C leucine for twenty-four hours; during the first 12 hours the daily food ration was given in 12 equal hourly portions and during the remaining 12 hours the subjects fasted. Protein synthesis and oxidation at night were significantly reduced compared to the daytime value. Because Golden and Waterlow (1977) had observed no change in protein metabolism over 24 hours when food was given continuously, it was concluded that the diurnal cycle observed resulted from the discontinuous intake of food. Oxidation of protein contributed more energy consumption by day than by night but such variations in the rate of protein oxidation, and hence of urea synthesis, were not reflected

in the rate of urea excretion in the urine because the large size of the urea pool smoothed out fluctuations in the rate of excretion. Such results agree with those described here, in that urea excretion is lower in the night. Presumably, because urea is distributed uniformly throughout total body water, there will not be delay in excretion once it is formed. In subjects on normal oral diet with a normal sleep pattern, diurnal rhythm could be explained by discontinuous intake of meals, reduced fluid intake at night, changes in antidiuretic hormone secretion, and possibly changes associated with sleep itself, but in the subjects described here, and of course especially those on continuous intravenous nutrition, most of these do not appertain.

Although subjects slept, they were woken at 3 hourly intervals to pass urine. Fluid restriction did not apply, and in particular the changes cannot be attributed to discontinuous intake of food. As far as can be ascertained, this diurnal rhythm in nitrogen and urea excretion in urine with constant intravenous infusion of amino acids has not been described before.

Prominent circadian rhythms in urinary water and electrolyte excretion have been known for over 100 years (Roberts, 1980). Moore-Ede et al., (1975) have shown a urinary potassium rhythm as one component of a circadian variation in potassium distribution between body compartments. During the day there is net efflux of potassium out of cells (mainly muscle and red cells) into extracellular fluid, and influx in the reverse

direction at night. Such fluxes are counterbalanced by a circadian variation in urinary potassium excretion so that fluctuations in plasma potassium are minimised. Moore-Ede et al., (1978) later compared the response of normal men to an infusion of potassium chloride at midday and midnight. Plasma potassium was 40% higher at midnight than midday. However, urinary potassium excretion was higher at midday than midnight, indicating reduced renal responsiveness to a rise in plasma potassium at midnight compared with midday. Changes in aldosterone secretion were not a major determinant of the differing renal response. Hence, circadian variations in potassium homeostasis influence the response to exogenous potassium loads. Such a circadian variation in potassium excretion, with urinary excretion being lower at midnight than at midday is similar to the findings described here of a lower urinary urea excretion in the early hours of the morning and a higher excretion at 1200-1500 hours.

Similar results were also obtained by Steffee et al., (1981) who observed a diurnal variation in urinary urea excretion in healthy young adults eating four meals daily. As in the results described here, there was a rise in excretion during the daylight hours with the nadir occurring during the early morning at about 0630 hours. With intermittent meals throughout the day it might be assumed that increased urinary urea excretion simply reflects degradation of physiologically excessive dietary protein. However, this would not explain the results with continuous intravenous amino acids.

Nor would it explain the findings of Steffee et al. who still found a diurnal rhythm at protein intakes of 0.38 and 0.1 g/Kg/d. as well as 1.5g/Kg/d. One would not expect a basic rhythm to ingested protein to persist at grossly inadequate levels of protein intake.

The data of Feigin et al, (1967) suggest that blood amino acid rhythms are relatively independent of food intake. Feeding different protein loads produced changes only in the amplitude of the oscillations but not the frequency or wave-form. Similar conclusions were reached by Wurtman et al. (1968). However, a diurnal rise in the concentration of many amino acids has been shown to occur in daytime, and these rhythms, especially that of tyrosine, have been shown to persist in subjects maintained even on an almost protein-free diet. Furthermore, hepatic degradative enzymes such as tyrosine transaminase exhibit a similar rhythm (Wurtman, 1970).

It is not unreasonable to expect an acceleration of protein turnover during daily activity. Further evidence for a circadian regulation of protein turnover has been obtained with the chair-acclimatized squirrel monkey. Negative nitrogen balance and weight loss are less when the monkeys are fed intravenously during the dark period, as compared to intravenous feeding during the light period or continuously (Finn et al/. 1982). Similarly, physically active human volunteers gained weight when they ate 2000 Kcal (8400 KJ) in the evening as compared to when they ate the same amount in the morning (Goetz et al., 1976). Apfelbaum et al. (1976) found that urinary urea excretion in adults on a low-calorie diet was greater with

"breakfast-only" or "dinner-only" schedules than when the same diet was provided in four isocaloric meals, suggesting a greater proteolytic effect from the day-only than from the night-only feeding.

Changes in urine volume during the twenty-four hours might be partly responsible for the difference between urinary urea excretion during daytime and at night. Increase in urea excretion during daytime might result partly from a rise in urine volume due to enhanced fluid intake. Steffee et al., found that this occurred, and also that, especially at night, the rate of urine flow fell at times to less than 2 ml/min; the so-called augmentation limit. In these instances an increased renal tubular reabsorption of urea and a decreased excretion might occur. However, this possibility is much less likely in our subjects receiving 3 litres daily of fluid intravenously, and who were woken three-hourly during the night to pass urine.

In conclusion, it seems that a diurnal rhythm of excretion exists, but the physiological basis for the rhythm remains unclear. There is some evidence that there is a circadian variation in protein metabolism even at a cellular and subcellular level. For example, the amount of nonhistone protein in the nucleus of liver cells of rats fed ad libitum and kept under a 12-hour light/ 12-hour dark schedule shows a rise during the dark period and a decline during the light period (Gaub, 1976). Also, a circadian rhythm of protein synthesis is found in liver and kidney slices taken from normal rats killed at various times (Pocknee and Heaton, 1978).

If the findings described here and those of Steffee et al. do represent a diurnal rhythm of protein metabolism as manifested by a change in urinary urea excretion, there may be implications for clinical nutrition in terms of cyclical parenteral nutrition to optimise anabolism.

JF - Urinary Total N.g.

