SOME STUDIES ON THE INFLUENCE
OF ENERGY SOURCE AND INTAKE ON
NITROGEN METABOLISM IN CHRONIC
RENAL FAILURE

- by -

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Being a thesis presented in accordance with the regulations governing the award of the degree of Master of Philosophy in the University of Surrey

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SUMMARY

1. Nitrogen balance studies on eight uraemic patients on intakes of 45 - 55 mg nitrogen per kg body weight, and an average of 44 - 57 Calories per kg body weight show a tendency to become decreasingly negative with an increasing energy intake. This trend is also shown with a decreasing faecal nitrogen when expressed as a percentage of dietary nitrogen.

2. Net protein utilization is assessed from nitrogen balance data and it is suggested that this is related to the combined potential of the urea pool size and the energy available, and that the energy intake can be a limiting factor in anabolism. A suggested assessment of the partitioning of the energy supplied is given.

3. Varying proportions of fat to carbohydrate and polysaccharides to mono and disaccharides have been used as the energy source. The results of such variations, over a wide range show no influence on nitrogen balance in these studies.

4. A preponderance of carbohydrate energy appears to be necessary to ensure a constant supply of tricarboxylic acid cycle intermediates. A plentiful and palatable supply of dietary energy is essential, and long term acceptance of novel foods can present a major problem in the dietary treatment of uraemic patients.
1. **RENAL FUNCTION**

In irreversible chronic renal failure a persistently elevated blood urea represents the end result of extensive damage to the kidneys. The process may have a multiplicity of causes but the final clinical picture is very similar, irrespective of the preceding disease process. The primary disease is therefore sometimes difficult to define clearly.

The common basis of progressive renal failure is a total reduction in the number of functioning nephrons, the result of which is a reduced filtration rate and an increased level, amongst others, of blood urea; but the filtration per nephron may in fact be normal or even increased. Platt, Roscoe and Smith (1952) showed, in rats, that it was necessary to remove the whole of one kidney and a large part of the second before an increase in blood urea occurred. A similar kind of reserve exists in man, although the reserve in the rat is much greater than in man.

The most ready evidence of renal failure is an increased blood urea. This is however a very crude estimate of renal function.

In progressive renal failure, as more nephrons become atrophied, the limited number remaining become hypertrophied and individually more active than normal, so that the amount of solute cleared by each is increased. Hypertrophy of cells seems to depend on the integrity of the anterior pituitary, and probably on the secretion of growth hormone, as this does not occur in hypophysectomised animals.

**a. Stages in the development of renal failure**

An early stage of chronic renal insufficiency may occur, when only biochemical abnormalities are seen without symptoms.
The composition of the extracellular fluid is little changed, glomerular filtration rate is only 50 - 30 per cent of normal values namely 60 - 40 ml per minute. Further small decreases in the glomerular filtration rate incur only minute increases in serum urea or creatinine, and values are not much greater than normal at this stage. Failure to concentrate urine efficiently then leads to a slight increase in urine volume; and the excretion of urine at night becomes similar to day time levels. These changes are manifested by the presence of nocturia and is in fact a compensatory polyuria.

For the purposes of this study chronic renal failure may be conveniently divided into three stages.

**Stage I renal failure**

Chronic renal failure may be said to be present when the glomerular filtration rate (see appendix) becomes less than 20 per cent of normal, i.e. (20 - 10 ml per minute). The blood levels of creatinine, sulphate and phosphate all rise with an associated metabolic acidosis and this is accompanied by a decrease in the serum bicarbonate level. Varying degrees of proteinuria are observed in chronic renal failure and this can exacerbate the appearance of oedema. Proteinuria tends to decrease with further reduction of glomerular filtration rate, but may still be significant in advanced renal failure and care should be taken when calculating the protein requirements of patients on low protein diets, as for example 2 g protein represents 10 per cent of a 20 g protein diet. However, at this stage no dietary restriction is required unless oedema is very marked, when a reduced sodium intake may be necessary or dietary protein increased if serum albumin is low.
Stage II renal failure

Stage II renal failure occurs when the glomerular filtration rate is between 10 - 5 ml per minute, and a modest protein restriction to approximately 30 - 40 g per day is necessary. Patients generally have no symptoms and the blood urea concentration is normal.

Stage III renal failure

Terminal or end stage renal failure is reached when the glomerular filtration rate is less than 5 ml per minute. A strictly controlled Giordano-Giovannetti regime is required at this stage and the blood urea controlled at around 50 to 150 mg per 100 ml when a reduction of the urea to creatinine ratio from 20 : 1 to 10 : 1 is anticipated. After one or two weeks on this diet patients show clinical improvement with less nausea and vomiting. Acidosis may be a problem, also retention of sodium may appear, and require dietary restriction.

b. Renal function

The kidney contains over a million excretory units, the nephrons, and normally there is a wide variation in size, structure and functional capacity of individual nephrons. It follows therefore that clearance values and secretory and reabsorptive maxima are always average values.

The elimination of nitrogenous end products of protein metabolism is one of the major homeostatic mechanisms of the kidney. In normal subjects the blood urea concentration may vary between 12 - 40 mg per 100 ml of blood, depending on the protein intake. When patients take diets containing only small amounts of protein the blood urea may fall to about 20 per cent of the level on a normal protein intake, so that evaluation of renal function in these circumstances would be
unrealistic. A more reliable indication of renal function is generally considered to be the urinary creatinine excretion, although the constancy of this is questioned by Ismail, Khan and Bender (1972), for they have found that it can vary considerably. The combined values of serum creatinine and the 24 hours creatinine clearance, can together give a reasonable estimate of renal functioning mass.

Uric acid excretion depends on the intake of nucleo-proteins and purines. In normal individuals the concentration of uric acid in the plasma is approximately 4.0 - 5.0 mg per 100 ml in women and 5.0 - 6.5 mg per 100 ml in men. The excretion of uric acid is the net result of filtration by the glomerulus, tubular reabsorption and also tubular secretion. In normal subjects a large proportion of uric acid is reabsorbed and therefore the renal clearance is lower than that of inulin.

c. Tubular reabsorption

In normal individuals if the total body water is 40 litres then approximately 170 litres of water are daily recycled through the renal tubular system by filtration and reabsorption of a variety of valuable components of the glomerular filtrate.

The normal glomerular filtration rate is around 120 ml per minute and the rate of urine flow is approximately 1 ml per minute. As the glomerular fluid passes into the proximal convoluted tubule there is an active reabsorption of sodium which is accompanied by an obligatory reabsorption of water to maintain osmotic equilibrium. This isotonic fluid remains at around 300 mOs. per litre, until it is delivered into the thin descending limb of the loop of Henle where there is a passive diffusion of sodium into the tubule and of water out into the hypertonic interstitium. The tubular fluid
an urinary K⁺ derived from distal tubular secretion of K⁺

active reabsorption 75% NaCl

remaining 25% Na⁺ reabsorbed under influence Aldosterone

ion exchange Na⁺/K⁺ Na⁺/H⁺

CORTEX influence of A. D. H.
P increasingly -o hypotonic urine

Fig. 1 The elaboration of urine in the nephron, in terms of the mechanisms of active and passive exchanges of water and ions
becomes progressively hypertonic as it passes down the descending limb reaching a very high osmolar concentration at the bend of the loop of Henle. In the ascending Henle's loop (impermeable to water) sodium is actively pumped into the interstitial tissues of the medulla, creating an area of high osmolality. As the descending limb and the interstitium are, in contrast to the ascending limb freely permeable to water and sodium ions there is a resulting passive diffusion of water and sodium into the descending limb. A concentration gradient is established between the tubular fluid in the ascending limb of Henle's loop, and the interstitium with a graded decrease in the osmolality of the tubular fluid so that a hypotonic urine is delivered to the distal convoluted tubule. This operation is called a countercurrent multiplication of concentration. The longer the loop of Henle the more efficient the concentrating mechanism. (See Figure 1).

The rate of tubular urine flow also influences urine concentration. A more rapid transit of tubular fluid i.e. increased GFR decreases the tubular concentrating ability, whilst a decreased GFR is associated with more back diffusion of urea into the medulla, thus increasing medullary osmolality and resulting in a more concentrated urine. The excretion of urea can therefore have an important effect on the concentrating mechanism. A decreased excretion of urea and a lower capacity for concentrating urine has been demonstrated in dogs fed on low protein diets (Bricker, Davey, Lubowitz, Stokes and Kirkengaard, 1959).

In the new born baby, the loops of Henle are much shorter than in later life, and the facility to produce a highly concentrated urine is limited. Unless a sufficiently plentiful supply of water is given it is possible that an osmotic diuresis can easily occur, accompanied by a rapid loss of body water and severe dehydration;
the plasma becomes dangerously hypertonic.

In chronic renal failure when excess urea is delivered to
the concentrating sites, the concentrating mechanism might be
expected to be enhanced as the osmolality of the medulla is
increased. However, this is offset by the greater urine flow
per nephron i.e. compensatory polyuria showing the osmotic diuretic
effect of urea.

d. Facultative reabsorption

Fluid entering the distal convoluted tubule is hypotonic
to the surrounding interstitium, but facultative reabsorption can
take place, by the independent reabsorption of water and solutes
the osmotic pressure of the urine can be varied. The process is
largely determined by the presence of anti-diuretic hormone (A.D.H.)
in the blood. This hormone secreted by the posterior pituitary,
regulates the permeability of the tubular cells to water. A.D.H.
secretion is responsive to a one per cent change in the tonicity of
the body fluids. The renin-angiotensin aldosterone axis is a system
which also plays a major role in the homeostatic control of the
extracellular fluid volume. Renin release is triggered by a
decreased arterial pressure and the angiotensin system is then
activated and has a direct vasopressor effect on the peripheral
arterioles. The biosynthesis of aldosterone is also stimulated and
this increases the reabsorption of sodium in the distal tubule.

The diluting mechanism is normally ensured by the reabsorption
of sodium from the distal system, which is impermeable to water in
the absence of A.D.H., and hence a more dilute urine is formed. In
chronic renal failure a defect in the diluting mechanism appears
secondarily to an inability in concentrating urine. This is shown
by a limitation in the ability to dilute urine efficiently after
drinking a large volume of water. This limitation in the capacity to form a dilute urine could most likely be the result of an increase in the rate of urine flow and an increase in solute load per nephron - reabsorption of sodium in the distal system cannot be an efficient function in such conditions.

e. Osmotic diuresis

In normal individuals a load of solute increases urine flow, solute will therefore be incompletely reabsorbed, and contribute to osmotic activity and prevent water reabsorption. The resulting urine volume is accordingly increased and its concentration decreased. This represents a limitation in the concentrating mechanism, and explains the accelerated dehydration which follows the drinking of large volumes of salt water.

In chronic renal failure a large proportion of nephrons are destroyed and the increased flow of solute and water in the remaining nephrons limits the amount of sodium reabsorbed in the loop of Henle, due to a reduced transit time, and an increase in fluid volume. These changes result in an osmotic diuresis in which the osmolality of the urine becomes relatively fixed (isothienuria). It is concluded that in chronic renal failure, the remaining intact nephrons retain an efficient concentrating and diluting mechanism in the distal system, but a large increase in the solute and water load imposes a condition of solute diuresis, which a reduced nephron population is unable to modify.

f. Urinary sodium

In chronic renal failure the excretion of sodium can be modified to a limited extent. Whilst some patients on a low salt intake will become sodium depleted, others on a normal salt intake will become salt and water overloaded. In the later stages of renal failure, when retention of sodium, and severe hypertension
may be present, dietary restriction of sodium is necessary. Conversely, patients with predominantly renal tubular lesions, for example, pyelonephritis, can show varying degrees of salt wasting in the earlier stages of chronic renal failure, and in this situation an increased dietary sodium is needed.

g. Urinary potassium

In normal individuals approximately 92 per cent of filtered potassium is reabsorbed proximally, but in conditions of excessive potassium intake it is secreted by the distal tubules. Berliner (1960) has shown that tubular secretion of potassium is a process of cation exchange for sodium, and he considers that potassium and hydrogen ion compete for the same transport mechanism. Potassium is not conserved as efficiently as sodium in conditions of depletion.

In chronic renal failure, impairment of the secretion of potassium does not as a rule occur until the terminal stages, when the glomerular filtration rate is very diminished. The secretion of potassium is then increased but the increase is not sufficient to prevent the hyperkalaemia i.e. \( \frac{C_k}{C_{IN}} > 1 \). Inability to secrete adequate potassium tends to occur more frequently in patients with renal tubular lesions.

h. Acid-base balance

Normally most diets produce a total daily load of non-metabolisable acid amounting to approximately 40 - 60 mEq. hydrogen ion. This is derived from the oxidation of sulphur containing amino acids to non-metabolisable sulphuric acid and of dietary phosphate to phosphoric acid. Large amounts of hydrogen ion are generated in the process of carbohydrate and fat metabolism to form carbonic acid which can be eliminated by the lungs. By the combined homeostatic activity of the lungs and kidneys the hydrogen ion concentration of the extracellular fluid is finely controlled at
around pH 7.4. Hydrogen ions combine with buffers in the body, and therefore are not available in the free form for excretion. Hence new hydrogen ions are generated by the kidney for excretion. The process is essentially an active reabsorption of sodium as bicarbonate in exchange for the secretion of hydrogen ions from the epithelium of the tubule and occurs throughout the nephron.

Carbonic acid is an available source of hydrogen ion and is produced by the hydration of carbon dioxide in the tubular cells by the action of the enzyme carbonic anhydrase. Carbonic acid is then ionised to bicarbonate and hydrogen ions

$$\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3$$

In this way bicarbonate is provided for the plasma and hydrogen ions for secretion into the urine. Buffers present in the urine take up the hydrogen ions and convert them to acid forms; the titratable acid of the urine rises to an extent equivalent to that of the bicarbonate supplied to the plasma. Ammonia formed from amino acids and glutamine also combines with hydrogen ion in the distal lumen to form ammonium ions and this too increases the excretion of hydrogen ion.

Thus acidosis can be prevented by distal tubular cation exchange $\text{K}^+ / \text{Na}^+$, $\text{H}^+ / \text{Na}^+$ and by producing an increase in titratable acid as acid phosphate $\text{Na}_2\text{HPO}_4 \rightarrow \text{NaH}_2\text{PO}_4$ and by increasing the excretion of ammonium salts formed from ammonia. It can be seen therefore that the transport of sodium in the renal tubule plays a very central role in the mechanism of elimination of hydrogen ions. In uraemic metabolic acidosis the formation of hydrogen ions in metabolic processes is in excess of the rate at which they can be eliminated in the urine.
The limitation in the elimination of acid becomes more evident as the glomerular filtration rate diminishes. A reduced glomerular filtration rate results in a reduction in the net amount of phosphate or sulphate buffer being presented to the acid secreting sites in the tubule and therefore in a reduction in the amount of titratable acid in the urine. Since the amount of sulphate and phosphate buffer filtered through the glomeruli is reduced, sodium is excreted with other anions. Even with an increased serum phosphate, when more phosphate is filtered per nephron the increase in titratable acid is inadequate.

In end stage renal failure the diminishing ability of the kidney to continue to excrete sufficient acid phosphate becomes very critical and it is important to bear in mind the possible effect of giving aluminium hydroxide which may be taken to reduce the high serum phosphate, by increasing its faecal excretion. This may further compromise the urinary acidifying mechanism by decreasing still more the tubular filtered phosphate. Where possible dietary construction should therefore avoid an excessive acid load, and base should predominate.