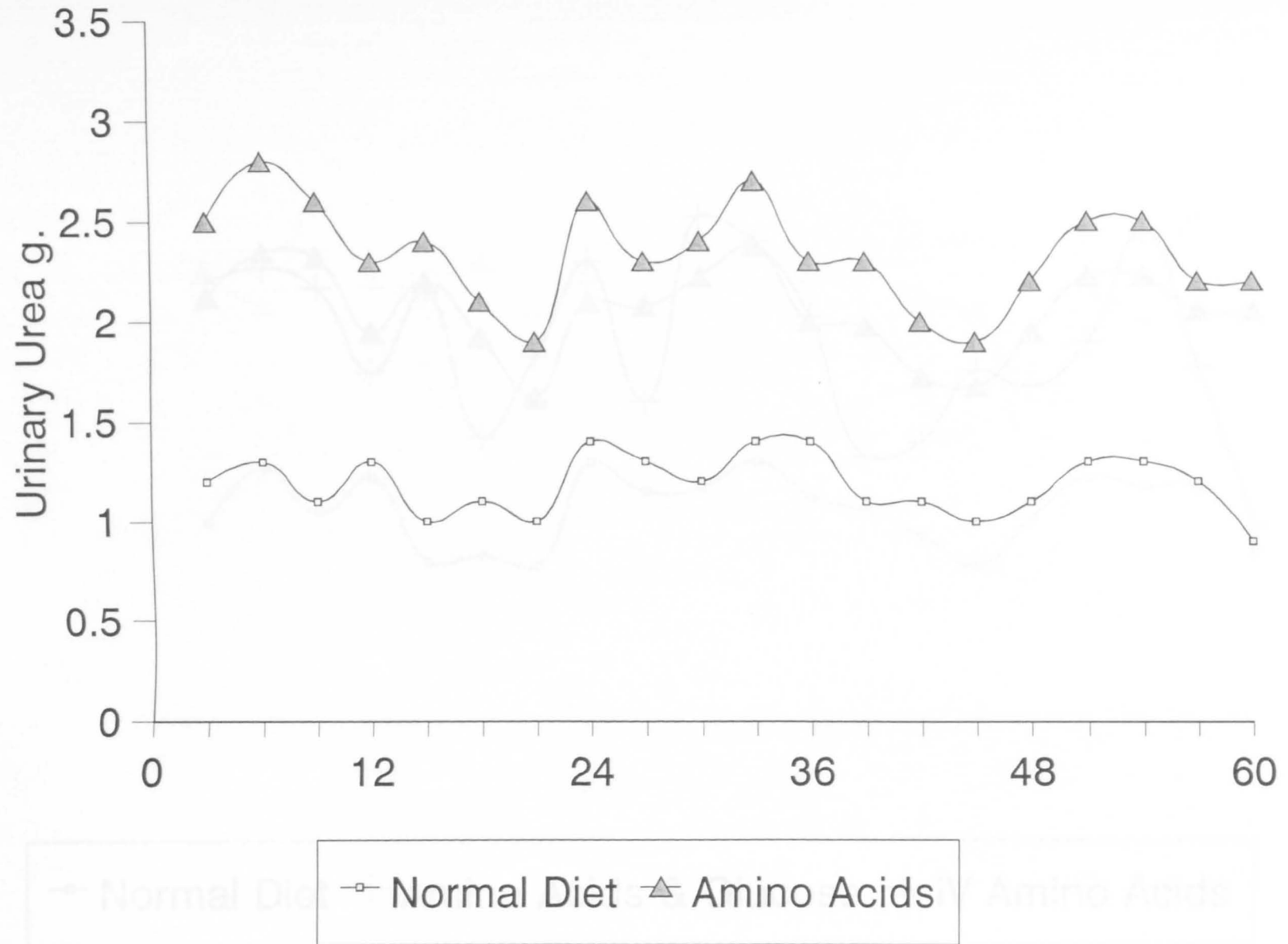


FIGURE XVI

JF - Urinary Urea g

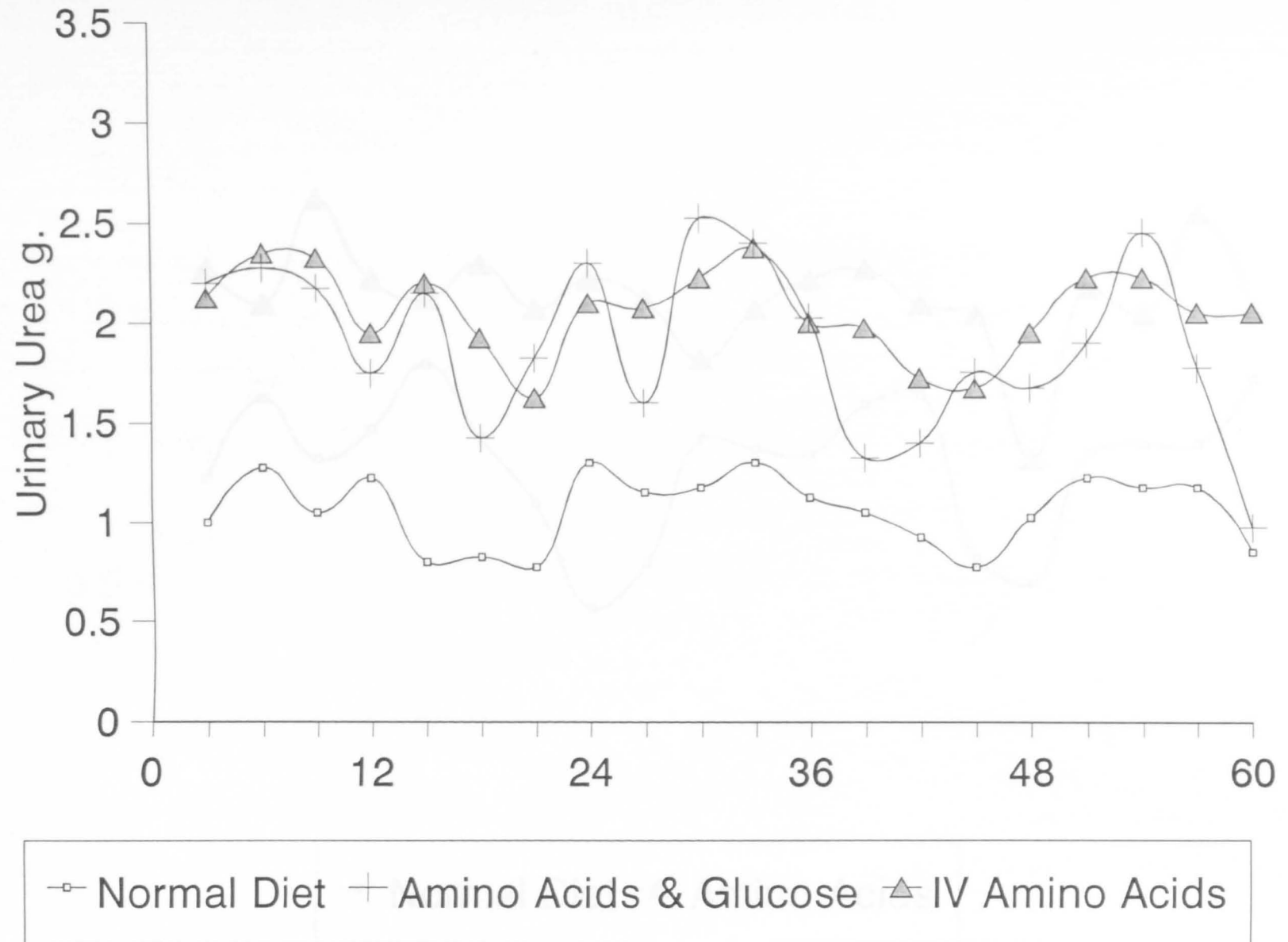


FIGURE XVII

JG - Urinary Urea

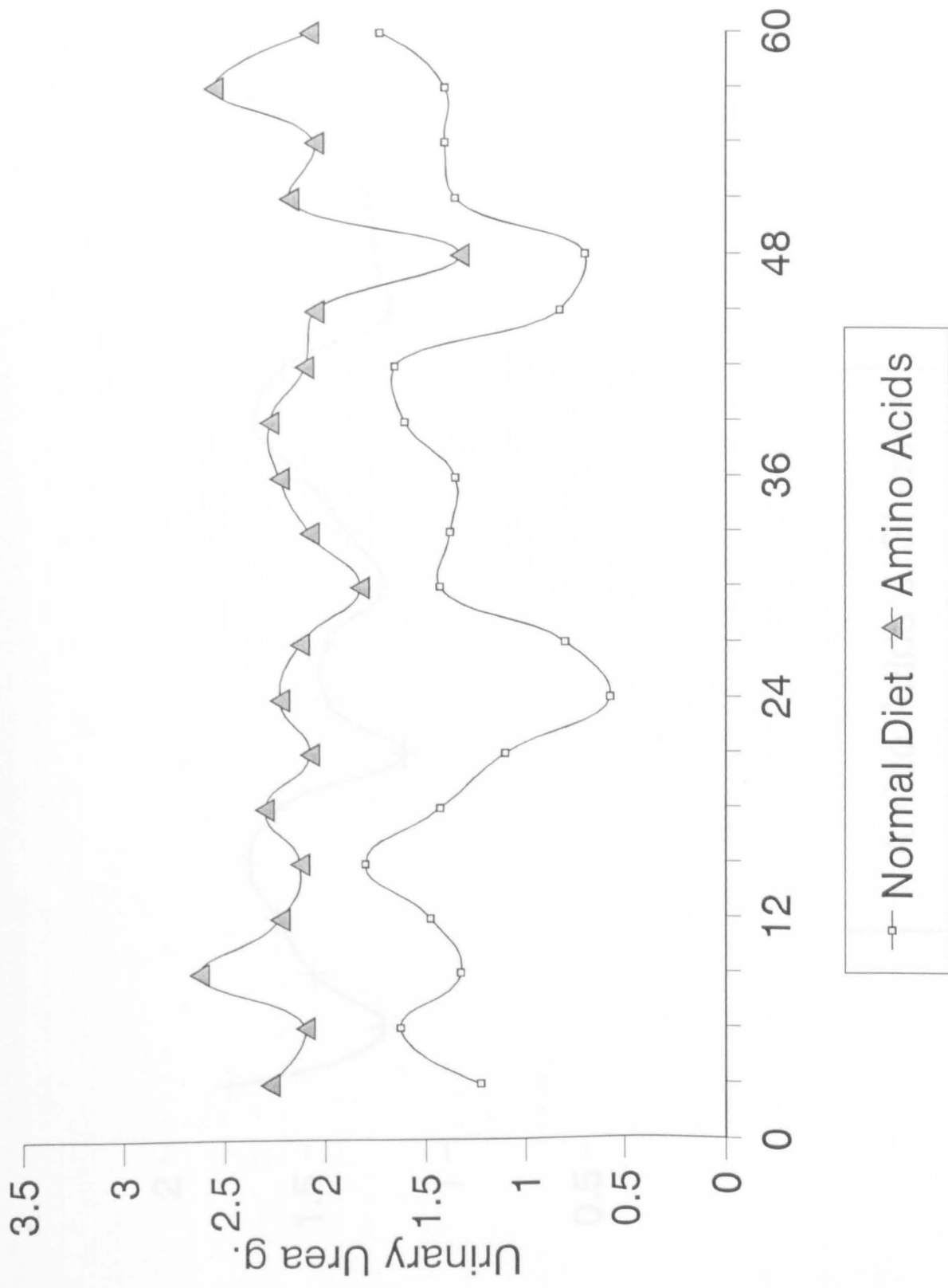


FIGURE XVIII

JG - Urinary Urea

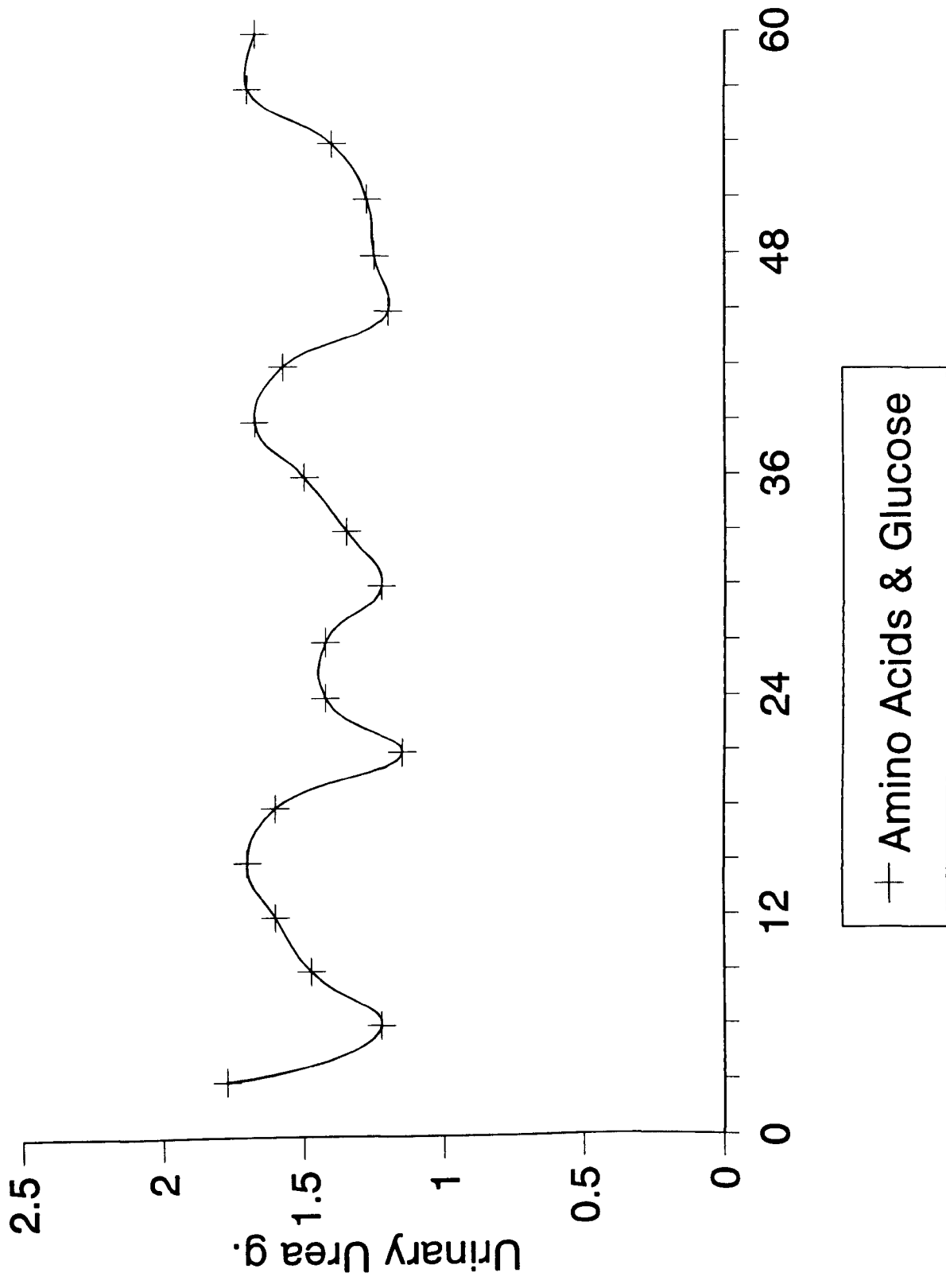


FIGURE XIX

WW - Urinary Urea

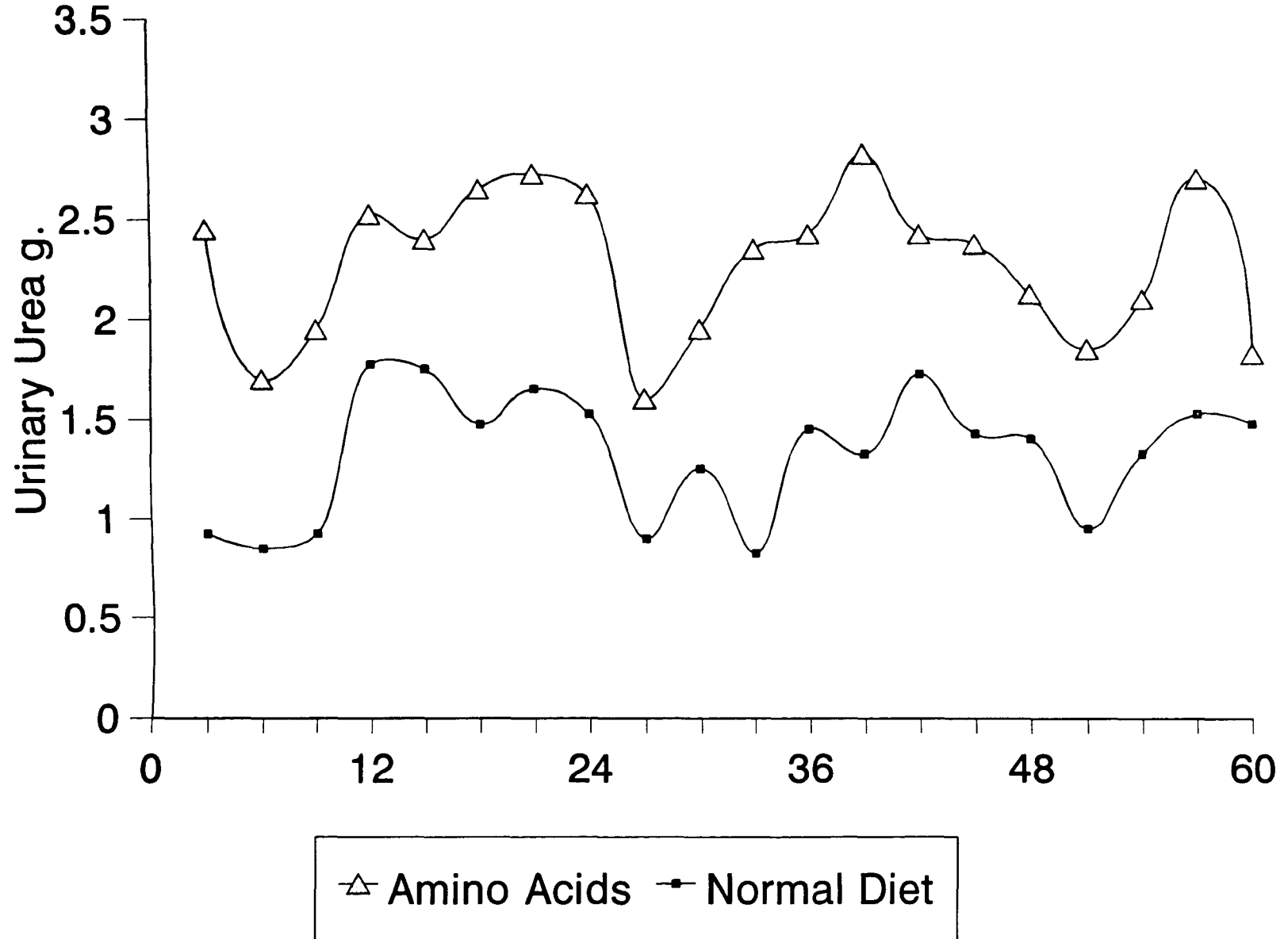


FIGURE XX

WW - Urinary Urea

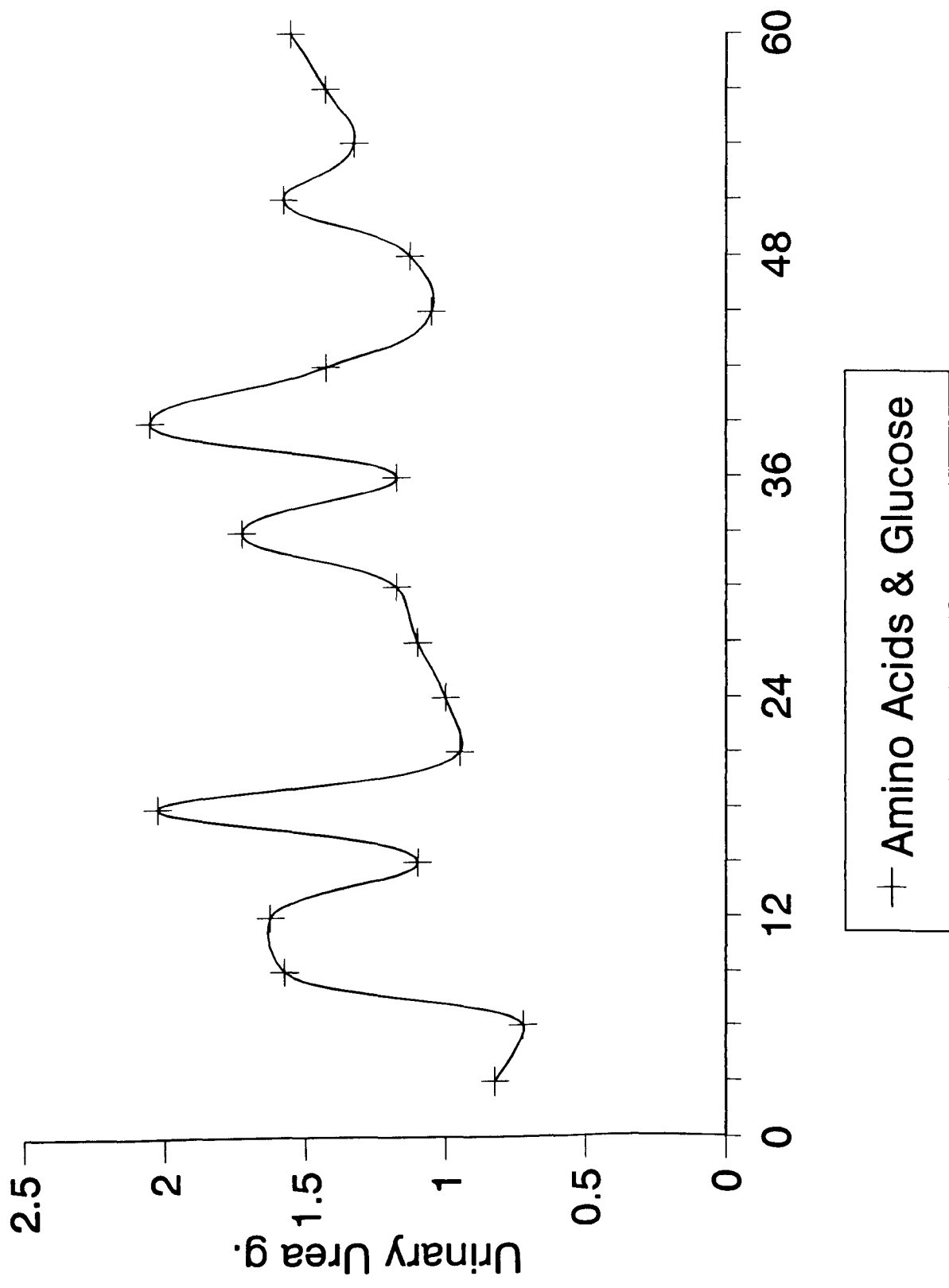


FIGURE XXI

DC - Urinary Urea

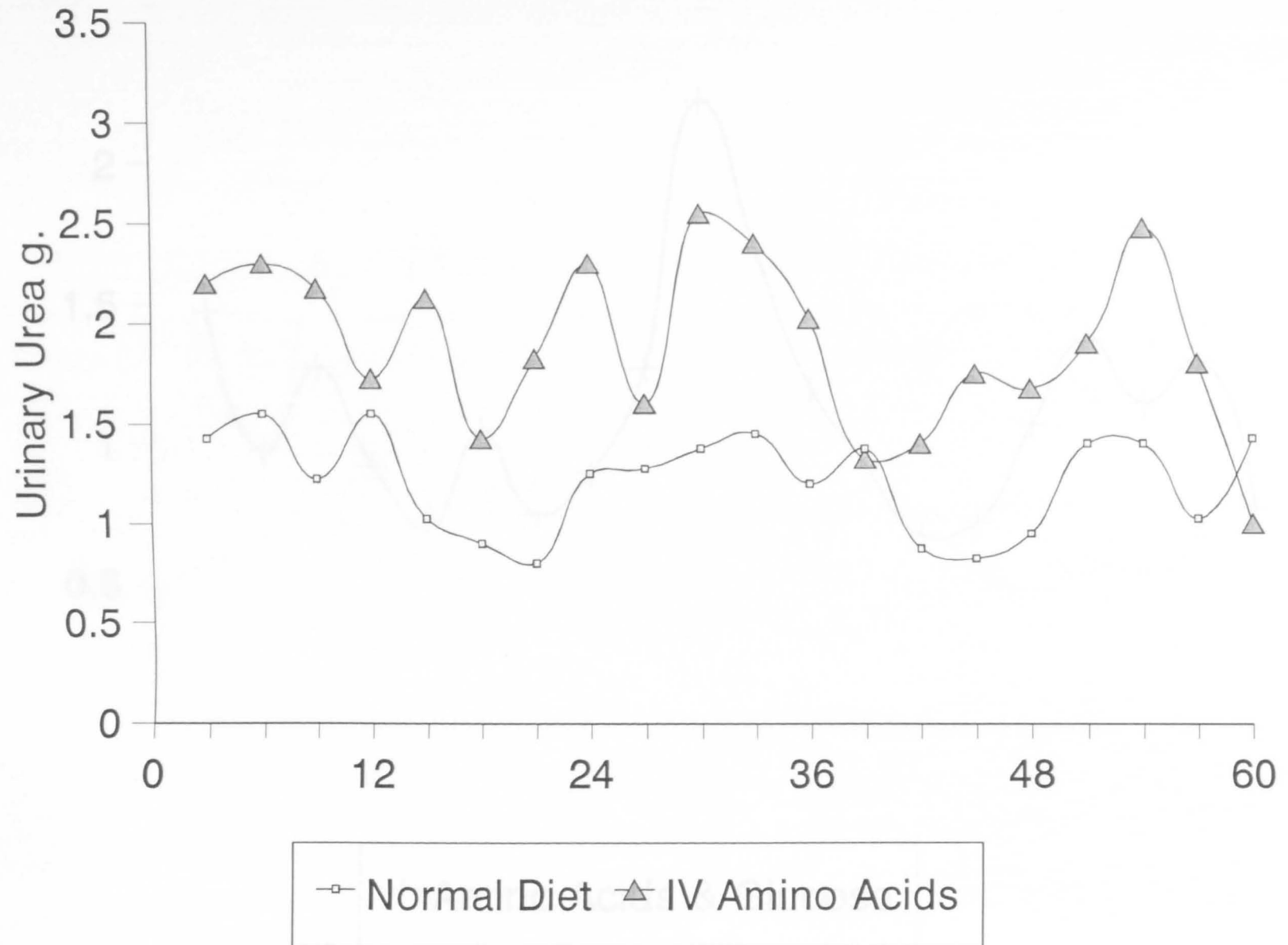


FIGURE XXII

DC - Urinary Urea

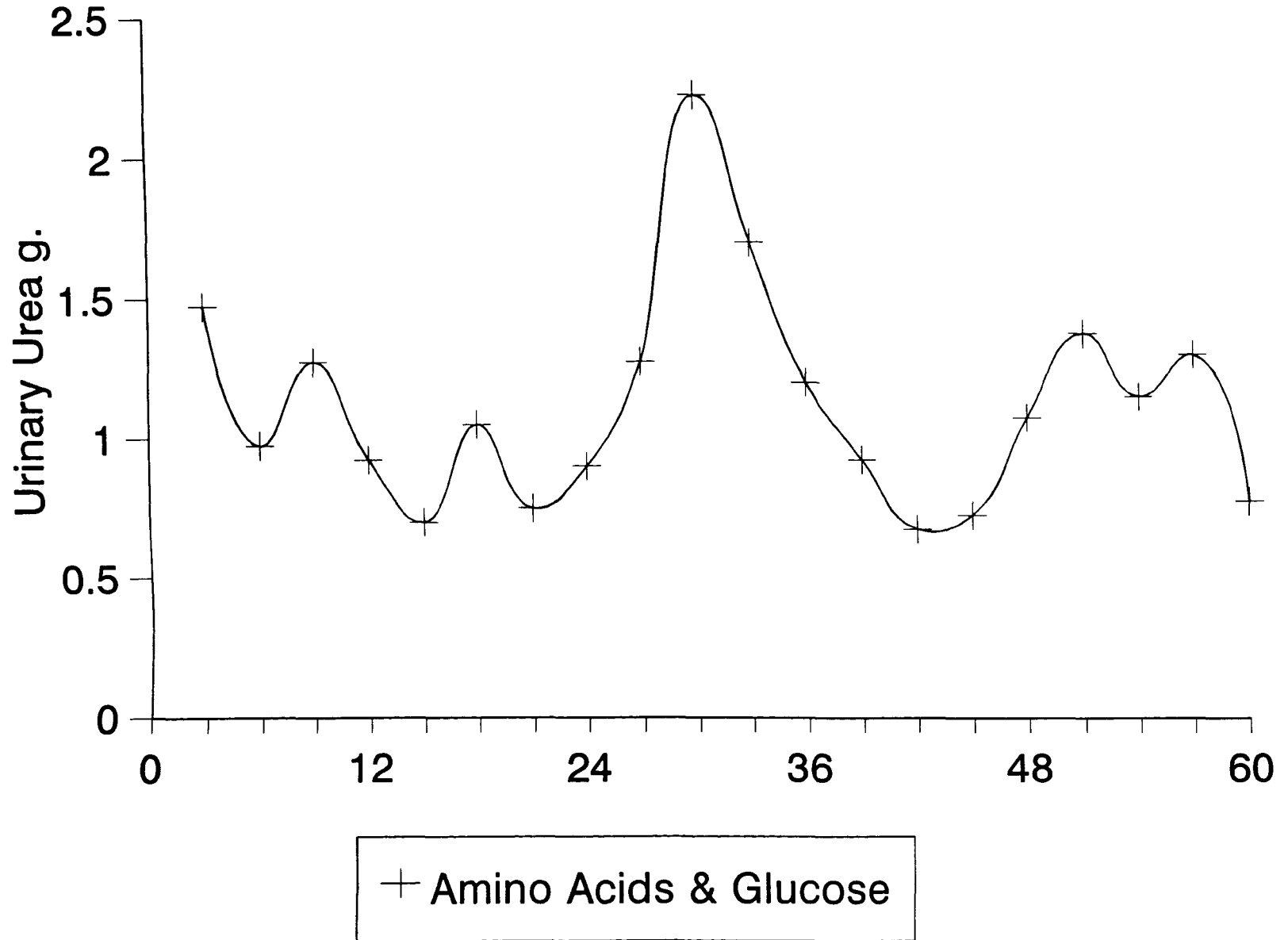


FIGURE XXIII

HK - Urinary Urea

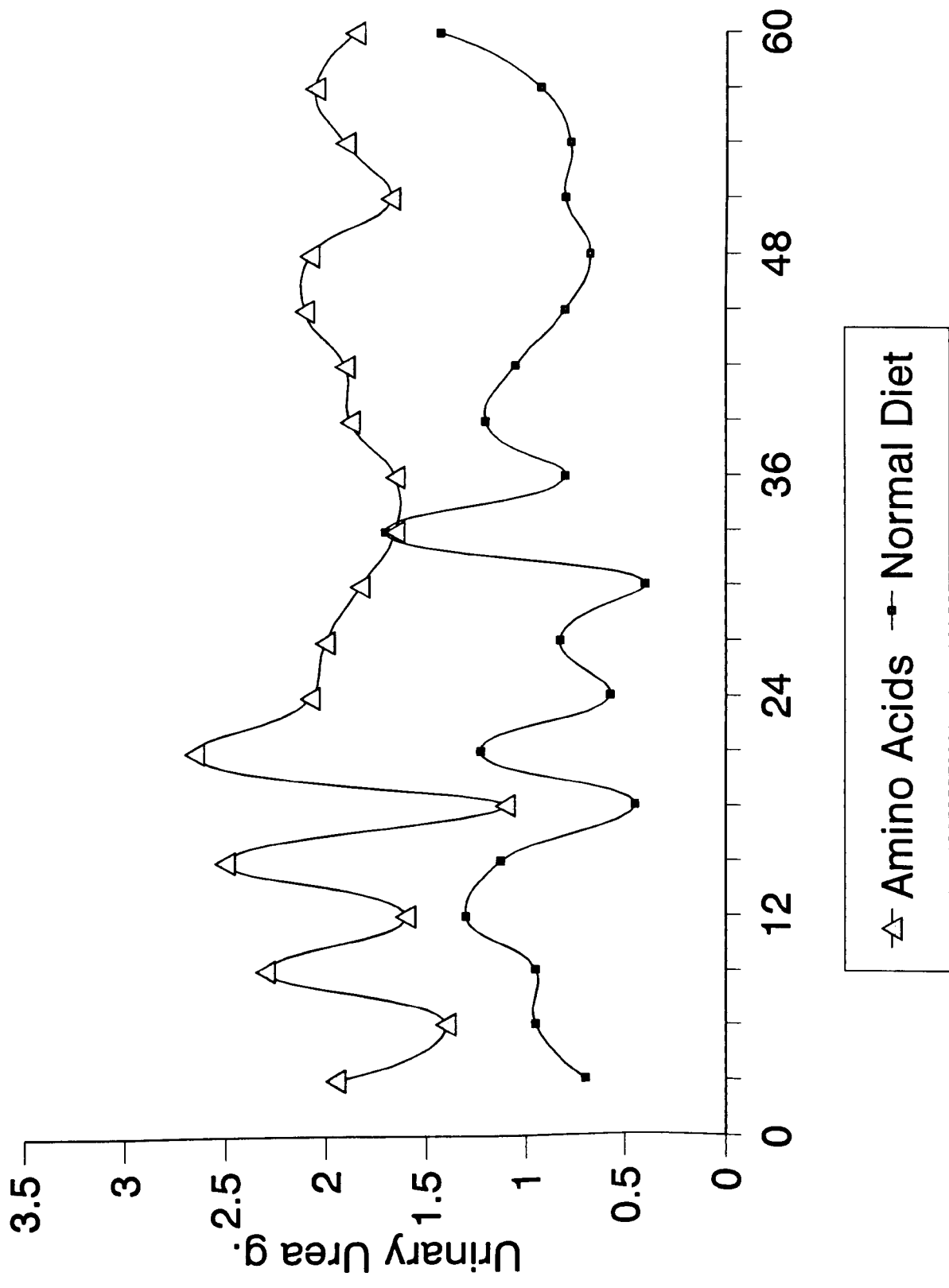


FIGURE XXIV

Means of JF, JG, WW, DC, HK

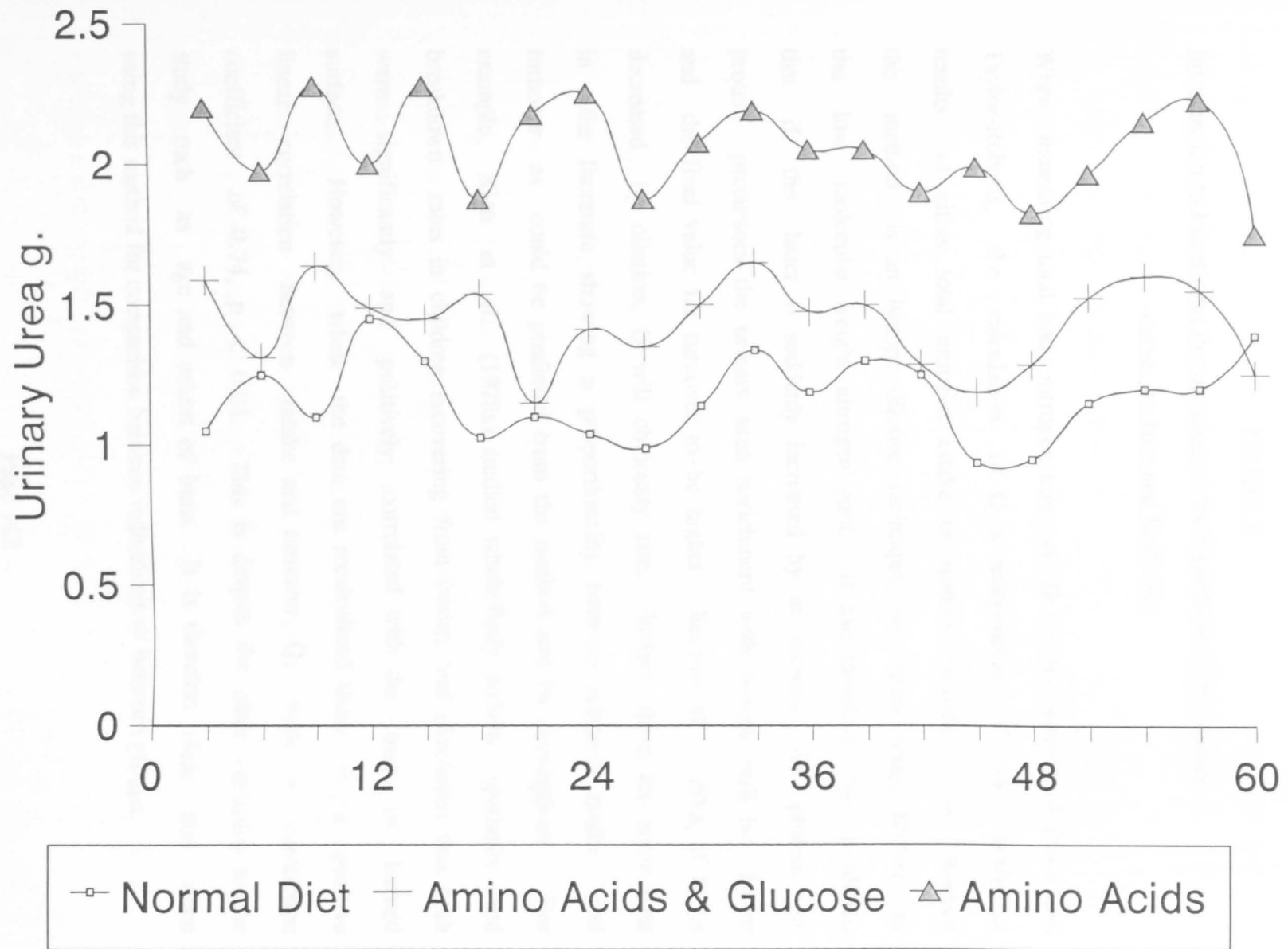


FIGURE XXV

STUDY II

Introduction to Intentional Perturbation of the Equilibrated ¹⁵N Glycine curve, by Diet and Exercise.

When measuring total body nitrogen turnover, Q , by the method of Picou and Taylor-Roberts, the calculation of Q is independent of the analytical results of either total nitrogen intake or urinary excretion. In essence the method is an isotope dilution technique, the tracer being diluted in the low molecular weight nitrogen pool. It can therefore be predicted that if the latter is suddenly increased by an increase in protein or protein precursors the urinary urea enrichment with isotope will be lower and the final value for turnover to be higher. Because $Q = d/S_a$, if S_a is decreased by dilution, Q will obviously rise. Indeed, there are some data in the literature showing a proportionality between nitrogen intake and turnover as could be predicted from the method and its assumptions. For example, Kien et al. (1978a) studied whole-body protein synthesis and breakdown rates in children recovering from burns, and concluded that both were significantly and positively correlated with the area of burned surface. However, when the data are recalculated there is a positive linear correlation between intake and turnover, Q , with a correlation coefficient of 0.74, $p < 0.01$. This is despite the other variables in the study such as age and extent of burn. It is therefore clear that when using this method for comparison between individuals or between groups,

nitrogen intake should remain comparable. If it is not constant, then the interpretation of results must include consideration of the fact that the intake alteration may have biased the change in Q.

In order to explore such relationships further, an initial study was conducted to intentionally perturb the equilibrated ^{15}N glycine curve.

i) Methods

Two young adult male volunteers, RF and CM, were studied, each subject on two occasions. Each study lasted 96 hours, after a preliminary stabilisation on the baseline diet for 48 hours. The baseline diet consisted of four meals, unequal in protein content, but in toto providing 1 g protein/Kg body weight and 35 Kcal (147 KJ) per Kg body weight daily. The meals were supplied as breakfast at 9am, lunch at 1pm, dinner at 5pm, and supper at 9pm. 48 hours into the study ^{15}N glycine solution was given by mouth every 3 hours to provide approximately 0.5 mg/Kg/d. On the second and fourth days of the study the total protein intake was doubled to provide 2g/Kg/d., and this was achieved by keeping the breakfast and supper meals constant but increasing the lunch and dinner contents. On the morning of the third study day each subject exercised on a bicycle ergometer for 30 minutes at 450 kilopond metres. This succeeded in elevating the pulse rate to c.150/min. in RF and c.125/min. in CM, taking approximately twenty minutes to return to normal.

ii) Results

The results of each study in terms of nitrogen intake, urinary nitrogen excretion and nitrogen balance are shown in Table 15.

RESULTS

R.F Aged 38 Study I

	Date	URINE Ng	INTAKE g	BALANCE g	Wt Kg	
Feb	5 - 6	8.8	11	2.2	61.2	} \bar{x} 59.5
	6 - 7	9.3	11	1.7	59.4	
	7 - 8	10.8	11	0.2	59.4	
	8 - 9	14.6	21.7	7.1	59.4	
	9 - 10	11.5	11	-0.5	59.7	
	10 - 11	15.4	21.7	6.3	59.6	

R.F Study II

Feb	19 - 20	8.4	10.9	2.5	61.2	} \bar{x} 59.5
	20 - 21	9.4	10.9	1.5	60.2	
	21 - 22	10.1	10.9	0.8	59.9	
	22 - 23	13.7	22	8.3	59.4	
	23 - 24	10.4	10.9	0.5	59.4	
	24 - 25	13.2	22	8.8	59.5	

C.M Aged 30 Study I

Mar	2 - 3	7.8	10.85	3.05	67.3	} \bar{x} 67.0
	3 - 4	10.2	10.85	0.65	67.1	
	4 - 5	8.5	10.85	2.35	—	
	5 - 6	13	21.8	8.8	67	
	6 - 7	11.2	10.85	-0.35	67	
	7 - 8	14.2	21.8	7.6	67	

C.M. Study II

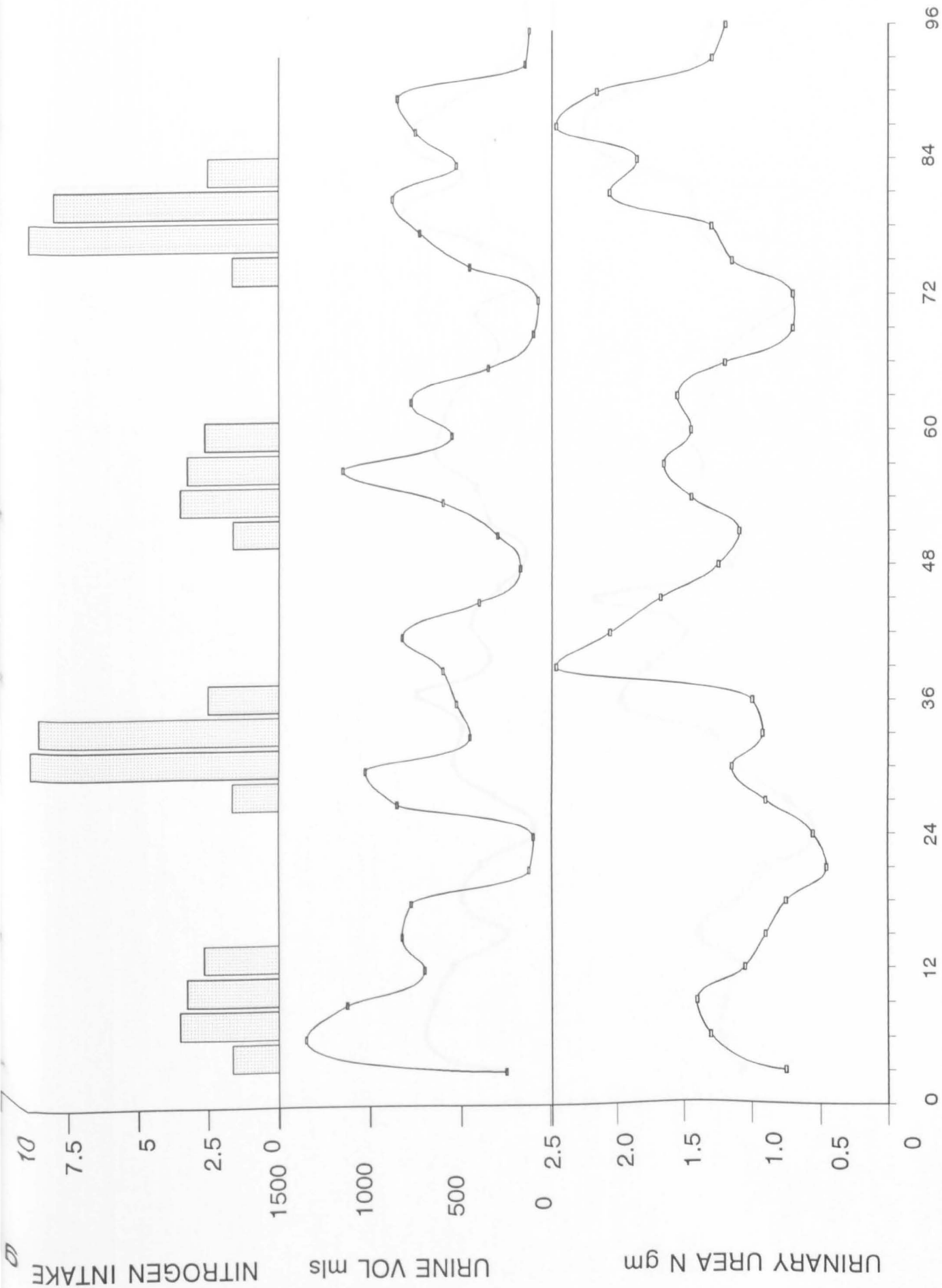
Mar	18 - 19	10	10.85	0.05	68.2	} \bar{x} 66.75
	19 - 20	10.05	10.85	0.35	67.2	
	20 - 21	9.9	10.85	0.95	66.8	
	21 - 22	24.2	21.8	7.7	66.8	
	22 - 23	22.8	10.85	-0.95	66.9	
	23 - 24	14.2	21.8	7.6	66.5	

Table 15

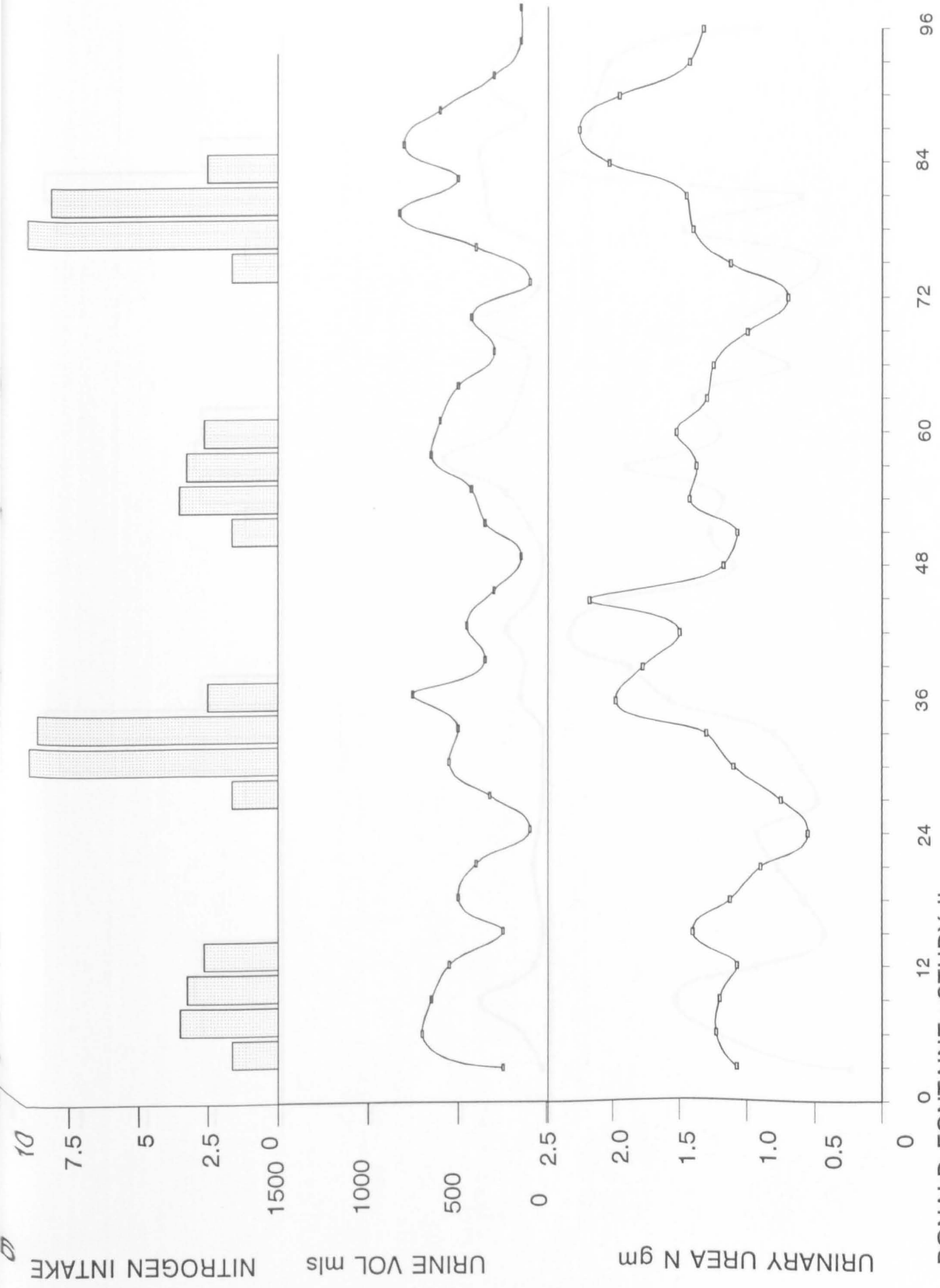
Results are plotted graphically to demonstrate the relationships between nitrogen intake, 3-hourly urine volumes and 3-hourly urinary urea excretion (Figures XXVI to XXIX), and the relationship between nitrogen intake and the curve ascending to plateau of enrichment of urinary urea with ^{15}N (Figures XXX to XXXIII).

It can be seen that on the baseline diet there is nitrogen equilibrium, the slight positive balance being accounted for by losses in faeces and skin. A temporary positive balance occurs on doubling of protein intake, even though this is accompanied by increased urinary excretion of nitrogen. There is a lag period of about 9 hours between doubling of protein intake and maximal level of excretion of urinary nitrogen. There is an obvious diurnal variation in urinary excretion as has been described previously, being lowest in the early hours of the morning (midnight to 8am) and highest in the mid to late afternoon on the baseline diet, with a subsequent lag period extending to 11pm - 5am when the protein intake is doubled, and which influences the urinary excretion into the following day by causing a slightly higher level than baseline. Whereas this diurnal variation can be explained mainly by the unequal distribution of meals when compared with continuous feeding, it bears a very close relationship to urine volumes.

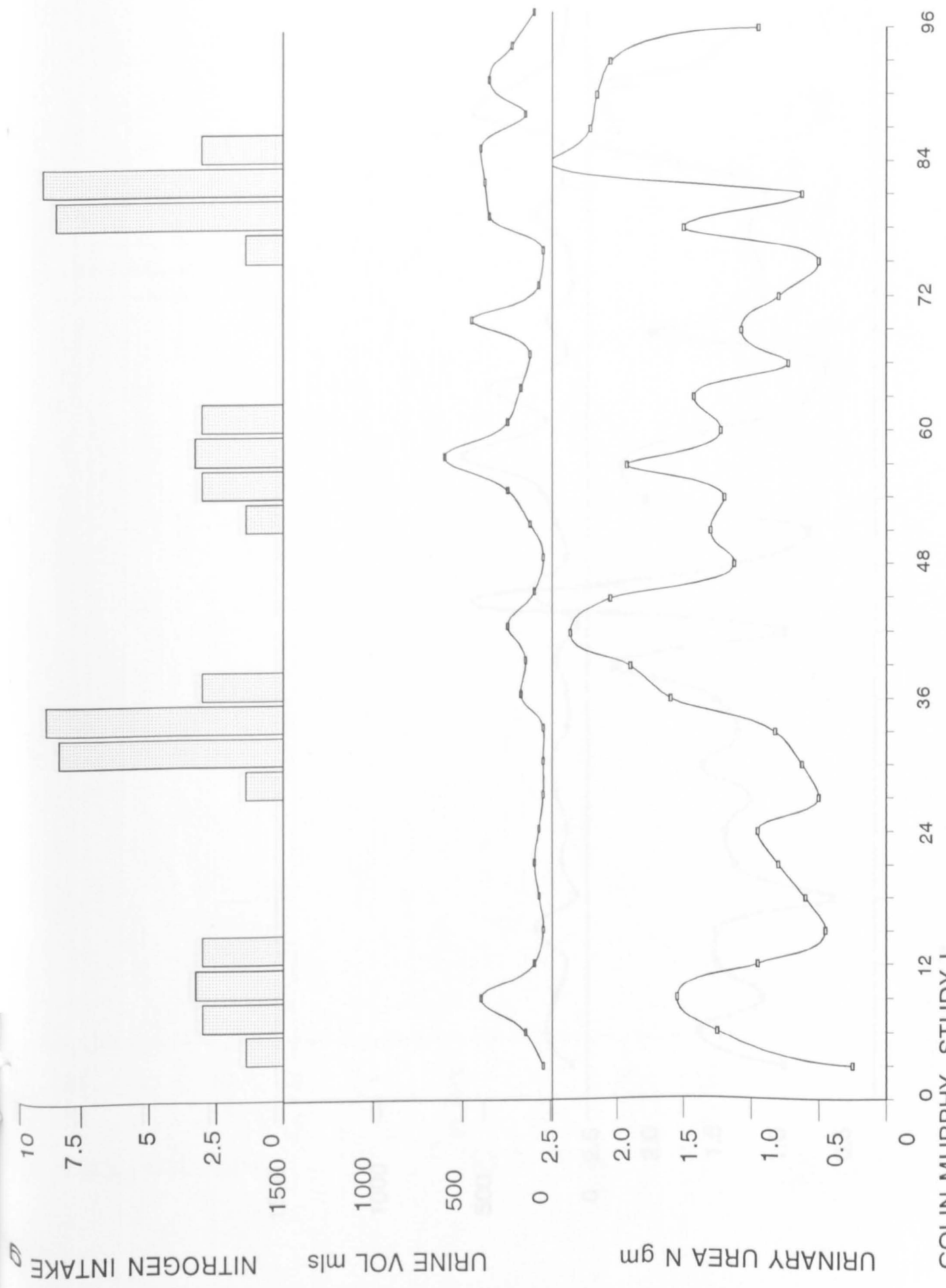
Attention to the graphical display of nitrogen intake and the ^{15}N glycine equilibration curves (Figures XXX to XXXIII) shows that the rise in enrichment on baseline diet is suppressed almost immediately following doubling of nitrogen intake and this persists for about 12 hours before the