In some cases base may be provided as sodium bicarbonate, but this would depend upon the salt requirements, and may be impossible if severely salt restricted. Base cannot always be given as a potassium salt because of the increased risk of hyperkalaemia. It would appear that there is a need to redefine the requirement of sulphur containing amino acids in uraemia.

i. Calcium and phosphate metabolism and bone disease in chronic renal failure

As the creatinine filtration rate decreases and renal failure progresses the plasma phosphate increases to high levels as the amount of phosphate filtered is not sufficient to counteract the
retention of dietary phosphate, serum calcium levels tend to be reciprocally depressed. Urinary calcium excretion is decreased and calcium absorption is negligible or non-existent. Parathormone activity is stimulated by the decreased level of serum calcium and the calcium phosphate product can exceed 75 and calcified foci in soft tissues is observed. Changes in calcium metabolism in chronic renal failure are widespread and complex. It is possible that bone metabolism becomes altered at a very early stage in renal failure. The gross changes of uraemic osteodystrophy become very evident in progressive renal failure. A picture of osteomalacia (or rickets in children) emerges; and superimposed on this a secondary hyperparathyroidism leading to increased rate of bone resorption or destruction. In some patients patches of increased bone density are also present (osteosclerosis). This may represent an increased calcitomin response. Bone lesions may become widespread. This complex situation may reflect the result of an attempt to adapt to a state of vitamin D resistant bone disease (Avioli, Birge, Lee, Slatopolsky, 1968).

i. Anaemia

Anaemia is a prevalent feature in chronic renal failure.

The major factor is a decrease in erythropoiesis due to a reduced secretion of renal erythropoietin by the diseased kidney. In addition other factors are involved, for a decreased red cell survival rate is considered to result from the retention of toxic metabolites resulting from protein metabolism. Shaw (1967) reported that red cell survival was related to blood urea concentration.

In the terminal phase of chronic renal failure haemolysis of the red blood cells occurs, and survival time is greatly shortened. It is generally accepted that there is a factor present in the
Plasma of patients with prolonged and severe chronic renal failure that has a haemolytic effect on the matured red cell (Desforges and Dawson, 1958). This factor has been separated as a dialysable substance and is probably a product of protein metabolism (Shaw, 1967) which accumulates in severe uraemia.

k. Disturbance of carbohydrate metabolism in patients with chronic renal failure

A mild degree of impairment in the utilization of carbohydrate in uraemic patients is generally recognised. It is manifested by a limited capacity to handle a glucose load, and the degree of uraemia appears to correlate with the degree of impairment.

The presence of insulin antagonists, as low molecular weight compounds, and as yet unidentified, has been suggested by numerous workers (Briggs, Buchanan, Luke, McKiddie, 1967; Alfrey, Sussman and Holmes, 1967; Spitz, Rubenstein, Bersohn and Lowy, 1970). The peripheral utilization of glucose appears to be affected, and results in a prolonged elevation of blood sugar and a decrease in the effective action of insulin after an oral glucose load. Evidence for the presence of a dialysable insulin antagonist in uraemic sera is supported by the fact that carbohydrate utilization rapidly improves after haemodialysis. This antagonism to the action of insulin in chronic renal failure is considered to be secondary to the widespread biochemical intracellular changes which occur.

Carbohydrate utilization has a direct relationship to plasma lipid levels and it is now recognised that patients with chronic renal failure have high plasma lipids. Lodowsky and Kenward (1968) reported that the fasting plasma triglycerides are elevated in chronic renal failure. Serum free fatty acids and cholesterol on the other hand are usually normal, intravenous glucose does however result in a fall in the elevated level of free fatty acids. In normal subjects glucose
inhibits lipolysis, but in these patients, there is a greater and more prolonged depression of free fatty acids. The authors therefore considered that glucose has an important sparing action on the mobilization of lipid stores.

It is therefore evident that in chronic renal failure despite a gross reduction in the functioning nephron population, the kidney is able to maintain a biochemical state compatible with life for some considerable time. A distinctive feature of chronic renal failure is the uniform pattern of progressive deterioration, no matter what the aetiology. A uniform pattern of functional adaption emerges and this appears to be conditioned by an increased solute load and an increased rate of urine flow per nephron.

1. Treatment

Ten years ago patients with terminal renal failure, whose kidney function was no longer able to maintain a near normal extracellular fluid, died. Dietary manipulation was generally considered to be an unnecessary hardship. Now the possibility of intermittent haemodialysis treatment and renal transplantation has revolutionised the future management of chronic renal failure. Two very different forms of management have become established. In the first, an artificial kidney substitutes for renal excretory function and diffusible solutes and water are transferred across a semipermeable membrane, thus normalising the composition of the extracellular fluid (and possibly also the intracellular fluid). In the second, the accumulation of exogenous toxic metabolites is limited by a controlled dietary intake.

m. Choice of treatment

The clinical suitability of the patient is an important criterion for acceptance onto a regular dialysis and transplant scheme, for not all patients with chronic renal disease are suitable candidates. For instance, some consider patients not suitable if chronic renal failure
secondary to another primary generalised disease process such as a metabolic disease, diabetes mellitus, amyloidosis or collagen disease, advanced cardiac disease, primary atherosclerosis or mental instability. The age of a patient can also be critical. There is some evidence to suggest that patients over 40 years of age do not respond so well to regular dialysis treatment and transplantation, as those who are younger. Deterioration in the vascular system is generally much more prevalent in the older age group.

For these reasons a large majority of patients with chronic renal failure are not eligible for treatment on intermittent haemodialysis and renal transplantation, even if resources and availability of donor kidneys would permit. A controlled diet is the alternative and this has been shown to improve the biochemical picture and alleviate many of the distressing features of terminal chronic renal failure which can be summarised as follows: lassitude, anorexia, nausea, vomiting, diarrhoea, pruritus, oedema, breathlessness, bleeding, visual disturbances, bone symptoms, stunting, pain, fractures, joint symptoms (pseudo-gout), neuropathy, fits. If these symptoms can be alleviated, then the aim of dietary therapy is achieved, and these patients are able to enjoy an almost normal and useful life, working until only a few days before dying in terminal renal failure.

Briefly, this form of dietary treatment is based on two principles, restriction of the dietary protein intake to the minimal (at present undefined) quantities of essential amino acids required by uraemic patients and the provision of adequate energy to avoid the breakdown of body tissues and to provide energy for the endogenous synthesis of non-essential amino acids and the re-utilization of urea.
The results of such a regimen are extremely encouraging, Shaw, Bazzard, Booth et al. (1965), Berlyne (1966), Berlyne and Hocken (1967). The patients are very well motivated as the clinical improvement is most convincing. They feel normal and have less anorexia and gastro-intestinal symptoms (inherent in other regimes).

Dietary therapy therefore represents a major step forward in the treatment of chronic renal failure and its therapeutic importance is firmly established.

2. METABOLISM OF UREA

a. Urea re-utilization

There is evidence that a number of species, including man, can convert non-protein nitrogen into body protein. Exogenous nitrogen as urea or an ammonium salt can be used for the synthesis of amino acids in vivo, and endogenous urea nitrogen can be re-utilized by continued re-cycling in the form of ammonia.

The evidence has been derived from two kinds of studies:

a) studies of growth in the young or nitrogen balance in the adult and b) the use of labelled $^{15}$N. Lardy and Feldott (1950) showed that there was an increase in the growth rate of young rats when ammonium citrate was added to a diet supplying the minimal essential amino acids and adequate total nitrogen for growth. Similar results were obtained by Rose, Smith, Womack and Shane (1949) when diets containing ample essential amino acids, and minimal non-essential nitrogen were supplemented with various sources of nitrogen including ammonium salts, L-glutamate, glycine and urea. In these experiments urea was utilized the least efficiently.

Snyderman, Holt, Dancis et al. (1962) made some studies in infants and found that the growth rate increased following a period of delayed growth due to a restricted milk intake, when a supplement
of urea nitrogen or glycine nitrogen was given. Moore, Lavietes, Wakeman et al. (1931) studying normal men taking an adequate diet showed that when the diet was supplemented with urea, the amount of additional nitrogen excreted approximately equal ed the intake, but when nitrogen intake was decreased, only a proportion of the urea nitrogen supplement was excreted. Giordano (1963) obtained similar evidence for the utilization of exogenous urea nitrogen in healthy individuals and in uraemic subjects he found that the endogenous body urea nitrogen pool was decreased. This suggested that the urea was being re-utilized for anabolic purposes. A positive nitrogen balance was indicative of this, but after the blood urea fell to below 80 mg per 100 ml a negative balance resulted. This became positive again when exogenous nitrogen as ammonium citrate was added to the diet. Giordano concluded that endogenous urea nitrogen can be re-utilized until the total body urea pool becomes reduced to below 80 mg per 100 ml.

This concept of urea re-utilization by uraemic patients was emphasized by Giovannetti and Maggiore (1964) who used minimal amounts of essential amino acids with a low protein diet for severe uraemic patients and noted a marked decrease in blood urea nitrogen. Gallina and Dominguez (1971) emphasized the importance of energy intake in the ability to utilize exogenous non-protein nitrogen. In their studies a negative nitrogen balance was induced by using low energy diets in which the milk protein was reduced. They then showed that nitrogen equilibrium could be restored when urea nitrogen was added to these diets. But if the energy intake was reduced still further, it was found that nitrogen equilibrium could not be regained, when supplements of urea nitrogen were given.

Compounds labelled with $^{15}$N have been used in a number of studies.
Thus Rittenburg, Schoenheimer and Keston (1939) found that a small proportion of \( ^{15}\text{N} \) given as ammonium citrate to normal adult rats on a stock diet was incorporated into the tissue amino acids.

Growing rats, when fed on normal or low protein diets containing \( ^{15}\text{N} \) labelled urea incorporated the label into tissue amino acids, and the amount of label retained was increased on a low protein diet (Rose and Dekker, 1956).

Snyderman et al. (1962) demonstrated the labelling of plasma proteins and haemoglobin in infants after initial protein deprivation and subsequent supplementation with \( ^{15}\text{N} \) urea. Giordano, de Pascale, Ballestrieri et al. (1968) and Richards, Metcalfe, Gibson, Ward et al. (1967) confirmed the retention of \( ^{15}\text{N} \) in the plasma proteins of normal and uraemic individuals.

b. The metabolism and recycling of urea

Leifer, Roth and Hempelmann, (1948) were possibly the first to obtain evidence that urea is metabolised. They found that after \( ^{14}\text{C} \) labelled urea was given to mice \( ^{14}\text{C} \) was recovered in the exhaled carbon dioxide. Further evidence that not all the urea produced in the body is excreted was obtained by Walser and Bodenlos (1959) who measured urea production and excretion rates by injecting labelled urea and showed that normally urea production exceeded excretion. They calculated that approximately 25 per cent of the total urea produced in 24 hours was broken down in the body. Evidence for the recycling of urea was obtained by Regoeczi, Irons, Koj et al. (1965) in rabbits from urea labelled with both \( ^{15}\text{N} \) and \( ^{14}\text{C} \). They calculated that approximately 20 per cent of the \( ^{15}\text{N} \) dose was reconverted to urea because of the amount by which the extended half-life of \( ^{15}\text{N} \) labelled urea exceeded the half-life of \( ^{14}\text{C} \) labelled urea.
Walser and Bodenlos (1959) had studied this matter in man by giving an intravenous dose of urea in which more than 95 per cent of the total molecules given contained $^{15}$N in both nitrogen atoms. By the use of mass spectrometry, urea N molecules of mass 28, 29 and 30 can be identified. Therefore almost all the Nitrogen molecules of mass 30 would be derived from the labelled urea as first introduced, but urea molecules of mass 29 would contain only one labelled nitrogen atom which therefore must have been recycled. In this way after injecting double labelled urea, the decrease in the ratio of $^{30}$N : $^{29}$N indicated the amount of urea nitrogen recycled.

c. Urea hydrolysis

Urea hydrolysis occurs in the human colon as the result of action by bacterial ureases. Levenson, Crowley, Horowitz et al. (1959) showed that when antibiotics were given to rats there was a marked decrease in urea breakdown. Also Wilson, Ing, Metcalfe-Gibson et al. (1968) and Wrong, Houghton, Richards et al. (1970) have shown that in man given antibiotics the urea concentration in the faeces was approximately the same as that in the blood and it was also shown that an increased urease production in the colon follows an increased blood urea.

Walser and Bodenlos (1959) showed that when labelled urea was injected into individuals pretreated with an antibiotic, the excretion of $^{15}$N increased from 70 per cent to about 100 per cent. It therefore appears that conversion of urea to ammonia by bacterial ureases is a necessary part of the process of the reutilization of urea.

In uraemic individuals re-utilization of urea nitrogen appears to take place when the minimum amount of essential nitrogen and maximum amount of energy are given.
Thus there would appear to be three anabolic pathways for the synthesis of non-essential amino acids. They can be synthesized from dietary protein and energy or they can be synthesized from the nitrogen of the urea pool and carbon skeletons made from non-protein precursors or the endogenous amino acid pool.

In uraemic individuals, net protein utilization is higher than that predicted as being possible in normal individuals from the exogenous protein and energy supplied. In normal individuals the theoretical net protein utilization can be predicted by the methods of Miller and Payne (see Appendix).

The concept of nitrogen balance in patients with uraemia requires modification to allow for an internal recycling of nitrogen from urea into a synthetic pathway, a process which depends primarily on the protein : energy ratio.

From what has been said, it may be concluded that there is an additional energy requirement for the provision of the adenosine triphosphate necessary for the reutilisation of a portion of the urea pool. The priority for this energy requirement and the partitioning of the energy consumption in the functional adaptation in the uraemic subject has not yet been defined. Energy also performs an anti-catabolic function i.e. protein-sparing and a normal synthetic function i.e. basal anabolism in which in the uraemic individual urea recycling and reutilization must be included.

Considering the additional importance of dietary energy to the chronic renal failure patient, it could well be argued that the nature of the source as well as its amount would play an important role.
3. DIETARY TREATMENT IN CHRONIC RENAL FAILURE

a. Considerations in relation to the diet for chronic renal failure patients.

It is evident that a metabolic adjustment to a deranged biochemistry occurs in patients with uraemia, and furthermore that criteria applied to normal individuals cannot be applied with any accuracy to these patients. It would be quite erroneous for example to assume that the normally constant relationship between dietary and faecal nitrogen found by Mitchell and Bert (1945) also applies in these patients.

There is no consensus of opinion on the minimal protein intake required by uraemic patients, and the optimal protein and energy intakes have not yet been determined with any degree of precision. Even in normal individuals the ideal protein requirements are debatable. Much of the experimental data available on this subject is derived from short term studies and it is doubtful whether full adaptation or a state of equilibrium is achieved. The importance of defining protein and energy intake on a body weight basis and of compensating for urinary protein loss is now being realised. This is particularly important in the uraemic subject in whom controlled studies have only recently been done. The possibility of the long term maintenance of these patients has only lately become a reality.

From the few controlled studies that have been made on such patients certain aspects are emphasized.

The maintenance of a metabolic and renal equilibrium is essential. When the amino acid supply is changed there is a short lag period of negative nitrogen balance, until equilibrium is achieved. This lag period reflects a complex readjustment of the synthetic processes within the cell. The readiness with which
equilibrium can be lost is a reflection of the precarious metabolic balance. Because of the dynamic state of interrelationship that exists between various metabolites, a deficiency of one may affect the functional integrity of the whole organism and result in an unstable situation. It is therefore of the utmost importance that there should be a preliminary transitional period to allow the adaptation to a specific dietary regimen.

Individuals vary in a way that may depend on their previous nitrogen and energy intakes. The protein content of some tissues such as liver or gut mucosa or the serum albumin concentration are sensitive to variations in nitrogen intake. Body protein may be depleted and the serum aminograms abnormal. In chronic renal failure it has been shown by Coles, Peters and Jones (1970) that considerable reduction of body protein may occur before this is reflected by the serum albumin. Deficiencies of particular enzymes may also occur, and these would all prolong the lag period in adaptation, and make comparative studies difficult.

The age of the patient may incur an altered rate and pattern of amino acid turnover. The older age groups may well be less able to adapt to an altered nitrogen intake. There is in fact some evidence that the transitional period in adapting to changes in dietary intake may be extended in the elderly (Kountz, Hofstatter and Ackerman, 1947). Watkin (1964) suggests that there may be a difference in the biological value of proteins in the aged, compared with younger age groups. There are many individual deviations and variables associated with the ageing process, which may influence the metabolic response in different ways, and be of importance in interpreting metabolic data.
The normal constant relationship between dietary and faecal nitrogen cannot be applied to uraemic individuals. If the expansion of the urea pool exceeds a certain value per unit mass it is conceivable that substrate inhibition can occur when the rate of urease activity does not proceed beyond a certain increase in substrate concentration (Dixon and Webb, 1964). In this instance urea reutilization would be limited as it can also be limited by the energy available. The efficiency of urea reutilization is then reflected in the faecal nitrogen and a relationship can be shown to exist between the percentage of faecal nitrogen to dietary nitrogen and the final nitrogen balance result.