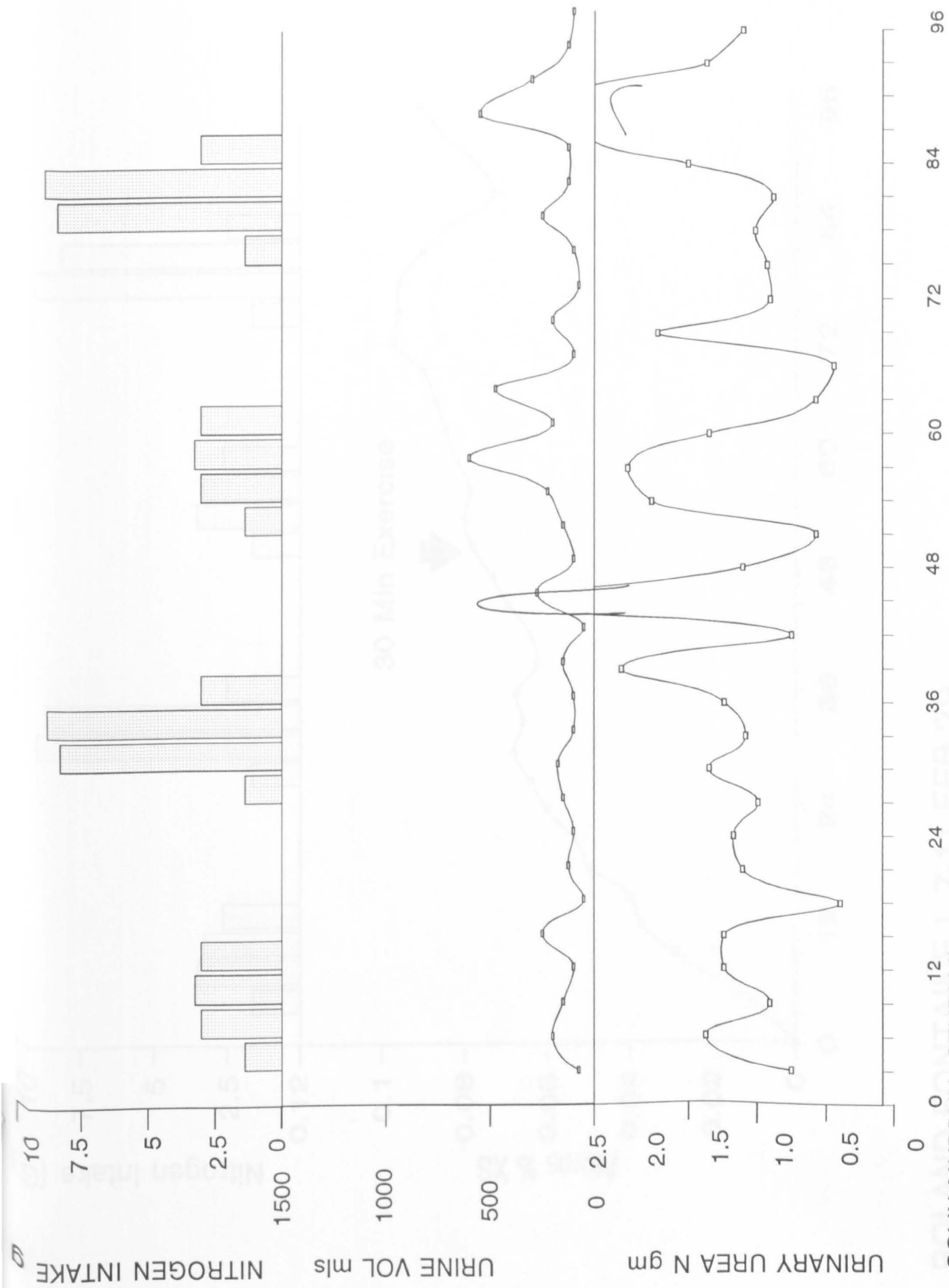
RONALD FONTAINE STUDY I
FIGURE XXVI



RONALD FONTAINE STUDY II
FIGURE XXVII

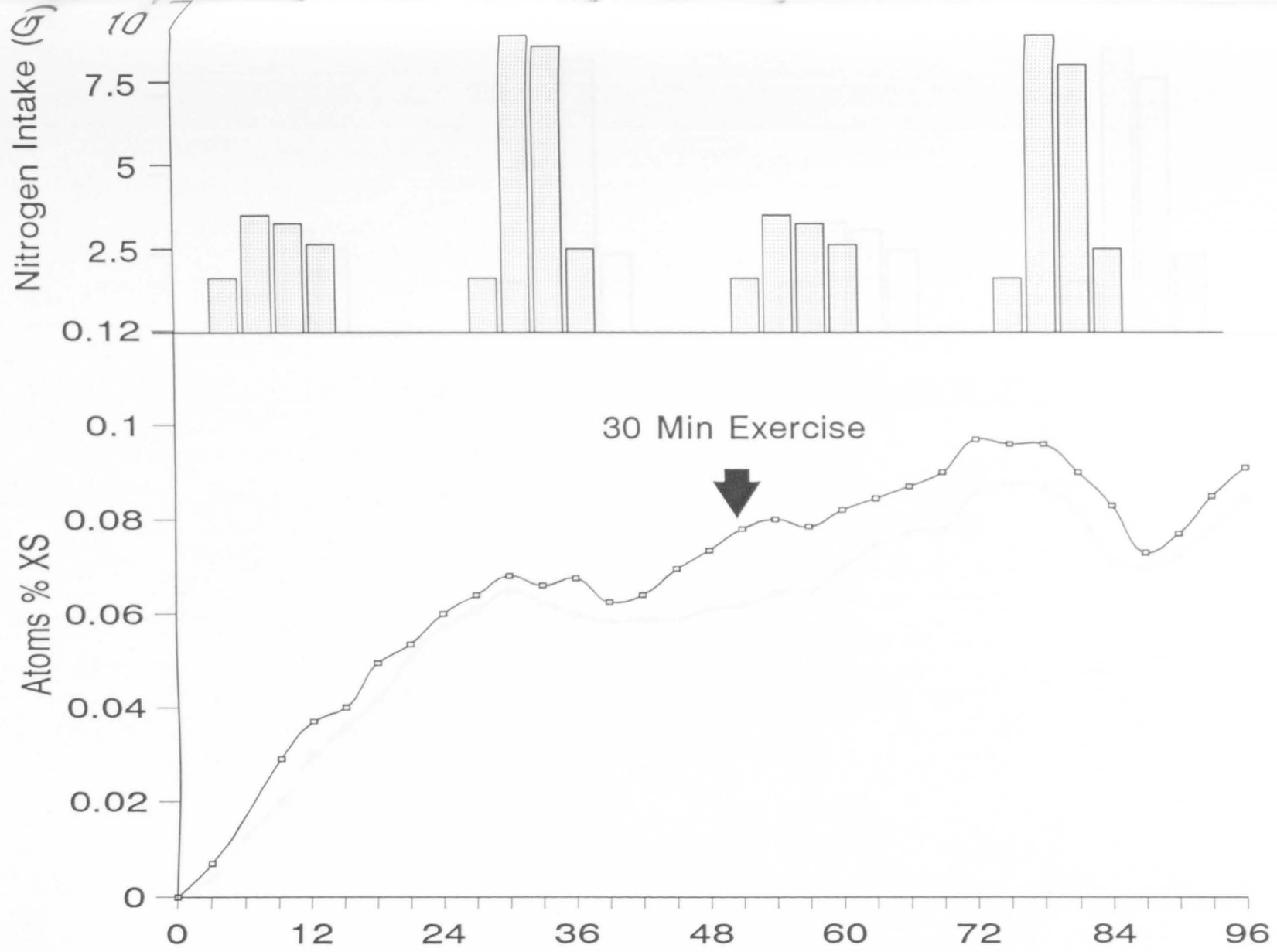


COLIN MURPHY STUDY I
FIGURE XXVIII



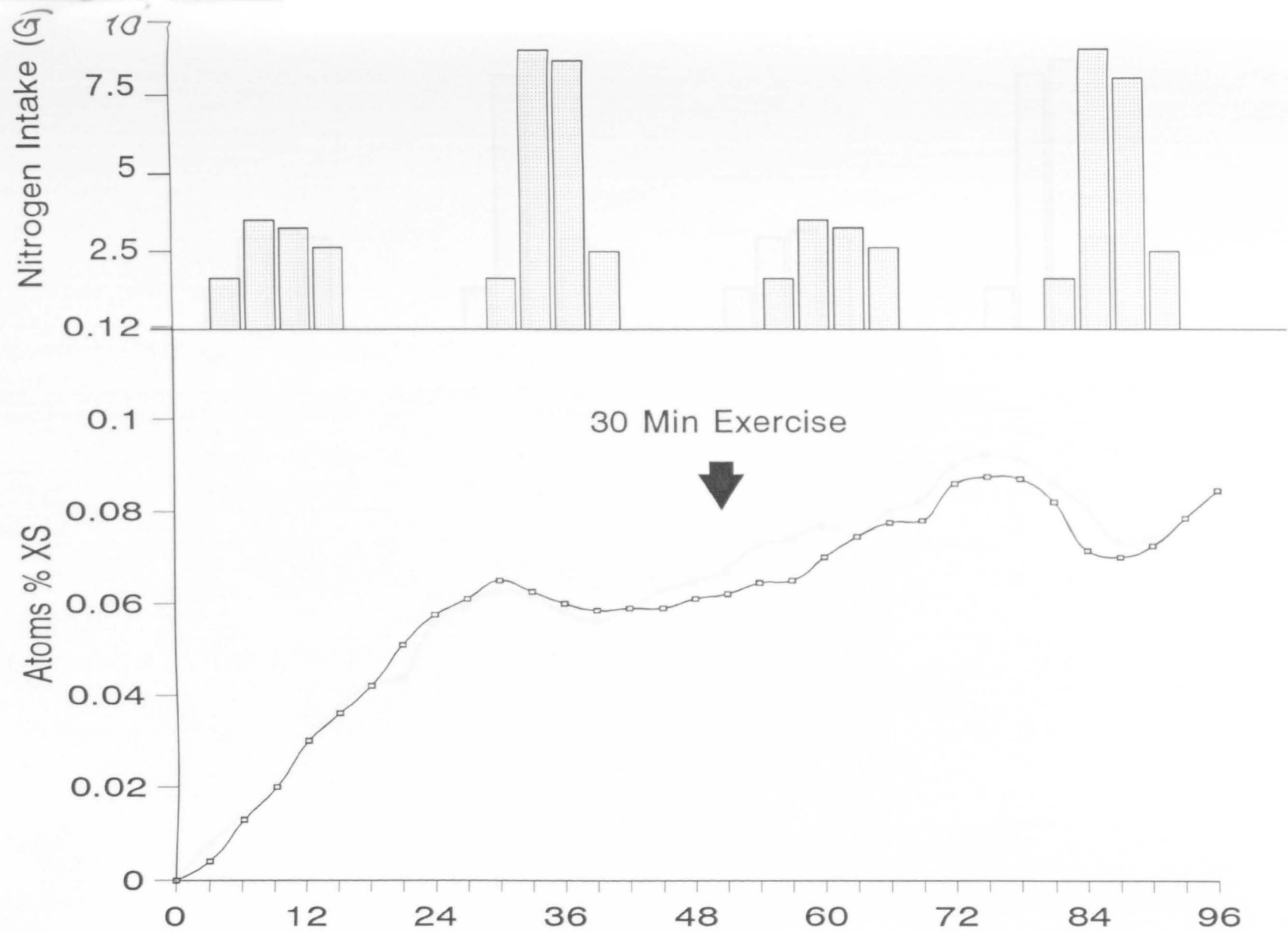
COLIN MURPHY STUDY II

FIGURE XXIX

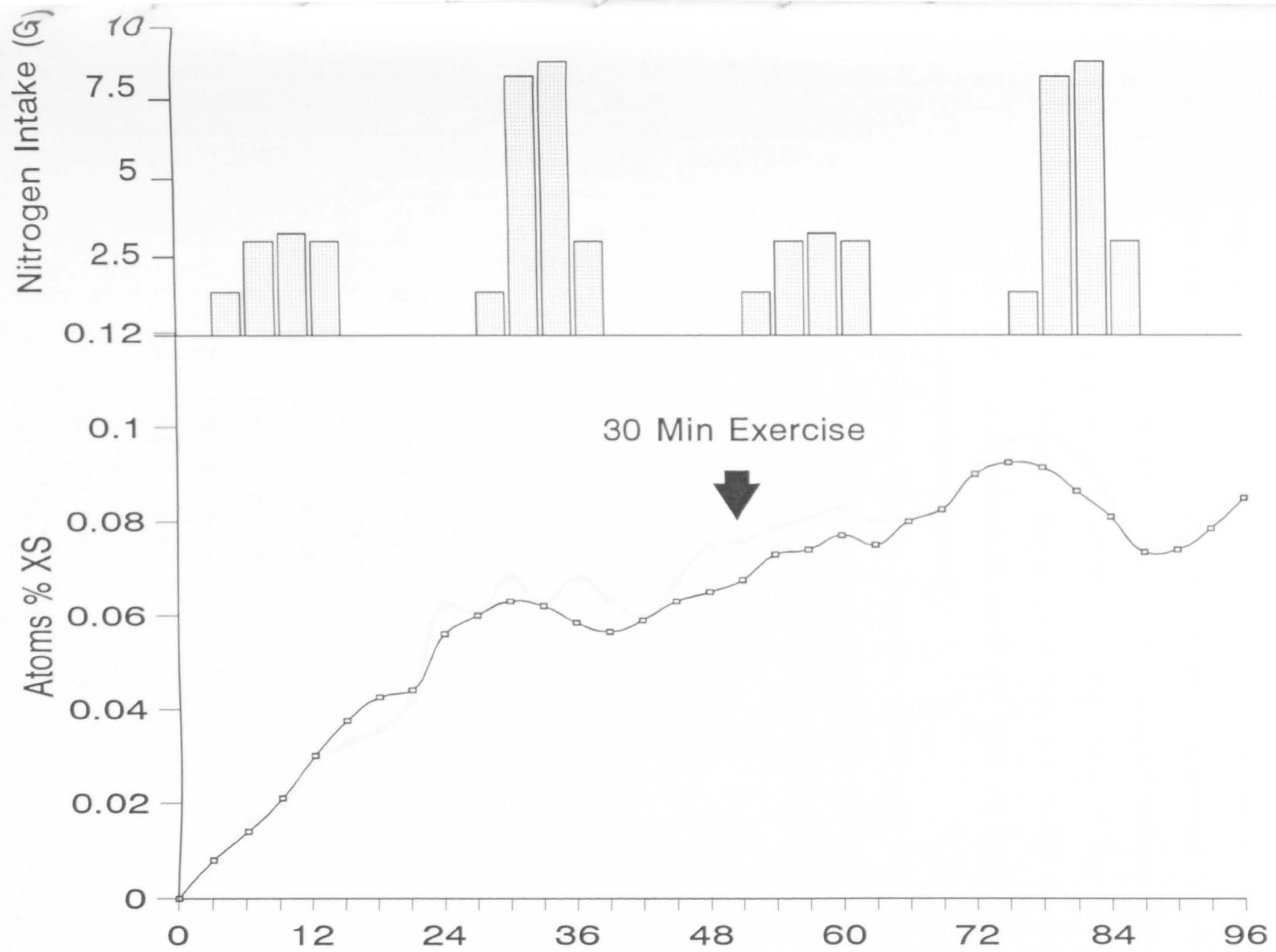


ROLAND FONTAINE I 7-11 FEB '79

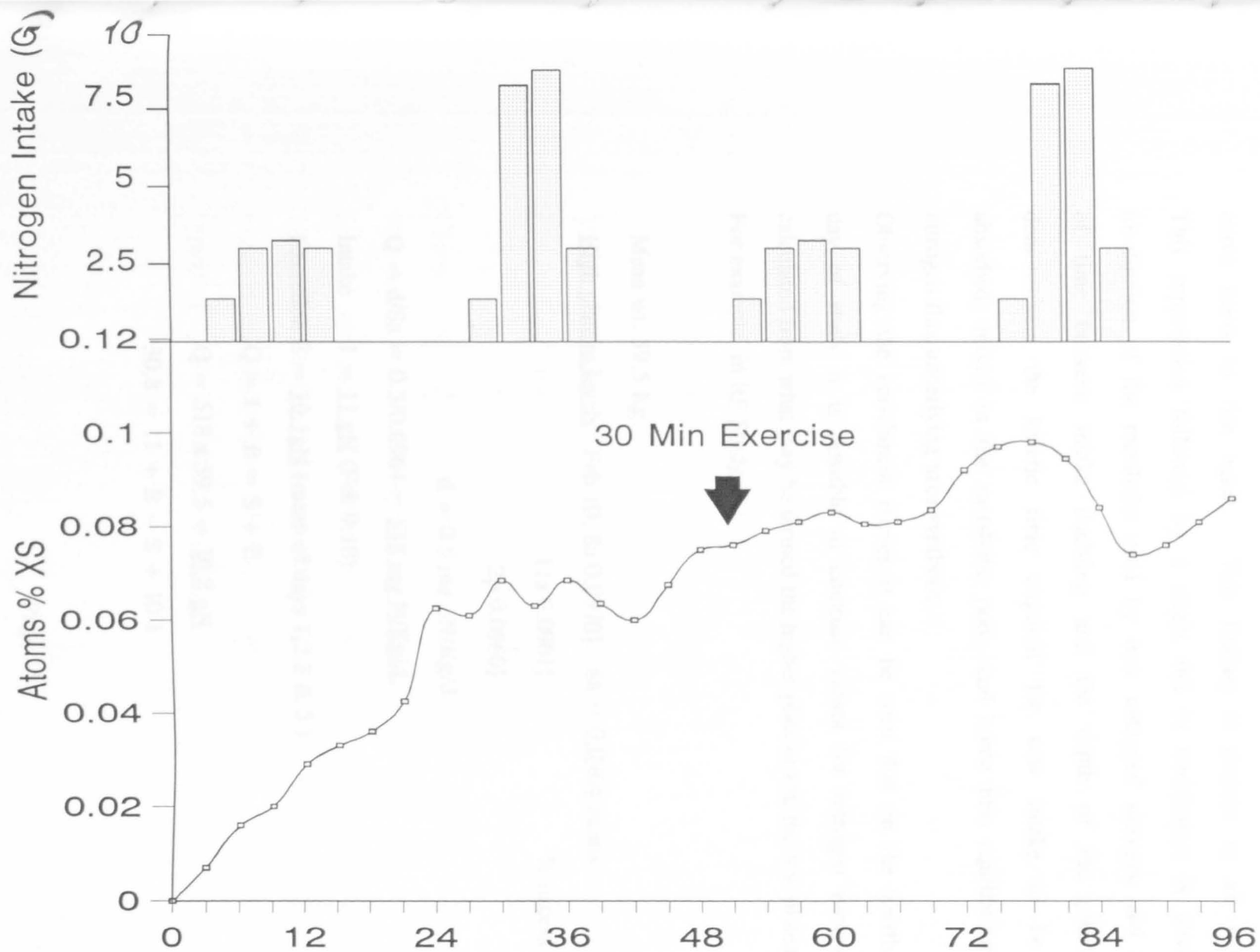
FIGURE XXX



ROLAND FONTAINE II 21-25 FEB '79
FIGURE XXXI



COLIN MURPHY I 4-8 MAR '79 79
FIGURE XXXII



COLIN MURPHY II 20-24 MAR '79
FIGURE XXXIII

curve starts to rise again. This finding is present in all four studies. This suppression followed by a slight fall in enrichment is obviously due to dilution of the metabolic pool by new untagged nitrogen and the length of time between intake doubling and the depth of the plateau change demonstrates the kinetic time required for new intake to be digested, absorbed, mixed in the metabolic pool, and come into equilibrium with the nitrogen flux underlying urea synthesis.

Observing the enrichment curves it can be seen that on the fourth and final day of study it is possible to compare values for nitrogen turnover, Q, as calculated from what may be termed the higher plateau and the low plateau.

For example, in RF Study 1:-

Mean wt. 59.5 Kg.

High plateau levels Feb 10, 8a 0.0970} Sa = 0.0964 atoms

11a 0.0961} % excess

2p 0.0960}

d = 0.5 mg ¹⁵N/Kg/d.

Q = d/Sa = 0.5/0.0964 = 518 mg N/Kg/d.

Intake I = 11 gN (Feb 9-10)

Excretion E = 10.1gN (mean of days 1,2,3 & 5)

$$Q = I + B = S + E$$

$$Q = 518 \times 59.5 = \underline{30.8 \text{ gN}}$$

$$30.8 = 11 + B = S + 10.1$$

$$\dots B = 19.8\text{gN} \equiv \underline{2.08 \text{ g protein/Kg/d.}}$$

$$\& S = 20.7\text{gN} \equiv \underline{2.17 \text{ g protein/Kg/d.}}$$

Similarly, with the low plateau

$$\text{Feb 10 11 p 0.0732 } \} \text{ 0.0753}$$

$$\text{11 2A 0.0773 } \}$$

$$Q = d/SA = 0.5/0.0753 = \underline{664 \text{ mg N/Kg/d.}}$$

$$\text{Intake I on doubling} = \underline{21.7 \text{ gN}}$$

$$\text{Excretion E (Feb 10-11)} = \underline{15.4 \text{ gN}}$$

$$Q = I + B = S + E$$

$$Q = 664 \times 59.5 = \underline{39.5 \text{ gN}}$$

$$39.5 = 21.7 + B = S + 15.4$$

$$\dots B = 17.8 \equiv \underline{1.87 \text{ g protein/Kg/d.}}$$

$$\text{and } S = 24.1 \equiv \underline{2.54 \text{ g protein/Kg/d.}}$$

The change in nitrogen intake I = 10.7 gN (21.7 - 11) and the change in

$$Q = 146 \text{ mgN} (664 - 518)$$

$$= \underline{8.7 \text{ gN}}$$

$$\text{Change in nitrogen excretion } E = \underline{5.3 \text{ gN}} (15.4 - 10.1)$$

Subtracting the change in urinary nitrogen output (5.3 gN) from the change in total nitrogen intake (10.7 gN), the figure of 5.4 gN is obtained; this can be called "the change in amount of available small molecular weight nitrogen in the body, after the diet change", or simply termed the "apparent metabolic pool size".

Similarly, in RF Study II Wt. 59.5 Kg.

High plateau Feb 24 8A 0.0861 }

11A 0.0873 } sa = 0.0868

2P 0.0871 }

$$Q = 0.5/0.0868 = \underline{576 \text{ mgN/Kg/d}}$$

Basal I = 10.9, E + 9.7 (mean of 8.4, 9.4, 10.1,10.4)

$$Q = 576 \times 59.5 = 34.3 \text{ gN.}$$

$$34.3 = 10.9 + B = S + 9.7.$$

$$B = 23.4 \equiv \underline{2.46 \text{ g protein/Kg/d.}}$$

$$\text{and } S = 24.6 \equiv \underline{2.58 \text{ g protein/Kg/d.}}$$

Low plateau Feb 24 8P 0.0718 }

11P 0.0703 } Sa = 0.0715

Feb 25 2A 0.0725 }

$$Q = 0.5/0.0715 = \underline{699 \text{ mgN/Kg/d.}}$$

Double intake I = 22, E = 13.2 (Feb 24-25)

$$41.6 = 22 + B = S + 13.2$$

$$B = 19.6 \text{ gN} \equiv \underline{2.06 \text{ g protein /Kg/d.}}$$

$$\text{and } S = 28.4 \text{ gN} \equiv \underline{2.98 \text{ g protein/Kg/d.}}$$

Treating the studies on subject CM in exactly the same fashion, but cutting out the intermediary steps, the following applies:-

CM Study I Wt 67 Kg.

High plateau Mar 7 8A 0.0903 }

11A 0.0922 } Sa 0.0912

2P 0.0913 }

$$Q = \underline{548} \text{ mgN/Kg/d.}$$

$$I = 10.85, E = 9.42 \text{ (mean of 7.8, 10.2, 8.5, 11.2)}$$

$$B = \underline{2.41} \text{ g protein/Kg/d.}$$

$$\text{and } S = \underline{2.54} \text{ g protein/Kg/d.}$$

Low plateau Mar 7 11P 0.0732}

8 2A 0.0739} 0.0736

$$Q = \underline{679} \text{ mg N/Kg/d.}$$

$$I = 21.8, E = 14.2 \text{ (Mar 7-8)}$$

$$B = \underline{2.21} \text{ g protein/Kg/d.}$$

$$\text{and } S = \underline{2.92} \text{ g protein/Kg/d.}$$

CM Study II Wt 66.75 Kg.

High plateau Mar 23 11A 0.0971}

2P 0.0982} Sa 0.0965

5P 0.0943}

$$Q = \underline{518} \text{ mg N/Kg/d.}$$

$$I = 10.85, E = 10.55 \text{ (mean of 10.0, 10.5, 9.9, 11.8).}$$

$$B = \underline{2.22} \text{ g protein/Kg/d.}$$

$$\text{and } S = \underline{2.25} \text{ g protein/Kg/d.}$$

Low plateau Mar 23 11P 0.0739}

24 2A 0.0760} Sa 0.0750

$$Q = \underline{667} \text{ mg N/Kg/d.}$$

$$I = 21.8, E = 14.2 \text{ (Mar 23-24).}$$

$$B = \underline{2.12} \text{ g protein/Kg/d.}$$

$$\text{and } S = \underline{2.84} \text{ g protein/Kg/d.}$$

iii) Discussion

Firstly, the individual variation between studies ranges from 10-18% in RF and 3-13% in CM with respect to synthesis and breakdown, and 2-11% with respect to flux. These results resemble those of Fern, Garlick, Sheppard and Fern (1984), although there is little other information about reproducibility in the same individuals. In all four studies, doubling of nitrogen intake creates a small fall in protein breakdown and a larger rise in synthesis (cf. results in Study III).

Any effect of the exercise on the ^{15}N urinary urea enrichment curve was minimal, but in three out of four experiments there appeared to be either a slight downward trend or flattening of the curve, either immediately following the exercise or delayed by three hours or so. Such changes were not reflected in any discernible difference in urinary urea excretion or urine volume. However, exercise was always done on the third day of study, during the upswing of the curve following the drop due to the previous day's doubling of intake, and there was always an increased urinary excretion of urea "carried over" into the third day which could have obscured any change due to exercise.

For many years it has been thought that exercise has no effect on nitrogen excretion or protein requirements, although the evidence for this has been conflicting. Millward et al. (1982) examined the effect of treadmill exercise for 3.75 h. at 50% VO_2 max. on whole-body protein turnover or nitrogen excretion. In the fed state exercise induced increased nitrogen

excretion of 71 mg/Kg. over 18 hours after exercise, and measurement of the time course of changes in $^{13}\text{CO}_2$ excretion from ingested ^{13}C leucine showed that all of this increased nitrogen production occurred during exercise. Because of reduced renal clearance and slow turnover of the urea pool, urea excretion lags behind urea production. The nitrogen loss resulted from an increase in protein breakdown and a decrease in protein synthesis. In a second series of experiments the authors gave glucose orally and found that the increase in protein breakdown was reduced, presumably due to suppression of gluconeogenesis. In exercise, increased muscle uptake of glucose is matched by increased hepatic production. Subsequently they investigated the relationship between intensity of exercise and extent of urea production by exercising for 30 minute periods on a bicycle ergometer at three successive work rates increasing from 25-89% VO_2 max. They found a highly significant correlation between leucine oxidation and the intensity of exercise. These last experiments are similar to those described in RF and CM in that the same duration of exercise of 30 minutes was used, on a bicycle ergometer, but the units of work were different (VO_2 max versus kilopond metres). Wolfe et al. (1982) also used leucine oxidation as an index of net protein catabolism, exercising subjects on a bicycle ergometer for 105 mins. at 360 kpm (heart rate c.110/min.) which was equivalent to 26-31% VO_2 max. Leucine oxidation increased, but urea concentration and production did not change. Wolfe et al. suggested that in exercise urea production is possibly inhibited.

However, Mole and Johnson (1971) found that nitrogen excretion was increased on the first or second day after 30 minutes of intensive exercise, when subjects were maintained on excess feeding. It is thus possible that some of the increased nitrogen excretion observed in RF and CM described here may have been due to the period of exercise, but "hidden" within the increased excretion due to the preceding day's doubling of protein intake. Certainly, if the exercise done by RF and CM is compared with that in Wolfe et al., which was expressed in both sets of units, they were probably exercising at 32-40% VO_2 max. which on the basis of the findings of Millward et al. should have resulted in an increase in leucine oxidation amounting to 25-30% of flux. This should have been sufficient to increase nitrogen excretion as in the treadmill experiments of Millward et al. which were carried out at 50% VO_2 max., and although for a longer period of time, the subsequent nitrogen excretion was prolonged for 18 hours.

Rennie et al. (1981) measured plasma amino acid levels during exercise and found that the BCAA, glutamine, glutamate, alanine, phenylalanine and tyrosine were raised but as exercise progressed all amino acid concentrations fell. At the end of exercise large falls were observed in almost all amino acids but particularly alanine, glutamine, glycine and the BCAA. Plasma amino acids were measured in RF and CM in each study on the day of exercise but unlike the results of Rennie et al. the only

significant changes were a fall in alanine following exercise in each study, and falls in glycine and aspartate and glutamine (measured together) in two out of the four studies. Interestingly, no changes were noted in the levels of the BCAA. This was noted also by Millward et al. (1982) despite the observation by them and by Wolfe et al. (1981) cited earlier, that during exercise leucine oxidation increases. Muscle is the main site of transamination of the BCAA and in exercise one would expect a net uptake of BCAA together with a net output of alanine and/or glutamine disposing of their nitrogen. Because alanine and possibly glutamine fall in plasma following exercise, and because these two amino acids are the only vehicles by which nitrogen released from deamination of the BCAA can be transported out of muscle in large quantities, it must be assumed that any increased production of them must be more than matched by increased removal.

The fact that Millward et al. (1982) could suppress protein catabolism during exercise by glucose is important. There is no doubt that athletes in training eat much more protein than normal adults. This may be of benefit psychologically but unless athletes have an increased protein turnover it seems unlikely to be of value once the basal requirements have been met. Furthermore, it is expensive for the individual and would be wasteful "ecologically" if practised on a wide scale. Stein et al. (1983) studied whole-body flux and protein synthesis rates in male oarsmen and

anthropometrically similar sedentary males using ^{15}N glycine as tracer and ^{15}N urinary ammonia as end-point. Subjects snacked hourly to eliminate perturbation by set meals. The oarsmen excreted more than twice as much nitrogen as the control group although, in fact, both groups were consuming excess protein when compared with USDA recommendations. The results indicated that the extra protein intake of the oarsmen was unnecessary for the maintenance of their basal protein synthesis, and it would seem that the role of increased nutrition among athletes is to provide additional fuel rather than work capacity.

There is clearly scope for further investigations of this nature. In retrospect, in the cases of RF and CM it would have been more sensible to have conducted the period of exercise on and following baseline diet, and for a longer period. In any account, the distorting effects of change in total protein intake and to a lesser extent exercise, on the equilibrium isotopic behaviour in these four studies were sufficient to prompt further study, the better to understand the effects of perturbation of intake. These subsequently conducted experiments form the next section.

STUDY III

Effects of Measured Intake Perturbation on the Equilibrated Urea Enrichment Curve; Comparison of Change in Nitrogen Turnover Rate with Change in Nitrogen Balance; the Basal Catabolic Rate.

The experimental design was to introduce measured perturbations during prolonged constant rate isotope infusion, observing the results of perturbation on configuration of the enrichment curve and calculation of the apparent turnover rate. It was perceived that such observations would be best made during a prolonged continuous study rather than by comparison of widely separated periods with individual isotope buildup curves and enrichment analysis.

Methods

1. Subjects

Normal male volunteers aged 18-30 were used for the studies. Procedures were outlined in detail, and carried out in conformity with the regulations of the Human Subjects Committees both of the Harvard Medical School and the Peter Bent Brigham Hospital. The subjects were cared for on The Clinical Center of the Peter Bent Brigham Hospital, a metabolic ward.

2. Intakes

Oral diets consisted of regular hospital food presented four times daily in equal quantities at equal intervals, with no food intake provided between

8 30 pm and 8 00 am during which time de-ionised water alone was provided. Diet aliquots were analysed. The baseline oral diet provided 1 gm. protein/Kg/day (160 mg N/Kg/d) with 3.2 g carbohydrate/Kg/day, 1.4 g.fat/Kg/day, providing 26.3 non-protein Kcal (110 KJ) per Kg/day, and a calorie:nitrogen ratio of about 120.

On what was termed Doubling, the nitrogen intake was increased for 48 hours to 2 g protein/Kg/d. (320 mg/Kg/d) with a slight change in non-protein calories, which lowered the calorie: nitrogen ratio from 120 to about 70.

On Dropout, no food intake was allowed, de-ionised water alone being given.

Intravenous intake was provided in the form of an amino acid mixture (Travasol 8.5%) given with appropriate amounts of glucose so that in the baseline intake it was exactly equivalent to that of the oral diet.

For Doubling nitrogen intake intravenously, the Travasol infusion rate was increased to provide a nitrogen intake equivalent to 2.0 g. protein/Kg/d with a calorie: nitrogen ratio of about 80.

Dropout of intravenous diet involved the intravenous infusion only of an equivalent fluid volume of balanced salt solution.

Intravenous feeding experiments were also carried out in which the calorie:nitrogen ratio was intentionally manipulated. Firstly, the amino acid intake was kept constant, without glucose ("PROT, O CAL") when amino acids alone were given at 160 mgN/Kg/d. Subsequently, calories without amino acids were given ("CAL, O PROT") in the form of a glucose infusion at 30 Kcal (126 KJ)/Kg/d. All intravenous infusions were given by central vein,

employing a cannula inserted via an antecubital vein into the superior vena cava as confirmed by chest xray.

3. Protocol Design

A. Oral Intake (Long Protocol-Baseline I, Doubling, Return to Baseline II, Dropout, Return to Baseline III).

In 4 subjects studies were carried out over 12 days. ^{15}N glycine was given orally at a constant rate throughout the twenty four hours. After four days at Baseline I intake dietary nitrogen was Doubled as indicated, for two days, then returned to Baseline II (identical with Baseline I). After two days of such re-equilibration, food was omitted ("Dropout") for two days, following which there was return to the starting intake (Baseline III) for one day.

B. Intravenous Intake (Short Protocol-Doubling or Dropout).

Observations were carried out over a shorter period, six days. The nutrition and ^{15}N glycine were both given continuously throughout. In all the intravenous intakes protocols a "priming" dose of ^{15}N glycine was given during the first three hours of infusion, to shorten the period of time required for equilibrium. Sim et al. (1981) used a prime of 0.4 mg $^{15}\text{N}/\text{Kg}$. before continuous infusion of isotope. Plateau was reached at 12-18 hours and results for turnover, synthesis and breakdown were similar to those obtained by the conventional technique. There was an initial period of Baseline I of two days, then two days of either intravenous Doubling or Dropout, followed by return to Baseline II.

C. Intravenous Intake (Short Protocol - Calorie: Nitrogen Manipulation

In five intravenous experiments there was manipulation of the calorie: nitrogen ratio as described above. In three of these, after Baseline I equilibrium the subject was given "CAL, O PROT" for two days, then returning to Baseline II. In the other two subjects after 48 hours of equilibrium, the "PROT, O CAL" was instituted for 48 hours followed by a single day of return to Baseline II.

4. ¹⁵N Glycine

The isotope was obtained and prepared for intravenous use as previously described. It was mixed with a small amount of fruit juice for oral use. In a few cases when priming was employed, an erroneous high dose was initially used, making it impossible to calculate the baseline turnover rate. Subsequent curve configuration was satisfactory however for calculation of the later perturbations in the protocol.

The isotope dose rate in all experiments was 0.5 mg ¹⁵N per Kg per day. Minor departures from this were due only to vagaries of oral ingestion or infusion rate, and are shown as such in the tables.

The plateau values were satisfactory for calculation in all the experiments; the variability amongst the points employed for plateau calculation is shown as a coefficient of variation in plateau urea ¹⁵N enrichment or specific activity (Sa) in the tables.

5. Sampling and Analyses

Venous blood samples were taken at regular intervals for determination of

amino acids by chromatography, as well as electrolytes, glucose and blood urea. Urine and faeces were collected and analysed for total nitrogen by macro-Kjeldahl and nitrogen fractions including urea, creatinine, creatine, uric acid and ammonia.

Urine was collected every three hours and subjects woken at night to void every three hours. In fact, the exact time of collection was not critical so long as the urea enrichment values were plotted on appropriate time co-ordinates. Urine samples for measurement of ^{15}N urea enrichment were treated as previously described.

6. Calculations

a) Nitrogen balance data are expressed both as grams N/day and as mgN/Kg/d., the latter so as to be comparable with the isotope data. No corrections were assumed for losses from skin or hair.

Change in Nitrogen balance ($\Delta \text{N bal}$) is based on the change in nitrogen balance between two successive periods. The basis for the calculation of the Q: N ratio (see below) is based on the change in turnover and nitrogen balance for the second 24 hours at one intake, to that of the second 24 hours of the next intake programme.

Amino acid changes were, as before, based on total amino acids (TAA), the sum of the branched chain amino acids (BCAA) and the ratio of the two (BCAA/TAA). All values for amino acids are given as mg/dl.

b) Whole body nitrogen turnover rate (Q) was determined as before, where $Q = d/S_a$. S_a at plateau was calculated from the mean of 5-8 points. The

nature of the plateau achieved is shown graphically in the charts. In the tables, the mean Sa and coefficient of variation are shown, for each plateau determination.

c) Change in nitrogen turnover rate (ΔQ) is based on the net change in Q between two successive diet periods. The Q value before the change is compared with that observed during the dietary alteration, basing the calculation of apparent Q for each occasion on the plateau enrichment during the second 24 hour period. Thus, the two values for Q were made 48 hours apart. The only exception was on the final day of each study (return to Baseline III) during which time observations were made only for 24 hours.

d) Synthesis and breakdown of body protein were calculated as before using $Q = I + B = S + E$.

7. Q: N Ratio - The Ratio of Change in Turnover Rate to Change in Nitrogen Balance

$$Q : N \text{ Ratio} = \frac{\text{Turnover}}{\text{Balance}} = \frac{\Delta Q}{\Delta N \text{ bal.}}$$

The interpretation of the Q: N ratio across induced dietary changes rests on four possible alternatives which are detailed in the Discussion.

Results

(to preserve continuity of the text as much as possible, the large number of Tables (16-29) and Figures (XXXIV-L) form the Appendix.

A. Oral Intake (Long Protocol)

1. Nitrogen Balance (Tables 16 & 17, Figure XXXIV)

Baseline I nitrogen balance on regular diet (4 days) was either zero or slightly positive in three out of four subjects; in the fourth (R.S) the balance was strongly positive on all 4 days. R.S. was 94 Kg, 30% heavier than the others. The mean nitrogen balance for the first four days on Baseline I intake was positive at 40 ± 13 mg/Kg/day. (this corresponds to about + 0.35 gm/day).

Doubling nitrogen intake (days 5 and 6) produced an increase in total body nitrogen content at a positive nitrogen balance rate of about half the increase in intake rate. Nitrogen balances ranged from + 85 to + 160 mg/Kg/d. with a mean of $+ 117 \pm 18$ mg/Kg/d. In all cases except one the nitrogen balance on the first day of Doubling was more strongly positive than on the second day. In the exception, balances for the two days were equal. On both days, total urinary nitrogen output was increased, but obviously not as much as the intake. The change in nitrogen balance ($\Delta N \text{ bal} = N \text{ bal Day 6} - N \text{ bal Day 4}$) ranged from + 28 to + 99, with a mean range of $+ 69.5 \pm 31$ (a significant increase, $p = 0.02$).

Following Doubling, restoration of intake to Baseline II reduced nitrogen balance to practically zero (mean $- 14 \pm 4$). The mean $\Delta N \text{ bal}$ was $- 97 \pm 29$ ($p < 0.01$).

Dropout of nitrogen intake produced large negative nitrogen balances in all subjects (-110 to - 192) with a mean of $- 146 \pm 23$ mg/Kg/d. The negativity

is almost exactly equal to the magnitude of intake reduction, and in all cases the negativity is greater on the second day of Dropout than on the first. The mean Δ N Bal was -160 ± 17 ($p < 0.001$). Restoration of intake to Baseline III for one day after Dropout produced zero balance (-21 to $+12$) in three subjects, but in R.S. a strongly positive balance ($+93$). The mean nitrogen balance on Baseline III was $+37 \pm 47$ but if R.S. is omitted the mean for the other three is -20 ± 12 . The Δ N Bal is $+180 \pm 33$ ($p = 0.001$).

Plasma amino acid concentrations (Table 18)

Baseline normal resting values were based on 18 determinations in 9 subjects. The normal values were: for total amino acids (N=18) 286 ± 37 mg/dl; for the sum of branched chain amino acids (BCAA), 43 ± 4.4 mg/dl; for the BCAA/TAA ratio 0.15 ± 0.02 .

In the experiments with oral intake the amino acid data were generally free of variation save for one constant finding observed at a high level of significance in all four experiments. Namely, that on Dropout, the value for total amino acids fell (from 285 ± 37 to 207 ± 19 , $p < 0.03$), while that for branched chain amino acids almost doubled (from 43 ± 4.4 to 71 ± 15 , $p < 0.05$). Thus there was a marked increase in the BCAA/TAA ratio (rising from 0.15 ± 0.02 to 0.34 ± 0.03 , $p = 0.05$). In nearly all cases these changes had returned to normal by the following day.

During Doubling, total amino acids fell slightly from 286 ± 37 to 247 ± 10 . In addition, there were insignificant rises in BCAA (from 43 ± 4.4 to $50 \pm$

1.5, $p = 0.08$) and BCAA/TAA ratio (from 0.15 ± 0.02 to 0.21 ± 0.01 , $p = 0.06$).

2. Isotope Equilibrium Behaviour (Figs. XXXV to XXXVIII inclusive, Table 19)

Initial isotope equilibrium behaviour is charted and tabulated following the first 48 hours of the unprimed buildup curve. In three out of four subjects (R.S., M.G., and L.F.) the behaviour was characteristic of that seen in previous experiments. A flat plateau of urea enrichment was attained, and equilibrium evaluated on 8 points, with coefficients of variation around the mean S_a being 4.4%, 2.9%, and 7.3%. In one subject (T.H) the curve appeared to be still rising, although the 7 points used for calculation showed a coefficient of variation of only 4.2%. On Doubling, all four subjects showed a similar and characteristic phenomenon not seen in any other circumstances. During the daylight hours of doubled intake (with a lag of 6-8 hours after the first expanded meal) there was a sharp drop in urea enrichment reaching a low point maintained during the first few nocturnal hours. Then, during the night, but before intake was given again in the morning, the specific activity again rose, reaching an early morning value about equal to Baseline I equilibrium level, despite continued absorption and metabolism of the doubled intake. On the second day this diurnal curve was repeated, but at a lower value for the highest point and at a slightly higher value for the lowest point. The points upon whose mean the plateau specific activity depended and the calculation of

the apparent turnover are based, are again the final points of the second day of doubling indicated by a straight line on the charts. As shown in Table 19, the variability here is notably larger than under any other circumstances, the coefficients of variation around the plateau being 6.4%, 6.2%, 9.4% and 8.0%.