Hence consistent and comparable data are essential in nitrogen balance studies. There may be a cumulative error for instance due to food in the diet being overestimated (minute quantities of food left on plate). Similarly, nitrogen excretion may be underestimated. Integumental and sweat nitrogen losses may be variable but are approximately 50 mg nitrogen daily in normal people (Voit, 1930; Mitchell and Hamilton, 1949; Consalazio, Nelson, Natěush et al., 1963). These integumental losses have not been allowed for in the present studies as no data is available on uraemic patients.

Carefully controlled and systematic uniformity in presentation of nitrogen balances, can provide reasonably accurate data for measuring overall trends, but the limitations of this method should be recognized. Errors are likely to be less when small nitrogen intakes are given. Constant errors will not modify the conclusions.

Evaluation of a long term dietary regimen should be supported by long term studies if possible. These may be difficult because many criteria are to be fulfilled in patients whose flexibility and adaptive capacity is limited to varying extents.
The protein-energy relationship is of considerable importance if maximal utilization of nitrogen is to be achieved.

b. Protein-energy relationships in normal individuals

The minimum protein requirement is that which results in the absorption of sufficient nitrogen to maintain nitrogen equilibrium when the energy is adequate. This amount is only sufficient for healthy subjects under physiologically stable conditions.

This minimal protein requirement would be equivalent to the total minimal nitrogen excretion when a nitrogen free diet is taken, and this endogenous nitrogen excretion is approximately 3.0 g nitrogen or 250 mg nitrogen/kg \(0.73\), Miller and Payne, (1962). The nitrogen balance is therefore dependent on the nitrogen intake, the amount of obligatory loss, and the efficiency of utilization which in turn is proportional to the amount of the nitrogen intake that is retained (Net Protein Utilization) (see Appendix).

Hence

\[ \Delta \text{BAL} = I \text{(intake)} \times (\text{N.P.U.})^M \text{(obligatory loss)} \]

Dietary factors that are involved in the efficiency of utilization of protein will now be considered.

An estimate of the minimal requirement for the essential amino acids has been made by Rose, Wixom, Lockhart and Lambert (1955) and from this the pattern of the essential amino acids contained in a particular protein can be translated into terms of the nutritive value of the protein.

From the work of Rose et al., an ideal amino acid pattern was proposed by F.A.O. 1957. From this ideal pattern the protein can be given a score relating to the amino acid present in the lowest proportion with reference to the pattern, and this denotes the nutritive value or "protein score". It has been shown (Miller and Domoso, 1963), that the level of sulphur-containing amino acids is the most limiting for animal
and human diets. Therefore methionine and cystine were considered to be critical in determining the ideal reference value. Accordingly a score has been derived by evaluating the total sulphur-containing amino acids in a diet.

It is important to realise that the protein score of a diet may not reflect a direct measure of its efficiency especially when very low nitrogen intakes are used; here the size of the endogenous pool is enormous compared with the very minute exogenous source, and the methionine content of this body pool would be very high (Allison, 1949).

In order to attain nitrogen equilibrium, the diet must supply, in addition to the eight essential amino acids, sufficient nitrogen for the in vivo synthesis of non-essential amino acids. This requires probably less than 2.55 g nitrogen per day (Rose and Wixom, 1955). For reference purposes the essential amino acid requirement is expressed as a proportion of the total nitrogen \( (E/T_N) \) ratio (Snyderman, Holt, Dancis, Roitman, Boyert, Balis, 1962).

Another factor influencing the efficiency of utilization of the intake nitrogen is the concentration of protein in relation to the energy content of a diet. The greater the proportion of protein above a certain level the greater will be the wastage due to oxidation (Miller and Payne, 1961a). Net Dietary protein Calories per cent \( (N.D.P. \text{ Cals.} \%) \) is a measure of the proportion of the total energy content of a diet which, as protein, is fully utilizable for anabolic functions. There is a large energy requirement in synthesizing and maintaining body tissues, and if this is not supplied the amount of protein available for anabolism will be reduced. A linear correlation between the efficiency of utilization and protein concentration has been shown to exist (Mitchell, 1923-4). A low energy intake can limit the efficiency with which a protein is utilized.
The timing of the intake of nutrients can also have an effect on protein utilization. Thus Berg and Rose (1929) showed that if young rats were fed a diet deficient in Tryptophan, and an additional supplement of Tryptophan was delayed, the more extended the time interval between the deficient diet and the supplement, the greater the growth retardation. This effect is less marked in man (Munro and Wikramanayake, 1954), where the time equivalence would be much less. It may nevertheless be of relevance to the efficiency of protein utilization, thus Munro and Wikramanayake, (1954) showed a slightly improved nitrogen retention when extra carbohydrate was eaten at the same time as protein, compared with the resulting nitrogen retention when eaten separately. Similarly, Cohen and Joseph (1959) and Fabry (1969) showed that rats having an intermittent pattern of feeding compared with continuous feeding retained less protein and had an increased excretion of urea in the urine.

The intake of minerals and vitamins can affect the protein efficiency of a diet (Morrison, Sabry and Campbell, 1962). Diets low in sodium and potassium have been shown to result in a diminished protein efficiency (Miller and Payne, unpublished data).

c. Energy relationships

There is a fundamental relationship between metabolic size and protein metabolism. Many workers, Terronne and Sorg-Mutter (1927); Sorg-Mutter (1928); Smuts (1935) and Brody (1945) have shown that the minimal nitrogen excretion (endogenous nitrogen) on a protein free diet is directly proportional to the basal metabolic rate, and both are directly related to the amount of metabolically active tissue. A value for this minimal excretion of nitrogen has been estimated by Brody (1945) to be approximately 2 mg nitrogen per basal Calorie. Brody has also compiled data from various species and this shows that the basal energy expenditure is proportional to
Fig. 2  The relationship between nitrogen balance and energy intake in normal individuals on a nitrogen intake of 2.4 - 4.5g nitrogen per day. Maximal protein utilization occurs at a total energy intake of 800 - 1,100 Calories per day (13 Cals/kg - 20 Cals/kg = body weight approximately) From Calloway and Spector (1954)
body weight \(0.73\). A constant relationship appears to exist between minimal nitrogen excretion and body weight \(0.73\) over a wide range of different species and body weights.

The question of the relationship between energy and protein intake in normal individuals was reviewed by Calloway and Spector, (1954). The response of nitrogen balance to a standard addition of protein to the diet is the same over a wide range of energy intakes, but this response is not elicited when the energy intake is below a critical level. A limiting energy level could be defined, beyond which increasing the energy without the protein, or increasing the protein without the energy is of no advantage; they found that on diets containing 3 g nitrogen per day, maximal nitrogen retention was obtained at around 900 Calories and no further nitrogen retention could be induced by an increase of the energy. (See Figure 2).

d. The problem of food acceptance

Palatability can be a problem, as food patterns are decided by the society in which the patient lives, and conventional foods appear to be essential and even comforting for some patients especially when ill. The diet can be adapted to a small extent to the eating habits of individual patients and then as the benefits of dietary therapy become evident - complete co-operation is ensured.

Prior to the introduction of the therapeutic principles inherent in the Giordano-Giovannetti diet, many physicians considered that dietary treatment was an unnecessary burden on uremic patients.

e. Evolution of dietary treatment in chronic renal failure

Attempts to establish nitrogen requirements in chronic renal failure are noteworthy. Thus in 1905 Folin showed that in normal individuals 400 g starch and 300 g cream (2,800 Calories) reduced the excretion of nitrogen in the urine to 4 g per day.
Keutman (1935) found that when patients who had a heavy proteinuria and hypoproteinaemia, and were eating 0.8 g protein per kg body weight, were given an increasing daily energy intake, nitrogen retention was also increased.

Atchley (1943) considered dietetic treatment to be of little therapeutic value in patients with chronic renal failure, because he thought that, to be effective, restriction of protein required such an increase of energy intake that it was rarely feasible.

Olbricht (1944) produced a negative nitrogen balance on uraemic patients by giving them 0.75 g protein and 30 Calories per kg body weight. He calculated that this lack of nitrogen equilibrium was due either to increased catabolism or decreased anabolism and Olbricht suggested that a protein intake sufficient in normal individuals is therefore inadequate in these patients. An increase in the intake to 2 g protein per kg body weight resulted in all patients retaining nitrogen. It is notable that no increased allowance was made for proteinuria, and the quality of the protein was not specified. Faecal nitrogen was not determined, and an average value was taken for all patients. An increased catabolism and a decreased synthesis of protein, leading to a negative nitrogen balance was probable in these studies and would be due to inadequate replacement of urinary protein, in both quantity and quality, combined with an insufficient energy intake.

Fishberg (1944) used a diet containing 20 g protein in the treatment of acute uraemia, for chronic renal failure a 40 g protein intake irrespective of body weight or renal function, was considered necessary, as these patients were chronically malnourished after long debilitating illness.

Kempner (1945) introduced his rice and fruit diet which provided 20 g of second class protein and 2,000 Calories irrespective of body
This treatment was used for patients with malignant hypertension and renal failure. Nitrogen balance could be maintained for several months and in most cases, the blood urea levels were decreased.

Gamble (1947) reported that the addition of 100 g glucose to survival rations, consumed by normal subjects reduced the amount of protein catabolised by 40 g. More glucose elicited no further effects.

Borst (1948) gave a diet of butter and sugar (see Appendix) which provided approximately 2000 Calories, and he noted that in uraemic patients the protein sparing effect was higher than in normal people. Borst maintained that an anuric patient on his diet might live for twenty three days before the blood urea reached 350 mg per 100 ml. Low protein bread was introduced by Borst and shown to have the same protein sparing effect as the rice or sugar/butter diet.

Bull and Joekes (1949) treated acute anuric uraemia with an intragastric drip feed (see Appendix).

Kolff (1952) used various alternatives for uraemic patients based on Borst's butter soup (see Appendix), following the principle of high fat, high carbohydrate, or he used frozen butter pills x 30 pills to provide 150 g butter containing 128 g fat and 1,150 Calories. A forced feeding regimen via a naso-gastric tube was used for acute renal failure, and a forced high energy diet was tried on some chronic renal failure patients, but considered unjustifiable as a long term policy.

Borst, Kempner and Kolff all believed that a daily diet containing 20 g second class protein sufficed, if the energy requirements were met.
with carbohydrate and fat, and Kempner demonstrated that a uraemic patient can be maintained in nitrogen equilibrium for several months on a diet containing 20 g second class protein as rice and fruit, and a total daily Calorie intake of 2,000. The protein and energy content of these diets was not graduated to body size.

The protein energy relationship was emphasized by Calloway and Spector (1954) who showed that in normal individuals a limiting energy level could be defined beyond which increasing the energy intake is of no advantage. They found that an intake of 3 g nitrogen daily resulted in a maximum nitrogen retention with approximately 900 Calories, and that no further nitrogen retention occurred by increasing the energy.

The importance of the quality of the protein was shown by Rose and Wixom (1955). All theoretical knowledge about the minimal protein requirements have been determined by nitrogen balance. In studies on normal individuals Rose and Wixom estimated the minimal amounts of essential amino acids, and an energy intake of 35/50 Calories per kg body weight was necessary to maintain nitrogen equilibrium in normal adults. These studies were done on healthy adults taking high protein intakes and although not applicable to uraemic patients Rose's estimates have been used for expediency, as a working basis.

Herndon et al. (1958) who studied five uraemic and one normal subject found that the uraemic subjects required 0.54 - 0.70 g protein per kg body weight daily to achieve nitrogen equilibrium; the one normal person required 0.49 g protein per kg. The daily energy intake given was 27 - 30 Calories per kg body weight. In this study the low energy intake probably limited the protein sparing effect and any urea recycling would be less efficient. These workers emphasized the importance of the amino acid pattern of the dietary protein in attempting to achieve nitrogen equilibrium.
Merrill (1960) introduced the idea of a minimal basal requirement, which he fixed at 0.5 g protein per kg body weight, with additional amounts related to the patient's own condition, for example for stress and urinary protein losses. The protein intake was to be related to the concentration of urea in the blood. Thus with a blood urea of 150 mg per 100 ml the intake recommended was 35 g protein per day, and this was increased to 50 g when the blood urea concentration was less than 100 mg per 100 ml. The quality of the protein was thought to be as important as its quantity. Merrill considered that an intake of 300 or 400 g of carbohydrate taken with the protein was essential to achieve maximum protein sparing. Although fat has a higher energy value than carbohydrate, Merrill considered a high fat diet to be nauseating and unsuitable for uraemic patients.

Giordano (1963, 1966) applied Rose's values for minimal essential amino acids requirements to the dietary treatment of uraemic patients in conjunction with minimal total nitrogen and high energy intakes. He introduced the concept of reutilization of urea for the synthesis of non-essential amino acids in vivo, because he observed that azotaemic patients, when so treated showed a reduced urea pool, with an improved nitrogen balance, and the total dietary nitrogen requirements were lower than those of normal subjects.

This concept of in vivo synthesis of amino acids and reutilization of endogenous urea nitrogen by giving a diet of high biological value protein but minimum non-essential nitrogen was then translated into a practical dietary regime containing 2000 - 3000 Calories daily by Giovannetti and Maggiore (1964), who used their diet in patients with severe chronic renal failure. The importance
of the energy intake was again emphasized by Shaw, Bazzard, Booth et al. (1965) who evolved a Manchester version of the Giovannetti diet, containing 0.26 g protein per kg body weight daily.

Ford et al (1969) considered that 0.5 g protein per kg body weight daily was required by chronic renal failure patients and that the type of protein or total energy had no significant effect on nitrogen balance. The data in this study cannot be comparative because a wide range of dietary constituents were used. Conclusions applied to the meat proteins, chicken, lamb and beef, would certainly be invalid for protein which is more completely absorbed and having a higher biological value. It is reasonable to expect that the net protein utilization would have been improved with less protein but of higher biological value and an increased energy intake. Protein intakes given were 0.23 g protein - 1.0 g protein per kg body weight. The proportion of high biological value protein to total protein varied between 40 per cent and 98 per cent and the range of Calories between 1,330 to 3,310 (23.6 - 59.0 Calories/kg) daily. It is significant that in studies using protein with biological values of less than 70 per cent the resulting nitrogen balances were more negative.

Richards, Metcalfe Gibson, Ward et al., (1967) confirmed the utilization of ammonia nitrogen for anabolic purposes and suggested the recycling of urea nitrogen. They studied four normal individuals and two uraemic patients to whom they gave $^{15}$N labelled ammonium chloride. The degree of labelling of plasma albumin was found to be increased during a state of protein depletion, and the total incorporation into the albumin pool was greatest in the uraemic patients who had previously taken a prolonged low protein intake.
They also showed that urea splitting organisms were involved in the mechanism of urealysis because when the patients were given antibiotics, the faecal concentration of urea became close to plasma levels.

This concept had previously been explored when Walser and Bodenlos (1959) showed that antibiotics given to mice and men increased the recovery of $^{15}$N labelled urea in the faeces by 100 per cent.

Robson, Kerr and Ashcroft (1967) and also Richards et al. (1967) have shown that the urea nitrogen reutilised in uraemic individuals is much higher than in normal subjects and that the amount reutilised increased with a rise in blood urea. Also an increased blood urea nitrogen may result in an increased faecal nitrogen, as urea is freely diffusible across the small intestine. Faecal nitrogen may therefore be related to nitrogen balance.

Until very recently few balance studies were done on uraemic patients, and there has been a lack of uniformity of procedure in such studies.

The importance of high biological value protein began to emerge with Giordano's work, and a very high energy intake was emphasized by him and others – Giovannetti and Maggiore (1964), Richards et al. (1967).