Upon restoration to Baseline II, all four subjects showed a similar phenomenon. On the first day, the specific activity slope rose to a level almost identical with that of the initial resting equilibrium on Baseline I. but on the second day of return to Baseline II, as if the period of Doubling had a persistent effect on nitrogen turnover, there was a further rise, and in three out of four subjects a clear and new higher plateau was reached, whose specific activity was quite constant, the mean of which was used for the calculation of the apparent Q and the Q:N ratios on return to Baseline II. Here, the coefficients of variation for these urea enrichment plateau calculations are at a lower level (3.2%, 4.3%, 4.8% and 5.0%).

On Dropout all four subjects showed similar equilibrium curves. On the first day a gradual rising slope began, reaching a plateau on the second day with a much higher specific activity than either Baseline I or Baseline II (and therefore, lower values for apparent Q). It is this plateau on the second day that is used for calculation of apparent Q, and the Q:N ratio. The plateau characteristics are satisfactory with coefficients of variation of 1.5%, 1.7% and 4.1%.

Finally, on return to regular diet (Baseline III) for one day only, the

four subjects showed similar phenomena. A sharp downward slope of urinary urea enrichment reached a plateau after 12-18 hours. The final plateau specific activity was significantly higher than that of the initial plateau (Baseline I) and that of the second return to normal diet (Baseline II), yielding a lower value for the final Q.

3. Whole Body Nitrogen Turnover Rate, Synthesis and Breakdown (Q, S and B)
(Table 19, Figure XXXIX).

The Baseline I control Q values on normal oral diet ranged from 452 - 529 mgN/Kg/d. (mean 486 ± 37). These values are consistent with previous observations in normal subjects (see earlier). Upon Doubling, the apparent Q values increased from 6-19%, ranging from 516 - 571 (mean 542 ± 27 mg N/Kg/d., $p < 0.01$), a mean increase of 11.5%. If the calculations were based on the lowest enrichment point observed during this period, the Q values would be about 10-12% higher. Contrariwise, if the apparent Q was based on the mean of the highest points observed, the value would be 10-12% lower.

Upon return to Baseline II the Q values fell to a range of 396 - 471 mgN/Kg/d. (mean 434 ± 30 , $p < 0.01$). This turnover is about 20% lower than that observed during Doubling and about 12% lower than the resting Baseline I values.

Upon Dropout, Q values fell to a range of 283 - 327 mg N/Kg/d. (mean 303 ± 18 , $p < 0.01$), a value 30% lower than that observed at Baseline II and

almost 50% lower than the mean of the control or Baseline I. This is what can be termed the Basal Catabolic Rate in these normal individuals, as described below.

Upon return to Baseline III for a single day, the Q value returned to 371 - 391 (mean 373 ± 12 , $p < 0.01$), about 14% lower than Baseline II, although a 23% increment over the lowest value observed during Dropout, and about 25% lower than the Q value for Baseline I. Statistical analysis of the three baseline values for Q showed no differences between I and II ($p > 0.05$), with III significantly lower than II ($p < 0.01$) and I ($p < 0.01$).

4. Synthesis and Breakdown

Basal rates of synthesis (338 ± 24) and breakdown (305 ± 38) on regular oral diet are shown in Table 19. The effect of doubling was a small reduction (10%) in the synthetic rate (338 ± 24 to 304 ± 20 , $p = 0.04$), accompanied by a considerable (33%) fall in breakdown (305 ± 38 to 198 ± 9.6 , $p < 0.01$).

Upon returning to Baseline II, the synthetic rate fell below its former level (265 ± 26 , $p < 0.05$), and the breakdown rate rose (198 ± 9.6 to 253 ± 36 , $p < 0.05$) but to a level still slightly below resting baseline.

With Dropout, the principal effect was exactly the reverse of that on Doubling, namely, that the synthetic rate fell about 60% (265 ± 26 to 143 ± 13 , $p < 0.01$), whereas breakdown rose insignificantly (253 ± 36 , to 303 ± 18 , $p = 0.05$).

Restoration to Baseline III returned the synthetic rate upwards (143 ± 13 to 229 ± 39 , $p < 0.01$) with a marked fall in the breakdown rate from 303 ± 18 to 193 ± 20 ($p < 0.01$). Both values are well below Baseline I.

In summary, these changes, taken together, suggest that increasing dietary intake has the principal effect of reducing breakdown rate, whereas reducing dietary intake has the principal effect of reducing the synthetic rate.

The consistency of the changes is remarkable in that all four subjects showed changes in the same direction during the four perturbations of the intake, as shown in Table 19. Comparing baseline data for synthesis and breakdown, the three baseline values for S are significantly different

(I vs II, $p < 0.05$; II vs III, $p < 0.05$; I vs III, $p < 0.05$), but for B, I and II are not significantly different ($p = 0.07$), I and Dropout are not significantly different ($p = 0.90$) while III is significantly lower than I ($p < 0.01$).

5. Q: N Ratio: Ratio of Change in Nitrogen Turnover to Change in Nitrogen Balance (Table 20, Figure XL)

The four values for the Q: N ratio on change from Baseline I to Doubling were all ratios of the same sign with a mean of 1.04 ± 0.75 . One was low (L.F, 0.34) and one high (R.S, 2.07).

On return to Baseline II from Doubling, the four values were 0.72, 0.89, 1.20 and 2.26, the high value again being subject R.S. The mean was 1.26 ± 0.69 . Omitting R.S. the mean is 0.92.

On change of Baseline II to Dropout, the four values were closely grouped and lower (0.85, 0.54, 0.75 and 1.00), with a mean of 0.79 ± 0.19 .

The final change, that upon returning to Baseline III from Dropout, showed low values in all subjects (0.31, 0.40, 0.41 and 0.45) with a mean of

$$0.39 \pm 0.06.$$

Statistical analysis showed that the Q: N ratios of the first three changes were not significantly different from 1.0, using the paired t-test by logarithm of the changes. The fourth change, Dropout to Baseline III, showed a significant difference from unity at 0.39 ± 0.06 ($p < 0.01$).

B. Intravenous Intake: Doubling and Dropout

1. Nitrogen Balance (Table 21, Figures XLI & XLII)

These studies were carried out on two subjects (J.P & K.K). Nitrogen balance showed a mean close to zero on all four occasions on Baseline I intake (Mean + 25). Upon Doubling, a strongly positive balance was found in both subjects (+ 106 and + 81, mean + 93), a change of + 68. Upon Dropout, a strongly negative balance was observed (-142, -138, mean - 140). In both cases, the balance change was more marked on the first than the second day. In all instances the return to Baseline II resulted in a balance close to zero (- 24 to + 34, mean +4).

On Dropout there was a marked increase in nitrogen loss, with a mean of -140; a mean negative change of -151. In Dropout the negative nitrogen

balance was more marked on the second day than the first. Upon restoration to Baseline II, the increase in nitrogen loading returned to a mean of + 30, an upward change of + 182.

Amino acid changes upon Doubling showed a rise in total amino acids from 286 ± 37 to 332 ± 44 ($p < 0.05$), with a concomitant rise in BCAA (43 ± 4.4 to 54 ± 4.3 , $p < 0.05$) and no significant change in the ratio (0.15 ± 0.02 to 0.17 ± 0.07 , $p > 0.05$). Upon return to baseline the changes returned to normal. (Table 18).

Upon Dropout there was a fall in total amino acids but unlike with oral intake the drop was minor (286 ± 37 to 281 ± 18). However, the rise in BCAA was almost the same as in the oral group (43 ± 4.4 to 71 ± 1 , $p < 0.05$).

The rise in the BCAA/TAA ratio was not significant, from 0.15 ± 0.02 to 0.25 ± 0.06 , $p > 0.05$).

Upon return to baseline intake from Dropout a phenomenon was seen quite distinct from any previously observed, namely that the total amino acid values continued to rise upon restoration of intravenous intake (from 281 ± 18 to 330 ± 3 , $p < 0.05$), while the BCAA fell (71 ± 1 to 41 ± 0 , $p < 0.05$). The BCAA/TAA ratio remained in the normal range (0.13 ± 0.01).

2. Isotope Equilibrium Behaviour (Table 22, Figs XLIII & XLIV).

Due to inadvertent overprime, the Baseline I data are not available. The subsequent isotope equilibrium behaviour on these four intravenous

experiments (Doubling and Dropout) showed flat, stable plateaux. The coefficients of variation around the mean Sa values were small (3.2 - 4.5%). In these experiments the intermediate points between the plateau periods were not determined. On return to baseline intake from Doubling, there was a rise in specific activity, as expected. On return to baseline intake from Dropout, there was a striking lack of change in both plateau values, responsible for the anomalies in the Q: N ratio.

3. Q Values (Whole Body Nitrogen Turnover Rate) (Table 22, Figure XLV).

Q values in these four experiments showed a fall in both instances when the intake returned to Baseline II from Doubling (422 to 367 in J.P., 420 to 362 in K.K). Baseline I data are not available due to overprime. In the return from Dropout to Baseline II, Q changes were inconstant, as would be expected from the equilibrium data mentioned above, showing a very small drop (367 to 351) in J.P. and no change in K.K. (337 and 341).

4. Synthesis and Breakdown (Table 22)

Upon return to Baseline II from Doubling, the synthesis rate fell slightly in both instances, while breakdown rose significantly in both instances. Upon return to Baseline from Dropout, synthesis rose in one and remained unchanged in the other, breakdown falling in both. These changes are all in the same direction as in the oral intake study, but of lesser magnitude.

5. Q: N Ratio (Table 23, Figure XLVI).

Q: N ratio on return to Baseline II from Doubling showed values of 0.49

and 0.70 (mean 0.59) in J.P and K.K. On return to Baseline II from Dropout the figures were very low in both cases.

C. Intravenous Intake: Calorie and Nitrogen

Manipulation (Tables 24, 25, 26, and Figures XLV, XLVI, XLVII-L).

Three experiments were done exploring the change from baseline to CAL, O PROT (J.P., W.T.I and W.T 3). Two experiments were done from baseline to PROT, O CAL (W.T 2 and M.C). In all five instances the return to baseline was also noted.

CAL, O PROT (subjects J.P., W.T.I, W.T3).

On baseline there was zero balance. There was no isotope data in J.P because of overprime. On changing to CAL, O PROT there was loss of nitrogen (mean - 82, range - 75 to - 91) which was restored on return to Baseline II. In J.P. the isotope equilibrium behaviour showed a flat plateau with a slight drop in urea enrichment upon return to regular diet. Accordingly, Q, very low on CAL, O PROT at 264, rose only to 279 on return to Baseline II. The Q:N ratio was very low at $+15/+140 = 0.10$.

In W.T.I, there was a failure to achieve equilibrium on the first two plateaux. Baseline Q was 545, falling 20% to 423 on CAL, O PROT, and falling lower still to 389 on return to Baseline II. The Q: N ratio for the first change was $-122/-69 = 1.9$, a high value; upon return to Baseline the Q:N ratio was anomalous, being of a different sign ($-34/+126$).

In W.T.3 there were inconstant plateaux on the first two occasions with

slowly rising points from which an arithmetic mean was constructed. On Baseline II a flat plateau was observed. Initial Q was high at 597, falling to 512 on CAL, O PROT and continued to fall on return to Baseline II, to 453.

The Q: N ratio from Baseline I to CAL, O PROT WAS $-85/-97 = 0.83$, but upon return to Baseline II with a positive nitrogen balance (+121) but a negative Q change (-60) the situation is that of a Q:N ratio with both numbers significant but of a different sign.

PROT, O CAL (Subjects W.T 2, M.C).

On Baseline I there was essentially zero nitrogen balance. With change to PROT, O CAL a strongly negative balance ensued, more marked on the second than the first day. Upon restoration of diet to Baseline II there was continued negative nitrogen balance.

In W.T 2 isotope behaviour showed flat plateaux for all three determinations, the values showing very little difference in the mean, and hence only small changes in Q- from 579, to 612 and finally 587. The Q:N ratios are anomalous (+ 33/-117 and - 25/+25) and uninterpretable.

In M.C. there was an unstable plateau on the regular diet, normal plateau on Baseline II, but again an unstable plateau upon return to regular diet from PROT, O CAL. Q fell from an initial 579, to 501, and finally 451. Q: N ratio across the first change was $-78/-117 = 0.66$, but on the second

change was -50/+59, both figures significant but of opposite sign.

Discussion. (see also Tables 27,28,29).

The widespread use of isotope equilibrium methods in clinical studies makes it essential to quantify the effect of sudden changes in metabolic regimen. In this study, measured changes in intake were used as the test model, in a ¹⁵N glycine - urea enrichment system. It is evident that changes in intake produce alterations in the isotope plateau of urea nitrogen enrichment within a matter of hours. It is also clear that return to the starting point or Baseline intake is not accompanied by an immediate return to the same plateau, but instead by return to a different level indicating a response of the body's protein synthetic mechanisms and nitrogen metabolism, including urea disposal pathways, to the prior alteration in intake.

1. Interpretation of the Q: N Ratios.

These studies have enabled a comparison to be made of the change in apparent nitrogen turnover rate (as measured by isotope kinetics) with the change in body content of nitrogen (as measured by conventional nitrogen balance). This relationship is expressed as the Q: N ratio. Both numerator and denominator have dimensions of "change of rate", the units are expressed in mg N/Kg/d., and the sign of both numerator and denominator can be either positive or negative, depending upon whether the turnover rate and body content of nitrogen are increasing or decreasing across the change in diet.

The simplest model, referred to as Doubling, is when large amounts of new dietary nitrogen are added (as protein), but with the isotope dose kept constant. This unlabelled "cold" nitrogen, when given orally, enters the gastrointestinal tract and is distributed to the liver and other tissues, finally entering the urea precursor pool where it produces a fall in the total S_a of the intake, in the ^{15}N enrichment throughout the system and, on excretion, an appropriate fall in the urinary urea enrichment. Since the whole body nitrogen turnover rate has a reciprocal relationship to the urinary urea nitrogen specific activity, the dose of isotope (d) being constant, the value for apparent Q will rise. The typical change in these experiments with doubled intake was for synthesis to remain constant or nearly so, while breakdown was markedly decreased. In the pilot studies using subjects R.F and C.M synthesis rose more than the fall in breakdown.

The two sets of experiments are not strictly comparable because in R.F and C.M the isotope was only given for twenty four hours before the change in diet was begun.

When the intake was returned back to Baseline, exactly the reverse set of circumstances obtains. On the first day of this return the specific activity is not as high as it is on the second day, suggesting that a metabolic unsteady state is still operating, despite only two days of the doubled intake.

In the converse situation of Dropout, when nitrogen intake is zero, but

with constant intake of ^{15}N glycine, the specific activity suddenly increases because of lack of dilution by "cold" intake, and the value for Q apparently drops. This new Q value, on short-term cessation of intake, represents the basal level of breakdown of the body, (the basal catabolic rate). In these experiments, typically, cessation of intake was associated with a sharp decrease in the synthesis rate, while breakdown remained the same or increased slightly.

A. Implications of Q: N Ratio Unity.

Across the first three dietary changes in the four subjects on oral intake, the Q:N ratios clustered around unity (mean 1.03). Statistically none of the first three Q:N ratio means differed from 1.0.

If the change in nitrogen balance is the same as the change in total body nitrogen turnover, as measured by the isotope, the balance data validate the absolute magnitude of the isotope turnover calculation, and suggest that the latter is measuring the daily total nitrogen flux with dimensions directly analogous to the chemical measurement of nitrogen intake and output.

$$\text{If } Q = I + B = S + E$$

$$\text{and } \Delta \text{ N Bal} = \Delta (I - E)$$

Then when Q : N ratio = 1.0

$$\Delta Q = \Delta \text{ N Bal}$$

it follows that $\Delta Q = \Delta (I - E)$

Also, if $I + B = S + E$

then $I - E = S - B$

and therefore $\Delta Q = \Delta (S - B)$.

That is, when $\Delta Q / \Delta N = 1.0$ the change in whole body nitrogen turnover is wholly explicable by a change in body protein content; it is not necessary to postulate a second or extracellular phase pool. This provisional conclusion depends upon demonstrated constancy of extracellular fluid concentrations of nitrogen exchange moieties.

B. Implications of a low Q: N Ratio

In the group of four observations (oral intake) on return to the single final baseline intake day, the Q: N ratio was low (about 0.4), a significant departure from the other three changes. In several of the other (intravenous) experiments, returning to Baseline also gave very low values for the Q: N Ratio (ie 0.33 or lower). Such a result suggests that the change in body nitrogen content has been large, relative to the change in nitrogen turnover rate; the change in urea enrichment has been very small. The simplest interpretation of this finding is that protein synthesis is utilising the substrate offered from the diet at a rapid rate, and that continued excretion of protein degradation products over 24-48 hours represents the breakdown of recently tagged protein containing the same ratio of tagged to untagged nitrogen (ie S_a) as that observed in

protein synthesized during the previous 24 hours. The fact that this was most characteristically observed upon returning to Baseline after withdrawal of nitrogen intake, or on distortion of calorie: nitrogen ratios, suggests that the sudden increase in synthesis with a declining breakdown rate, involves the breakdown largely of protein recently synthesized (ie very short turnover rate proteins).

This "re-feeding" depression in Q:N ratio (here observed in normal individuals after a short - 48 hour - interruption of intake) provides an interesting model with which the re-feeding of chronically starved subjects might be compared. In the oral intake protocol the subjects had been on a continuous dose of ^{15}N glycine for 12 days and there had been ample opportunity to tag large fractions of rapid turnover proteins, those whose continued breakdown during the short final period of observation would be responsible for the maintenance of specific activity at its former level, producing a low Q: N ratio. In re-feeding after chronic starvation, with anabolism strongly favoured, this Q: N ratio might be even lower, enabling the extent of the response to re-feeding to be quantified. This simultaneous increase in synthesis and decline in breakdown might be even more marked in re-feeding after chronic (ie "adapted") starvation, and the relationship between Q, S and B, with a low Q:N ratio, might be an index of the means of optimizing the re-feed diet.

C. Implications of high Q: N Ratio.

In none of the subjects was a consistently or repeatedly elevated Q: N ratio observed, although R.S did show a ratio of 2 across his first two (oral) dietary changes. W.T 1 showed a ratio of 1.9 on his intravenous change from Baseline I to CAL, O PROT. Were an elevated Q: N ratio to be a persistent finding, it would suggest that there is some stimulus to body nitrogen turnover, such as recycling of amino acids, that is far out of proportion to any net change in total body nitrogen content.

D. Implications of Q: N Ratio of Different Sign.

This anomalous finding was most commonly seen after calorie: nitrogen manipulation, when it was recorded five times out of ten dietary-regimen changes. These results are not interpretable and suggest rearrangements of nitrogen recycling without appropriate change in intake or output.

2. Basal Rate of Breakdown ("Basal Catabolic Rate").

In the six "Dropout" experiments (four on oral intake, two on intravenous intake) it was possible to observe the basal rate of breakdown in a normal subject suddenly deprived of nitrogen intake. This has interesting mathematical implications:

$$\text{If } Q = I + B = S + E$$

$$\text{then, when } I = 0$$

$$\text{it follows that } Q = B$$

$$\text{and } S = Q - E = B - E$$

Stated otherwise, when intake is zero, nitrogen turnover rate is equivalent to the total breakdown of body protein. This can be termed the "Basal Catabolic Rate". In these normal subjects ($n = 6$) the mean value was 321 ± 33 mg N/Kg/d. In Table 27 are shown all the data on "Basal Catabolic Rate". The values cluster around 325 mg N/Kg/d.; about one-half of this catabolised protein yields products which are re-synthesized (ie basal re-synthesis rate on zero intake = 145 - 150 mg N/Kg/d). This re-synthesis rate at only one-half the basal catabolism produces the typical negative nitrogen balance on short-term Dropout of 150 mg N/Kg/d. The Q: N ratio across this change is characteristically 1.0, ie change in turnover approximates change in body nitrogen content. This measurement of the "basal catabolic rate" may be of use in studying altered metabolic states such as trauma, sepsis, late starvation etc.

With intravenous intake the "basal catabolic rate" levels were slightly (about 15%) higher than with oral intake. This suggests that when there is no gut pool of exchangeable nitrogen and the intravenous intake is suddenly freed of cold nitrogen dilution, the change in urea enrichment is proportionally less and the apparent basal catabolism higher.

E. Calorie: Nitrogen Manipulation

The most bizarre results were obtained on manipulation of the calorie: nitrogen ratio. In the five instances on intravenous intake (three on "CAL, O PROT" and two on "PROT, O CAL") there were several notable findings. First, when calories were maintained for two days without amino

acid intake (subjects J.P, W.T.I,W.T 3) upon restoration of normal intake the subject quickly resumed protein synthesis, (compensating for the loss of the previous two days). In contrast, when amino acids were given alone (PROT, O CAL) (subjects W.T 2, M.C) the day of resumption of normal intake was characterised by continued brisk loss of nitrogen. This suggests that when the energy requirement for protein synthesis is lost by caloric restriction, synthesis rates cannot be normalised by one day of caloric supply.

The values for turnover rate were inconsistent, but showed higher continued turnovers on PROT,O CAL suggesting that under this circumstance giving amino acids without energy support is associated with continued brisk catabolism. By contrast those given calories without amino acids (CAL, O PROT) showed normal or slightly lowered turnover rates. The most anomalous Q:N relationships were observed in the calorie: nitrogen manipulations, particularly in those having amino acid administration without calories (PROT, O CAL).

F. Isotope Plateau Behaviour

a) Plateau Calculation of Urea Enrichment (Sa)

The means and coefficients of variation of plateau Sa values are shown in the tables. Graphical portrayal of each plateau is shown in the charts.

In most instances, the plateaux were flat and the calculations present little difficulty (C.V. of multiple points less than 5%). In no instance

was the mean coefficient of variation for the group larger than $\pm 7\%$, even in those instances where by visual inspection the 'plateau' level appeared unstable or steadily rising.

b) Enrichment Instability on Diet Doubling:

Circadian Fluctuations in Urea Enrichment.

When nitrogen intake was Doubled on the oral diet, no food was given at night and a circadian rhythm was noted. With a lag time of 4-6 hours, the newly ingested cold nitrogen during daytime meals diluted the urea enrichment curve to low levels observed in the late evening or early morning. In the absence of intake at night, the enrichment curve rose again, reaching a peak in the early morning before breakfast. This occurred in all four subjects. It was not observed in the subjects on intravenous infusion in whom intake was constant throughout the twenty four hours. This is in contradistinction to urinary excretion of urea in which there was a circadian rhythm, as described in detail earlier.

Presumably, when subjects ingest the amount of protein and energy to which they are accustomed, but then have their intake suddenly doubled, the body handles the new nitrogen "passively", accumulating "cold" nitrogen in urea very rapidly. In a normal subject the mere feeding of additional nitrogen does not result in any increase in body protein content. Muscle is not built by diet alone. Over the initial period of several days for enzyme induction to deal with the added load, there will be a "factitious" increase in nitrogen loading, partly due to accumulation of small molecular

weight nitrogen compounds in body water. The circadian fluctuations of the specific activity during Doubling by mouth suggests that the isotope behaviour is reflecting changes in intake hour-by-hour, and the inability of the body over the short term of 48 hours either to synthesize protein more rapidly or to induce the enzymes required for smooth excretion of the added intake. This is reminiscent of the early balance studies on sudden supranormal nitrogen loading by Albright et al. (1946).

c) The Dropout Phenomenon: Uniform Rise in Urea

Enrichment with New Plateau Attainment.

The specific activity curves with sudden cessation of nitrogen intake were similar in all four subjects on oral intakes. None demonstrated any circadian fluctuation. For the first day there was a steady rise in the ^{15}N enrichment curve, attaining a new, much higher plateau, associated with the basal catabolic rate and a lower value for Q.

d) The Problem of ECF Nitrogen Changes: Amino Acid Concentrations

Any interpretation of the Q: N ratio depends upon the validity of the nitrogen balance determinations. Data on nitrogen balance are valid as a measure of body protein only in the absence of significant "storage" or body water accumulation of low molecular weight nitrogen compounds.

In a normal adult male with 55% body weight as water, there are approximately 5 gm. of urea nitrogen in body water. It is assumed that urea is distributed uniformly throughout body water (Dunegan et al., 1978).

A decline of this value to 15-30% of its starting level would result in an

apparent loss of body nitrogen (not represented by a change in protein content) of 1-2 gms. nitrogen per 70 Kg. per day. In the units employed here, this would be a "false negative" nitrogen balance of - 15 Mg N/Kg/d. (ie a very small decrement devoid of the significance of body protein loss). The only circumstance where this might have occurred would be in the two experiments of calorie: nitrogen manipulation where calories were maintained without protein precursors (CAL, O PROT). In both of these cases, upon restoration to Baseline II there was a strongly positive change in nitrogen balance (+ 140 and + 121). Changes in urea concentration would have been an inconsequential component; in none of the experiments was any significant change in blood urea nitrogen noted.

In a normal adult male there is approximately 44 mg. of amino acid nitrogen per litre of body water, or about 1.7 g. of amino nitrogen in total body composition, assuming no significant concentration gradients across the cell. For some individual amino acids this assumption is not correct, but for the entire array of amino acids the assumption does not carry any significant interpretive error.

In starvation there is a transient increase in plasma amino acid nitrogen, especially of the essential amino acids, followed later by a fall (Cahill, 1970; Cahill et al., 1966). This fall is more profound if glucose infusions are superimposed (Wolfe et al., 1977). Thus, a "false negative" nitrogen balance as great as -75 mg. N/Kg/d. might theoretically be accountable on the basis of normalisation of plasma amino acid nitrogen

level when intravenous amino acids were discontinued. This might have been a factor in the intravenous experiments during Dropout, were it not for the negligible changes observed. Similarly, rapid infusion of amino acids is associated with a false positive nitrogen balance as high as + 25 mgN per Kg per day due to rising plasma amino acid concentrations (Tweedle et al., 1977). Only in those receiving amino acids without glucose (PROT, Q CAL) could such an artefactual positive nitrogen balance be expected.

Viewed in the light of the above theoretical considerations the observed changes in amino acids in these experiments are of interest. They are summarised in Table 29. Doubling the oral intake produced a slight drop in total amino acids and a slight rise in branched chain amino acids suggesting brisk uptake but not sufficiently rapid to incorporate all the available BCAA into protein. The changes were minor, however, when compared with the analogous change with Dropout. Here, there was a marked lowering of total amino acids, and a sharp rise in branched chain amino acids with a change in the ratio to over twice normal. This sharp rise in branched chain amino acids in the early hours after cessation of intake has previously been described by Cahill et al., (1970, 1966) and here can be viewed with the same interpretation offered by Wolfe et al., (1977), namely that synthetic incorporation of amino acids is inadequately supported by calories and a sharp rise in BCAA/TAA results.

CONCLUSION

Although the original purpose of this research was to explore the effect on isotope kinetics of sudden alterations in protein intake, so as to better understand random variations that might be observed in clinical studies, it is clear that intentional curve perturbation may have value as a research method in itself. Such studies would be interesting in subjects "adapted" to prolonged malnutrition such as in third world countries, and in morbid obesity, and possibly during administration of growth hormone.

Certainly, such perturbations of the isotope equilibrium curve towards plateau have been largely ignored by most other workers in the field. This is understandable because in the clinical situation prolonged studies in a steady state are difficult to achieve, and the research worker is more concerned with obtaining a result as quickly as possible. Hence there has been an increasing tendency to use ammonia as an end-product, shortening the collection period to as little as 9-12 hours.

However, a few workers have explored the method, in particular Clugston and Garlick (1982). They studied obese women who were given continuous intravenous infusions of $1-^{14}\text{C}$ leucine for 24 hour periods, which were divided into two 12 hour periods of feeding and fasting; food being provided hourly during the feeding period. During the feeding period the plateau S_a in plasma and respiratory CO_2 was reached in 6 hours and maintained until the last portion of food was given. When feeding ceased

there was an abrupt rise in S_a to a new plateau so that protein synthesis at night was calculated as 71% of the day-time value. Protein breakdown remained constant. 30% of protein intake was immediately deposited whereas the remaining 70% was immediately oxidised. At night the rate of protein oxidation fell to 38% of its daytime value. The authors concluded that the changes reflect the normal, discontinuous pattern of food intake and the need during feeding to store protein in tissues for use in subsequent periods of fasting. For comparison, very similar results were obtained by Rennie et al., (1982) but here fed and fasted adults were studied on separate occasions, so that perturbation of the equilibrium curve was not observed. However, they also found increased protein synthesis on feeding, largely accounted for by that in muscle, which doubled. Whole body protein breakdown was not significantly different in the fed and fasted states.

Finally, Stein et al., (1976) noticed that the urinary ^{15}N excretion profile was very sensitive to perturbation during continuous infusion of ^{15}N glycine. If no meal was consumed the curve was smooth. They observed on several occasions that diabetic patients receiving either TPN or intravenous glucose exhibited dips in the ^{15}N urea enrichment curve following even small doses of insulin. Insulin decreases hepatic gluconeogenesis by decreasing alanine uptake (Felig, 1973) and inhibits hepatic proteolysis (Mortimer and Mondon, 1970). This should lead to a decrease in the size of the urea precursor pool in the liver and therefore a decrease in the rate of urea synthesis and of S_a in the newly synthesized

urea. Stein et al., only observed the latter, and therefore suggest that the major effect of insulin is on the origin of the urea precursor amino nitrogen rather than on the actual rate of synthesis. Similarly, the method has also been used by Halliday et al., (1983) to observe the effect of insulin on previously untreated diabetics.

Summary & Conclusions of Perturbation Expts.

1. By intentional perturbation of the equilibrium curve of ^{15}N glycine-urinary urea enrichment, using prolonged constant isotope infusion, it is possible to compare the change in total body nitrogen turnover rate revealed by isotope kinetics, with the change in nitrogen balance measured by conventional chemical methods.

2. Experiments were carried out in normal human volunteers. In four instances a prolonged ^{15}N constant glycine intake (12 days) included perturbations caused by: Doubling total oral protein intake from Baseline I, return to Baseline II, total cessation (Dropout) of intake, and finally, return to Baseline III. In nine instances, shorter (intravenous) protocols were used, on four occasions studying the Doubling or Dropout of nitrogen intake, and on five occasions studying induced alterations in the calorie: nitrogen ratio of the intake.

3. The baseline value for turnover (Q) on oral intake was 575 ± 22 mgN/Kg/d; on intravenous intake 486 ± 27 . For the total of all observations in normal subjects on both intakes ($n = 8$), $Q = 530 \pm 55$, synthesis (S) = 391 ± 63 , and breakdown (B) = 366 ± 72 .

4. Fluctuations in blood amino acid concentrations were in no instance large enough to bias factitiously the nitrogen balances; a constant finding was the rise in BCAA with fall in TAA, and a sharp rise in BCAA/TAA during cessation of intake.

5. Withdrawal of intake for 48 hours permits the measurement of the Basal Catabolic Rate during the second 24 hour period. This is the Q value expressed as MgN/Kg/d. The mean values for normal adult males were 303 ± 18 MgN/Kg/d. for subjects who had been on oral intake, and 356 ± 20 mg N/Kg/d. for subjects who had previously been on intravenous intake. The mean for the group, including both forms of intake ($n=6$) was 321 ± 33 mgN/Kg/d.

6. Sudden Doubling of the total nitrogen intake with constancy of isotope infusion was followed by circadian fluctuations in the urea enrichment; complete cessation of intake produced a slowly rising curve with a plateau on the second day.

7. Calorie: nitrogen manipulation produced bizarre alterations in these relationships. The most consistent finding was that with Dropout of intravenous amino acid intake, but maintenance of caloric intake, the body can quickly resume normal protein synthesis upon restoration to normal diet. By contrast, the continuation of amino acid intake unsupported by exogenous calories is accompanied, when calories are added, by continuation of net catabolism.

8. The Q: N ratio is the ratio of the change in nitrogen turnover rate as measured by the isotope to the change in body content of nitrogen as measured by nitrogen balance. Although there was considerable variability, the data clustered around a Q: N ratio of 1.0 for the first three dietary changes on oral intake suggesting that the isotope-measured value for whole body nitrogen flux has a clear dimensional basis in the daily changes of body nitrogen content measured chemically.

9. Curve perturbation could become a useful adjunct to the study of starvation, re-feeding, injury and infection.

STUDY IV
WHOLE BODY NITROGEN TURNOVER RATES, PROTEIN SYNTHESIS
AND BREAKDOWN IN PATIENTS.

Finally, studies were carried out on four hospitalised patients to document the effect of trauma and sepsis on nitrogen turnover, protein synthesis and breakdown. Because only four patients were studied, with different pathological conditions, and on different diets in changing clinical situations it is not appropriate to group them together as with normal control volunteers, and hence each patient will be considered separately.

Patient 1, G.P Aged 52. Admission weight 75.8Kg.

This man was admitted with 54% second and third degree burns. The head and both hands were 100% burned.

1st ¹⁵N glycine study conducted 17 days post-burn.

Mean intake $I = 29.9 \text{ gN/d.}$

Mean excretion $E = 18.0 \text{ g N/d.}$

$$d = 0.477 \text{ mg } ^{15}\text{N/Kg/d.}$$

$S_a = 0.0547$ (mean of 5 figures at plateau).

$$Q = 0.477/0.0547 = \underline{872 \text{ mg N/Kg/d.}}$$

$$= \underline{62.61 \text{ g N/d}} \equiv \underline{5.45 \text{ g protein/Kg/d.}}$$

$$Q = I + B = S + E$$

$$62.61 = 29.9 + B \dots B = \underline{2.85 \text{ g. protein/Kg/d}}$$

$$62.61 = S + 18 \dots S = \underline{3.78 \text{ g. protein/Kg/d.}}$$

2nd ¹⁵N glycine study, 30 days post-burn.

Mean weight 71.0 Kg.

Mean intake I = 30.45 g N/d.

Mean excretion E = 20.6 g N/d.

$$d = 0.437 \text{ }^{15}\text{N/Kg/d.}$$

Sa = 0.0584 (mean of 5 figures).

$$\begin{aligned} Q &= d/Sa = 0.437/0.0584 = \underline{748 \text{ mg N/Kg/d.}} \\ &= 53.11 \text{ g N/d.} \\ &= \underline{4.67 \text{ g protein/Kg/d}} \end{aligned}$$

$$B = \underline{1.99 \text{ g protein/Kg/d.}}$$

$$\text{and } S = \underline{2.9 \text{ g protein/Kg/d.}}$$

Patient 2, A.B., aged 40. Admission weight 78.8 Kg.