This matter was studied in the course of the present investigations, and the results have been published (Hyne, Fowell and Lee, 1972). However, neither in this work nor in any of the previous work mentioned was any serious consideration given to the source of the energy, carbohydrate compared with fat, or polysaccharide compared with mono or disaccharide. This could be important from the practical point of view in devising a palatable diet for patients
with chronic renal failure, for any advantage resulting from a constraint on the selection of energy would have to be assessed against the disadvantage of the limitations imposed in the choice of foods, and food preferences play a very important role in the patient's acceptance of diet therapy.
CHAPTER 2

METHODS

Details of individual patients on whom these studies were carried out are given in Tables 1 - 9.

The investigations in these studies entailed strictly controlled dietary intakes in a Metabolic Unit.

The patients were all volunteers who gave their informed consent to these investigations.

The complete co-operation of the patient was considered essential. To ensure a well motivated volunteer, prior to the study, each patient was given a careful explanation of the objectives of the investigation. In this way a clearer understanding and a feeling of participation in the investigation, enlisted a greater co-operation and minimised food rejection.

Strict discipline of procedure and precise control of timing and environment were considered indispensable, in order to limit variables and obtain a reasonably true evaluation of the results on the data obtained.

1. PROCEDURES AND OBSERVATIONS

A standardized protocol was observed in which record keeping was performed routinely and punctually. Patients were under fairly close supervision, up and dressed in a fairly even temperature. Exercise taken was reasonably constant at around the same time each day, and patients were non-infected. Body weights and temperatures were recorded daily at the same time.

A routine was established for collecting and labelling specimens; faeces and urine by the patient, and blood by the laboratory staff. Faecal collections were discarded until the first carmine marker was identified, and saved until the last carmine marker collected. Each
faecal specimen was labelled by the patient with name, date and time.

Diet

Details of each diet used are given in Table 2 for each patient. Before beginning the balance study the subjects followed a low protein Giovannetti-Giordano type diet as outpatients, although they were not strictly controlled.

An equilibration period of a minimum of 4 days on the exact experimental diet always preceded the commencement of the balance.

A detailed dietary history was always taken on each patient and the experimental diet adapted to individual preferences where possible.

Strictly controlled constant Giovannetti-Giordano type diets were then given, divided into four meals daily taken at the same time intervals. The amount of dietary protein allowed was increased by the amount of proteinuria present. A uniform and meticulous system of food preparation and method of production was followed. Examples of this are that bread was always crustless, milk always homogenised, the weight of one whole egg always increased by the addition of egg white only, or reduced by decreasing egg white only, tea always strained, tomatoes were always peeled etcetera. Plates and cups were inspected after each meal to verify complete consumption of food. The food was always presented as attractively as possible.

The diet provided at least 0.25 g protein per kg body weight, the proportion of high biological value protein to total protein was greater than 75 per cent, with one exception which was 72 per cent.

The total energy was as specified and divided into aliquots throughout the day, providing at least 60 Calories per gram of protein. The source of the energy is given in Table 9.

Water and sodium intakes were adjusted according to losses and the height of the blood pressure.
All diets contained very low levels of potassium, calcium, phosphorus and magnesium.

The essential amino acid content of the diet was estimated from tables prepared by Orr and Watt (1957) and it was found that the minimal requirements as estimated by Rose and Wixom et al. (1955) on normal individuals, were fulfilled with the exception of Methionine. No Methionine supplements were given.

An example of the essential amino acid content of the diet eaten by S.Y. (calculated from Orr and Watt) is shown in Table 4 S.Y. The nutrient content of foods was calculated throughout from food tables (McCance and Widdowson (1967).

The following vitamin supplements were given: Vitamin C and compound Vitamin B (thiamine 30 mg, riboflavin 12 mg, pyridoxine 12 mg, nicotinic acid 120 mg).

All food rejects were weighed and recorded individually. They were then sealed, labelled and stored at 4°C. Aliquots representing one day's total intake were prepared exactly as the food presented to the patient. This was then homogenised and stored at 4°C for future analysis.

Analyses

Nitrogen balances were divided into four day periods, and continued for a minimal total period of 16 days, and generally for 24 days.

Prior to carrying out the balance, two 24 hour urine specimens were collected for analysis of urinary protein, urea and electrolytes, whilst the subjects were following the dietary protocol which was to be taken during the balance. Urinary collections were made over 48 hour periods and kept at 4°C. Faecal collections were made over 4 day periods. Carmine was given at the commencement of each 4 days...
to delineate individual balance periods. Fasting blood samples were taken on the first day and thereafter at 4 day intervals and finally on the morning following the last day of the balance.

These specimens were analysed for urea, electrolytes, total protein, albumin and haemoglobin.

The following determinations were done by the Biochemistry Department of the Portsmouth Group hospitals:

Urea was measured by the diacetyl monoxine method (A.C.P. technical bulletin No. 9) and creatinine by an automatic colorimetric method (Technicon autoanalyser method N116.) Sodium and potassium were measured by flame photometry (Technicon autoanalyser method N-21 A). Serum bicarbonate was measured by the phenolphthalein method (Technicon autoanalyser method N-8 B) and total serum protein by the biuret method. Albumin was estimated by the bromocresol green method (A.C.P. technical bulletin number 11). Haemoglobin measured on a Coulter S automatic counter.

The total volume of the 48 hour urine collection was measured. Four day faecal collections were homogenised and 24 hour duplicate diets which were prepared for each four day period, were homogenised. All food rejects and vomit were homogenised for each four day period. These were then analysed for total nitrogen by the micro Kjeldahl method (Ingram, 1962).

Determination of total nitrogen using the Micro Kjeldahl method

Reagents
Conc. sulphuric acid
Mercuric sulphate (catalyst)
Hydrochloric acid 0.01 N
Sodium hydroxide 30% solution + sodium thiosulphate 5%
Indicator : Methyl red + Bromcresol green
Boric acid 2%
Validation of method

To ensure reproducibility of results and to verify complete
digestion, a standard in triplicate was performed prior to each
series of nitrogen estimations.

Examples as follows:

To determine the concentration of ammonium chloride in a
standard solution, (standard solution contained 350 mg ammonium
chloride per litre). The ammonia was expelled from 10 ml of ammonium
chloride solution and titrated with 0.01 N hydrochloric acid.

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<tr>
<td></td>
<td>6.5452</td>
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5 ml ammonium chloride solution were titrated with 0.01 N hydrochloric
acid.

<table>
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<td>(ii)</td>
<td>3.26</td>
</tr>
<tr>
<td></td>
<td>3.25</td>
</tr>
</tbody>
</table>

Calculation

A. 10 ml contains $6.52 \times 0.14 = 0.9128 \text{ mg nitrogen}$

$0.9128 \times \frac{53.46}{14} = 3.486 \text{ mg ammonium chloride in 10 ml}$

$\therefore 348.6 \text{ mg ammonium chloride in 1 litre}$

B. 10 ml contains $6.5452 \times 0.14 = 0.9163 \text{ mg nitrogen}$

$0.9163 \times \frac{53.46}{14} = 3.4909 \text{ mg ammonium chloride in 10 ml}$

$\therefore 349.9 \text{ mg ammonium chloride in 1 litre}$
C. 5 ml contains $3.25 \times 0.14 = 0.455$ mg nitrogen

\[
\frac{0.455 \times 53.46}{14} = 1.73745 \text{ mg ammonium chloride in 5 ml}
\]

\[
3.47438 \text{ mg ammonium chloride in 10 ml}
\]

\[
\therefore 347.4 \text{ mg in 1 litre.}
\]

A blank was performed using 10 ml distilled water. This was distilled over for 10 minutes.

Results

A pale pink end point. Therefore no nitrogen was present.

Procedure for balances

a) Forty eight hour collection of urine
b) Four day collection of faeces
c) One day duplicate diet (for each four day period)
   a) was measured
   b) and c) were homogenised and weighed.

A sample was then oxidised by heating with conc. sulphuric acid in the presence of the catalyst and the resulting ammonium sulphate decomposed by the addition of excess sodium hydroxide. The ammonia was distilled and nitrogen determined.

Dilutions

The faeces, which were divided into the balance periods were made up to 1500 g with distilled water and homogenised. The 24 hour duplicate diets were made up to 4000 g with distilled water and homogenised. The total 48 hour urine volumes were measured.

Into the weighed Kjeldahl flasks were added:

a) 2 ml of undiluted urine, 2 ml of faeces or diet homogenised and diluted as above and the flasks then reweighed.

b) Conc. sulphuric acid was added as follows:
   5 ml added to faeces
   5 ml added to urine
   10 ml added to diet
c) an amount of catalyst which will just cover the end of a spatula was added to each flask.

The digestion was always performed in duplicate. They were then heated in a fume cupboard for several hours as follows. Initially the heat regulator was set at 2. This was gradually increased and when the mixture showed an orange discoloration it was increased to 10 (maximum). When finally the mixture cleared and became colourless, the heat was turned off and the mixture allowed to cool. Digestion mixtures were then diluted to a known volume with distilled water in a graduated flask (to give a concentration of 1 in 10 acid); i.e. faeces and urine were made up to 50 ml and diet was made up to 100 ml.

10 ml of the diluted sample was taken, and poured through the funnel into the flask of the distillation apparatus. 15 ml of sodium hydroxide were added. Two distillations were done from each flask i.e. a total of four for each specimen. Liberated ammonia was passed into a receiver containing 5 ml of 2% boric acid and indicator (the condensor tube was well below the level of the liquid in the receiver to avoid loss of ammonia). Distillate was collected for exactly 3 minutes after the indicator appeared green. The receiver was then lowered so that the end of the condensor tube was above the level of the liquid to ensure complete drainage. After 4 minutes in this position the process was stopped, the receiver removed, and heat discontinued.

Before use, and after every fourth distillation, the apparatus was steamed through; and after each distillation the apparatus was washed through with distilled water.

The contents of the receiver were titrated with 0.01 N hydrochloric acid. Colour change is from bluish green to the end point of pale pink (A standard was performed prior to each series of distillations).
Calculation

Each ml of 0.01 N HCl neutralised by the ammoniacal distillate corresponds to 0.14 mg nitrogen.

**URINE** - made up to 50 ml

\[
\frac{\text{Vol}}{2} \times 5 \times t \times 0.14 = 0.35 \times v \times t
\]

**FAECES** - made up to 50 ml (total weight faeces = 1,500 g)

\[
\frac{1500}{\text{wt sample}} \times 5 \times t \times 0.14 = 1.05 \times \frac{t}{\text{wt specimen}}
\]

**DIETS** - made up to 100 ml (total weight = 4000 g)

\[
\frac{4000}{\text{wt sample}} \times 10 \times t \times 0.14 = 5.6 \times \frac{t}{\text{wt specimen}}
\]

2. **PRESENTATION OF DATA**

In these metabolic balance studies the results obtained on one patient were compared with those of other patients in the investigation.

The basic data obtained in all studies is presented in tabular form, and a modified Reifenstein plot used to illustrate the accumulated data.

The modified Reifenstein plot: based on the procedure of Reifenstein, Albright and Wells (1945).

The scale of nitrogen intake and nitrogen balance in g per 24 hours is shown on the ordinate. The horizontal line shown at 0 is the base line, referring to nitrogen intake and nitrogen balance. The time scale, divided into four day balance periods is shown on the abscissa.

Measurements are made on collections of 4 day specimens of excreta, and intake nitrogen excluding rejects and vomit. These are
represented as collective averages over four days, in amounts per 24 hours.

The nitrogen intake is indicated as the line covering the area from the base line to the bottom of the plot.

The faecal nitrogen is shown as a dotted area extending from the bottom level of the intake towards the top of the plot.

Urinary nitrogen is shown as a hatched area extending from the upper level of faecal nitrogen towards the base line 0.

Changes in blood urea nitrogen are graphed above the Reifenstein plot.

Total body urea was corrected for changes in body weight at the commencement of each balance period.

The final summary of the internal nitrogen balance as influenced by fluctuations in blood urea is indicated by arrows and thus external nitrogen balance can be shown to be modified by this mechanism, and is expressed as $\Delta B$.

Positive nitrogen balance is represented below the base line, either as a white area above the upper extent of the excretion line, or else as an adjusted final balance line (arrows) resulting from a reduced blood urea.

A negative nitrogen balance is represented if the excretion line (hatched area) is shown above the base line, or if the final balance line is modified (arrows) to a level above the base line.

In these studies energy values are generally expressed both in terms of Calories and k.Joules in the Tables, but in the text Calorie values only are given. The conversion factor is as follows,

$1 \text{ Calorie (k.cal.)} = 1 \times 4.2 \text{ k.Joules}$. 
The results on the patient S.Y. have been omitted from all graphs and from the theoretical assessment of the energy partitioning, as these results indicated that a state of equilibrium was never achieved in this study.

In conjunction with the present study Dr. S. Lavender and Miss A. Waterfall investigated the effect of aluminium hydroxide upon calcium and phosphorus balance, serum calcium and phosphorus, tubular reabsorption of phosphorus and aluminium absorption.

The results of their study are still being analysed.
The diagnosis was stage 3 chronic renal failure with membranous glomerulonephritis, the glomerular filtration rate being 3.0 ml. per minute at the time of the study.

The patient worked as a precision electronic fitter. He was married with three children and his family history showed nothing of relevance to his illness.

Albuminuria was first discovered in this man in 1963 when he had a partial gastrectomy for a chronic gastric ulcer, from which he made a satisfactory recovery.

Albuminuria was again discovered on an insurance examination in 1965; at that time he had normal renal function tests and a normal I.V.P. He had no urinary symptoms and felt very well. Albuminuria was 3.51 g per 24 hours. In 1967 the first signs of ankle oedema were evident, with swelling of the feet and hands and marked pitting oedema of the legs. He still had no urinary symptoms and no return of any gastric ulcer symptoms. Later that year his blood urea was 32 mg per 100 ml., total serum protein 4.6 g per 100 ml., serum albumin 1.2 g per 100 ml. He was instructed to take a high protein diet and given diuretics.

Later that year he was referred to the Renal Unit. Urinary protein was found to be 8.5 g of protein per 24 hours.

At the end of 1967 he had a renal biopsy to find the cause of his severe nephrotic syndrome, and this showed a membranous glomerulonephritis. Serum albumin was then 1.4 g per 100 ml., globulin 3.2 g per 100 ml., cholesterol 373 mg per 100 ml., serum creatinine 1.8 mg per 100 ml., creatinine clearance 47 ml. per minute and a 24 hour urine collection contained 7.58 g of protein. Glycosuria was found to be present on several occasions. He was told to take an 80 g protein, 23 mEq. sodium
diet, his oedema subsequently decreased. He was also started on steroid therapy.

He continued through 1968 with a steady weight and very little ankle oedema. He felt generally fit and had a good appetite. The proteinuria was unchanged. His serum creatinine appeared to be slightly reduced. Later in 1968 the treatment with immunosuppressants and steroids was slowly withdrawn, as no beneficial effect was evident. The 80 g protein, 40 mEq. sodium diet was continued. In mid 1969 the patient experienced cramps in the legs at night and occasionally nocturia with thirst at night. No oedema was present. It was considered that his renal function was deteriorating rapidly and therefore his protein intake was reduced to 50 g protein, and later that year to 40 g protein, 22 mEq. sodium. He was also beginning to feel tired and to feel some weakness in his legs.

In the autumn of 1969 this patient was admitted as an emergency. He was found to have uraemic pericarditis. His blood urea was 315 mg per 100 ml. (on a 40 g protein diet). He was given a Giordano Giovannetti diet and Shohl's solution to combat acidosis. After peritoneal dialysis he was discharged on a Giordano Giovannetti diet.

He subsequently made routine visits to the Out-Patient Clinic and he continued with no further complications. He felt fine, had no oedema and continued on a Giordano Giovannetti, low sodium diet. In 1970 he was having nocturia once nightly, but was still in full-time employment and managing the Giovannetti diet very well. At the end of that year he continued to look well and was working full-time. Although he was now having pruritus, his progress was so satisfactory at this time that he was given a 30 g protein diet.

In 1971 he was admitted for a nitrogen balance study to which he
consented. Pruritus was the only complaint and he was in a generally fit state. At this stage his chronic renal failure appeared to be slowly progressing.

Details of the study on S.Y.

The nitrogen balance study on S.Y. was of 24 days duration and divided into six balance periods of 4 days. An equilibration period of 4 days was observed. The total blood urea nitrogen pool was assessed by adjusting for changes in body weight at the beginning of each balance period. Urinary protein was 1.7 g protein per 24 hours. Complete details of this balance study can be seen in Table 1 S.Y. to Table 8 S.Y.

The data of this study is divided into A and B. A representing the first three balance periods (12 days) and B the last three balance periods (12 days).

The total energy intake as presented to the patient was 50 Calories per kg body weight for the first half of the total balance (A) and this was almost totally consumed by the patient.

The total energy given during the final three balance periods (B) was 64 Calories per kg body weight, but the actual Calories taken averaged 59 Calories per kg body weight. The average energy intake over the total 24 days was 55 Calories per kg body weight. The proportion of the total energy provided by fat was 37 per cent for both (A) and (B) and carbohydrate provided 61 per cent of the total energy. Of the fat butter and double cream provided the major portion. A much smaller proportion was contributed by egg yolk and milk. The carbohydrate comprised 43 per cent polysaccharides and 57 per cent mono or disaccharides during the first half of the total balance (A). During the second half (B) when the energy was increased polysaccharides comprised 33 per cent of the total carbohydrate and mono or disaccharides 67 per cent.
of this. The polysaccharides were supplied as low protein bread, low protein flour, potatoes, Caloreen, Hycal. The mono and disaccharides were given as sweetened fruit, marmalade, jam, Hycal, sugar and a very small amount as lactose in milk.

The high biological value protein consisted of egg, double cream, milk, butter, and the small quantity of non-essential nitrogen was contained in fruit and vegetables, low protein flour and bread.

Table 3 S.Y. shows the mineral content and acid base of the diets. From this table it can be seen that the intake of calcium is much lower than 800 mg calcium, as recommended daily in the Report on recommended allowances (1969). Uraemic individuals are considered to be insensitive to Vitamin D. (Avoli and Slatopolaki, 1968; Avoli et al., 1967). However, this low calcium intake would not appear to have a significant influence within a short term context.

The magnesium intake is also low, a normal intake being approximately 200 mg daily. The iron intake of 6.17 mg daily is lower than the recommended intake of 12.0 mg. Supplements of ferrous sulphate are given to patients with chronic renal failure when taking a Giovannetti diet, but these are discontinued during the period of the balance study because of obliteration of the faecal carmine markers. Copper intake is normally around 2.0 mg daily and the intake of 1.11 mg is low but as this is a short term study, and the copper intake would tend to increase when an unweighed and more liberal Giovannetti diet is taken on an Out-Patient basis, this may not be of significance. Copper is involved in a group of enzymes the ferroxidases. F.W. Heaton (1969) considers that excretion of copper is regulated by feed back control by the liver.

The acid base content of the diet shows an excess of base which is an advantage in patients whose renal capacity for excreting the hydrogen ion is limited.
Table 4 S.Y. shows the essential amino acid content of the diet. All essential amino acids were supplied in amounts exceeding the absolute minimal for a man of 52.2 kg body weight, with the exception of the sulphur containing amino acids which were below the required absolute minimal estimation.

The Giovannetti diet was originally supplemented with Methionine 250 mg daily (Berlyne and Hocken, 1968) but this was discontinued because it was found that the problem of acidosis in these patients was accelerated by the additional sulphur content of the diet as this is mostly retained because as the G.F.R. diminishes there is a gross reduction in titratable acid excretion.

In Table 5 S.Y. are shown the serum values at the commencement of each balance period.

At the beginning of the fourth balance period the blood urea shows a decline from 200 to 130 mg per 100 ml., after which a 25 per cent increase is shown. A decrease in blood urea reflects decreased exogenous and endogenous protein catabolism. Serum bicarbonate shows a decrease from 18 mEq. per litre at the beginning to 14 mEq. per litre at the sixth balance period. This would indicate a tendency to a gradually increased acidosis.

Serum creatinine is shown to remain fairly stable during the balance and renal function would appear to be steady throughout the duration of the balance. There would seem to be no significant variation in the serum values of chloride, sodium, potassium, haemoglobin and calcium. The serum phosphate shows a slight overall increase from 7.2 to 8.3 mg per 100 ml. This elevated level may reflect increased parathyroid activity because of an inability to maintain the serum calcium which is lowered. Administration of aluminium hydroxide at the beginning of the fifth balance period does not appear to have influenced
the serum phosphate level.

Serum albumin became slightly lowered from 4.8 g per 100 ml. to 4.0 g per 100 ml. during the first half of the balance and thereafter it remained around this level. This is considered to be an anticipated physiological response to a low protein diet.

Weight appeared to be reasonably steady throughout the period of the balance.
Results and discussion on the balance data from patient S.Y.

The nitrogen balance data is shown in tabulated form in Table 6 S.Y. and presented in the modified Reifenstein plot, Figure 3 S.Y.

The net protein utilization is estimated theoretically and also calculated from the nitrogen balance data. This is shown in Table 7 S.Y.

During the first three balance periods when the patient took 50 Calories per kg body weight, the negative balance gradually became less marked, but equilibrium did not appear to be achieved. When the energy was increased to 66 Calories per kg body weight, nitrogen balance immediately became increasingly more negative and blood urea nitrogen increased, also faecal nitrogen showed a marked increase. The elevation of blood urea continued during the fifth balance period. During the final balance period the patient found it impossible to take the total energy. At this time nitrogen balance became less negative and the blood urea became more stable at around 150 mg per 100 ml., faecal nitrogen was slightly decreased.

The total faecal nitrogen was very high in this patient and represented 59 per cent of the nitrogen intake.

These results indicate that a steady metabolic state was never attained. The equilibration period may not have continued for a long enough period as this patient was taking a 30 g protein, 1,700 Calorie intake, immediately preceding admission. The age of the patient was 48 years. This was older than the majority of patients studied, and it may be that older age groups are less resilient to a change in dietary protein intake. In nitrogen balance studies on elderly men Kountz et al., (1949) emphasize the sluggish response to a change in protein intake. A stable metabolic state was not encouraged in this
balance because the energy was increased after the first half.

Considering the fact that this patient had a partial gastrectomy in the past, adaptation to an increased energy intake in the middle of the balance study would be extremely difficult, especially as the bulk of this energy was given within a period of eight hours.

A mild degree of malabsorption may be possible, but did not appear evident as diarrhoea was not present. Unfortunately the total weight of faeces was not recorded. Doig and Girdwood (1960) state that faecal nitrogen output is increased in a fifth of post-gastrectomy subjects. Eveson (1952) suggests that some of these subjects have impaired absorption of protein.

During the second half of the balance, an increased faecal nitrogen and an increasingly negative nitrogen balance are not the response which would be expected from an increased energy intake. Both temperature and weight remained constant throughout. It is therefore suggested that this additional energy was not utilized.

From these results it would appear evident that a steady state was never achieved, and a metabolic equilibrium not possible. An increased energy supply in the middle of the total balance, and given within a comparatively short time span during the day would not contribute to a steady balance.

Factors which now appear to be emphasized from this study are that an entirely constant intake throughout the total period of the balance should be observed, and the period of time during which the energy is consumed should be as extended as possible.
### TABLE 1 S.Y.

Protein and energy content of diets given to S.Y. male aged 48 years

<table>
<thead>
<tr>
<th></th>
<th>Diet A</th>
<th>Diet B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total nitrogen</strong></td>
<td>2.6768 (gN/kg body weight)</td>
<td>2.6768 (gN/kg body weight)</td>
</tr>
<tr>
<td><strong>(g per 24 hours)</strong></td>
<td>0.0512</td>
<td>0.0512</td>
</tr>
<tr>
<td><strong>Net average nitrogen</strong></td>
<td>by analysis (g per 24 hours)</td>
<td>by analysis (g per 24 hours)</td>
</tr>
<tr>
<td></td>
<td>2.96</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>0.0567 (gN/kg body weight)</td>
<td>0.0559 (gN/kg body weight)</td>
</tr>
<tr>
<td><strong>Total energy</strong></td>
<td>2,650 (11130 kJ)</td>
<td>3,380 (14196 kJ)</td>
</tr>
<tr>
<td>(Cals. per 24 hours)</td>
<td>50 Cals/kg body weight (210 kJ)</td>
<td>64 Cals/kg body weight (267 kJ)</td>
</tr>
<tr>
<td><strong>Average energy</strong></td>
<td>2,640 (11088 kJ)</td>
<td>3,120 (13104 kJ)</td>
</tr>
<tr>
<td>(Cals. per 24 hours)</td>
<td>50 Cals/kg body weight (210 kJ)</td>
<td>59 Cals/kg body weight (248 kJ)</td>
</tr>
<tr>
<td><strong>High biological value</strong></td>
<td>protein as % of total protein</td>
<td>protein as % of total protein</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td><strong>Fat as % of total</strong></td>
<td>energy</td>
<td>energy</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td><strong>Carbohydrate as % of</strong></td>
<td>total energy</td>
<td>total energy</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>60</td>
</tr>
<tr>
<td><strong>Polysaccharides as % of</strong></td>
<td>total CHO energy</td>
<td>total CHO energy</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>33</td>
</tr>
<tr>
<td><strong>Mono and disaccharides as % of</strong></td>
<td>total CHO energy</td>
<td>total CHO energy</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>67</td>
</tr>
<tr>
<td>Specimen diet taken by S.Y.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
<td>---------------------------------</td>
<td></td>
</tr>
<tr>
<td>(Amount in g per 24 hours unless otherwise stated)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diet A (first 12 days)</th>
<th>Diet B (last 12 days)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetened grapefruit</td>
<td>Sweetened grapefruit</td>
<td>60</td>
</tr>
<tr>
<td>Marmalade</td>
<td>Marmalade</td>
<td>20</td>
</tr>
<tr>
<td>Pancake</td>
<td>Potato cake</td>
<td>230</td>
</tr>
<tr>
<td>Carrots</td>
<td>Runner beans</td>
<td>30</td>
</tr>
<tr>
<td>Sweetened peaches</td>
<td>Sweetened peaches</td>
<td>60</td>
</tr>
<tr>
<td>Watercress</td>
<td>Watercress</td>
<td>5</td>
</tr>
<tr>
<td>Potatoes</td>
<td></td>
<td>120</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Lettuce</td>
<td>10</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>Tomatoes</td>
<td>45</td>
</tr>
<tr>
<td>Jam</td>
<td>Jam</td>
<td>20</td>
</tr>
<tr>
<td>Sweetened pears</td>
<td>Sweetened pears</td>
<td>60</td>
</tr>
<tr>
<td>Low-protein bread</td>
<td>Low protein bread</td>
<td>120</td>
</tr>
<tr>
<td>Butter</td>
<td>Butter</td>
<td>50</td>
</tr>
<tr>
<td>Sugar</td>
<td>Sugar</td>
<td>70</td>
</tr>
<tr>
<td>Caloreen</td>
<td>Caloreen</td>
<td>60</td>
</tr>
<tr>
<td>Double cream</td>
<td>Double cream</td>
<td>110</td>
</tr>
<tr>
<td>Homogenised milk</td>
<td>Homogenised milk</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>as</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>milk</td>
</tr>
<tr>
<td>Hycal</td>
<td>Hycal</td>
<td>241</td>
</tr>
<tr>
<td>Strained tea</td>
<td>Strained tea</td>
<td>900</td>
</tr>
<tr>
<td>(distilled water)</td>
<td>(distilled water)</td>
<td>ml.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>900</td>
</tr>
</tbody>
</table>
### TABLE 3 S.Y.

Mineral content of diets taken by S.Y.

(Amounts in mg per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th></th>
<th>Diet A</th>
<th></th>
<th>Diet B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>275</td>
<td></td>
<td>249</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>347</td>
<td></td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>215</td>
<td></td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>1307</td>
<td></td>
<td>1274</td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>74</td>
<td></td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>6.17</td>
<td></td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>1.11</td>
<td></td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td>Sulphur</td>
<td>188.0</td>
<td></td>
<td>178.0</td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>463.0</td>
<td></td>
<td>442.0</td>
<td></td>
</tr>
</tbody>
</table>

**Acid Base Balance**

- **Acid** = 11.5 mEq
- **Base** = 24.2 mEq

**Acid** = 11.5 mEq

**Base** = 23.2 mEq
### TABLE 4 S.Y.

**Essential amino acid content of diets A and B taken by S.Y.**

(mg amino acids per 24 hours)

<table>
<thead>
<tr>
<th>In diet</th>
<th>Recommended amino acid requirement for 52.2 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute minimal</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>234</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>844</td>
</tr>
<tr>
<td>Lysine</td>
<td>1079</td>
</tr>
<tr>
<td>Methionine</td>
<td>416</td>
</tr>
<tr>
<td>Threonine</td>
<td>747</td>
</tr>
<tr>
<td>Valine</td>
<td>1102</td>
</tr>
<tr>
<td>Leucine</td>
<td>1356</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>982</td>
</tr>
<tr>
<td>Cystine</td>
<td>255</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>662</td>
</tr>
</tbody>
</table>

**Total 'S' containing amino acids in diet = 671 mg**
TABLE 5 S.Y.

Blood chemistry at the commencement of each balance period for patient S.Y.

(values per litre or per 100 ml serum unless otherwise stated)

<table>
<thead>
<tr>
<th>Balance period</th>
<th>Weight kg</th>
<th>Urea mg per 100 ml</th>
<th>Creatinine mg per 100 ml</th>
<th>Na mEq/l.</th>
<th>K mEq/l.</th>
<th>Cl mEq/l.</th>
<th>CO₂ mEq/l.</th>
<th>Ca mg per 100 ml</th>
<th>PO₄ mg per 100 ml</th>
<th>Alb. g per 100 ml</th>
<th>Hb. g per 100 ml blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>52.45</td>
<td>200</td>
<td>13.0</td>
<td>140</td>
<td>3.5</td>
<td>105</td>
<td>18</td>
<td>6.6</td>
<td>7.2</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>51.4</td>
<td>175</td>
<td>13.0</td>
<td>135</td>
<td>3.6</td>
<td>102</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>51.3</td>
<td>160</td>
<td>13.0</td>
<td>134</td>
<td>3.5</td>
<td>102</td>
<td>16</td>
<td>6.2</td>
<td>8.1</td>
<td>4.1</td>
<td>9.2</td>
</tr>
<tr>
<td>4.</td>
<td>52.2</td>
<td>130</td>
<td>14.5</td>
<td>133</td>
<td>3.6</td>
<td>102</td>
<td>15</td>
<td>6.4</td>
<td>8.2</td>
<td>4.0</td>
<td>8.9</td>
</tr>
<tr>
<td>5.</td>
<td>52.7</td>
<td>140</td>
<td></td>
<td>130</td>
<td>3.5</td>
<td>102</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td>9.3</td>
</tr>
<tr>
<td>6.</td>
<td>52.65</td>
<td>155</td>
<td>13.0</td>
<td>135</td>
<td>3.7</td>
<td>103</td>
<td>14</td>
<td>6.8</td>
<td>7.6</td>
<td>4.1</td>
<td>8.8</td>
</tr>
<tr>
<td>end</td>
<td>52.6</td>
<td>150</td>
<td>15.0</td>
<td>132</td>
<td>3.7</td>
<td>107</td>
<td>16</td>
<td>6.5</td>
<td>8.3</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.Y. (A)</td>
<td>S.Y. (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>---------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net nitrogen intake (by analysis)</td>
<td>2.96 (0.0567 gN/kg body weight)</td>
<td>2.92 (0.0559 gN/kg body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average faecal nitrogen</td>
<td>1.69 (0.0323 gN/kg body weight)</td>
<td>1.79 (0.0342 gN/kg body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faecal nitrogen as % of nitrogen intake</td>
<td>56.5</td>
<td>60.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net nitrogen balance</td>
<td>-1.4883 (-0.0285 gN/kg body weight)</td>
<td>-1.9466 (-0.0372 gN/kg body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A = first three balance periods

B = last three balance periods
TABLE 7a S.Y.