This patient was found in an alcoholic stupor in a burning building. He sustained a 70% burn involving the full thickness of his back, trunk, thighs, calves and upper arms, and partial thickness to hands, anterior thighs, arms and face.

1st ¹⁵N glycine study, 5 days post-burn.

Intake I = O

Excretion E = 19.25 g N/d.

$$d = 0.496 \text{ }^{15}\text{N/Kg/d.}$$

$$Sa = 0.0752 \pm 0.0011$$

$$\begin{aligned} Q &= d/Sa = 0.496/0.0752 = \underline{660 \text{ mg N/Kg/d.}} \\ &= \underline{52 \text{ g N/d.}} \equiv \underline{4.12 \text{ g protein/Kg/d.}} \end{aligned}$$

$$B = \underline{4.12} \text{ g protein/Kg/d.}$$

$$S = \underline{2.60} \text{ g protein/Kg/d.}$$

2nd ¹⁵N glycine study, 38 days post-burn.

$$\text{Mean intake } I = 13.5 \text{ g N/d.}$$

$$\text{Mean excretion } E = 12.53 \text{ g N/d.}$$

Mean weight 64 Kg (weight loss of 14.4 Kg in 33 days).

$$d = 0.502 \text{ Mg } ^{15} \text{ N/Kg/d.}$$

$$S_a = 0.0513 \text{ (mean of 5)}$$

$$\begin{aligned} Q &= d/S_a = 0.502/0.0513 = \underline{979 \text{ mgN/Kg/d}} \\ &= 62.66 \text{ g M/d} \\ &= \underline{6.12} \text{ protein/Kg/d.} \end{aligned}$$

$$B = \underline{4.80} \text{ g protein/Kg/d.}$$

$$S = \underline{4.90} \text{ g protein/Kg/d.}$$

Patient 3, D.P. aged 25.

This man was transferred from another hospital. Following what seemed to be an uncomplicated appendicectomy he presented ten days later with signs of peritonitis. At re-operation a large intra-abdominal abscess was found. The small bowel mesentery was damaged during mobilisation of adherent bowel and therefore a segment of ileum excised and a gastrostomy tube inserted. Postoperative fever continued and he then developed small bowel fistulae, a large gastric fistula and massive haemorrhage from gastric erosions requiring transfusion of 40 units of blood. He was transferred to PBBH and underwent surgery to ligate multiple gastric bleeding points and

splenectomy because of multiple splenic infarcts.

1st ¹⁵N study commenced on 4th postoperative day.

Mean weight 54 Kg.

Mean intake I = 24.6 g N/d. (3 litres of TPN daily).

Mean excretion E = 21.3 g N/d.

$$d = 0.579 \text{ mg } ^{15}\text{N/Kg/d.}$$

$$S_a = 0.672 \pm 0.0009$$

$$\begin{aligned} Q &= d/S_a = 0.579/0.0672 = \underline{862} \text{ mg N/Kg/d/} \\ &= \underline{46.95} \text{ g N/d.} \\ &\equiv \underline{5.43} \text{ g. protein/Kg/d.} \end{aligned}$$

$$B = \underline{2.56} \text{ g protein /Kg/d.}$$

$$S = \underline{2.94} \text{ g. protein/Kg/d.}$$

2nd ¹⁵N study conducted 7 weeks later. Patient still febrile, with both gastric and small bowel fistulae.

Mean weight 51.5 Kg.

Mean intake I = 29.3 g N/d. (4 litres TPN).

$$E = 14.8 \text{ g N/d.}$$

$$d = 0.583 \text{ mg } ^{15}\text{N/Kg/d.}$$

$$S_a = 0.0700 \text{ (mean of 5).}$$

$$\begin{aligned} Q &= d/S_a = 0.583/0.0700 = \underline{833} \text{ mg N/Kg/d.} \\ &= \underline{42.73} \text{ g N} = \underline{5.19} \text{ g protein/Kg/d.} \end{aligned}$$

$$B = \underline{1.96} \text{ g.protein/Kg/d.}$$

$$S = \underline{3.41} \text{ g.protein/Kg/d.}$$

Patient 4, M.W. aged 82.

This man was known to have complete heart block and had a demand pacemaker. On the day of admission he suddenly developed severe abdominal pain. At laparotomy the bowel was found to be non-viable from about 25 cms. distal to the duodeno-jejunal flexure to about 20 cms. proximal to the ileo-caecal valve. The involved bowel was excised and a jejunostomy and distal mucous fistula created. His immediate postoperative condition was good. Parenteral nutrition was started with 2 litres of a peripheral vein mixture and 1 litre of Intralipid.

One ¹⁵N study carried out commencing 8 days after admission.

Mean weight 80.35 Kg.

Mean intake I = 17 g N/d.

Excretion E = 13 G N/d.

$$d = 0.521 \text{ mg }^{15}\text{N/Kg/d.}$$

$$S_a = 0.0986$$

$$Q = d/S_a = 0.521/0.0986 = \underline{528 \text{ mg/Kg/d.}}$$

$$B = \underline{1.98} \text{ g protein/Kg/d.}$$

$$S = \underline{2.29} \text{ g protein/Kg/d.}$$

DISCUSSION

Although each patient has been considered separately, general conclusions can be drawn from them as a group. The most striking finding is a markedly increased whole-body nitrogen turnover, ranging from 528 - 979 mg N/Kg/d., when compared with the normal volunteers on both normal diet and the various intravenous regimens of 395 - 584 mg N/Kg/d. The lowest figure of 528 mg N/Kg/d., falls within the normal range, but this was in the oldest patient, aged 82, and eight days after a major operation when he was making a satisfactory recovery, and was afebrile. The other patients were suffering from severe burns or major sepsis, so-called "hypermetabolic" situations in the sense that they are accompanied by fever, tachycardia, increased resting metabolic expenditure, increased catecholamine excretion in urine, and increased catabolism. Intuitively, one might therefore expect to find increased nitrogen turnover. However, because turnover $Q = I + B = S + E$, Q could be increased as a result of increased nitrogen intake even if protein breakdown B remained unchanged. It is well known that such patients lose their body cell mass rapidly and hence they are always treated, from the outset, by hyperalimentation, either by nasogastric tube or intravenously. Thus, in subject GP, whereas nitrogen turnover is almost doubled when compared to normal, nitrogen intake is high at 30G (187.5g protein), and even at 30 days post-burn the same applies. Hence, this increased turnover could be attributed to increased intake alone. However, in AB, a similarly burned patient, nitrogen intake was

zero at five days and yet nitrogen turnover was still raised at 660 mg N/Kg/d. , but not to the same extent as in GP. AB was in marked negative nitrogen balance due entirely to increased protein breakdown. Indeed, in his case, protein synthesis was greater even than in those normal volunteers on low-dose glucose in Study I, otherwise the highest levels seen in the studies from these laboratories. When he was studied later at 38 days post-burn, this time on a nitrogen intake of 13.5 g. which would otherwise be regarded as normal, turnover was even more markedly elevated at 979 mg N/Kg/d. Thus, it appears that in severe burns nitrogen turnover is genuinely increased as a result of the insult. In this latter situation nitrogen equilibrium was practically achieved, but accompanied by a more than doubling of protein synthesis and breakdown compared to normal. Nevertheless, by this stage the patient had lost 14.4 Kg. in 33 days.

Similarly with DP nitrogen turnover was elevated at 862 mgN/Kg/d. but intake was also very high at 24.6 gN. 7 weeks after admission the patient was still febrile, with intestinal fistulae, but had a positive nitrogen balance, although still receiving massive amounts of nitrogen. Nevertheless, the patient had only lost 2.5 Kg. in weight during this seven weeks. However, in this particular instance it is not possible to tell whether the increased turnover was due to the high intake alone as opposed to the clinical condition.

Total body nitrogen turnover is said to be low in the elderly compared to young adults (Young et al., 1975; Golden and Waterlow, 1977). In MW aged

82, turnover was the lowest observed in the patients, although still at the upper end of the normal range. This may have been partly due to the high intake for an old man of 17 g N. It does seem that for valid comparisons of nitrogen turnover to be made between patients, intakes should be normal for their size and age. For example, as already discussed in the Introduction to Study II, Kien et al (1978a) showed an increase in synthesis and breakdown in children with burns greater than 60% of body surface area compared with those with burns less than 25%, or elective surgical patients. However, turnover was positively correlated with intake, and hence may have been just as much a result of increase in intake, as well as being related to the degree of injury. Nevertheless, because synthesis and breakdown were also increased compared to normals, this suggests that increased turnover was not entirely due to increased intake. In a similar publication, the same group (Kien et al., 1978b) described a decrease in protein synthesis of 15% following reconstructive surgery after burns in children, but nitrogen intake decreased also.

Birkhahn et al. (1980) compared eight healthy subjects with six patients who had sustained major skeletal trauma. All subjects received glucose only (600 Kcal, 2508 KJ) for 72 hours, and hence nitrogen intake was zero. Despite this, nitrogen turnover increased as did synthesis by 50% and breakdown by 79%, in the injured subjects. These findings are different from those of Crane et al. (1977) and O'Keefe et al (1974) who found

decreased synthesis after elective surgery, and in whom intake fell postoperatively. Similarly, Long et al. (1977) studied three septic patients and two normal subjects using pulse injections of ^{15}N alanine and ^{15}N urea. Once again, low dose glucose alone was given for two days before study and both synthesis and breakdown were raised in the septic patients compared to the normals.

Garlick et al. (1980) studied six volunteers who were vaccinated against typhoid/cholera. In all subjects there was fever on day one, associated with increased creatinine excretion, and turnover was higher on day one; synthesis was increased by 37% and breakdown by 55%.

Powell-Tuck et al. (1984) studied 19 undernourished patients with inflammatory bowel disease using ^{15}N glycine and with ^{15}N ammonia as end-product. Synthesis and breakdown were much higher in those patients with ESRs of 100 than those with ESRs of only 10. There was no correlation between nitrogen and energy intakes and turnover suggesting that intakes were adequate and that the increases in protein kinetics were genuine.

Clague et al. (1983) studied the effects of nutrition and elective surgery on whole-body protein metabolism. As previously discussed, turnover and synthesis increased with increased protein intake pre-operatively, but breakdown remained unchanged at all levels of intake. However, both synthesis and breakdown rose postoperatively commensurate with the degree of trauma, but breakdown rose more than synthesis, thus accounting for the negative nitrogen balance. It was concluded that synthesis responded more

to substrate availability but breakdown was largely related to the severity of the injury. The patient studies described here are in accord with the results of Clague et al. Similarly, Shaw and Wolfe (1989) showed that in the basal state, traumatised patients show significantly elevated rates of whole-body protein breakdown, partially balanced by a lesser increase in synthesis.

Of the various studies quoted, opinion seems to be divided as to whether synthesis increases after trauma (Kien et al., 1978a; Birkhahn et al., 1980; Long et al., 1977; Lowry et al, 1986; Clague et al, 1983; Shaw and Wolfe 1989) or whether it decreases (Ward, 1992; O'Keefe et al 1974; Crane et al, 1977; Kien et al 1978b). Clearly, nutritional factors account for much of the disparity, and probably the fact that at least six different methodologies were used. Harrison et al, (1989a) tried to resolve the matter using primed constant infusion of $1\text{-}^{13}\text{C}$ leucine in patients having surgery for colon cancer, studied 3 and 7 days postoperatively, and receiving 5% dextrose only. No significant change in protein metabolism was found in the early study, but at 7 days the results, while showing a wide range, suggested that the overall effect was one of increased turnover with both synthesis and breakdown increased. Although the studies were carried out under comparable conditions, there was no nitrogen intake, and subjects were receiving low dose glucose at even a lower dose than described in this thesis in normal volunteers. The study would have been more valuable if isonitrogenous diets had been used, especially in view of

the doubts expressed earlier in this thesis about the validity of the method in short-term studies in starvation. The study suggested that the previously expressed opinion that protein synthesis is reduced after surgery should be re-examined. It could be argued that when the negative effects of nutrient withdrawal on protein synthesis are excluded, the predominant effect of stress is anabolic. The response is characterised by production of acute phase proteins, immunoglobulins and the proteins required for tissue repair. Most of these proteins have a short half-life and the increased plasma levels are associated with high rates of protein turnover. In the absence of an exogenous source of nitrogen, because nitrogen reutilisation is less than 100% efficient, the increase in synthesis must be matched by an even greater level of nitrogen release. These changes result in the observed negative nitrogen balance. However, it can be argued that such an increase in turnover, despite its adverse effect on nitrogen economy, is advantageous as it ensures that there can be a rapid response to the variable demands for protein synthesis that characterise the period after injury.

This subject will be considered further in the Final Discussion which follows.

FINAL DISCUSSION

In this Final Discussion an attempt will be made to combine the various studies described in this thesis, to bring the subject up to date with reference to recent publications, and to speculate about possible directions in which research into surgical metabolism and nutrition might take in the future, with particular emphasis upon the potential value of further studies using the ^{15}N glycine method to measure total body protein turnover, synthesis and breakdown.

The purpose of the research described in this thesis was to maximise the effectiveness of supportive nutritional treatment by a better understanding of the metabolism of body fuels as they are influenced by starvation, sepsis and trauma; specifically, to determine the most appropriate mix of infused substrates as based on metabolic, endocrine and protein turnover measurements, initially in normal humans.

To this end, the most striking result in Study I was that addition of high dose glucose to amino acids improved nitrogen balance by an increase in whole-body protein synthesis, breakdown remaining unchanged, when compared with amino acids alone. The mechanism is obscure. Presumably inhibition of gluconeogenesis alone would have led to decreased breakdown, certainly in skeletal muscle, but this was not observed, and the whole-body results were reinforced by no evidence of change in 3-methyl histidine output from forearm muscle (Sim et al., 1979). Insulin might have been responsible, as insulin levels in the group given glucose were significantly higher than

on oral diet or amino acids alone. However, although insulin is the anabolic hormone par excellence, its actions are complex. For example, Fukagawa et al., (1985) found that insulin decreases leucine flux in a dose-dependent manner in normal volunteers, indicating a reduction in protein breakdown. However, some of the insulin levels produced in this study were grossly unphysiological. Several workers have shown that insulin stimulates protein synthesis and inhibits breakdown in skeletal muscle in vitro (Fulks, Li and Goldberg, 1975; Frayn and Maycock, 1984; Jefferson et al., 1974; Lundholm et a., 1981; Jefferson, 1980; Stirewalt, Low and Staiby, 1985) but the actual contribution of these actions to its anabolic effects in man is unknown. As a result of forearm perfusion experiments Gelfand and Barrett (1987) suggested that in normal man physiological elevations in insulin promote muscle anabolism by reducing protein breakdown rather than by stimulating synthesis. Castellino et al., (1987) came to the same conclusion, but like the study of Fukagawa et al., the level of hyperinsulinaemia achieved (70-80 μ u/ml) was far greater than would be observed in the physiological situation. In the studies described in this thesis, and in those of Wolfe et al., (1977), insulin levels greater than 45 μ u/ml. were never encountered even with a glucose dose of 740 g/day. Thus, whereas the in vitro studies cited above can be criticised for being somewhat artificial, the human studies are spoiled by producing unrealistic insulin levels. Hence, at the present time it is still not clear whether the observed effect of glucose in Study I in

stimulating protein synthesis was due to the effect of glucose as an energy source, due to insulin secretion, or due to some specific configuration of the glucose molecule, as previously suggested. Because whole-body protein synthesis and breakdown were estimated, it is still possible that protein synthesis was stimulated elsewhere than skeletal muscle eg. liver, but the 3-methyl histidine measurements would still indicate no change in breakdown from skeletal muscle.

Addition of high dose glucose to amino acids as described above resulted in nitrogen equilibrium in normal humans. The only other combination which had the same result as in Study I was amino acids, low dose glucose and fat emulsion (AA+ LDG + FE). As previously mentioned, at first sight it is difficult to understand why, in terms of nitrogen balance, this combination should be more effective than AA + LDG. The reason may lie in the fact that in the former group, the fat is introduced directly into the circulation so that there is a choice between utilisation or storage as fat, whereas in the AA + LDG group, endogenous fat may not be mobilised sufficiently, even though insulin levels were not significantly different between groups. Whatever the reason, this result indicated that nitrogen equilibrium could be attained using a combination of nutrients tolerated by peripheral veins, at least in normal individuals, and by extrapolation, in malnourished patients not suffering from major sepsis or trauma. Since then, peripheral TPN has become a practical possibility but has usually not been used for more than a week or so because of thrombophlebitis. Recently

very fine bore silicone catheters have been described, which allow prolonged peripheral TPN with minimal phlebitis when fat emulsion is used as 70% of nonprotein energy and a final osmolality of 1000 mOSM/l. (Kohlhardt and Smith, 1989).

Despite the fact that fat emulsions have been available in Europe for thirty years, and in North America since 1975, the fat versus glucose debate is still alive. Baker et al., (1984) studied ITU patients on ventilators and found that when TPN was alternated between 75% lipid: 25% dextrose and vice-versa that there were no differences between whole body protein synthesis or breakdown, and on 1g protein/Kg/d. all groups were close to nitrogen equilibrium. As recently as 1992 Smith et al., published a study indicating that lipid and glucose regimens were equivalent in terms of nitrogen balance in non-septic patients following gastrointestinal surgery, the lipid regimen being given by the peripheral route. Such results, coupled with the knowledge that patients suffering from trauma, sepsis, pancreatitis and burns display an enhanced reliance on fat as an energy substrate, an impaired capacity to oxidise plasma glucose, a continued loss of protein despite use of TPN (Shaw and Wolfe, 1989), and an inability to use protein at intakes above about 1.5g/Kg/d. (Shaw, Wildbore, Wolfe, 1987) make the concept of peripheral TPN more attractive, avoiding the potential complications of central vein feeding. This may be especially so in the future if the osmolality of infusates is reduced by the use of dipeptides, and hypocaloric feeds helped by addition of

growth-hormone (see later.).

The circadian variation in urinary nitrogen excretions, and by extrapolation, protein metabolism, outlined in Study IA deserves further consideration. Whether or not such a rhythm would have implications for clinical nutrition is debatable. Based on the findings of Study IA and those of Steffee et al., (1976), Berrizbeitia and Moore (1983), and Finn et al (1982), optimum utilisation of substrates may require intravenous feeding confined to the night. This may prove impractical for hospitalized patients, and the potential benefits marginal, but the small number of patients on home TPN probably use this method anyway, allowing daytime freedom of movement.

It is interesting that in sepsis, burns and severe trauma, whereas whole-body protein breakdown is increased, so is synthesis, but to a lesser extent, leading to the negative nitrogen balance. Turnover is increased if nitrogen intake is adequate or above normal. This seems to be a wasteful process and one can only speculate as to the teleological basis for it. Similarly, because growth involves net deposition of protein, synthesis must be greater than breakdown. The most economical way of achieving this would be by a reduction in breakdown, but this is not in fact what happens (Waterlow, 1984). During rapid growth breakdown is greater than in the non-growing state. In premature infants, for example, breakdown is twice that of one-year old children (Pencharz et al., 1983).

Following trauma the acute phase proteins are increased and needed for

tissue repair. Many have short half-lives, and it can be argued that an increased nitrogen turnover, despite its adverse effect on nitrogen economy, is advantageous because it ensures rapid response to variable demands for protein synthesis after injury. It seems that the greater the importance of a particular protein in the regulation of metabolism (enzymes, hormones) the faster its rate of turnover. Rapid protein turnover enables a limited pool to be used with optimal efficiency. Liver consists of several hundred different proteins, most of which are enzymes. Rather than maintain maximum levels of all possible enzyme systems all the time, those that are not immediately needed are maintained at a relatively low level, but as a result of this rapid turnover process, the concentration of a particular enzyme system can be rapidly increased if required, by changing the relative synthesis and breakdown rates.

From what is known about the biochemistry of protein synthesis, the calculated energy cost based on 4 mmol ATP + GTP per mole of peptide bond would be about 3.6 KJ per gram of protein of average composition. Since synthesis is accompanied by equivalent breakdown, some of which is ATP dependent, the figure of 3.6 must be a minimum. On this basis whole body protein turnover would account for 15-20% of resting metabolic rate. When turnover increases as a result of sepsis, burns and trauma, energy expenditure should also increase, partly because of this but also because of catecholamine secretion, pyrogens etc. In fact some workers have felt that increased protein turnover in burns provides the main reason for the

hypermetabolism observed, but Wolfe et al., (1987) have shown that increased substrate cycling contributes to the increased thermogenesis. In this situation opposing, nonequilibrium reactions catalysed by different enzymes are active simultaneously, eg. lipolysis and re-esterification of triglycerides. Such cycling follows beta-adrenergic stimulation.

These studies in man refer to whole-body protein metabolism, and it is difficult to study individual tissues other than muscle. Rennie and Harrison (1984) measured amino acid exchange across leg tissues in well-nourished patients, malnourished patients, cachectic cancer patients, and patients after elective surgery and concluded that the negative nitrogen balance across muscle is not associated with increased breakdown, since the efflux of 3-methyl histidine was lower, not higher, than normal. They suggested that there may be a fall in muscle turnover at the same time as a rise in visceral protein turnover. If liver export protein synthesis and gut protein breakdown rise sufficiently they may in terms of whole-body protein turnover swamp the opposing changes in muscle protein turnover, while adding to them in terms of nitrogen loss. However, the specificity of 3-methyl histidine as an index of skeletal muscle actomyosin breakdown is now in doubt. These results do not tally with the overwhelming view that net skeletal muscle proteolysis occurs following injury. Glutamine and alanine form 40% of total amino acid efflux, the BCAA 6%, and others 54% (Johnson and Wilmore, 1985). The stimuli for events are complex and may be related to hormones, altered perfusion, and inflammatory mediators (see

later.).

Whole-body protein kinetics have been discussed in normal volunteers, and in patients following elective surgery, sepsis and burns. Although no patients with cancer were described in this thesis, for completeness it does seem appropriate to discuss some studies which have been done on such patients. Carmichael et al (1980) reported that protein turnover measured by continuous infusion of $1-^{14}\text{C}$ leucine increased with advancement of the disease in patients with colorectal tumours (some had metastases). Similarly, Norton et al. (1981) using ^{15}N glycine found that certain patients with tumours had abnormally high turnover rates. In neither study were repeat measurements made to see if these high rates of turnover persisted after removal of the tumour. On the contrary, Glass et al. (1983) found no difference in turnover, synthesis and breakdown measured just before and twelve weeks after surgery in patients with colorectal cancer (Dukes A-C) using oral ^{15}N glycine and either urea or ammonia as end-product. Harrison et al (1989b) found the same. However, Ward et al. (1985) did find an approximately 50% increase in turnover in postoperative patients with disseminated cancer when compared to those with either benign disease or localised malignant disease (623 ± 64 , 462 ± 42 and 431 ± 36 respectively). Thus, there is evidence that some patients with advanced tumours have high turnover rates. If this is so, it might be because as patients become more depleted it is the slower turning over muscle proteins that are lost, and hence the mean turnover rate of the remaining proteins,

which will include a greater than normal contribution from the faster turning over visceral proteins, will be increased, especially if the tumour is very active. Alternatively, cancer patients may have an underlying metabolic derangement.

At the time that the experimental work described in this thesis was being conducted, the author was also part of a Parenteral Nutrition Team responsible for management of all patients on such treatment in the hospital. Whereas members of such teams have little doubt that TPN is beneficial when deemed indicated, and can quote case histories of patients who they believe would not have survived without it, there is still only limited objective evidence of its efficacy in terms of reduced morbidity and mortality in severely ill patients (Mullen et al., 1980; Mullen et al., 1982; Skillman, 1983; Koretz, 1983). Reference to subjects AB and DP in Study IV indicates that almost certainly neither would have survived without TPN. Nevertheless, in both cases the draught on the lean body mass was either merely just held in check or reduced, rather than reversed.

The knowledge gained about changes in whole-body protein turnover, synthesis and breakdown as described in this thesis has not led to an ability to increase synthesis or decrease breakdown in severely illpatients, by manipulation of the various substrates alone. Indeed, whereas in the 1970s it was fashionable to use large amounts of nitrogen and energy, the tendency in recent years has been to prescribe more modest quantities. There are several reasons for this. Firstly, in septic

and burned patients the massive increase in gluconeogenesis cannot be reversed by increasing infusions of glucose, unlike in starvation. The explanation for the increased gluconeogenesis remains unclear, but is no longer thought to be adaptive to meet increased energy requirements. Only moderate increases in energy expenditure occur in such cases, and these can easily be met by endogenous energy sources other than protein; for example measurements of body composition and indirect calorimetry show that fat oxidation is the most important energy source after injury, with protein oxidation accounting for no more than 20-30% of total energy requirements. The relative inability of exogenous glucose to inhibit this gluconeogenesis may be partly explained by the fact that in the injured patient rates of uptake of glucose are already significantly increased and the glucose intolerance of such patients is not due to reduced peripheral utilisation. In fact, studies in recent years have found that there is a physiological maximum to the amount of exogenous glucose that can be oxidised (Wolfe, Allsopp and Burke, 1979). Exceeding the optimal glucose infusion rate creates fat deposits in the liver and increases rates of CO₂ production (Burke et al., 1979). Such increases in CO₂ load and ventilatory drive may make it difficult to wean ITU patients from mechanical ventilators (Elwyn et al., 1981). In addition, although energy expenditure is increased in trauma and sepsis, it rarely exceeds 2500 Kcal (10.4 MJ) daily (MacFie, 1984). Also, the maximum stimulation to anabolism seems to be achieved when protein is provided at about 1.5 g protein/Kg/d., excess being converted

into urea and excreted (Shaw, Wildbore, Wolfe, 1987).

Consequently, because nutritional support does not appear to prevent considerable loss of lean body mass during critical illness, attempts have been and are still being made to attenuate the catabolic response, by various other means. One can consider such approaches under three main headings:- reduction of the stress response, provision of specific nutrients, and administration of growth factors.

1) Reduction of the stress response.

The mediators of the stress response are hormones, cytokines and lipid mediators. Sepsis and trauma are followed by a series of neuro-hormonal events, described in an earlier section, but which include activation of the sympathetic nervous system, stimulation of the hypothalamo-pituitary-adrenal axis, and an increase in glucagon secretion relative to insulin. Infusion of cortisol, glucagon and catecholamines into healthy volunteers can produce many of the responses that occur following injury, including increased nitrogen loss (Bessly et al., 1984). It is hence reasonable to propose that neurohormonal blockade may attenuate the metabolic response to trauma. Indeed epidural anaesthesia to T4 does reduce the cumulative postoperative nitrogen loss in abdominal hysterectomy (Brandt et al., 1978). However, a combination of epidural analgesia and beta blockade in dogs caused reduction in skeletal muscle breakdown, but total nitrogen excretion was unchanged, indicating probable increased visceral protein breakdown (Hueton et al., 1985). There is no doubt that

epidural analgesia is used much more frequently now than in the past, and possible modification of the metabolic response is one of the several reasons for this, but hormonal blockade is not practised except in certain rare instances, eg. anaesthesia for removal of a pheochromocytoma. Potentially the most exciting means to manipulate the response to infection and inflammation to the benefit of the host is by blocking certain cytokines. Despite the tremendous efforts by surgeons during the last half century or so since the discovery of antibiotics, the mechanisms underlying severe sepsis remain incompletely understood. Whereas invading micro-organisms were originally thought to be wholly responsible for host responses in sepsis, it later became clear the endotoxin from dead bacteria could be lethal. It is now realised, paradoxically, that when the host succumbs to bacterial invasion, it is the host's own body which sows the seeds of destruction. As long ago as 1919 Cannon concluded that in addition to haemorrhage and infection, some unknown factor was at work in causing fatal shock in wounded soldiers at the front line in World War I. Subsequently, Moore (1959) proposed that infected or damaged tissue elaborates circulating factors ("woundagons") that initiate the fever, cardiovascular and neuroendocrine responses in critical illness. In the early 1980s several groups of workers described mobilisation of amino acids from peripheral tissues in animal models by the plasma of septic patients, due to the presence of a low-molecular weight glycopeptide factor; (Clowes et al., 1983; Baracos et al, 1983; Yang et al., 1983). It has now been

shown that such products are produced by the reticuloendothelial system, and are collectively called cytokines (Dinarello and Mier, 1987). Of the various cytokines, ten interleukins have been described and the list is growing all the time, but the role of some of them remains unknown. Cytokines interleukins 1,2 and 6, tumour necrosis factor (TNF) and interferon gamma, when given intravenously, are generally all capable of inducing virtually the full spectrum of host responses associated with sepsis in a dose-dependent fashion. The most exciting seems to be TNF which is the only cytokine to fulfill Koch's postulates. It is found in the bloodstream at the onset of severe sepsis, although its presence declines rapidly. TNF levels will predict with remarkable accuracy whether death will follow (Girardin et al., 1988; Grave et al., 1989). Administration of TNF initiates changes identical to those after septicaemia such as circulatory collapse, renal failure and disseminated intravascular coagulation. When given as treatment to cancer patients it produces a spectrum of changes otherwise observed in infection, ranging from a mild 'flu-like illness to 'shock lung', depending on the dose. (Michie and Wilmore, 1990). Sublethal injections in dogs initiated the characteristic hormonal and metabolic abnormalities associated with sepsis. There is growing evidence that TNF is the initial signal in lethal sepsis, and neutralisation of TNF prevents death from otherwise lethal gram-negative septicaemia (Tracey et a., 1988; Mathison, Wolfson, Ulevitch, 1987). However, TNF has been preserved throughout mammalian

evolution suggesting that it may have an as yet unidentified beneficial function.

Of the lipid mediators, prostaglandin blockade with nonsteroidal anti-inflammatory agents such as ibuprofen or diclofenac has been shown to reduce the metabolic abnormalities that occur following endotoxic shock (Urbascheck, 1985; Slotman, Burchard, Yeltin, 1985) and to reduce the toxic effects of interleukin-2 treatment in cancer patients (Eberlein et al., 1989). Shaw and Wolfe (1988) assessed the effect of a variety of forms of metabolic intervention on both energy and protein metabolism in 44 severely ill surgical patients. Patients were studied either in the basal state or receiving TPN, and metabolic effects were assessed using the primed-constant infusion of a combination of stable isotopes and radioisotopes. The following perturbations were used: somatostatin infusion to decrease insulin, growth hormone, and glucagon available; naloxone infusion to decrease endorphin availability; and two forms of prostaglandin blockade using either diclofenac or dypyrindamole. The rationale for the use of ranitidine and naloxone is that Brackett et al., (1985) have shown that the use of H₁ and H₂ histamine receptor antagonists reduced haemodynamic and metabolic abnormalities in endotoxaemia in the rat, and blocking release of endorphins from the brain with the use of naloxone improves survival in animals suffering from experimental shock (Holoday and Faden, 1978). Infusion of somatostatin, ranitidine or naloxone resulted in a significant decrease in the rate of net protein

catabolism in severely ill surgical patients both in the basal state and during TPN. The agents did not influence glucose kinetics in any major way. Prostaglandin antagonists caused increase in insulin levels and reduction of glucose turnover, but no change in net protein catabolism.

2) Provision of specific nutrients.

It has not been part of the research described in this thesis to examine the effects of a change in composition of amino acid solutions. There is probably little to choose between the various proprietary amino acid solutions, although in theory if disproportionately large amounts of one or more amino acids are relied upon to provide sufficient nitrogen, there may be increasing urinary losses of nitrogen and increasing blood levels of urea and ammonia. Otherwise, no firm recommendations for the composition of a parenteral amino acid solution can be given, apart from following the aminogram of egg protein which has one of the highest biological values (WHO/FAO report, 1986). However, in critically ill patients with catabolic disease the requirements for specific nutrients may be altered. For example, some workers have argued for the beneficial effects of a high concentration of the branched chain amino acids (BCAA). There are two reasons for believing that such solutions may help - firstly, because they are mainly oxidised in skeletal muscle they may spare such protein loss in postoperative and septic patients; secondly, solutions with a high BCAA/low aromatic AA may benefit patients with liver disease who could not tolerate high intakes of normal amino acid mixtures. On this basis the use

of solutions with high (40-50%) BCAA content has been proposed. Such solutions are 2-5 times more expensive than standard amino acid solutions.

Results of controlled clinical trials have not been promising and the conclusion of a consensus meeting (ASPEN, 1985) was that until greater benefits could be demonstrated supplemented BCAA formulations should be considered only as a research tool (Brennan et al., 1986). The BCAA appear to sensitise muscle protein synthesis to the action of insulin (Garlick & Grant, 1988) and hence such solutions may be more valuable in patients who are insulin-resistant.

Another possible approach is additional supply of glutamine. This is the most abundant free amino acid and previously considered to be non-essential. However, glutamine is a specific fuel for mucosal cells of the small and large bowel, and cells of the immune system, and malnutrition with sepsis or stress causes a fall in the intracellular amino acid pool in skeletal muscle where it normally constitutes 60% of total. This fall is not prevented by conventional TPN, and in severely ill ITU patients correlates with survival (Roth et al., 1982). A triple hormone infusion of adrenaline, cortisol and glucagon simulating surgical stress depresses the glutamine concentration after six hours (Wernerman et al., 1987), and after elective surgery a fall is present within 12 hours (Essen et al., 1988).

For pharmaceutical reasons, commercially available amino acid solutions do not contain glutamine. Glutamine is unstable in aqueous solutions at high concentration, and must be added immediately before administration. An

alternative is to use the stable dipeptide, alanyl-glutamine, which undergoes hydrolysis after infusion.

In one study, postoperative administration of glutamine-supplemented TPN improved nitrogen balance and enhanced synthesis of polyribosomes, an effect attributed to an increase in synthesis of skeletal muscle protein (Hammarquist et al., 1989). Similar results were later obtained by the same group using alanyl-glutamine as a stable means of providing glutamine (Hammarquist et al., 1990).

In conclusion to this section, it seems that history is repeating itself in that there is increasing interest in the use of short-chain protein hydrolysates for TPN. In addition to any possible metabolic advantage such as that described above with alanyl-glutamine, the lower osmolarity of short-chain protein hydrolysates and dipeptides could make peripheral TPN a much more practical possibility than heretofore (Grimble et al., 1988).

3) Administration of growth factors.