Diet A

Prediction of theoretical net protein utilization on patient S.Y. (A)

Protein score of diet:

671 mg = total S containing amino acids
2.68 gN = total dietary nitrogen

Therefore 250 mg S containing amino acids per g total nitrogen

\( \frac{250}{270} \times 100 = 92.59 = \text{score} \)

\( \varphi = S \left(1 - k \text{ P Cals} \% \right) \)

\( k = 0.019 \) (Miller and Payne)

Net Cals in = 50 Cals/kg. Total Calories = 2,640 Weight = 52.2 kg.
Average nitrogen in = 2.96 gN/24 hours (urinary leak = 1.7 g protein/
24 hours = 0.272 gN.)

Therefore net available for anabolism = 2.688 gN. (16.8 g protein =
67 Calories)

67 as % of 2640 = 2.5%

\( \varphi = 92.59 \left(1 - 0.019 \times 2.5 \right) \)

\[ = 1.0 - 0.0475 = 0.9525 \]

\[ 92.59 \times 0.9525 = 88.2 \] theoretical N.P.U.

From nitrogen balance data

\[ \Delta \text{Bal/kg}^{0.73} = I/kg^{0.73} \times \varphi - N \] (200 mg N)

\[ - 83.01 \text{ mg N/kg}^{0.73} = 149.9 \text{ mg N/kg}^{0.73} \times \varphi - 200 \]

\[ 116.99 = 149.9 \times \varphi \]

\[ \varphi = 0.78 \]
TABLE 7b  S.Y.

Diet B

Prediction of theoretical net protein utilization on patient S.Y. (B)

Protein score of diet = 92.59

Net Calories in = 59 Cals/kg. Total Calories = 3,120

Average nitrogen in = 2.92 gN/24 hours (urinary leak = 1.7 g protein/
24 hours = 0.272 gN.)

Therefore net available for anabolism = 2.648 gN. (16.6 g protein =
66 Calories)

66 as % of 3,120 = 2.1%

\[ \Theta = 92.59 \times (1 - 0.019 \times 2.1) \]

= 1.0 - 0.0399 = 0.9601

92.59 x 0.9601 = 88.9 theoretical N.P.U.

From nitrogen balance data

\[ \text{Bal/kg}^{0.73} = I/kg^{0.73} \times \Theta - M \] (200 mg N)

- 108.57 mg N/kg^{0.73} = 147.7 mg N/kg^{0.73} \times \Theta - 200

91.43 = 147.7 \times \Theta

\[ \Theta = 0.619 \]
TABLE 8 S.Y.

Drugs taken by S.Y. during the period of the balance study

Ascorbic Acid 50 mg t.d.s.
Aneurine Co. forte 2 tablets  5 mg Aneurine
                          2 mg Riboflavin  x 2
                          20 mg Nicotinic Acid
                          2 mg Pyridoxine

Chromium sesquioxide 1.5 g daily
Aluminium hydroxide 6 tablets (Evans) 3 g daily commencing
at the beginning of the fifth balance period.
Methyl Dopa 250 mg  4 x daily.
Correction for B. U. N.

KEY: applying to modified Reifenstein plots used in all the following studies

- urinary N
- faecal N

(210 KJ) (277 KJ) (252 KJ) (218 KJ)
The diagnosis was stage 3 chronic renal failure with gouty nephropathy. Glomerular filtration rate was 1.5 ml per minute.

R.M. was first referred to the Renal Unit in May 1970 with a diagnosis of idiopathic familial advanced renal failure. She had been known to have hyperuricaemia for twenty years. Her gout had been treated with various uricosuric agents and Allopurinol.

In 1962 renal involvement was confirmed when her blood urea was 90 mgs per 100 ml. In 1963 blood urea was 150 mg per 100 ml. She had been given a 40 g protein diet in 1968.

In 1969 blood urea had risen to 200 mg per 100 ml.

In May 1970 she was referred to the Renal Unit. Serum values recorded in April were as follows: urea 315 mg per 100 ml., chloride 101 mEq. per litre, bicarbonate 17 mEq. per litre, sodium 138 mEq. per litre, potassium 6.5 mEq. per litre, total protein 6.6 g per 100 ml.

When referred she had no bleeding or haematuria. She was pigmented and itching. Her symptoms were few, except that she had pain in the hips when walking, and she tired easily. At the age of 25 years she had acute arthritis in the first metatarsal-phalangeal joint but had experienced no acute arthritis since that time. She had an episode of skin trouble in 1968 thought to be erythema multiforme which had resolved spontaneously.

Her family history was as follows.

She had a twin sister who had gout at the same age as R.M. and who died of chronic renal failure in 1968. Another sister also died of chronic renal failure.

R.M. had three daughters the first of whom was unaffected. The
second and third daughters both had hyperuricaemia. A niece, daughter of one of the above, had chronic renal failure. R.M.'s father died of tuberculosis & gout. R.M.'s mother died aged 80 & renal failure.

Serum values in May 1970 were as follows: urea 250 mg per 100 ml., chloride 95 mEq. per litre, bicarbonate 17 mEq. per litre, sodium 130 mEq. per litre, potassium 6.1 mEq. per litre, total protein 6.5 g per 100 ml., creatinine 13 mg per 100 ml. and haemoglobin was 7.7 g per 100 ml.

Treatment was commenced with Allopurinol and iron supplements. She was next seen in June 1970. Osteodystrophy of the lumbar spine was confirmed with an increased plasma phosphate of 9.2 mg per 100 ml. and 8.2 mg of calcium per 100 ml. She was found to have marked arterial calcification in the abdomen and limbs. Treatment with Aluminium hydroxide and a 30 g protein diet was commenced.

At the beginning of August 1970 she was seen again and her serum values at this time were:

Urea 365 mg per 100 ml., chloride 93 mEq. per litre, bicarbonate 17 mEq. per litre, sodium 138 mEq. per litre, potassium 4.6 mEq. per litre, total protein 6.8 g per 100 ml.

At this time her Allopurinol was stopped and she was instructed to take a Giovannetti diet and to come into hospital if vomiting or unable to manage the Giovannetti diet at home.

Her next visit to the outpatient clinic was on 1st September, 1970 when she was feeling much better with no vomiting, and managing the diet at home. Her skin was better with no itching.

Serum values at this time were:

Urea 305 mg per 100 ml., chloride 96 mEq. per litre, bicarbonate 15 mEq. per litre, sodium 139 mEq. per litre, potassium 5.8 mEq. per
litre, total proteins 6.5 g per 100 ml., calcium 7.4 mg per 100 ml.,
phosphate 11.1 mg per 100 ml.

She looked pale and pigmented and had minimal ankle oedema.

By the end of September 1970 serum levels were as follows:

- Urea 210 mg per 100 ml., chloride 103 mEq. per litre, bicarbonate
  17 mEq. per litre, sodium 143 mEq. per litre, potassium 7.3 mEq. per
  litre, total protein 6.1 g per 100 ml. Her response to the Giovannetti
diet had been good but her intake of fluids had not been restricted.
She was now oedematous, also serum potassium had risen. She was
therefore admitted.

After treatment with resonium enemas, restricted fluid intake and
a Giovannetti diet she was discharged. Serum values at this time were:

- Urea 200 mg per 100 ml., chloride 101 mEq. per litre, bicarbonate
  17 mEq. per litre, sodium 139 mEq. per litre, potassium 5.0 mEq. per
  litre, total proteins 5.5 g per 100 ml., haemoglobin 6.1 g per 100 ml.,
  and serum creatinine of 16.5 mg per 100 ml.

At the end of October 1970 a sore throat had developed, chest pain,
and nausea and vomiting and she was readmitted for peritoneal dialysis.

Her urea was 215 mg per 100 ml., haemoglobin 5.8 g per 100 ml.,
serum calcium 7.5 mg per 100 ml., phosphate 8.8 mg per 100 ml., albumin
3.9 g per 100 ml. and serum creatinine 13.5 mg per 100 ml.

X-rays of the lumbar spine and pelvis at this time showed bone
changes compatible with secondary hyperparathyroidism, with alternating
bands of rarefaction and increased density. Bone resorption of the
middle phalanges and erosion of the terminal phalanges was noted.
Extensive calcification within the aorta and iliac vessels was present.

After peritoneal dialysis R.M. felt better. She was up and about
and the oedema had disappeared.

A nitrogen balance study was commenced.
On completion of the nitrogen balance study R.M. was discharged on a strictly controlled Giovannetti diet of 44 mg nitrogen per kg body weight and 53 Calories per kg body weight. She adhered to this regime very well at home. She was seen at fairly frequent intervals.

Soon after Christmas she felt very well and enjoyed herself over Christmas. Her only symptom was itching.

Her serum creatinine at this time was 30 mg per 100 ml.
Details of study on R.M.

A preliminary equilibration period of 4 days was allowed before urine and faecal collections were started.

The balance on this patient was divided into two studies (1 and 2) each of which continued for 12 days and was divided into 3 balance periods each of 4 days duration.

Balance study (1)

Body weight at the beginning of the study was 51.65 kg and at the end of the third balance period it was 50.8 kg. Urinary protein was 0.7 g per 24 hours. Details of this study are shown in Table 1 R.M. to Table 8 R.M. The total energy content of the diet was 40 Calories per kg body weight per 24 hours and fat and carbohydrate supplied 50 per cent and 47 per cent respectively.

The energy intake over the 3 balance periods was as follows:

<table>
<thead>
<tr>
<th>Balance period 1 (4 days)</th>
<th>39 Calories per kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balance period 2 (4 days)</td>
<td>38 Calories per kg body weight</td>
</tr>
<tr>
<td>Balance period 3 (4 days)</td>
<td>36 Calories per kg body weight</td>
</tr>
</tbody>
</table>

The average Calorie intake over the period of 12 days was thus 38 Calories per kg body weight. The net nitrogen intake (as determined by analysis) was 1.89 g Nitrogen per 24 hours.

A large proportion of the fat was supplied as double cream and butter, with smaller amounts as egg yolk. The polysaccharides were contributed by wheat starch, potatoes, Giovannetti bread, Caloreen and Hycal. The mono and disaccharides were supplied as grapefruit, peaches, peas, marmalade, jam and Hycal. The high biological value protein was given as egg, double cream and butter and the second class protein (non-essential nitrogen) as grapefruit, peaches, peas, marmalade, jam, potatoes, Giovannetti bread, wheat starch, carrots, lettuce, tomato and onion.
Balance study (2)

The body weight at the commencement was 50.8 kg and at the end of the final balance 51.5 kg.

The total energy content of the diet was increased to 54 Calories per kg body weight per 24 hours and a rather higher percentage of the Calories (57 per cent) came from carbohydrate and correspondingly a lower percentage (41 per cent) from fat.

The energy intake over the last 3 balance periods was as follows:

- Balance period 4 (4 days) 51 Calories per kg body weight
- Balance period 5 (4 days) 54 Calories per kg body weight
- Balance period 6 (4 days) 54 Calories per kg body weight

The average Calorie intake of the final period of 12 days was 53 Calories per kg and an increase of 15 Calories per kg body weight over that in period (1). The net nitrogen intake (determined by analysis) was 2.248 g Nitrogen per 24 hours.

The sources of the fat were the same as before. The Polysaccharides were contributed by wheat starch, corn starch, potatoes, Caloreen, arrowroot, with no Giovannetti bread or Hycal. Mono and disaccharides supplied as before by grapefruit, peaches, pear but with the addition of honey and glucose in place of Hycal and preserves. The sources of high biological value protein were as before. The second class (non-essential nitrogen) was made up of wheat starch, maize starch, grapefruit, peach, honey, potatoes, lettuce, tomatoes and lemon juice.

Table 3 R.M. shows the mineral content and acid base of the diets. From this table it can be seen that the calcium magnesium, iron and copper intakes are all low, this is a feature of all the diets in this series of studies. The diets also show a slight excess of base.

Table 4 R.M. shows the essential amino acid content of the diet.

All the essential amino acids were present in excess of the absolute minimal theoretical requirement for a woman of 51.0 kg body
weight, with the exception of the sulphur containing amino acids.

Table 5 R.M. shows the serum values at the commencement of each balance period.

The blood urea shows a fairly sharp rise during the first two balance periods of 30 mg per 100 ml. It is stable during the third period but shows another rise of 15 mg per 100 ml. during the fourth and fifth periods, but during the final balance period there is a rapid decline of 65 mg per 100 ml. This would appear to be related to sodium balance. The serum sodium fell from 135 mEq. sodium per litre to 118 mEq. per litre during the first half of the balance. As the serum sodium became gradually corrected to a near normal level during the final balance period, the blood urea showed a marked reduction when sodium balance was achieved. The serum chloride follows the same pattern as the sodium. Body weight remains fairly stable throughout the balance, the lowest weight being recorded at the beginning of the fourth period.

It is noted that the bicarbonate is also related to the sodium balance. In a state of sodium depletion, less sodium can be reabsorbed as sodium bicarbonate. An inevitable consequence of a reduced glomerular filtration is a reduction in filtered phosphate and sulphate and more bicarbonate is excreted as sodium bicarbonate.

Serum albumin and haemoglobin remain fairly steady. Serum potassium shows no marked change throughout the balance. The low serum calcium levels vary between 7.5 mg per 100 ml. and 8.4 mg per 100 ml. and inorganic phosphate is increased and values are between 8.6 mg per 100 ml. and 10.6 mg per 100 ml. Administration of aluminium hydroxide does not appear to influence the serum phosphate. This would reflect increased parathyroid activity stimulated by a lowered serum calcium. The serum creatinine is high at the beginning
of the first balance period being 13.5 mg per 100 ml and this increased to 15.0 mg per 100 ml. by the second balance period and again to 17.5 mg per 100 ml. by the fifth balance period. Nine days after the end of the balance study creatinine was as high as 27.0 mg per 100 ml. This indicates a rapid deterioration in renal function.

Results and Discussion on data from R.M.

The nitrogen balance data on R.M. is shown in the modified Reifenstein plot (Figure 3 R.M.) and in tabulated form in Table 6 R.M.

Balance 1

The net average nitrogen intake was 2.06 g nitrogen per 24 hours for the first three balance periods (the first twelve days).

Balance 2

The net average nitrogen intake was 2.09 g nitrogen per 24 hours for the final three balance periods covering the last twelve days.

The net average faecal nitrogen in Balance 1 averaged 0.977 g nitrogen per 24 hours and this represented 52 per cent of dietary nitrogen. In Balance 2 the average faecal nitrogen was reduced to 0.673 g nitrogen per 24 hours. The net nitrogen balance was - 1.303 g nitrogen per 24 hours in Balance 1 and - 0.442 g nitrogen per 24 hours in Balance 2.

During Balance 1 blood urea rose from 160 mg per 100 ml. to 215 mg per 100 ml. but it fell to 150 mg per 100 ml. during the final 4-day balance period.

The average Calorie intake during Balance 1 was 38 Calories per kg and during Balance 2 was 53 Calories per kg.

The interpretation of this balance data is complicated by the fact that the patient was salt depleted and this was especially marked during the first three balance periods, when the serum sodium fell.
from 135 mEq. sodium per litre to 118 mEq. sodium per litre (Table 5 R.M.)
The sodium balance was gradually corrected by increasing the dietary
sodium and by giving intravenous hypertonic saline. The serum sodium
became almost normal at 129 mEq. sodium per litre during the final
balance period.

The serum chloride showed the same trend as the sodium and reached
the lowest value of 80 mEq. chloride per litre at the commencement of
the fourth balance period, just prior to the saline infusion, when it
increased to 86 mEq. per litre.

The 24 hour urine volume in this patient ranged from 830 ml. to
1,060 ml. Her total fluid intake was 1,100 ml.

This patient showed no obvious clinical evidence of oedema, except
for slight oedema of the feet, following the infusion of hypertonic
saline. Extracellular fluid volume did not therefore appear to be
enlarged.

Sodium intake during the first three balance periods was 31 mEq.
sodium daily and during the last three 40 mEq. sodium. It is interesting
to note that dietary sodium prior to hospital admission was approximately
46 mEq. per day. During the balance urinary sodium excretion was 86 - 90
mEq. sodium per 24 hours. The low serum sodium in this patient would
therefore appear to be an indication of depletion of body sodium as no
retention of fluid is evident, in fact body weight was reduced.

Hyponatraemia due to dilution and overhydration would be accompanied by
weight gain. It is likely that the sodium depletion occurred as the
result of poor tubular reabsorption and the requirement of additional
sodium for "buffering" acids.