In the past application of the well known anabolic effects of exogenous growth hormone was limited by its lack of availability, being derived from human pituitary extracts, although one study examining its effects on the metabolic response to trauma was conducted as long ago as 1967. (Soroff et al.). The advent of recombinant - DNA technology has made human growth hormone more freely available, although it remains expensive. Normal subjects receiving recombinant human growth hormone (r-hGH) maintained positive nitrogen balance even when receiving only 150g. glucose daily as

non-protein energy (Manson and Wilmore, 1986). Subsequently, nitrogen balance was maintained postoperatively in patients undergoing major elective gastrointestinal surgery, using a combination of growth hormone and hypocaloric nutrition (Ponting et al., 1988; Jiang et al., 1989). In both studies protein kinetics were measured using ^{15}N glycine and in both cases turnover, synthesis and breakdown were markedly increased, but synthesis increased more than breakdown. In normal volunteers, Wolfe et al., (1992a) demonstrated an increase in whole-body and skeletal muscle protein net balance with r-hGH and insulin administration alone, as well as a further increase when r-hGH and insulin are combined. The combined effect seemed to be mediated by an increase in protein synthesis associated with r-hGH and a decrease in protein breakdown observed with insulin. The same group obtained the same results in cancer patients (Wolfe et al., 1992b).

It is now thought that growth hormone may exert its anabolic effects by stimulating the endogenous synthesis of insulin-like growth factor I., whose hypoglycaemic action is about 7.5% as potent as that of insulin on a molar basis, but it also has potent anabolic and growth-promoting effects in animals, (Gular et al., 1988). Its effects in patients have not yet been studied.

Finally, it might be expected that androgenic steroids would have a weak anabolic effect, but this does not appear to have been extensively investigated, although there is anecdotal evidence that they have an effect

additive to that of growth hormone in normal volunteers.

In conclusion, it is necessary to evaluate the role of the ^{15}N glycine method used to measure whole-body nitrogen turnover, protein synthesis and breakdown in the context of the 1990s. Unlike the radioisotope methods, when ^{15}N glycine is used, children can be studied, and repeat measurements can be ethically conducted on the same individuals under different conditions. The oral or intravenous route can be used equally effectively. Studies can be shortened by primed constant infusion, and by using ammonia as end-product. In longer studies either ammonia or urea can be used as end-product, or a mean taken of the two. Results have been obtained in normal volunteers both on normal diet and various intravenous substrates; normal adults of different age groups; children in differing states of nutrition; adults and children after surgery, burns, sepsis, cancer; and the method has also been used in animals in which, of course, ^{15}N incorporation into various tissue proteins can be measured. Thus, the method has been used extensively, but the burst of enthusiasm marked by the number of publications in the late 1970s and early 1980s has died down. Among the reasons for this are undoubtedly the fact that different groups of workers have sometimes obtained different results with similar groups of subjects, and that clearly for valid results to be obtained subjects should have similar and constant dietary intakes. Also, whole-body results are obtained, and in man the only solid tissue which can easily and ethically be studied is skeletal muscle, either by biopsy or forearm aminoacid

exchange. This limits the value of the information obtained.

Results of the studies have, however, made those concerned with surgical metabolism and nutrition realise that a negative nitrogen balance is not always due to "proteolysis" or increased protein breakdown, but may be (and usually is) due to decreased synthesis.

Nevertheless, despite the large number of studies conducted, the knowledge gained about changes in protein synthesis and breakdown has not had a major therapeutic impact in terms of nutritional intervention. Hence, it could be argued that the method has been exploited to the full and should now fall into disuse. However, there has been a resurgence of interest in recent years, now that attention has been focused on other methods of trying to reduce the catabolic response to trauma, or increase the effectiveness of certain nutrients. Examples are the exciting work being done with recombinant human growth hormone, and the effects of certain drugs on protein kinetics as described by Shaw and Wolfe (1988).

An entirely new approach was recently described when the method was used in rats to demonstrate that an increased inspired oxygen concentration could increase protein synthesis (Mimura et al., 1990).

Whereas the efficacy of BCAA and glutamine-enriched solutions has been assessed using patients, it would seem more sensible to compare them with standard amino acid solutions in normal fasting volunteers, either during the same study or at a later date, and studying protein kinetics using ^{15}N glycine. Similarly, the normal fasting human volunteer would seem the

ideal experimental model to further study the effects of recombinant human growth hormone, insulin-like growth factor I (recombinant), somatostatin, anabolic steroids, ranitidine, and even the combination of insulin and growth hormone. It was mentioned in study III that intentional perturbation of the ^{15}N urea enrichment curve might become a research tool in itself. Certainly, any perturbation induced by the hormones mentioned above would be interesting, especially that of growth hormone whose effect on muscle energetics appears to precede any effect on protein synthesis (Jian et al., 1989). The effects of exercise also need to be clarified, and Study II needs to be repeated but with more prolonged, vigorous exercise. The effects of differing inspired oxygen levels on protein kinetics, briefly mentioned above, also need to be repeated, but in normal man.

Future studies could include measurement of protein kinetics in cancer patients, before and during chemotherapy and radiotherapy. Attempted attenuation of the catabolic response by the methods previously outlined will need to be measured in patients, and in surgical patients the simplest experiments will follow standard elective operations of some magnitude, such as colectomy. Studies in critically ill, septic patients, need to continue to be performed as such patients continue to pose the biggest therapeutic challenge, but because of variations in clinical conditions, drug therapy and fluid and electrolyte replacement, they will always be the most difficult to interpret. It is in such patients that long and repeated

studies using constant infusion of ^{15}N glycine may well give the most valuable information, rather than short-term observations.

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APPENDIX

Nitrogen Balance Oral Protocol

Subject	TH (70 Kg)	LF (64 Kg)	RS (93 Kg)	MG (62 Kg)	MEAN					
A - BASELINE I										
Day 1		Gm	Mg/Kg/d	Gm	Mg/Kg/d	Gm	Mg/Kg/d	Gm	Mg/Kg/d	Mg/Kg/d
	In	11.5	164	11.3	176	18	193	11.6	187	
Day 2	Out	8.9	127	4.3	67	13.7	147	9.2	148	
	Bal	2.6	37	7	109	4.3	46	2.4	39	
	In	11.5	164	11.3	176	18	193	11.6	187	
Day 3	Out	9.1	130	10.8	168	11.9	127	9.3	150	
	Bal	2.4	34	0.5	8	6.1	66	2.3	37	
	In	11.5	164	11.3	176	18	193	11.6	187	
Day 4	Out	11.1	158	10.8	168	11.8	126	8.6	139	
	Bal	0.4	6	0.5	8	6.2	67	3	48	
	In	11.5	164	11.3	176	18	193	11.6	187	
Day 5	Out	8.7	124	10	156	12.7	136	10.5	169	
	Bal	2.8	40	1.3	20	5.3	57	1.1	18	33.8 ± 18
	Nbal	2.1	30	2.3	36	5.5	59	2.2	35	40 ± 13
\bar{X}_{1-4}										
B - DOUBLING NITROGEN INTAKE										
Day 5	In	22.4	320	22.3	348	30.5	328	23.3	375	
	Out	14.3	204	15.4	241	18.7	201	13.3	215	
	Bal	8.1	116	6.9	107	11.8	127	10	160	
Day 6	In	22.4	320	22.3	348	30.5	328	23.3	375	
	Out	15.1	216	15.4	241	22.6	243	16	258	
	Bal	7.3	104	6.9	107	7.9	85	7.3	117	103.3 ± 13
$\bar{X}_{5,6}$	NBal	7.7	110	6.9	107	9.9	106	8.9	144	117 ± 18
NITROGEN BALANCE CHANGE - BASELINE I TO DOUBLING										
Δ NBal	4, 6	4.5	64	5.6	87	2.6	28	6.2	99	69.5 ± 31

Table 16

**Nitrogen Balance
Oral Protocol**

Subject		TH (70 Kg)		LF (64 Kg)		RS (93 Kg)		MG (62 Kg)		MEAN
C - BASELINE II										
		Gm	Mg/Kg/d	Gm	Mg/Kg/d	Gm	Mg/Kg/d	Gm	Mg/Kg/d	Mg/Kg/d
Day 7	In	11.5	164	11.3	176	18	193	11.6	187	
	Out	12.8	182	13.2	206	17.2	184	12.9	208	
	Bal	-1.3	-18	-1.9	-30	0.8	9	-1.3	-21	
Day 8	In	11.5	164	11.3	176	18	193	11.6	187	
	Out	10.8	154	11.3	176	15	161	11.3	182	
	Bal	-0.7	-10	0	0	3	32	0.3	5	4
$\bar{X}_{7,8}$	NBal	-1	-14	-0.9	-15	1.9	20	-0.5	-8	- 14 ± 4.3
NITROGEN BALANCE CHANGE - DOUBLING TO BASELINE II										
	NBal 6,8	-8	-114	-6.9	-107	-4.9	-53	-7	-112	- 97 ± 29
D - OMISSION (DROPOUT) OF NITROGEN INTAKE										
Day 9	In	0	0	0	0	0	0	0	0	
	Out	8.2	117	9.7	152	10	110	8.7	140	
	Bal	-8.2	-117	-9.7	-152	-10	-110	-8.7	-140	
Day 10	In	0	0	0	0	0	0	0	0	
	Out	11.8	169	12.3	192	11.2	120	10.3	166	
	Bal	-11.8	-169	-12.3	-192	-11.2	-120	-10.3	-166	- 161.8 ± 30
$\bar{X}_{9,10}$	NBal	-10	-143	-11	-171	-10.6	-115	-9.5	-153	- 146 ± 23
NITROGEN BALANCE CHANGE - BASELINE II TO DROPOUT										
	NBal 8,10	-11.1	-159	-12.3	-192	-14.2	-152	-10.6	-171	- 169 ± 17
E - BASELINE III (24 HRS)										
Day 11	In	11.5	164	11.3	176	18	193	11.6	187	
	Out	10	143	10.5	164	9.4	100	10.9	176	
	Bal	-1.5	-21	0.8	12	8.6	93	-0.7	-11	37 ± 47
NITROGEN BALANCE CHANGE - DROPOUT TO BASELINE III										
	Nbal 10,11	10.3	148	13.1	204	19.8	213	9.6	155	180 ± 33

Table 17

EFFECT OF DIETARY CHANGES ON NITROGEN BALANCE IN 4 NORMAL SUBJECTS

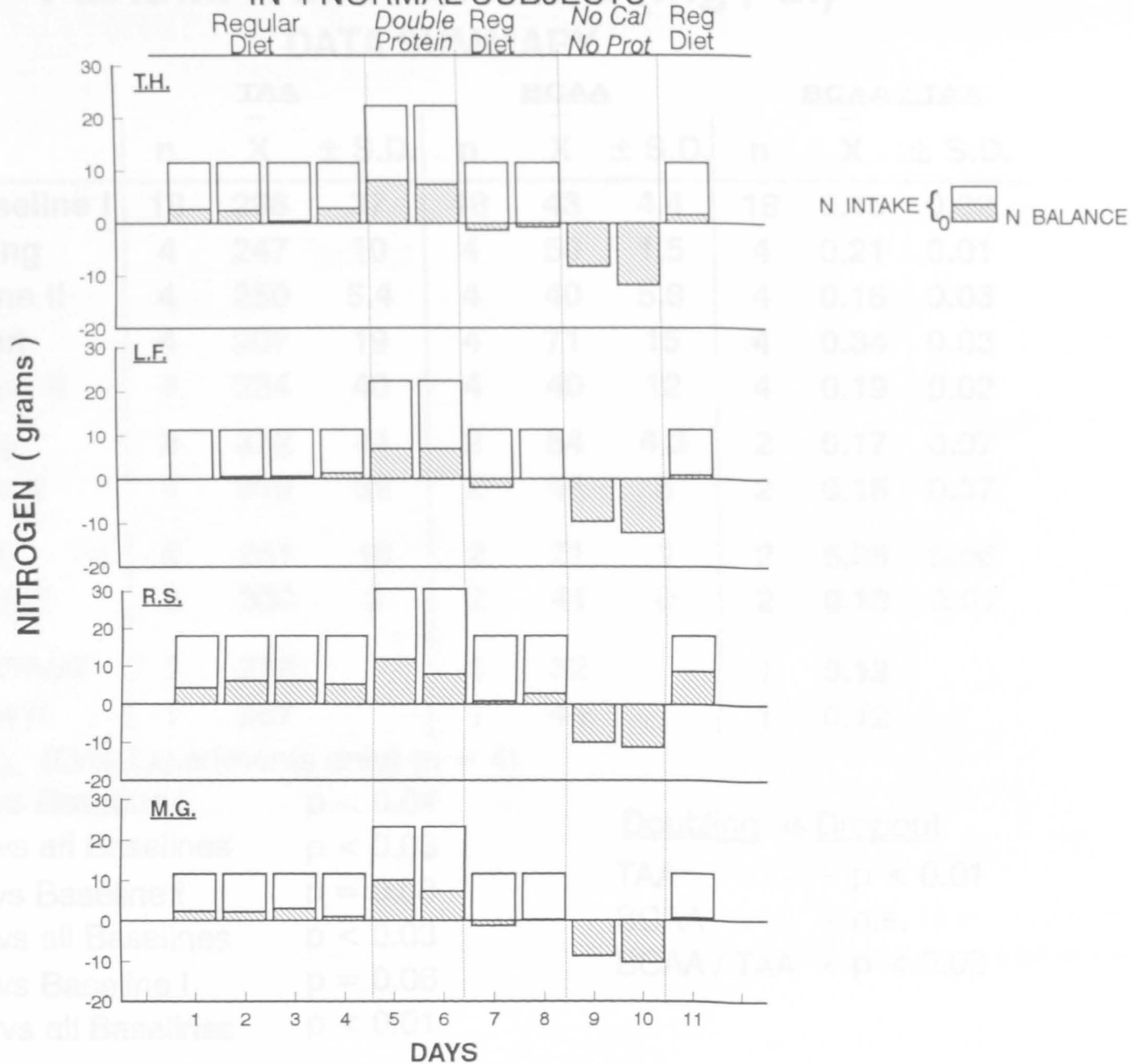


FIGURE XXXIV

Nitrogen balances, four subjects, oral intake, long protocol.

PLASMA AMINO ACIDS (mg / dl)

DATA SUMMARY

	<u>TAA</u>			<u>BCAA</u>			<u>BCAA / TAA</u>		
	n	\bar{X}	\pm S.D.	n	\bar{X}	\pm S.D.	n	\bar{X}	\pm S.D.
Resting Baseline I	18	286	37	18	43	4.4	18	0.15	0.02
Oral Doubling	4	247	10	4	50	1.5	4	0.21	0.01
Oral Baseline II	4	250	5.4	4	40	5.8	4	0.16	0.03
Oral Dropout	4	207	19	4	71	15	4	0.34	0.03
Oral Baseline II	4	234	40	4	40	12	4	0.19	0.02
I.V. Doubling	2	332	44	2	54	4.3	2	0.17	0.07
I.V. Baseline II	2	316	32	2	46	5	2	0.15	0.07
I.V. Dropout	2	281	18	2	71	1	2	0.25	0.06
I.V. Baseline II	2	330	3	2	41	0	2	0.13	0.01
I.V. CAL O PROT	1	278		1	32		1	0.12	
I.V. Baseline II	1	367		1	44		1	0.12	

Significance Levels (Oral Experiments only) (n = 4)

TAA Doubling vs Baseline I p < 0.04

Dropout vs all Baselines p < 0.03

BCAA Doubling vs Baseline I p = 0.08

Dropout vs all Baselines p < 0.03

BCAA/TAA Doubling vs Baseline I p = 0.06

Dropout vs all Baselines p < 0.01

Doubling vs Dropout

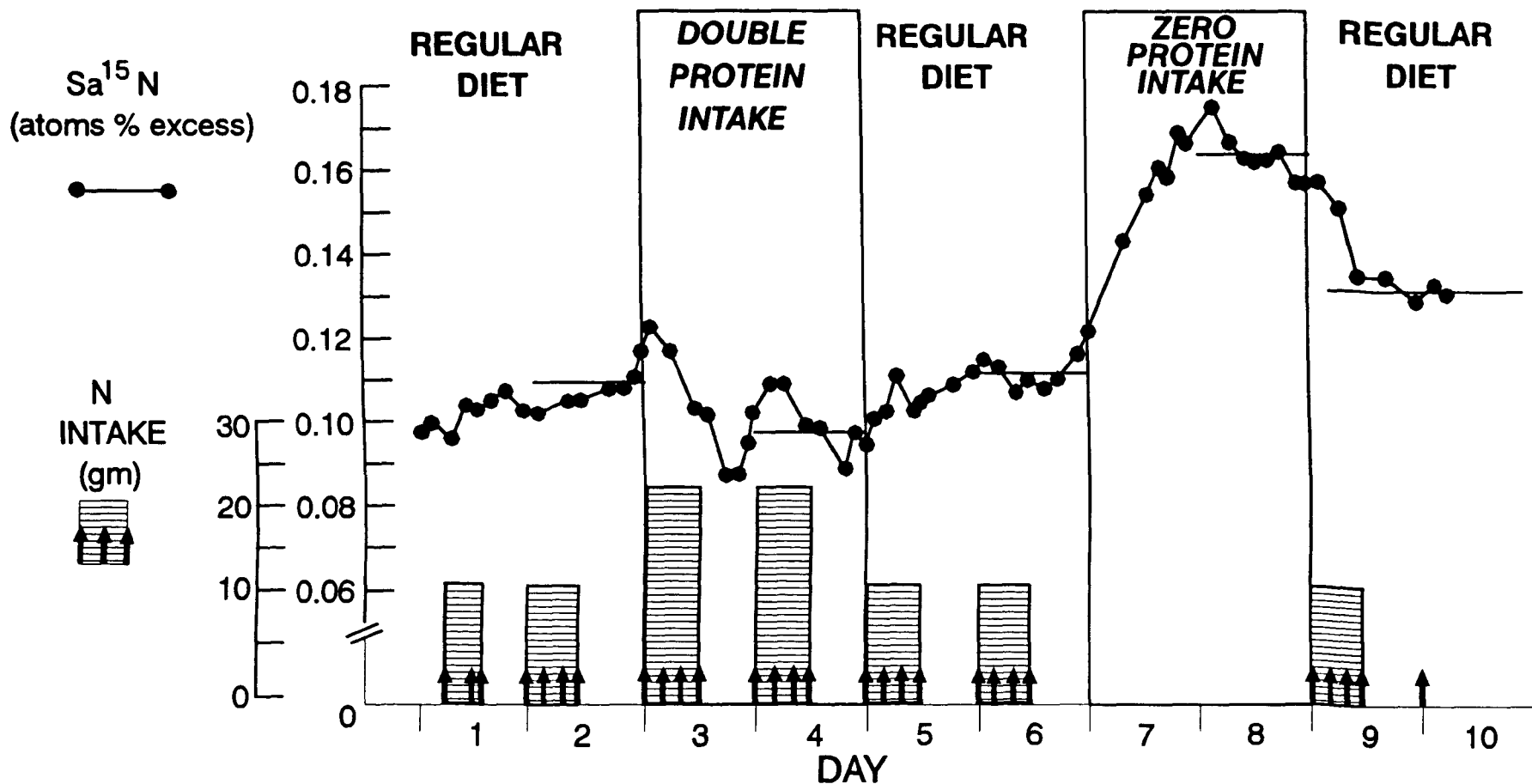
TAA - p < 0.01

BCAA - n.s.

BCAA / TAA - p < 0.02

TABLE 18

DIETARY NITROGEN INTAKE CHANGES
EFFECT ON ^{15}N GLYCINE EQUILIBRIUM
 T.H. - Normal Subject (^{15}N and Diet p.o.)

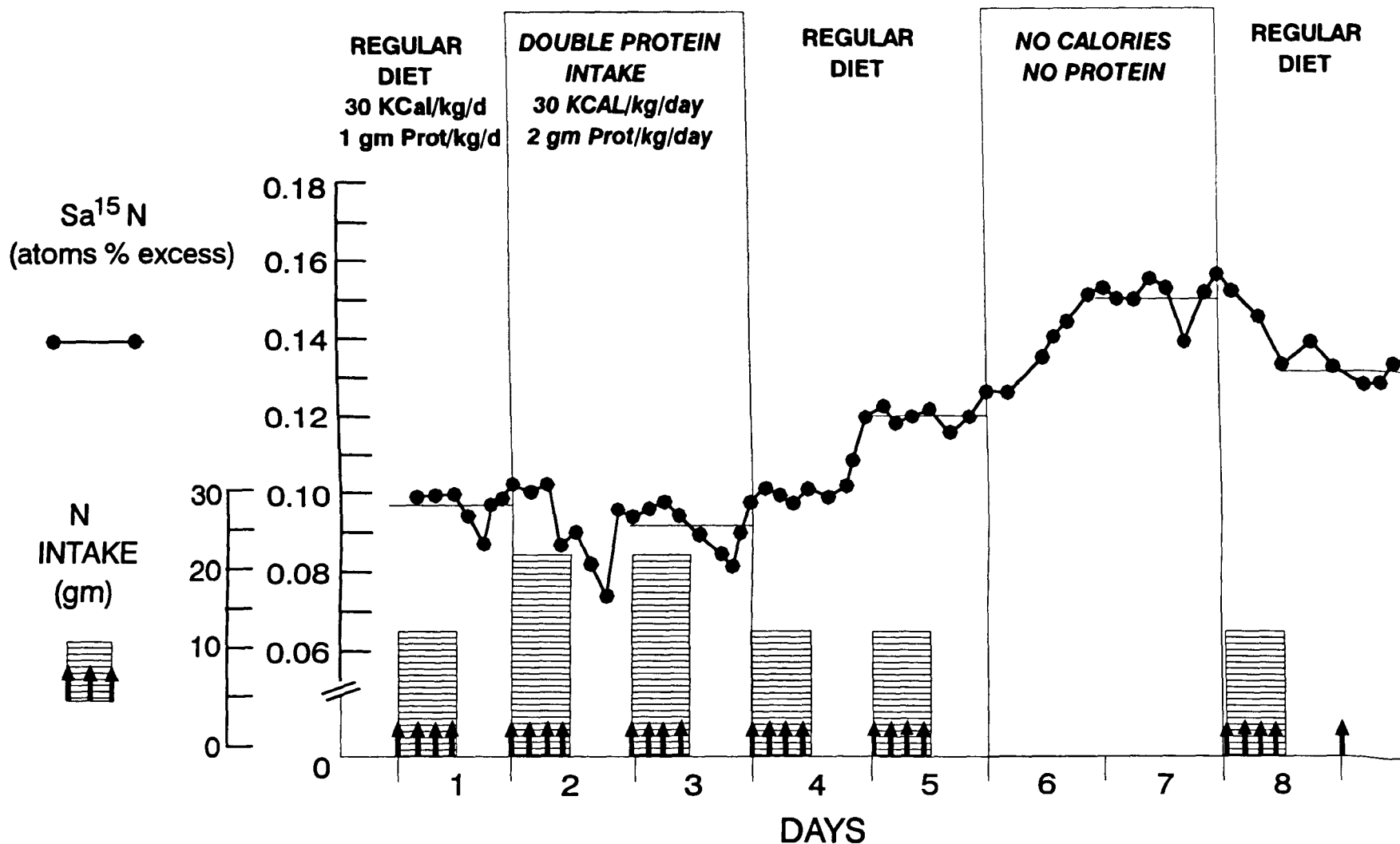


Here, and in Figures XXXVI to XXXVIII are shown the sequential values for urea ^{15}N enrichment in the four subjects on oral intake, long protocol. In the lower part of the chart are shown proportional crosshatched areas to indicate the intake changes. The above curve is for subject T.H.

FIGURE XXXV

NITROGEN TURNOVER, CATABOLISM AND SYNTHESIS USING ^{15}N GLYCINE IN NORMAL SUBJECTS ON ORAL DIET
 EFFECT OF SUDDEN CHANGE IN PROTEIN INTAKE

L.F. - N Dose (0.5 mg/kg/day) and Diet p.o.



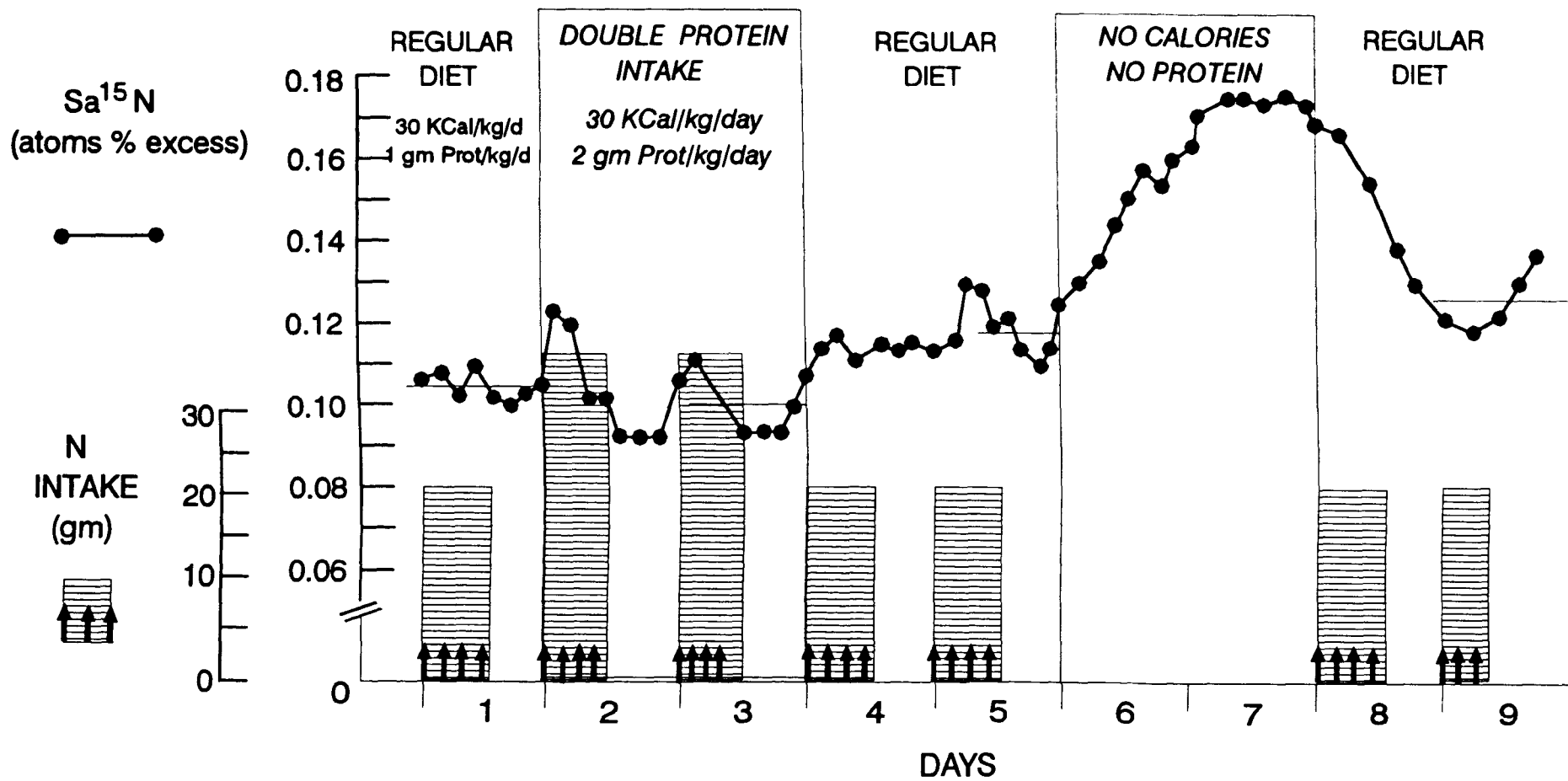
Urea ^{15}N enrichment. Subject L.F.

FIGURE XXXVI

NITROGEN TURNOVER, CATABOLISM AND SYNTHESIS USING ^{15}N GLYCINE IN NORMAL SUBJECTS ON ORAL DIET

EFFECT OF SUDDEN CHANGE IN PROTEIN INTAKE

R.S. - ^{15}N Dose (0.5 mg/kg/day) and Diet p.o.



Urea ^{15}N enrichment. Subject R.S.

FIGURE XXXVII

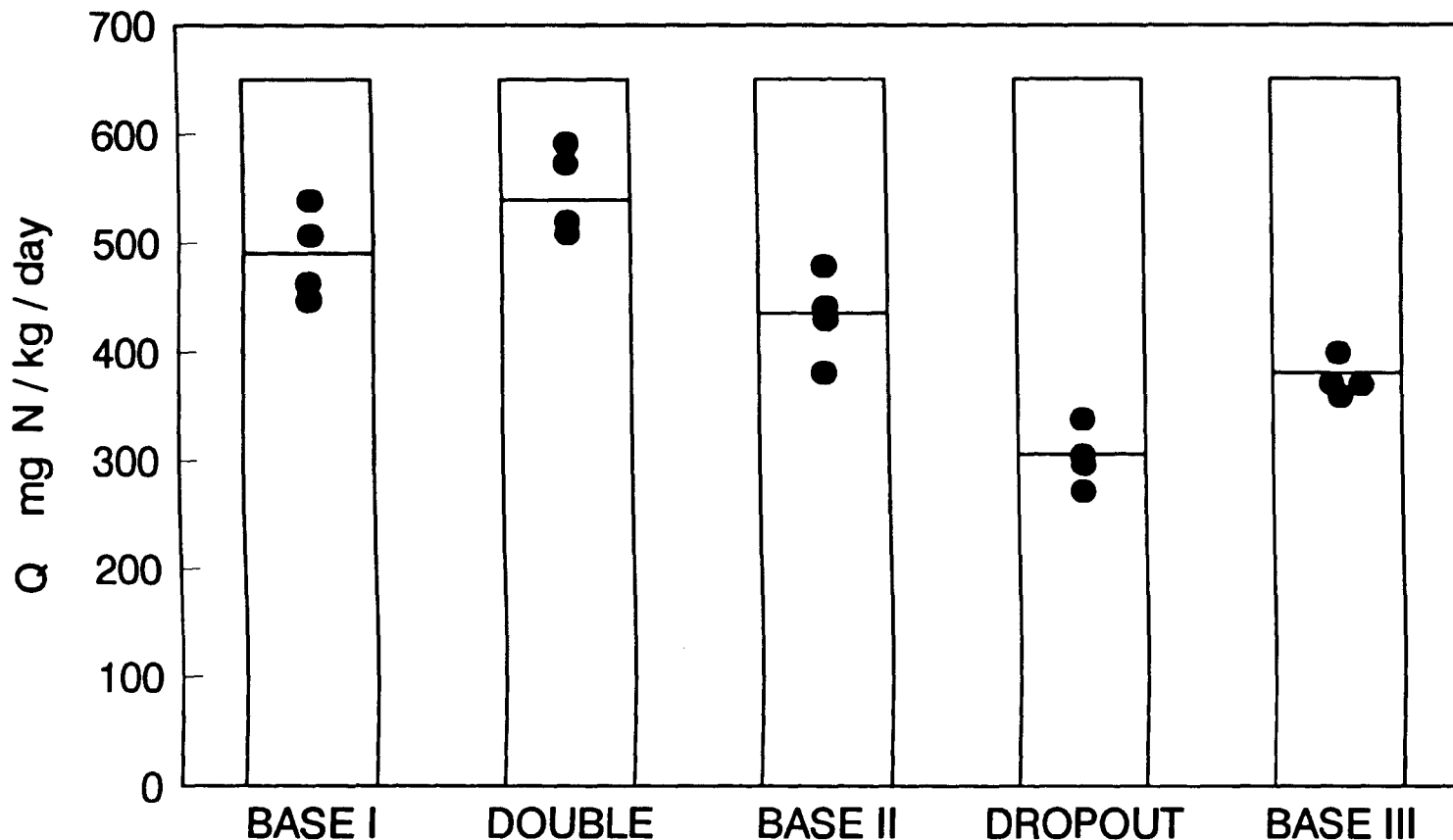
ISOTOPE EQUILIBRIUM PLATEAUX DATA AND CALCULATION OF Q, S AND C ORAL INTAKE - LONG PROTOCOL*

Subject	TH (70 Kg)	LF (64 Kg)	RS (93 Kg)	MG (62 Kg)	MEAN
Baseline I (Hrs)	48 - 72	48 - 72	48 - 72	48 - 72	
X Sa ± S.D.	0.1108 ± 0.0046	0.0946 ± 0.0042	0.1092 ± 0.0032	0.0991 ± 0.0072	
± C.V. (n)	± 4.2% (7)	± 4.4% (8)	± 2.9% (8)	± 7.3% (8)	
Q (Mg/Kg/d)	452	529	458	505	486 ± 37
S (Mg/Kg/d)	319	372	324	335	338 ± 24
C (Mg/Kg/d)	286	352	265	318	305 ± 38
Doubling I (Hrs)	24 - 48	24 - 48	24 - 48	24 - 48	
X Sa ± S.D.	0.0960 ± 0.0062	0.0895 ± 0.0056	0.0968 ± 0.0091	0.0876 ± 0.0070	
± C.V. (n)	± 6.4%	± 6.2% (8)	± 9.4% (8)	± 8.0% (8)	
Q (Mg/Kg/d)	521	559	516	571	542 ± 27
S (Mg/Kg/d)	306	319	274	315	304 ± 20
C (Mg/Kg/d)	201	209	187	193	198 ± 9.6
Baseline II (Hrs)	24 - 48	24 - 48	24 - 48	24 - 48	
X Sa ± S.D.	0.1138 ± 0.0049	0.1162 ± 0.0038	0.1262 ± 0.0060	0.1230 ± 0.0061	
± C.V. (n)	± 4.3% (8)	± 3.2% (7)	± 4.8% (8)	± 5.0% (8)	
Q (Mg/Kg/d)	439	430	396	471	434 ± 30
S (Mg/Kg/d)	284	253	235	289	265 ± 25.7
C (Mg/Kg/d)	274	253	203	284	253 ± 36
Dropout (Hrs)	24 - 48	24 - 48	24 - 48	24 - 48	
X Sa ± S.D.	0.1647 ± 0.0067	0.1529 ± 0.0063	0.1765 ± 0.0025	0.1668 ± 0.0028	
± C.V. (n)	± 4.1% (8)	± 4.1% (7)	± 1.5% (7)	± 1.7% (8)	
Q (Mg/Kg/d)	304	327	283	300	303 ± 18
S (Mg/Kg/d)	140	136	163	135	143 ± 13
C (Mg/Kg/d)	304	328	283	300	303 ± 18
Baseline III (Hrs)	9 - 27	12 - 29	12 - 27	9 - 27	
X Sa ± S.D.	0.1347 ± 0.0022	0.1278 ± 0.0035	0.1348 ± 0.0058	0.1382 ± 0.0048	
± C.V. (n)	± 1.6% (6)	± 2.8% (5)	± 3.4% (5)	± 3.5% (6)	
Q (Mg/Kg/d)	371	391	371	362	373 ± 12
S (Mg/Kg/d)	236	227	269	186	229 ± 34
C (Mg/Kg/d)	207	214	177	175	193 ± 20

* In all cases the isotope dose is 0.5 Mg ¹⁵N/Kg/d; all data for Q,S and C are in MgN/Kg/d. Isotope enrichment (Sa) is given as the absolute fraction(¹⁵N / Total N); after each coefficient of variation (cv) is given the number of points on which the plateau calculation was based.

TABLE 19

WHOLE BODY PROTEIN TURNOVER RATE " Q "
A. LONG PROTOCOL (ORAL)



Values for whole body nitrogen turnover rate ("Q") in mgN/Kg/day, for the four subjects on oral diet (long protocol). These bar diagrams show the individual values for Q under the five dietary conditions studied, as well as a linear display of the mean. Statistical significance of differences are indicated in the text.

FIGURE XXXIX

**CHANGES IN Q AND N BALANCE AND Q : N RATIOS
ORAL INTAKE - LONG PROTOCOL (Q and N Bal in Mg/Kg/day)**

SUBJECT	TH	LF	RS	MG	MEANS
- A - CHANGE FROM BASELINE I TO DOUBLING N INTAKE					
BASLINE I Q	452	529	458	505	
DOUBLING Q	521	59	516	571	
Δ Q	69 (15%)	30 (6%)	58 (13%)	66 (13%)	56 ± 18
BASELINE I N BAL	40	20	57	18	
DOUBLING N BAL	104	107	85	117	
Δ N Bal	(+) 64	(+) 87	(+) 28	(+) 99	(+) 70 ± 31
Q : N RATIO	1.08	0.34	2.07	0.67	1.04 ± 0.75
- B - RETURN FROM DOUBLING TO BASELINE II					
DOUBLING Q	521	559	516	571	
BASELINE II Q	439	430	396	471	
Δ Q	- 82 (16%)	-129 (30%)	- 120 (23%)	- 100 (18%)	- 96 ± 41
DOUBLING N BAL	104	107	85	117	
BASELINE II N BAL	-10	0	32	5	
Δ N Bal	(-) 114	(-) 107	(-) 53	(-) 112	(-) 96 ± 29
Q : N RATIO	0.72	1.2	2.26	0.89	1.26 ± 0.69
- C - CHANGE FROM BASELINE II TO DROPOUT (ZERO N)					
BASELINE II Q	439	430	396	471	
DROPOUT Q	304	327	283	300	
Δ Q	- 135 (31%)	- 103 (24%)	- 113 (29%)	- 171 (40%)	- 130 ± 30
BASELINE II N BAL	-10	0	32	5	
DROPOUT N BAL	-169	-192	-120	-166	
Δ N Bal	(-) 159	(-) 192	(-) 151	(-) 171	(-) 168 ± 18
Q : N RATIO	0.85	0.54	0.75	1	0.79 ± 0.19
- D - RETURN TO BASELINE III (24 HRS) FROM DROPOUT					
DROPOUT Q	304	327	283	300	
BASELINE III Q	371	391	371	362	
Δ Q	57 (22%)	64 (20%)	87 (31%)	62 (20%)	130 ± 30
DROPOUT N BAL	-169	-192	-120	-166	
BASELINE III N BAL	-21	12	93	-11	
Δ N Bal	(+) 148	(+) 204	(+) 213	(+) 155	180 ± 33
Q : N RATIO	0.45	0.32	0.41	0.4	0.39 ± 0.06

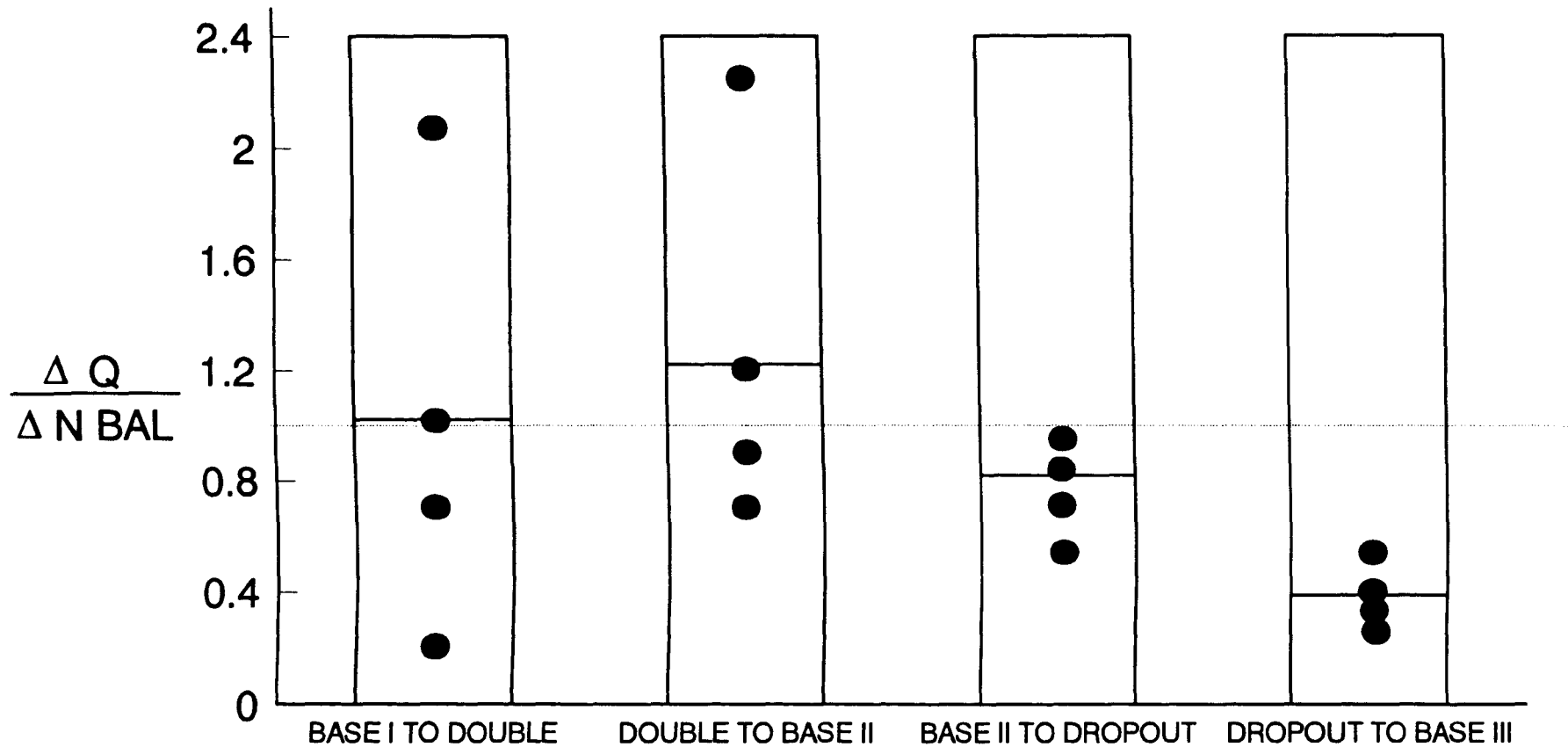
Q : N Ratio means: All 48 - hr Equilibria (1st 3 changes) (n=12) : 1.03 ± 0.57

TABLE 20

By subjects (48 - hr Eq) : TH - 0.88 ± 0.18; LF - 0.69 ± 0.45; LS - 1.69 ± 0.82; 0.85 ± 0.17

$$Q : N \text{ RATIO } \left(\frac{\Delta Q}{\Delta N \text{ BAL}} \right)$$

A. LONG PROTOCOL (ORAL)



Q : N ratio, four subjects, long protocol, oral intake. The ratio of the change in whole body protein turnover rate (Q) to the change in chemically measured nitrogen balance is shown for each of the four changes sequentially made between the five dietary regimens.

Probability of significant departure from 1.0 for each set of changes is indicated in the text.

FIGURE XL

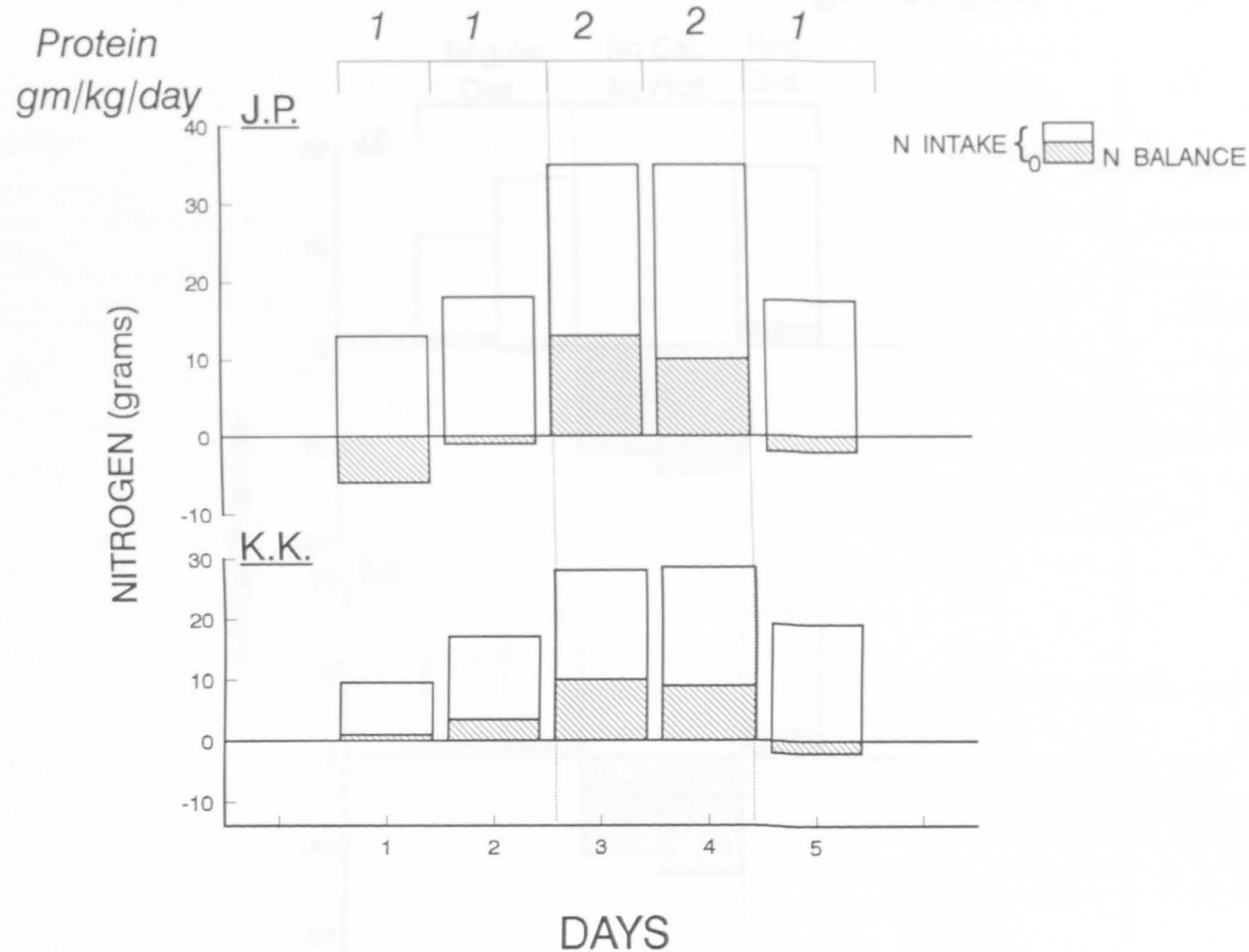
NITROGEN BALANCE - INTRAVENOUS INTAKE
DOUBLING AND DROPOUT

SUBJECT		JP (104 Kg)		KK (91 Kg)		JP (104 Kg)		KK (91 Kg)	
A - BASELINE I						A - BASELINE I			
		GM	11/6-9 Mg/Kg/d	GM	12/15-19 Mg/Kg/d	GM	11/27-1 Mg/Kg/d	GM	11/14-18 Mg/Kg/d
DAY 1	In	12	115	8.4	92	11	205	9.8	107
	Out	18.2	175	8.3	91	10.6	102	7.3	80
	Bal	-6.2	-60	0.8	7	0.4	3	2.5	27
DAY 2	In	17	163	14.9	163	15.8	150	14.3	156
	Out	18.3	175	11	121	15.9	152	13.8	150
	Bal	-1.3	-12	3.9	43	-0.1	-2	0.5	5
$\bar{X}_{1,2}$	Bal	-3.4	-33	2.4	25	0.2	3	1.5	16
B - DOUBLING N INTAKE					B - DROPOUT N INTAKE				
DAY 3	In	35	336	26.8	294	0	0	0	0
	Out	22.1	213	16.9	185	12.8	123	12.1	132
	Bal	13.1	123	9.9	109	-12.8	-123	-12.1	-132
DAY 4	In	35	336	26.6	291	0	0	0	0
	Out	25.7	247	21.6	237	16.9	162	13.4	146
	Bal	9.3	89	5	54	-16.9	-162	-13.4	-146
$\bar{X}_{3,4}$	Bal	11.2	106	7.4	81	-14.8	-142	-12.7	-138
CHANGE : BASELINE I TO DOUBLING					CHANGE : BASELINE I TO DROPOUT				
Δ N Bal _{2,4}		10.6	101	1.1	11	-16.8	-160	-13.9	-151
C - BASELINE II					C - BASELINE II				
DAY 5	In	17	163	16.2	178	17	163	14.6	160
	Out	19.4	187	18.8	206	14.5	139	11.2	122
	Bal	-2.4	-24	-2.6	-28	2.5	24	3.4	37
CHANGE : DOUBLING TO BASELINE II					CHANGE : DROPOUT TO BASELINE II				
Δ N Bal _{4,5}		-11.7	-113	-7.6	-82	19.4	186	16.8	183

TABLE 21

EFFECT OF PROTEIN DOUBLING ON NITROGEN BALANCE IN NORMAL SUBJECTS

Constant I.V. Diet : 30 K Cal/kg/day

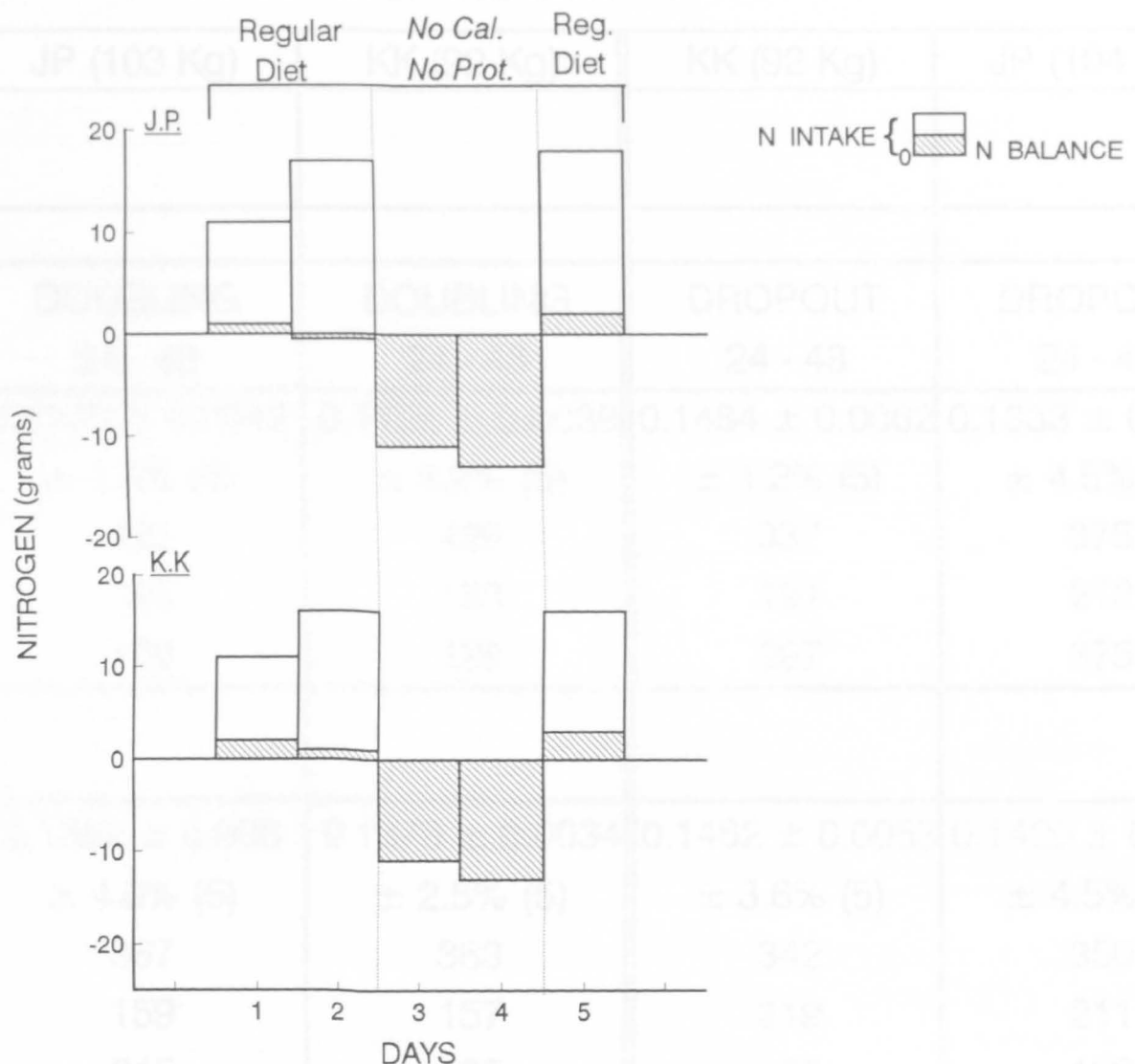


Nitrogen balance, two subjects (J.P. and K.K.) intravenous intake, doubling of intake, and return to baseline.

FIGURE XLI

EFFECT OF CALORIE AND PROTEIN WITHDRAWAL ON NITROGEN BALANCE IN NORMAL SUBJECTS

Regular Diet: Constant I.V. { 30 KCal/kg/day
1 gm Prot/kg/day



Nitrogen balance, two subjects (J.P. and K.K), intravenous intake, cessation of macronutrients, and return to baseline.

FIGURE XLII

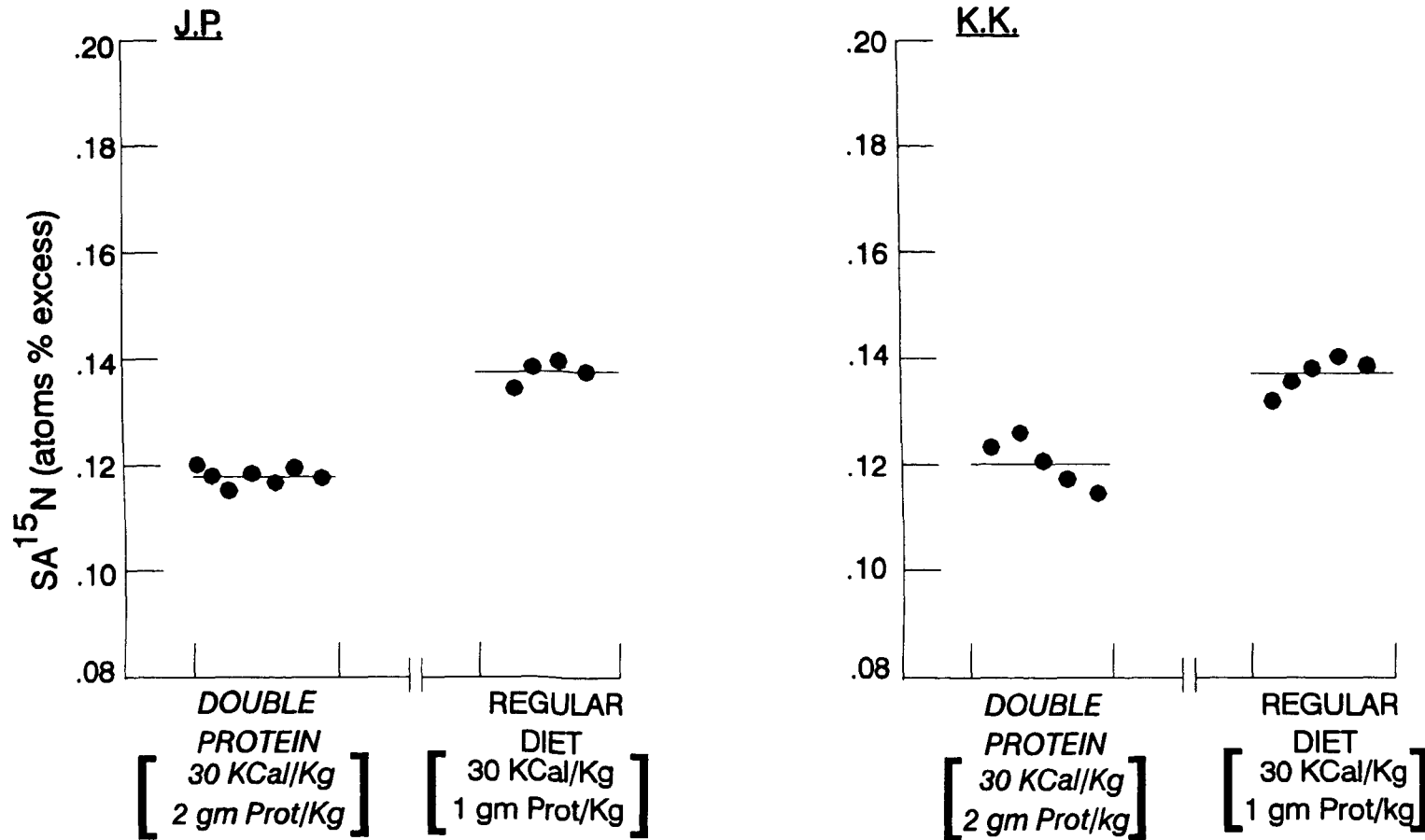
**ISOTOPE EQUILIBRIUM PLATEAUX DATA AND CALCULATION OF Q, S, AND C
INTRAVENOUS INTAKE**

1 - DOUBLING AND DROPOUT

Subject:	JP (103 Kg)	KK (92 Kg)	KK (92 Kg)	JP (104 Kg)
Baseline I (OVERPRIME)				
CHANGE:				
DOUBLING (Hrs)	DOUBLING 24 - 48	DOUBLING 24 - 48	DROPOUT 24 - 48	DROPOUT 24 - 48
X Sa ± S.D. ± C.V. (n)	0.1185 ± 0.0042 ± 3.4% (5)	0.1192 ± 0.0039 ± 3.2% (5)	0.1484 ± 0.0062 ± 4.2% (5)	0.1333 ± 0.0060 ± 4.5% (5)
Q (Mg/Kg/d)	422	420	337	375
S (Mg/Kg/d)	173	183	191	213
C (Mg/Kg/d)	103	128	297	375
Baseline II (Hrs)				
X Sa ± S.D. ± C.V. (n)	0.1362 ± 0.006 ± 4.0% (5)	0.1376 ± 0.0034 ± 2.5% (5)	0.1462 ± 0.0053 ± 3.6% (5)	0.1429 ± 0.0064 ± 4.5% (5)
Q (Mg/Kg/d)	367	363	342	350
S (Mg/Kg/d)	159	157	219	211
C (Mg/Kg/d)	215	185	182	193

TABLE 22

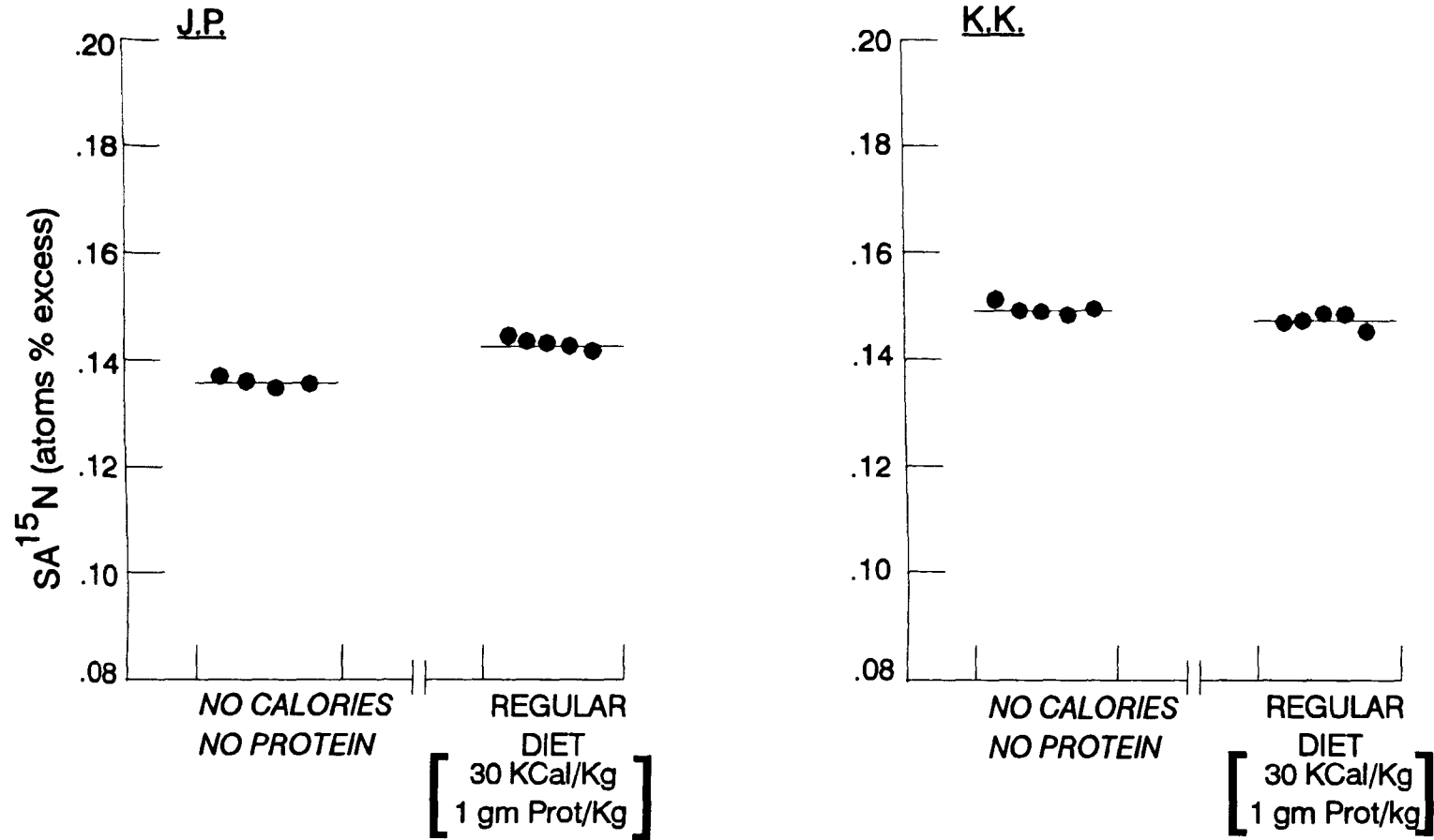
EFFECT OF PROTEIN DOUBLING
ON ^{15}N GLYCINE EQUILIBRIUM
Constant I.V. Diet and ^{15}N Glycine



Here, and in Figures XLVI, XLIX, and L, are shown the urine urea ^{15}N enrichment points at equilibrium, for the subjects on intravenous intake. The intervening connecting points (shown in Figures XXXV - XXXVIII for oral intake) were not measured in these instances. Here are shown the plateau ^{15}N urea enrichments for doubling protein and return to regular diet.

FIGURE XLIII

EFFECT OF CALORIE AND PROTEIN WITHDRAWAL
ON ^{15}N GLYCINE EQUILIBRIUM
Constant I.V. Diet and ^{15}N Glycine

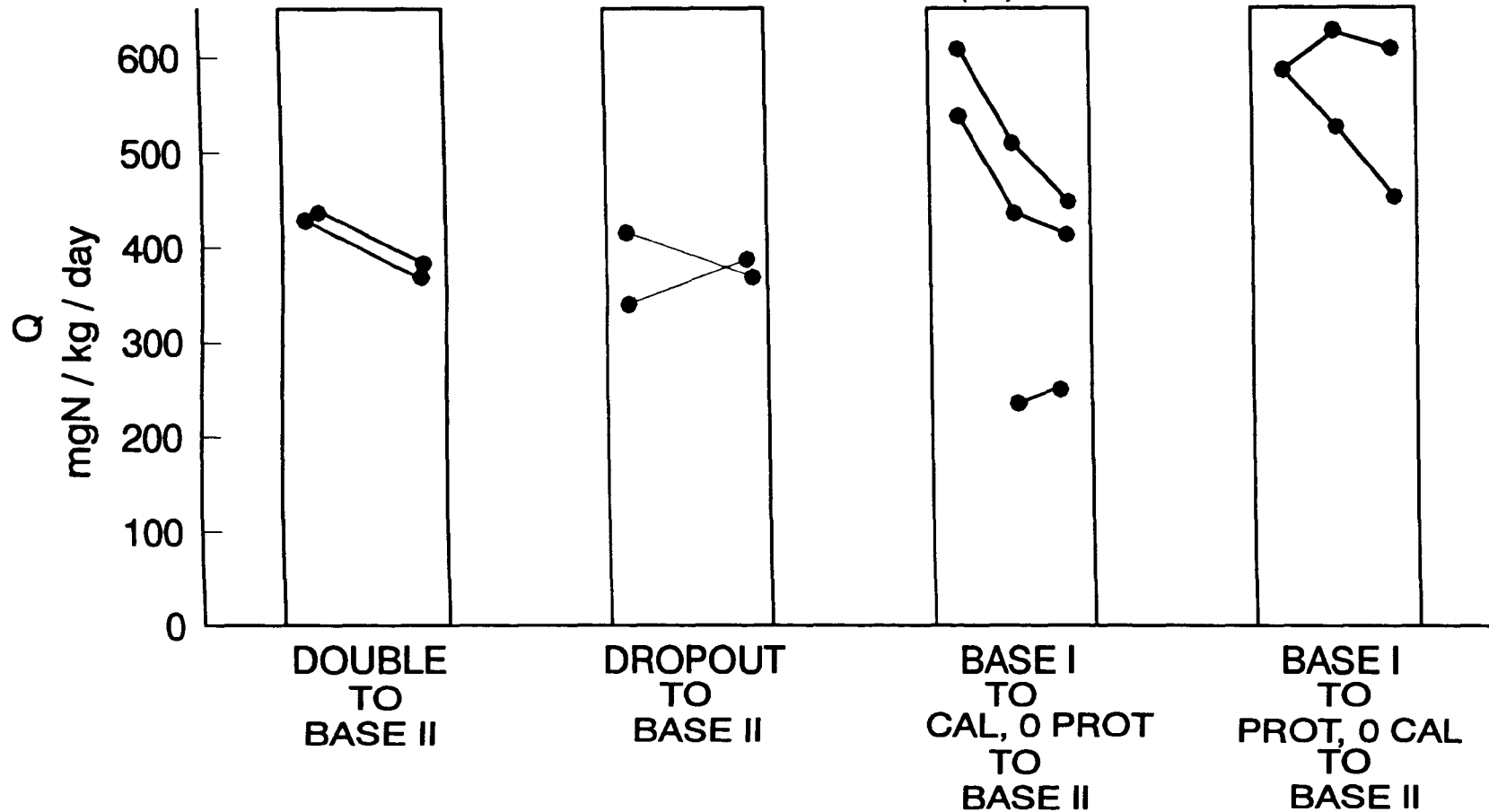


See Figure XLIII. Here are shown the plateau ^{15}N urea enrichments on dietary macronutrient withdrawal in two subjects on intravenous intake, followed by return to regular diet.

FIGURE XLIV

WHOLE BODY PROTEIN TURNOVER RATE "Q"

B. SHORT PROTOCOL (I.V.)



Changes in body nitrogen turnover rate ("Q") in mgN/Kg/day for nine experiments based on intravenous intake. From left to right the bar diagrams show (1) changes for return to Baseline from Doubling, (2) return to Baseline from Dropout, (3) three stages (before, during and after) of caloric provision without amino acids (4) (last set to the right) the three stages (before, during and after) of administration of amino acids without calories. In each of the last two instances, the central point of the three represent the calorie-nitrogen manipulation and the points to the left and right indicate the baseline before and after the change.

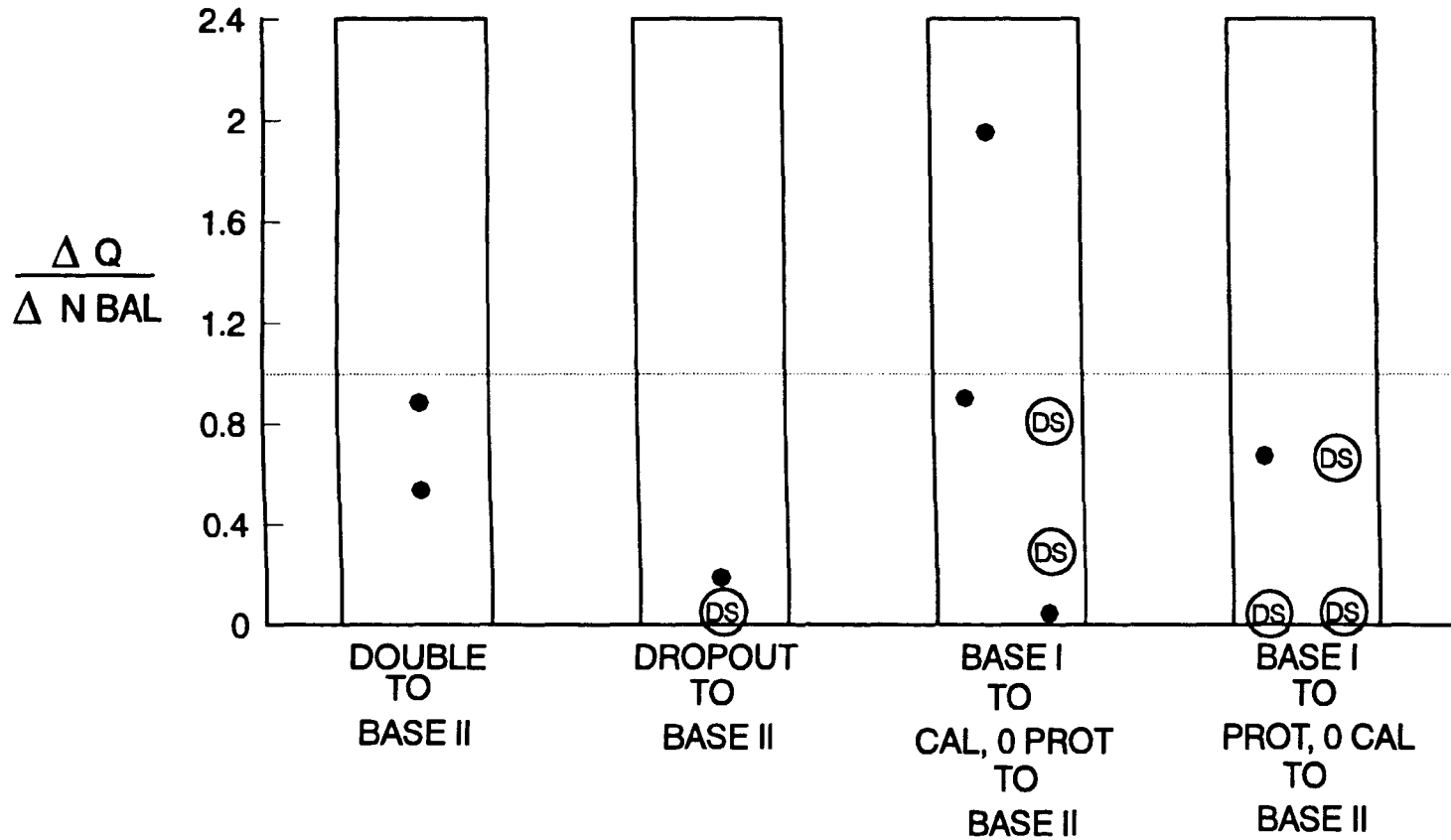
FIGURE XLV

CHANGES IN Q AND N BALANCE AND Q : N RATIO
 INTRAVENOUS INTAKE
 DOUBLING AND DROPOUT

SUBJECT	JP	KK	KK	JP
BASELINE I (OVERPRIME)				
	DOUBLING TO BASELINE II		DROPOUT TO BASELINE II	
Q ₂	422	420	376	337
BASELINE II Q	636	363	351	341
Δ Q	-55	-57	-25	4
N BAL ₂	89	54	-162	-146
BASELINE II N BAL	-24	-28	24	37
Δ N BAL	(-) 113	(-) 82	(+) 186	(+)183
Q : N RATIO	0.49	0.7	DS (very low)	0.02

TABLE 23

Q : N RATIO $\left(\frac{\Delta Q}{\Delta N \text{ BAL}} \right)$
B. SHORT PROTOCOL (I.V.)