From the data presented it is to be noted that during the fourth
and fifth balance periods the blood urea continued to rise, in spite of
increased energy intake, but when sodium balance was finally achieved
during the last balance period there was an immediate response in nitrogen
balance.

The apparent instability of this balance would appear to underline two factors, the unstabilizing effect of the lack of sodium balance during the first half, and the advantage of additional energy intake during the second half. The impression is that a continuation of this study after the sixth period may have shown a steadily improved nitrogen balance.

This study emphasized the interdependence of nutrients. This interrelationship was demonstrated by an apparent effect on nitrogen balance when sodium balance was not achieved. Sodium is an extracellular ion and serves an integral function in cellular membrane transport mechanisms. Sodium depletion may possibly affect the efficiency of energy utilization.

It is noteworthy that Miller and Payne (unpublished data 1964) have shown a reduced net protein utilization in the sodium deficient rat.

The active entry of amino acids into the cell nucleus has been shown to be very dependent on the presence of sodium ions (Allfrey and Mirsky, 1961). Mitochondria also contain fairly high concentrations of sodium as well as other ions. Sodium ions also function as enzyme activators (e.g. for ketohexokinase). They can also act as an enzyme inhibitor.
### TABLE 1 R.M.

Protein and energy content of diets for patient R.M. female aged 46 years

<table>
<thead>
<tr>
<th></th>
<th>Diet I</th>
<th>Diet II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total nitrogen</strong></td>
<td>2.064 (0.039 N/kg body weight per 24 h)</td>
<td>2.098 (0.041 N/kg body weight per 24 h)</td>
</tr>
<tr>
<td></td>
<td><strong>Net average nitrogen by analysis</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.87 (0.0363 N/kg)</td>
<td>2.248 (0.0437 N/kg)</td>
</tr>
<tr>
<td><strong>Total energy</strong></td>
<td>2,080 (8736 kJ) (40 Cals/kg body weight/24 h)</td>
<td>2,760 (11592 kJ) (54 Cals/kg body weight/24 h)</td>
</tr>
<tr>
<td><strong>Average energy</strong></td>
<td>1,950 (8190 kJ) (38 Cals/kg body weight/24 h)</td>
<td>2,726 (11449 kJ) (53 Cals/kg body weight/24 h)</td>
</tr>
<tr>
<td><strong>High biological value protein as % total protein</strong></td>
<td>78</td>
<td>75</td>
</tr>
<tr>
<td><strong>Fat as % total energy</strong></td>
<td>50</td>
<td>41</td>
</tr>
<tr>
<td><strong>Carbohydrate (CHO) as % total energy</strong></td>
<td>47</td>
<td>57</td>
</tr>
<tr>
<td><strong>Polysaccharides as % total CHO energy</strong></td>
<td>67</td>
<td>52</td>
</tr>
<tr>
<td><strong>Mono and disaccharides as % total CHO energy</strong></td>
<td>33</td>
<td>48</td>
</tr>
</tbody>
</table>
### TABLE 2 R.M.

**Summary of diet as eaten by R.M.**

(Amount in g per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Diet 1 (first 12 days)</th>
<th>Diet 2 (last 12 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetened grapefruit</td>
<td>Waffle</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Marmalade</td>
<td>Sweetened grapefruit</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>Jam</td>
<td>Honey</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Pancake</td>
<td>Custard</td>
</tr>
<tr>
<td>140</td>
<td>195</td>
</tr>
<tr>
<td>Carrots</td>
<td>Sweetened peaches</td>
</tr>
<tr>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Sweetened peaches</td>
<td>2 pancakes</td>
</tr>
<tr>
<td>60</td>
<td>125</td>
</tr>
<tr>
<td>Boiled potatoes</td>
<td>Boiled potatoes</td>
</tr>
<tr>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Butter (L. salt)</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Tomato</td>
<td>Lettuce</td>
</tr>
<tr>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Spring onion</td>
<td>Tomatoes</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Sweetened pears</td>
<td>Spanish onion</td>
</tr>
<tr>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>Giovannetti bread</td>
<td>Sweetened pears</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Double cream</td>
<td>Double cream</td>
</tr>
<tr>
<td>130</td>
<td>90</td>
</tr>
<tr>
<td>L. salt butter</td>
<td>(Caloreen</td>
</tr>
<tr>
<td>20</td>
<td>as</td>
</tr>
<tr>
<td>Caloreen</td>
<td>(Sucrose</td>
</tr>
<tr>
<td>45</td>
<td>drink</td>
</tr>
<tr>
<td>Hycal</td>
<td>(Glucose</td>
</tr>
<tr>
<td>170 ml.</td>
<td>100</td>
</tr>
<tr>
<td>Strained tea</td>
<td>(Lemon juice</td>
</tr>
<tr>
<td>(distilled water)</td>
<td>50 ml.</td>
</tr>
<tr>
<td>600 ml.</td>
<td>Strained tea</td>
</tr>
<tr>
<td></td>
<td>450 ml.</td>
</tr>
</tbody>
</table>
### TABLE 3 R.M.

**Mineral content of diet eaten by R.M.**

(Amounts in mg per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>183</td>
<td>176</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>242</td>
<td>252</td>
</tr>
<tr>
<td>Sodium</td>
<td>167</td>
<td>169</td>
</tr>
<tr>
<td>(± 23 mEq. added) total = 30 mEq.</td>
<td>(± 34 mEq. added) total = 41 mEq.</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>1005</td>
<td>1034</td>
</tr>
<tr>
<td>Magnesium</td>
<td>57</td>
<td>53</td>
</tr>
<tr>
<td>Iron</td>
<td>6.91</td>
<td>5.42</td>
</tr>
<tr>
<td>Copper</td>
<td>3.2</td>
<td>1.81</td>
</tr>
<tr>
<td>Sulphur</td>
<td>138.0</td>
<td>136</td>
</tr>
<tr>
<td>Chloride</td>
<td>347</td>
<td>316</td>
</tr>
</tbody>
</table>

**Acid Base Balance**

- **Acid = 10.2 mEq.**
- **Base = 16.9 mEq.**

- **Acid = 16.3 mEq.**
- **Base = 16.3 mEq.**
TABLE 4  R.M.

Essential amino acid content of diet 1 and 2 (R.M.)

(mg amino acids per 24 hours)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>In diet</th>
<th>Recommended amino acid requirement for 51.7 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absolute minimal</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>189</td>
<td>186</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>688</td>
<td>802</td>
</tr>
<tr>
<td>Lysine</td>
<td>846</td>
<td>595</td>
</tr>
<tr>
<td>Methionine</td>
<td>354</td>
<td>802</td>
</tr>
<tr>
<td>Threonine</td>
<td>591</td>
<td>362</td>
</tr>
<tr>
<td>Valine</td>
<td>691</td>
<td>595</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>791</td>
<td>517</td>
</tr>
<tr>
<td>Leucine</td>
<td>1081</td>
<td>802</td>
</tr>
<tr>
<td>Cystine</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>541</td>
<td></td>
</tr>
</tbody>
</table>

Total 'S' containing amino acids in diet = 567 mg
<table>
<thead>
<tr>
<th>Balance period</th>
<th>Weight kg</th>
<th>Urea mg per 100 ml</th>
<th>Creatinine mg per 100 ml</th>
<th>Na mEq/l</th>
<th>K mEq/l</th>
<th>Cl mEq/l</th>
<th>CO₂ mEq/l</th>
<th>Ca mg per 100 ml</th>
<th>P0₄ mg per 100 ml</th>
<th>Alb g per 100 ml</th>
<th>Hb g per 100 ml blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>51.65</td>
<td>160</td>
<td>13.5</td>
<td>135</td>
<td>5.8</td>
<td>91</td>
<td>17</td>
<td>8.4</td>
<td>8.6</td>
<td>3.4</td>
<td>6.4</td>
</tr>
<tr>
<td>2.</td>
<td>51.25</td>
<td>170</td>
<td>15.0</td>
<td>127</td>
<td>5.2</td>
<td>88</td>
<td>19</td>
<td>8.4</td>
<td>9.9</td>
<td>3.3</td>
<td>6.8</td>
</tr>
<tr>
<td>3.</td>
<td>51.40</td>
<td>190</td>
<td>121</td>
<td>4.6</td>
<td>85</td>
<td>15</td>
<td>7.7</td>
<td>10.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>50.8</td>
<td>190</td>
<td>118</td>
<td>4.2</td>
<td>80</td>
<td>15</td>
<td>7.9</td>
<td>10.6</td>
<td>3.1</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>51.4</td>
<td>200</td>
<td>128</td>
<td>3.2</td>
<td>86</td>
<td>21</td>
<td>7.5</td>
<td>8.8</td>
<td></td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>51.8</td>
<td>215</td>
<td>129</td>
<td>4.0</td>
<td>85</td>
<td>18</td>
<td>8.2</td>
<td>9.8</td>
<td>3.9</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>end</td>
<td>51.5</td>
<td>150</td>
<td>129</td>
<td>4.1</td>
<td>84</td>
<td>20</td>
<td>7.2</td>
<td>9.5</td>
<td>3.1</td>
<td>5.9</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 6 R.M.

**Nitrogen Balance Data**

(Amounts are averages in g per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th></th>
<th>R.M. (1)</th>
<th>R.M. (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Net Nitrogen intake</strong></td>
<td>1.87 (0.0363 gN/kg body weight)</td>
<td>2.248 (0.0437 gN/kg body weight)</td>
</tr>
<tr>
<td>(by analysis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average faecal Nitrogen</strong></td>
<td>0.977 (0.0190 gN/kg body weight)</td>
<td>0.673 (0.0111 gN/kg body weight)</td>
</tr>
<tr>
<td><strong>Faecal Nitrogen as % of</strong></td>
<td>52</td>
<td>30</td>
</tr>
<tr>
<td><strong>nitrogen intake</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Net nitrogen balance</strong></td>
<td>-1.3 (0.0255 gN/kg body weight)</td>
<td>-0.442 (-0.0087 gN/kg body weight)</td>
</tr>
</tbody>
</table>

(1) = first three balance periods

(2) = last three balance periods
TABLE 7a R.M.


Weight = 51.65 kg.

Protein score of diet =
567 mg = total S containing amino acids
2.06 g N = total dietary nitrogen
275 mg S containing amino acids per g total nitrogen
\[
\frac{275 \times 100}{270} = 101.85 = \text{score}
\]

Net Cals in = 38 Cals/kg. Total Cals = 1,950

Average nitrogen in = 2.06 gN/24 hours Urinary leak = 0.7 g protein
(0.112 gN)

Therefore net available for anabolism = 1.948 gN = 12.175 g protein (49 Cals)

\[\frac{49}{1950} = 2.56\]

\[\varphi = 101.85 (1 - 0.019 \times 2.56) = 96.876\]

N.P.U. = 97% (theoretical)

From Nitrogen balance data

\[\Delta \text{Bal/kg}^{0.73} = \frac{I}{kg^{0.73}} \times \varphi - M (200 \text{ mg N})\]

- 73.16 mg N/kg^{0.73} = 109.3 mgN/kg^{0.73} \times \varphi - 200

126.84 = 109.3 \times \varphi

\[\varphi = 1.16 \text{ more than } 100\%\]
TABLE 7b R.M.


Weight = 50.8 kg

Protein score of diet =
567 mg = total S containing amino acids
2.0976 g N = total dietary Nitrogen
Score = 100

Net Cals in = 53 Cals/kg. Total Cals = 2,690

Average nitrogen in = 2.0976 gN/24 hours. Urinary leak = 0.112 gN.

net available for anabolism = 1.9856 gN = 12.41 g protein (50 Cals)

50 as % of 2690 = 1.86

\[ \Theta = 100 \times (1 - 0.019 \times 1.86) = 96.466 \]

N.P.U. = 96% (theoretical)

From Nitrogen balance data

\[ \Delta \text{Bal/kg}^{0.73} = I/\text{kg}^{0.73} \times \Theta - M \text{ (200 mg N)} \]

\[ 25.13 \text{ mg} = 112.88 \times \Theta - 200 \]

\[ 174.87 = 112.88 \times \Theta \]

\[ \Theta = 1.549 \quad \therefore \text{more than 100%} \]
TABLE 8 R.M.

Drugs taken by R.M. during the period of the balance study

Ascorbic Acid 50 mg t.d.s.
Aneurine Co. forte 2 tablets t.d.s.
Aluminium hydroxide 15 ml. (Wyeth) x 4 daily (4 g)
commencing at the beginning of the fourth balance period.
Fig. 3. R. M. Nitrogen balance study on R. M. \( \Phi \) age 46

Modified Reifenstein plot

blood urea mg per 100 ml

<table>
<thead>
<tr>
<th>Days</th>
<th>G. N. per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>+39 Cals/kg</td>
</tr>
<tr>
<td>8</td>
<td>+38 Cals/kg</td>
</tr>
<tr>
<td>12</td>
<td>+36 Cals/kg</td>
</tr>
<tr>
<td>16</td>
<td>+51 Cals/kg</td>
</tr>
<tr>
<td>20</td>
<td>+54 Cals/kg</td>
</tr>
<tr>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Calories per kg:
- 439 Cals/kg: (164 kJ)
- 438 Cals/kg: (160 kJ)
- 436 Cals/kg: (151 kJ)
- 51 Cals/kg: (214 kJ)
- 54 Cals/kg: (227 kJ)
The diagnosis was stage 2 chronic renal failure. She had a history of recurrent urinary tract infection and bilateral renal calculi with some parathyroid hyperplasia for which a partial parathyroidectomy had been performed ten years earlier. The glomerular filtration rate was 5.5 ml. per minute at the time of the study.

In 1959 M.S. had pain in the left loin for three weeks. An intravenous pyelogram was performed and two small staghorn calculi were visible in the left kidney. The right kidney was normal. Her blood urea was then 24 mg per 100 ml.

In April 1960 she was admitted to hospital with recurrent left loin pain and urinary infection and was given a course of Furadantin. Later the same month she underwent a left partial nephrectomy with removal of numerous stones. A subsequent x-ray showed that some opacities still remained.

In early 1961 she gave birth to a son. It was a normal delivery but was premature. This was her only child. During this pregnancy she had episodes of urinary infection and more stones appeared to have formed in her right kidney.

In March 1961 an intravenous pyelogram revealed a staghorn calculus in her right kidney and a right very dilated hydroureter. There was also a smaller calculus in her left kidney. Renal function appeared to be reasonable.

In April her blood urea was 34 mg per 100 ml. and serum calcium and phosphate were within normal limits.

In May the blood urea was still 30 mg per 100 ml., but the serum calcium was 5.2 mg per 100 ml. and phosphate 9.3 mg per 100 ml. Later
in May she was admitted because of a continuous right loin pain for one week, which affected her sleep. She was also getting some vomiting. At that time she went into spasm with extended back and stiff limbs. Her serum calcium was still low.

The possibility of a parathyroid tumour was considered and it was therefore decided to explore her neck; she was admitted to hospital the following August.

When admitted she was having a nagging bilateral loin pain fairly continuously with periodic exacerbations over the previous three months. This accentuated her feeling of tiredness. Her appetite was fair, but she had lost 2 stones in weight since the birth of her son.

The family history of M.S. revealed that she had one aunt who had renal stones. Her mother's mother had been deaf and dumb and had died with kidney disease at the age of 45 years.

Investigations in August 1961 indicated that her renal function had deteriorated and her blood urea was now between 98 to 120 mg per 100 ml. Serum ionised calcium was found to be high. Serum creatinine was 3.6 mg per 100 ml.

The clinical suspicion of hyperparathyroidism was confirmed when her neck was explored. Two hyperplastic parathyroid glands were removed. Another parathyroid gland was found to be normal, but the right lower parathyroid gland was not identified.

For the next few years she was entirely symptom free and was kept free from infection. Thereafter she continued to get intermittent urinary infections and renal calculi recurred.

In 1968 her blood urea was 43 mg per 100 ml. and around this time she had an episode of pleurisy and became depressed. Her depression was treated with Valium.