See Figure XL. Relationship of change in whole body nitrogen turnover rate to change in chemically measured nitrogen balance (Q:N ratio) in subjects on intravenous intake. To the left are shown the data for the two changes from Doubling to Baseline and from Dropout of macronutrients to Baseline. To the right are shown the two sets of changes involved with calorie-nitrogen manipulation. "DS" indicates ratios of different sign, the relative heights above the line being an approximation of the magnitude of the ratio.

FIGURE XLVI

**NITROGEN BALANCE - INTRAVENOUS INTAKE
CALORIE : NITROGEN MANIPULATION**

SUBJECT	JP (104 Kg)		WT ₁ (82 Kg)		WT ₃ (83 Kg)		WT ₂ (84 Kg)		MC (83 Kg)	
A - BASELINE I										
	GM	Mg/Kg/d	GM	Mg/Kg/d	GM	Mg/Kg/d	GM	Mg/Kg/d	GM	Mg/Kg/d
DAY 1 In	10.4	100	8.9	108	7.7	93	10.2	124	8.2	99
Out	11.4	109	6.8	83	8	97	9.7	117	11.8	143
Bal	-1	-9	2.1	25	-0.3	-4	0.5	7	-3.6	-44
DAY 2 In	16	154	11.3	137	11.2	135	13.9	168	11.9	144
Out	14.9	143	10.9	132	8.7	105	11.7	142	11.3	136
Bal	1.1	11	0.4	5	2.5	30	2.2	26	0.6	8
$\bar{X}_{1,2}$ Bal	0	0	1.2	15	1.1	13	1.3	16	-1.9	-23
B - CALORIES WITHOUT PROTEIN							B - PROTEIN WITHOUT CALORIES			
DAY 3 In	0	0	0	0	0	0	12.2	147	12	145
Out	8.8	84	9.7	188	7.6	92	14.6	176	19	230
Bal	-8.8	-84	-9.7	-188	-7.6	-92	-2.4	-2	-7	-85
DAY 4 In	0	0	0	0	0	0	12.9	156	11.9	144
Out	6.9	66	5.3	64	5.6	67	20.4	247	21.6	261
Bal	-6.9	-66	-5.3	-64	-5.6	-67	-7.5	-91	-9.7	-117
$\bar{X}_{3,4}$ Bal	-7.8	-75	-7.5	-91	-6.6	-80	-4.9	-59	-8.4	-102
CHANGE : BASELINE I TO CAL, 0 PROT							CHANGE : BASELINE I TO PROT, 0 CAL			
N Bal _{2,4}	-8	-77	-5.7	-69	-8.1	-98	-9.7	-107	-10.3	-125
C - BASELINE II							C - BASELINE II			
DAY 5 In	16.9	162	11.8	143	11.4	138	12.3	149	12.3	149
Out	9.2	88	6.7	81	6.9	83	17.8	215	17.1	207
Bal	7.7	74	5.1	62	4.5	55	-5.5	-66	-4.8	-58
CHANGE : CAL, 0 PROT TO BASELINE II							CHANGE : PROT, 0 CAL TO BASELINE II			
N Bal _{4,5}	14.6	140	10.4	126	10.1	122	2	25	4.9	59

TABLE 24

ISOTOPE EQUILIBRIUM PLATEAUX DATA AND CALCULATION OF Q, S, AND C
INTRAVENOUS INTAKE
CALORIE : NITROGEN MANIPULATION

Subject	JP (104 Kg)	WT ₁ (83 Kg)	WT ₃ (82 Kg)	WT ₂ (82 Kg)	MC (83 Kg)
<u>Baseline I</u>					
\bar{X} Sa \pm S.D. \pm C.V.(n)	(overprime)	0.0906 \pm 0.0025 \pm 2.8% (4)	0.0837 \pm 0.0030 \pm 3.6% (8)	0.0863 \pm 0.0038 \pm 4.5% (5)	0.0863 \pm 0.0059 \pm 6.9% (5)
Q (Mg/Kg/d)		545	597	579	579
S (Mg/Kg/d)		413	492	437	437
C (Mg/Kg/d)		408	461	410	430
	CAL, 0 PROT	CAL, 0 PROT	CAL, 0 PROT	PROT, 0 CAL	PROT, 0 CAL
<u>CHANGE:</u>					
\bar{X} Sa \pm S.D. \pm C.V.(n)	0.1896 \pm 0.0010 \pm 0.5% (5)	0.1182 \pm 0.0066 \pm 5.5% (5)	0.0976 \pm 0.0016 \pm 1.6% (8)	0.0817 \pm 0.0015 \pm 1.9% (5)	0.0999 \pm 0.0020 \pm 2.1% (5)
Q (Mg/Kg/d)	264	423	512	612	501
S (Mg/Kg/d)	197	359	444	362	227
C (Mg/Kg/d)	264	423	512	454	349
<u>Baseline II</u>					
\bar{X} Sa \pm S.D. \pm C.V.(n)	0.1795 \pm 0.0082 \pm 4.5% (5)	0.1286 \pm 0.0017 \pm 1.3% (5)	0.1104 \pm 0.0034 \pm 3.0% (5)	0.0852 \pm 0.0023 \pm 2.7% (5)	0.1108 \pm 0.0065 \pm 5.8% (7)
Q (Mg/Kg/d)	279	389	453	587	451
S (Mg/Kg/d)	190	413	369	367	235
C (Mg/Kg/d)	116	409	314	437	296

TABLE 25

CHANGES IN Q AND N BALANCE AND Q : N RATIOS
INTRAVENOUS INTAKE
CALORIE : NITROGEN MANIPULATION

SUBJECT	JP	WT	WT	WT	MC
	BASELINE I TO CAL, 0 PROT			BASELINE TO PROT, 0 CAL	
Q BASELINE I	(OVERPRIME)	545	597	579	579
Q ₂	264	423	512	612	501
ΔQ		-122	-85	33	-78
NBAL BASELINE	0	5	30	26	8
NBAL 2	-66	-64	-67	-91	-117
ΔNBAL	(-) 77	(-) 69	(-) 97	(-) 117	(-) 125
Q : N RATIO		1.9	0.83	DS	0.66
	CAL, 0 PROT TO BASELINE II			PROT, 0 CAL TO BASELINE II	
Q BASELINE II	264	423	512	612	501
Q ₂	279	389	453	587	451
ΔQ	15	-34	-59	-25	-50
NBAL BASELINE II	-66	-64	-67	-91	-117
NBAL ₂	74	62	55	-66	-58
ΔNBAL	(+) 140	(+) 126	(+) 122	(+) 25	(+) 59
Q : N RATIO	0.1	DS (low)	DS	DS (insig)	DS

DS = Different Sign for Q : N Ratio

TABLE 26

EFFECT OF PROTEIN WITHDRAWAL ON NITROGEN BALANCE IN NORMAL SUBJECTS

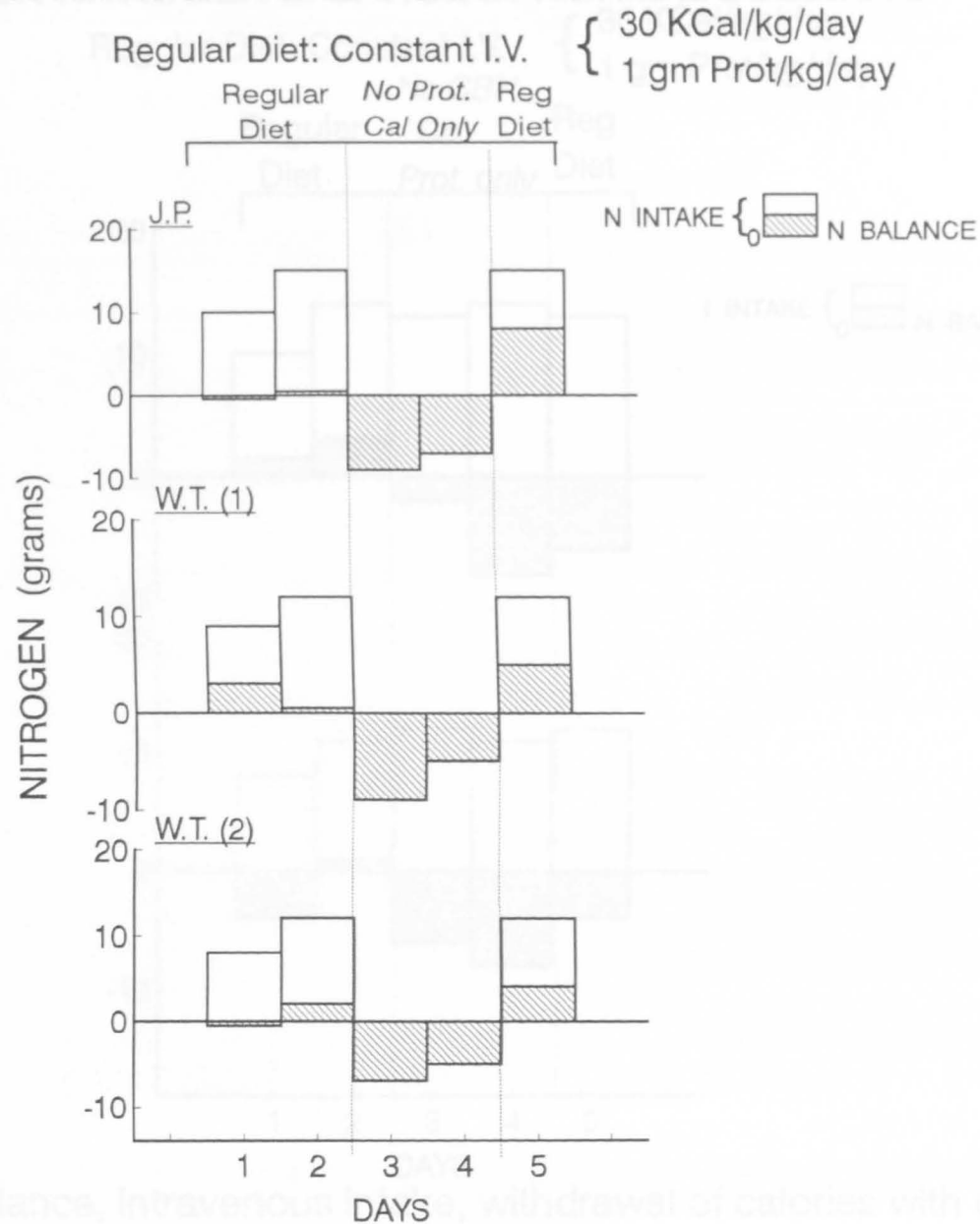
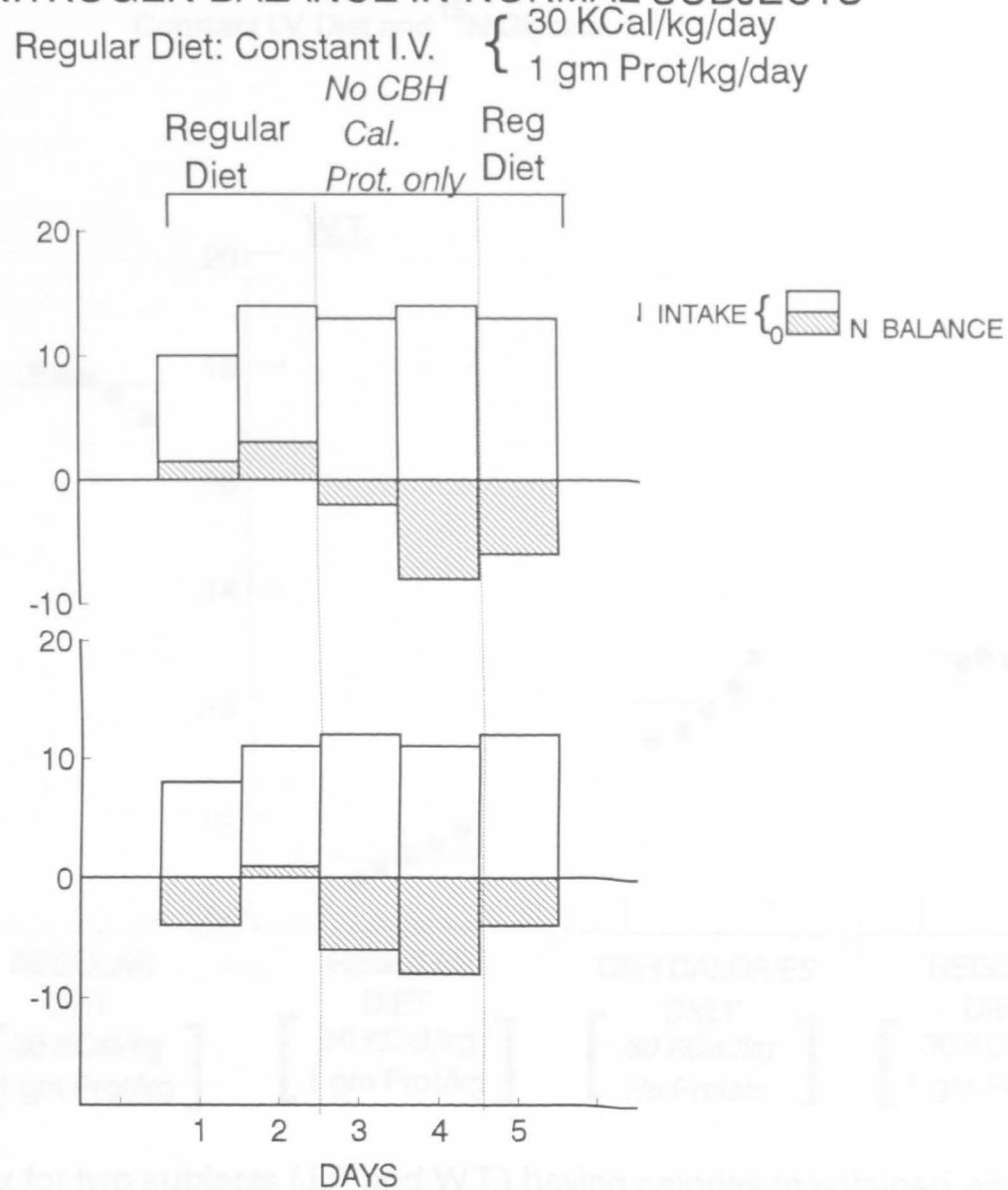


FIGURE XLVII

Nitrogen balance, intravenous intake, withdrawal of amino acid infusion with continuation of calories only, three subjects (J.P., W.T., and W.T.₂)

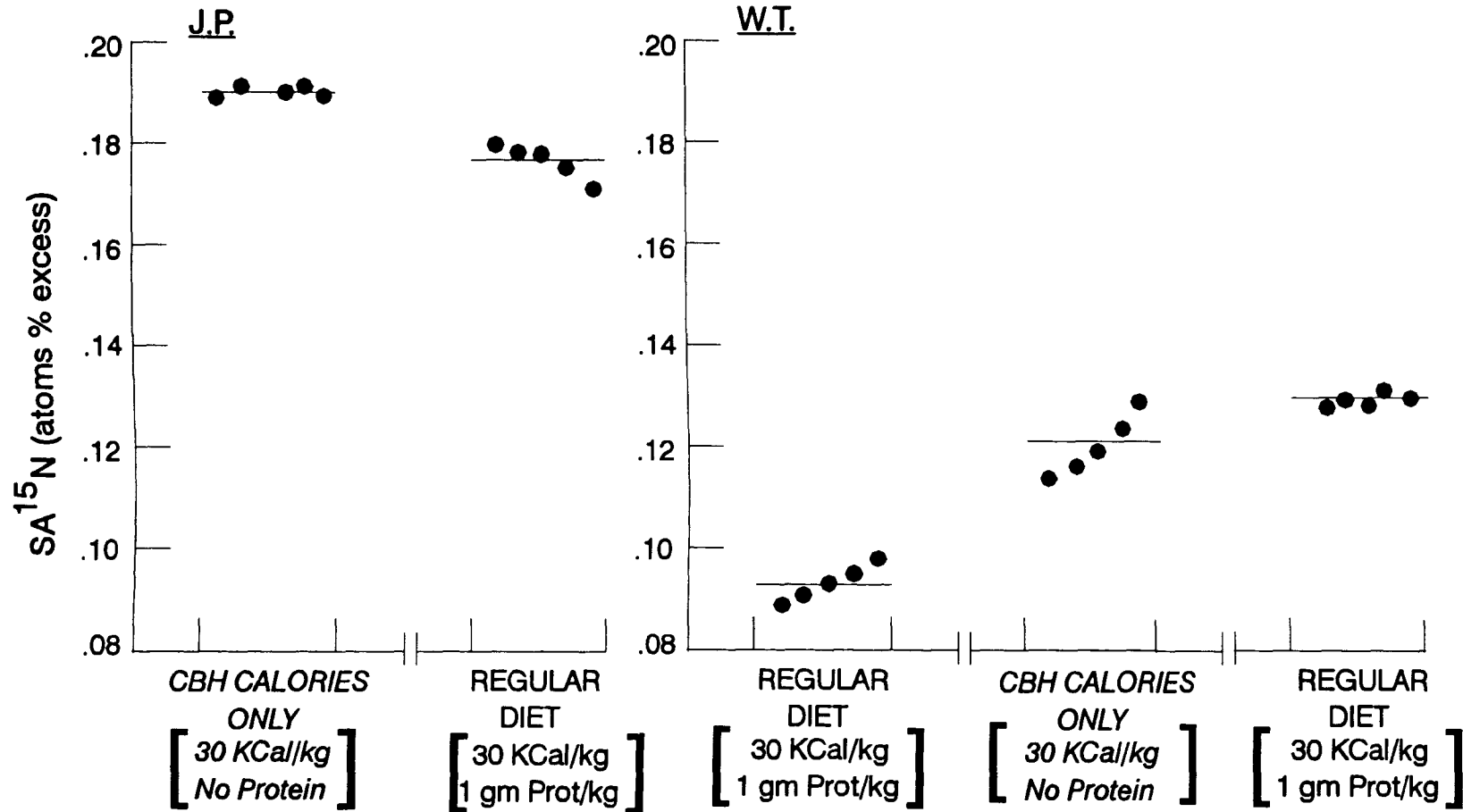
EFFECT OF CALORIE WITHDRAWAL ON NITROGEN BALANCE IN NORMAL SUBJECTS



Nitrogen balance, intravenous intake, withdrawal of calories with continuation of amino acid infusion only, two subjects (W.T. and M.C.).

FIGURE XLVIII

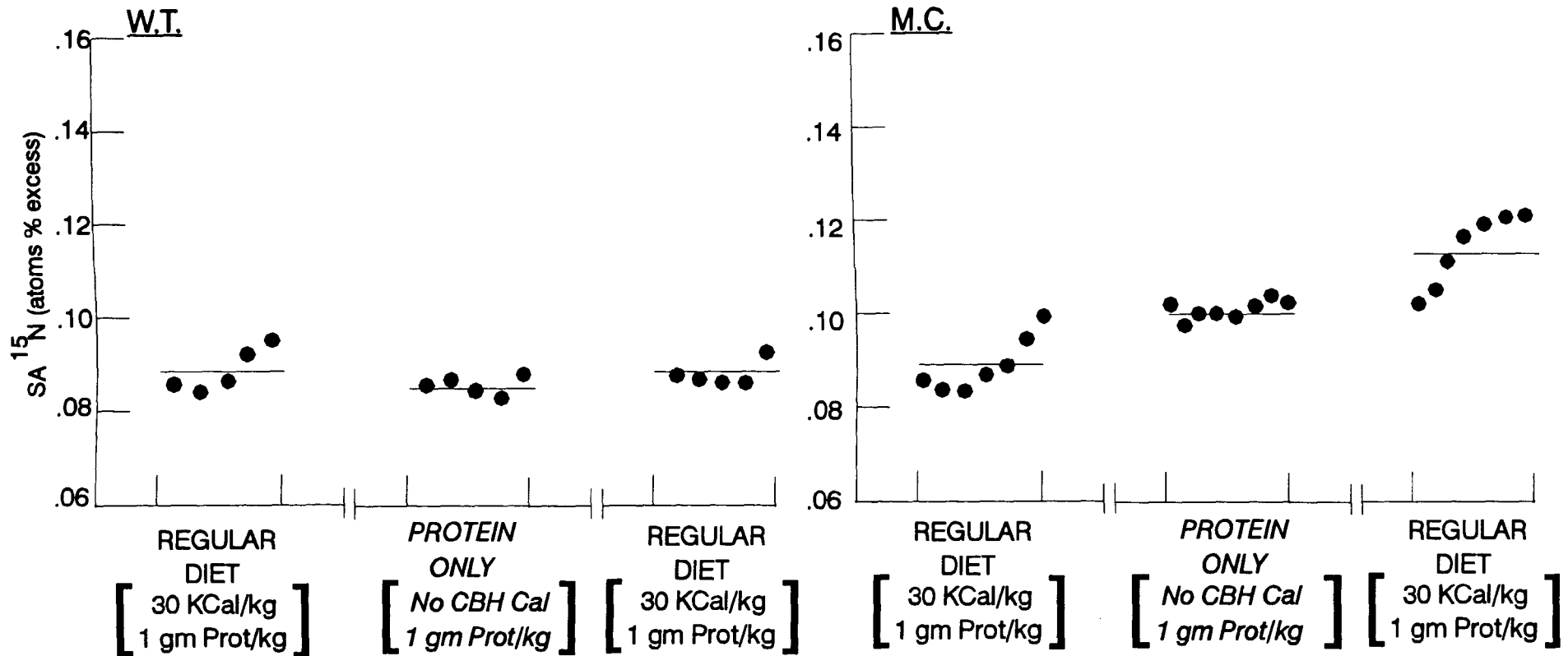
EFFECT OF SUDDEN WITHDRAWAL OF PROTEIN
ON ^{15}N GLYCINE EQUILIBRIUM
Constant I.V. Diet and ^{15}N Glycine



Urea ^{15}N enrichment plateaux for two subjects (J.P. and W.T.) having calories maintained while amino acid infusion was withdrawn

FIGURE XLIX

EFFECT OF CALORIE WITHDRAWAL
ON ^{15}N GLYCINE EQUILIBRIUM
Constant I.V. Diet and ^{15}N Glycine



Urinary ^{15}N urea enrichment curves for two subjects (W.T. and M.C.), intravenous intake having amino acids continued but calories withdrawn.

FIGURE L

**BASELINE Q, S AND C IN NORMAL MALE SUBJECTS
MEAN VALUES UNDER SEVERAL BASELINE CONDITIONS
(MgN/Kg/day)**

	n	Mean	± S.D.
Q, Baseline I Oral Diet	4	575	± 22
Q, Baseline I intravenous Intake	4	486	± 37
Q, Baseline I Oral or Intravenous Intake	8	530.5	± 55
S, Baseline I Oral Diet	4	337.2	± 24
S, Baseline I intravenous Intake	4	444.8	± 33
S, Baseline I Oral or Intravenous Intake	8	391.1	± 63
C, Baseline I Oral Diet	4	325.3	± 38
C, Baseline I intravenous Intake	4	427.3	± 25
C, Baseline I Oral or Intravenous Intake	8	366.3	± 72
Basal Catabolic Rate			
24 - 48 hrs after Cessation of Intake (I=0, Q=C)			
Cessation of Oral Intake	4	303.5	± 18
Cessation of Intravenous Intake	2	356	± 27
Cessation of Oral or Intravenous Intake	6	321	± 33
<u>Statistical Comparisons</u>			
Q, Baseline, Oral vs Intravenous		< 0.03	
S, Baseline, Oral vs Intravenous		< 0.01	
C, Baseline, Oral vs Intravenous		< 0.02	
Baseline Catabolic Rate, Oral vs Intravenous		n.s	

TABLE 27

CHANGES in Q, S, and C
Mean Changes Under Several Conditions (Oral Diet)

	n	Mean	± S.D.	P *
<u>Oral, Baseline to Doubling</u>				
Δ Q	4	55.5	± 18	< 0.01
Δ S	4	-34	± 20	< 0.05
Δ C	4	-107.8	± 31	< 0.01
<u>Doubling to Baseline II</u>				
Δ Q	4	-107.8	± 21	< 0.01
Δ S	4	-38.3	± 20	< 0.05
Δ C	4	56	± 33	< 0.05
<u>Baseline II to Dropout</u>				
Δ Q	4	-130.5	± 30	< 0.01
Δ S	4	-121.5	± 37	< 0.01
Δ C	4	47.8	± 30	< 0.05
<u>Dropout to Baseline III</u>				
Δ Q	4	70	± 12	< 0.01
Δ S	4	88	± 26	< 0.01
Δ C	4	-110.5	± 12	< 0.01

* p values based on comparison of the data before and after the change indicated.

TABLE 28

**PLASMA AMINO ACIDS (mg/dl)
DATA SUMMARY**

	<u>TAA</u>			<u>BCAA</u>			<u>BCAA/TAA</u>		
	n	\bar{X}	\pm S.D.	n	\bar{X}	\pm S.D.	n	\bar{X}	\pm S.D.
Resting Baseline I	18	286	37	18	43	4.4	18	0.15	0.02
Oral Doubling	4	247	10	4	50	1.5	4	0.21	0.01
Oral Baseline II	4	250	5.4	4	40	5.8	4	0.16	0.03
Oral Dropout	4	207	19	4	71	15	4	0.34	0.03
Oral Baseline II	4	234	40	4	40	12	4	0.19	0.02
I.V. Doubling	2	332	44	2	54	4.3	2	0.17	0.07
I.V. Baseline II	2	316	32	2	46	5	2	0.15	0.07
I.V. Dropout	2	281	18	2	71	1	2	0.25	0.06
I.V. Baseline II	2	330	3	2	41	0	2	0.13	0.01
I.V. CAL O PROT	1	278		1	32		1	0.12	
I.V. Baseline II	1	367		1	44		1	0.12	

Significance Levels (Oral Experiments Only) (n=4)

<u>TAA</u>	Doubling vs Baseline I	p < 0.03	<u>BCAA/TAA</u>	Doubling vs Baseline I	p < 0.05
	Dropout vs all Baselines	p < 0.04		Dropout vs all Baseline	p < 0.01
<u>BCAA</u>	Doubling vs Baseline I	p < 0.04	<u>Doubling vs Dropout</u>		
	Dropout vs all Baseline	p < 0.03	TAA	-	p < 0.01
			BCAA	-	n.s.
			BCAA / TAA	-	p < 0.02

TABLE 29

ADDENDUM

Statistical Methods

Statistical evaluations were made using paired t-tests where appropriate. In Study I comparisons between means was made using paired t-tests in the regimens normal oral diet, AA, and AA HDG because the same five subjects were used. Unpaired t-tests were used to compare the protein metabolism data in the remaining groups (AA LDG, AAFE, AA+LDG+FE and LDG) because 11 subjects were used in 16 studies, but the biochemical and hormonal data could be evaluated using paired t-tests because control and final results were compared in each case.

In Study III statistical analysis of the Q:N ratios between the various dietary changes was assessed as whether or not the difference was significantly different from 1.0, using the paired t-test by the logarithm of the changes.

Modern Methods of Measuring Body Composition

In the Introduction to this thesis it was stated that relationships between the known protein turnover in various tissues was coupled with knowledge about body composition to lead to the concept of Body Cell Mass (page 22). Until recently, the only direct method of measuring body cell mass in living man was by whole-body counting of naturally occurring radioactive potassium (^{40}K). Indirect methods involved densitometric, hydrometric and anthropometric techniques, as well as radioisotope dilution, to estimate major body components and compare results with cadaver dissection. However, in recent years the development of in vivo neutron-activation analysis (IVNAA) has made possible the direct determination of the absolute levels of several elements in humans. In a small number of centres around the world such facilities have been set up and are being used to measure changes in body composition during nutritional support and following surgery and trauma (Hill, 1992). The IVNAA facility consists of two plutonium-beryllium sources of neutron output placed above and below the patient who lies supine on a moveable couch. The body is irradiated completely, and the gamma radiation emitted by radionuclides induced by the neutrons is specific for various elements in the body eg. ^{48}Ca , ^{13}N , ^{13}C , ^{24}Na , ^{38}Cl and ^{28}Al (from P), and

measured by a whole-body counter. Hence, total body nitrogen and potassium can be measured to give skeletal mass, and in combination with estimation of total body water using tritiated water, the intra- and extracellular fluid spaces can be separated.

Cadaver analysis has shown the accuracy of the system when the two are compared (Knight et al., 1986). The combination of prompt gamma neutron-activation analysis for the measurement of total-body nitrogen and whole-body counting for the measurement of total-body potassium allows determination of muscle mass and non muscle lean tissue and their protein content. If both potassium and nitrogen are found in muscle and nonmuscle lean tissue in different concentrations, it is possible to determine the parameters of body composition. The underlying assumptions are that lipid from adipose tissue contains no potassium or nitrogen and the ratio of nitrogen to potassium is different in muscle and nonmuscle tissue. Studies in young men (20-29 yrs) have shown that the mass of the nonmuscle compartment is 44% of body weight, whereas the muscle compartment is 29% of body weight (Cohn et al., 1980). Skeletal muscle mass decreased 45% with age (compared with subjects aged 70-79) whereas the nonmuscle mass did not change significantly. Similarly, the protein content of muscle fell sharply. However, the total body protein content fell only 14% from age 20 to 80.

The second modality which promises exciting developments in body composition is nuclear magnetic spectroscopy. Conventional nuclear magnetic resonance (NMR), also known as magnetic resonance imaging (MRI), is used clinically as an anatomical imaging technique in radiology. However, the equipment can be modified to carry out NMR spectroscopy, presently used only as a research tool, but which is unique in being the only technique which provides noninvasive access to living biochemistry in situ.

Several biologically relevant chemical elements undergo magnetic resonance, but NMR imaging is almost always performed on the element hydrogen, the nucleus of which is a single proton. Not only is hydrogen by far the most abundant element numerically in the body, it also yields the strongest NMR signal per atom. In practical terms, it is the only element with which images can be produced with spatial resolution on the order of millimetres.

NMR spectroscopy involves separating the signal from a given element into its different chemical forms. This is possible because the magnetic field experienced by an atomic nucleus is "shielded" or modified by the fields produced by neighbouring atoms on the same molecule. This produces a "chemical shift" or small variation in the resonant frequency; a display of the NMR signal as a function of frequency is a spectrum, with different chemical forms of an element forming peaks at characteristic positions.

A hydrogen spectrum of biological tissue has two large peaks, for water and the $-CH_2$ -proton of fat; the myriad other chemical forms of hydrogen are present in concentrations over a thousand times smaller and are overwhelmed by dominant water and lipid signals.

Conventional NMR imaging is performed without spectroscopic separation; the intensity of each pixel is essentially the sum of the signals from all the chemical forms of hydrogen, which is, again, mainly water.

Several other elements can be used for spectroscopic study - including phosphorus - 31, carbon - 13, fluorine - 19, and sodium - 23 (Aisen and Chenevert, 1989).

The nucleus that has been used most widely for metabolic studies is ^{31}P , which is the naturally occurring phosphorus nucleus. ^{31}P spectra of brain or skeletal muscle include signals from ATP, phosphocreatinine, phosphodiesteres and inorganic phosphate, and have been widely used in research to study the metabolic changes associated with ischaemia (Gadian et al., 1993) and in the clinical situation to study muscle metabolism in peripheral vascular disease (Hands et al., 1986) and in the chronic fatigue syndrome (Wong et al., 1992).

The development of 1H NMR for metabolic studies in vivo has been slower than with phosphorus, partly for technical reasons. Although 1H NMR is about fifteen times more sensitive than ^{31}P NMR it is

technically more difficult because of the need to suppress the large signals from water and fats, and because of the large number of metabolites that produce signals in a narrow chemical shift range. However, techniques are now sufficiently well developed to study several metabolites of the citric acid cycle (Bottomley, 1989).

Carbon-13 is the stable isotope of carbon, with a natural abundance of about 1%, and which yields a weak NMR signal (carbon-12 does not undergo nuclear magnetic resonance). It has been used to study glycogen and fat metabolism, and presumably ^{13}C -labelled amino acids such as leucine could be tracked through their metabolic pathways, which could open up a whole new field of metabolic research.

Fluorine - 19 has 85% of the sensitivity of ^1H NMR, but there are no naturally occurring fluorine-containing compounds and so the role of ^{19}F NMR is the monitoring of exogenous ^{19}F containing molecules such as fluoro-carbon blood substitutes in the study of tissue perfusion.

Sodium-23 spectra show a single peak representing sodium ions in both intra- and extracellular spaces, but the relaxation properties of the nucleus may differ in the two environments permitting information to be obtained about oedema and the distribution of sodium between extra- and intracellular spaces (Gadian et al., 1993).

Thus, NMR spectroscopy will undoubtedly become established as a non-invasive method of studying metabolism in living systems, ranging from cellular suspensions to whole man, but is still in the process of development at the present time.

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