In 1970 she was admitted to the Renal Unit for investigations of her stone forming tendency.
She was getting nocturia once nightly and her weight was steady. Blood urea was 74 mg per 100 ml., electrolytes were within normal limits, total proteins were 6.1 g per 100 ml., serum creatinine was 3.0 mg per 100 ml. The serum calcium was within normal limits and she had no hypercalciuria but as she had a mild degree of chronic renal failure this would be expected. Whether her hyperparathyroidism was secondary to a derangement in renal function was not elucidated. No metabolic defect could be demonstrated as being responsible for her stone-forming tendency.

In 1971 more stones had formed and she had a right pyelolithotomy and more stones removed.

In April 1972 she was feeling well but looked pale. Her haemoglobin was 8.7 g per 100 ml., serum creatinine now 7.4 mg per 100 ml. and urea 188 mg per 100 ml. The following month she was instructed to take a 30 g protein diet.

At her next visit in June although the cramp in her legs had improved since taking the 30 g protein diet, she was having bad nights with tingling in her hands and legs. She was also troubled with headaches and a little vomiting. Serum creatinine was now 11.8 mg per 100 ml. and urea 122 mg per 100 ml. She was instructed to take a Giovannetti diet and agreed to come in for metabolic balance studies.

On her next visit later in the same month her urea had dropped to 78 mg per 100 ml. She had no vomiting. She had nocturia twice nightly, with a high fluid intake of approximately three litres daily. At the end of June she was admitted for balance studies.
Details of the study on M.S.

The total balance of twenty four days was divided into six balance periods each of four days duration. Weight at the commencement of the study was 53.45 kg and 55.0 kg at the conclusion of the study. Urinary protein was 4.2 g per 24 hours. Complete details of this study can be seen in Table 1 M.S. to Table 8 M.S. The protein and energy content of the diet are shown in Table 1 M.S.

The total energy content of the diet was 2,620 Calories (49 Calories per kg body weight per 24 hours). Fat supplied 36 per cent and carbohydrate 61 per cent of the total energy.

Actual energy intake over the six balance periods was as follows:

<table>
<thead>
<tr>
<th>Balance period</th>
<th>Duration</th>
<th>Calories per kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 days</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>4 days</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>4 days</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>4 days</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>4 days</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>4 days</td>
<td>45</td>
</tr>
</tbody>
</table>

The average Calorie intake over 24 days of the total balance period was therefore 46 Calories per kg.

Of the 36 per cent of the total energy provided by fat, the major portion was contributed by double cream, butter and egg yolk, a very small proportion was contributed by milk. Carbohydrate provided 61 per cent of the total energy and comprised 75 per cent polysaccharides and 21 per cent mono or disaccharides.

The polysaccharides were composed of potatoes, low protein flour as pancakes and ice cream or custard, also low protein bread and crispbread and Caloreen.

The mono and disaccharides were contained in grapefruit, marmalade,
peaches, glucose as lemon drink and ice cream, also a very small amount as lactose in milk.

The high biological value protein consisted of egg, double cream, butter, milk. The small proportion of non essential nitrogen was composed of grapefruit, marmalade, potatoes, carrots, low protein flour, peaches, lettuce, cucumber, tomato, lemon juice.

Table 3 M.S. gives the mineral content and acid-base of the diet. The intake of calcium, magnesium, iron and copper are all low and this is consistent with the other studies in this series. There is a slight excess of base in the diet.

Table 4 M.S. shows the essential amino acid content of the diet. All the essential amino acids are supplied in amounts higher than the absolute minimal required for the body weight of M.S., including the sulphur containing amino acids, and the following amino acids exceeded the safe minimal required, lysine, threonine, valine, leucine, isoleucine. This amino acid pattern is better than others in the series and is mainly due to the fact that M.S. was given a larger content of egg in her diet. This was possible because she had a large protein loss in her urine, which was compensated for in the diet with high biological value protein.

Table 5 M.S. records the serum values at the beginning of each balance period.

The blood urea can be seen to increase from 90 mg per 100 ml. to 122 mg per 100 ml. at the beginning of the fourth balance period, with a 10 per cent rise during the first two balance periods and a 20 per cent rise during the third balance period, and it finally returned to 90 mg per 100 ml. at the end of the last balance period. This seems to be related to her unstable blood pressure and to the fact that an additional loss of nitrogen was incurred during the first half of the balance due to menstruation.

Serum sodium becomes reduced from 136 mEq. per litre to 120 mEq.
per litre at the beginning of the fifth balance period, when the sodium intake was increased. This low serum sodium had been rectified by the sixth balance period. The serum chloride follows the same pattern as the sodium. Potassium values remain at a fairly steady level between 3.8 and 4.5 mEq per litre. The haemoglobin remains at a low value between 5.7 and 8.0 g per 100 ml. Serum albumin stays around the lower limit of normal at between 2.8 and 3.55 g per 100 ml.

Body weight is fairly stable but tends to increase slightly during the final three balance periods from 53.6 kg to around 55.0 kg. This would appear to follow an increased sodium intake at the beginning of the fourth balance period. Serum creatinine remains steady throughout the balance. Serum calcium ranges between 9.0 mg per 100 ml. at the commencement of the balance to 8.5 mg per 100 ml. at the beginning of the fifth balance period ending at 9.6 mg per 100 ml. Serum phosphate commences at 9.5 mg per 100 ml. and stays around that level until the beginning of the fifth balance period when it drops to 5.0 mg per 100 ml. and decreases to 3.9 mg per 100 ml. at the end of the balance. These results would seem to reflect the administration of aluminium hydroxide at the third balance period.

Results and discussion on the balance data from patient M.S.

The balance data on M.S. is shown in the modified Reifenstein plot Figure 3 M.S. and in tabulated form in Table 6 M.S.

Net average nitrogen intake throughout the balance on M.S. was 2.98 g N per 24 hours. (0.0557 g N per kg body weight). The net average faecal nitrogen was 0.79 g per 24 hours (0.0146 g N per kg body weight), and this represented 26 per cent of the net intake.

The net nitrogen balance was - 1.14 g N per 24 hours (- 0.0210 g N per kg body weight).

During the period the patient was studied the blood urea ranged from
90 mg per 100 ml. at the commencement of the first balance period, increasing to 122 mg per 100 ml. at the beginning of the fourth balance period and returning to 90 mg per 100 ml. at the end of the total balance. Energy intake averaged 46 Calories per kg body weight.

The data obtained from this study does not give the impression of the achievement of a steady state of equilibrium. This is especially noted in the first half (first three balance periods).

Prior to the study the patient followed a Giovannetti diet for approximately one month as an out-patient, and a mild degree of hypertension with a small gradually increasing oedema was present.

At the commencement of the balance the sodium intake was controlled at 30 mEq. sodium daily.

On the last day of the first balance period, blood pressure became increased, with an episode of epistaxis. Methyl Dopa was given on the first day of the second balance period, after which on the last two days of the second balance period sudden hypotension became a big problem and episodes of nausea and vomiting were experienced. Methyl Dopa was discontinued for 24 hours on the last day of the second balance period and subsequently the dose was reduced by two thirds.

At the beginning of the fourth balance period when the problem of hypotension had improved some salt depletion was evident, serum sodium was 123 mEq. per litre. Sodium intake was then increased to 50 mEq. sodium daily.

At the commencement of the fifth balance period serum sodium was 120 mEq. per litre, weight remaining fairly steady. Serum chloride followed the same pattern as sodium. The sodium intake was then again increased to 70 mEq. sodium for the duration of the whole of the fifth and sixth balance periods, after which serum sodium returned to normal values.

Another factor complicating the lack of metabolic equilibrium of
this study was that this patient was menstruating during the end of the first, the whole of the second and the first half of the third balance periods.

This source of nitrogen loss, which has been computed to be between 0.3 and 0.6 g nitrogen daily by Gillett, Wheeler and Yates (1918), was not analysed in this study. This factor would probably constitute an increased negativity during the second and third balance periods.

During the first half of this balance study, three factors must have contributed to the lack of equilibrium:

(a) hypotensive episodes with nausea and vomiting.

(b) an inability to maintain the dietary intake of nitrogen and energy.

(c) menstruation and accompanying additional nitrogen loss.

It may also be possible, that at this time renal function may have deteriorated more rapidly, although this was not confirmed by the data of the second half of the balance. This apparent lack of renal response may have been emphasized by the unstable metabolic state, and the impression appears to be improved during the second half.

The average nitrogen balance for the first half was \(-1.447\) g N per 24 hours (\(-0.0268\) g N per kg) but the average nitrogen balance for the second half was \(-0.8345\) g N per 24 hours (\(-0.0154\) g N per kg).

It would appear that a better equilibrium would have been possible after sodium balance was corrected.

The influence of sodium balance on the nitrogen balance has previously been emphasized in the balance study on R.M. The sodium ion apparently plays a very decisive role in nitrogen utilization.

During the second half of the balance study the energy intake
was more consistent, although not as high as some others in the series, but blood urea was reduced to around 90 mg per 100 ml. by the end of the study.

It is notable that the protein score for the diet given to this patient was the highest in the series, and an implied 100 per cent utilization would appear possible from the nitrogen balance data despite the complications of this difficult study.
### TABLE 1  M.S.

Protein and energy content of diets given to M.S. female aged 37 years

<table>
<thead>
<tr>
<th></th>
<th>3.4</th>
<th>(by calculation)</th>
<th>(0.0636 g N/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g per 24 hours</td>
<td>3.4</td>
<td>(by calculation)</td>
<td>(0.0636 g N/kg body weight)</td>
</tr>
<tr>
<td>Net average nitrogen</td>
<td>2.978</td>
<td>(by analysis)</td>
<td>(0.0557 g N/kg body weight)</td>
</tr>
<tr>
<td>g per 24 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy</td>
<td>2,620</td>
<td>(11004 kJ)</td>
<td>(49 Cals/kg body weight)</td>
</tr>
<tr>
<td>Cals per 24 hours</td>
<td></td>
<td></td>
<td>(2058 kJ)</td>
</tr>
<tr>
<td>Average energy</td>
<td>2,459</td>
<td>(4918 kJ)</td>
<td>(46 Cals/kg body weight)</td>
</tr>
<tr>
<td>Cals per 24 hours</td>
<td></td>
<td></td>
<td>(193 kJ)</td>
</tr>
</tbody>
</table>

High biological value protein as % total protein 82.7

Fat as % total energy 36.0

CHO as % total energy 61.0

Polysaccharides as % of total CHO energy 78

Mono and disaccharides as % total CHO energy 21
### TABLE 2 M.S.

**Specimen of diet taken by M.S.**

(Amount in g per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aproten Crispbread</td>
<td>20</td>
</tr>
<tr>
<td>Low protein bread</td>
<td>60</td>
</tr>
<tr>
<td>Butter</td>
<td>45</td>
</tr>
<tr>
<td>Homogenised milk</td>
<td>100</td>
</tr>
<tr>
<td>Lemon drink</td>
<td>973</td>
</tr>
<tr>
<td>Tomato</td>
<td>50</td>
</tr>
<tr>
<td>Cucumber</td>
<td>10</td>
</tr>
<tr>
<td>Lettuce</td>
<td>10</td>
</tr>
<tr>
<td>Peaches</td>
<td>60</td>
</tr>
<tr>
<td>Carrots</td>
<td>45</td>
</tr>
<tr>
<td>Egg (hard boiled)</td>
<td>45</td>
</tr>
<tr>
<td>Pancake</td>
<td>100</td>
</tr>
<tr>
<td>Potatoes</td>
<td>100</td>
</tr>
<tr>
<td>Marmalade</td>
<td>30</td>
</tr>
<tr>
<td>Grapefruit sections (sweetened)</td>
<td>100</td>
</tr>
<tr>
<td>Strained tea (distilled water)</td>
<td>900 ml</td>
</tr>
</tbody>
</table>
**TABLE 3 N.S.**

**Mineral content of diet**

(Amounts in mg per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>305</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>492</td>
</tr>
<tr>
<td>Sodium</td>
<td>393 (17 mEq.)</td>
</tr>
<tr>
<td>Potassium</td>
<td>1,355</td>
</tr>
<tr>
<td>Magnesium</td>
<td>88</td>
</tr>
<tr>
<td>Iron</td>
<td>6.9</td>
</tr>
<tr>
<td>Copper</td>
<td>0.8</td>
</tr>
<tr>
<td>Sulphur</td>
<td>295.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>685.0</td>
</tr>
</tbody>
</table>

**Acid Base Balance**

Acid = 21.5 mEq.

Base = 24.2 mEq.
<table>
<thead>
<tr>
<th>Essential amino acid content of diet taken by M.S.</th>
<th>(mg amino acids per 24 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommended amino acid requirement for 53.45 kg</td>
<td></td>
</tr>
<tr>
<td><strong>In diet</strong></td>
<td><strong>Absolute minimal</strong></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>324</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1158</td>
</tr>
<tr>
<td>Lysine</td>
<td>1428</td>
</tr>
<tr>
<td>Methionine</td>
<td>590</td>
</tr>
<tr>
<td>Threonine</td>
<td>1015</td>
</tr>
<tr>
<td>Valine</td>
<td>1509</td>
</tr>
<tr>
<td>Leucine</td>
<td>1846</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1354</td>
</tr>
<tr>
<td>Cystine</td>
<td>383</td>
</tr>
<tr>
<td>Tryosine</td>
<td>905</td>
</tr>
</tbody>
</table>

Total 'S' containing amino acids in diet = 973 mg
<p>| Balance period | Weight kg | Urea mg per 100 ml | Creatinine mg per 100 ml | Na mEq/l. | K mEq/l. | Cl mEq/l. | CO₂ mEq/l. | Ca mg per 100 ml | PO₄ mg per 100 ml | Alb. g per 100 ml | Hb. g per 100 ml | blood |
|----------------|-----------|-------------------|--------------------------|-----------|---------|----------|-----------|-----------------|-----------------|-----------------|------------------|
| 1.             | 53.45     | 90                | 12.8                     | 136       | 3.9     | 106      | 22        | 9.0             | 9.6             | 3.55            | 5.7              |
| 2.             | 53.3      | 92                | 127                      | 3.8       | 95      | 21       | 8.6       | 9.2             | 3.4             | 8.2             |
| 3.             | 53.6      | 98                | 128                      | 4.5       | 98      | 19       | 9.6       | 9.3             | 3.4             | 8.1             |
| 4.             | 54.65     | 122               | 123                      | 4.5       | 92      | 19       | 8.6       | 9.4             | 3.4             | 7.8             |
| 5.             | 55.0      | 100               | 11.6                     | 120       | 4.0     | 91       | 20        | 8.5             | 5.0             | 6.8             |
| 6.             | 54.8      | 107               | 128                      | 3.9       | 95      | 21       | 8.6       | 3.7             | 2.8             | 6.6             |
| end            | 55.0      | 90                | 12.2                     | 133       | 4.1     | 100      | 21        | 9.6             | 3.9             | 3.1             | 6.5             |</p>
<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net nitrogen intake</td>
<td>2.978</td>
<td>(0.0557 g N/kg body weight)</td>
</tr>
<tr>
<td>Average faecal nitrogen</td>
<td>0.79</td>
<td>(0.0145 g N/kg body weight)</td>
</tr>
<tr>
<td>Faecal nitrogen as % of nitrogen intake</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Net nitrogen balance</td>
<td>-1.141</td>
<td>(-0.0210 g N/kg body weight)</td>
</tr>
</tbody>
</table>
TABLE 7 M.S.

Prediction of theoretical net protein utilization on patient M.S.

Protein score of diet

\[ 972 \text{ mg = total } S \text{ containing amino acids} \]

\[ 3.4 \text{ g } N = \text{ total dietary nitrogen} \]

Therefore \( 286 \text{ mg } S \text{ containing amino acids per g total nitrogen} \)

\( (270 \text{ mg} = \text{ provisional pattern F.A.O.}) \)

\[ \frac{286 \times 100}{270} = 105.92 = \text{ score} \]

\( \varnothing (\text{N.P.U.}) = S \times (1 - \text{KP Cals}%) \quad (K = 0.019 \quad \text{ (Miller and Payne)}) \)

Net Cals in = 46 Cals/kg. Total Calories = 2,459 Weight = 58.45kg.

Average nitrogen in = 2.98 g N/24 hours (urinary leak = 4.2 g protein/24 hours = 0.672 g N)

Therefore net available for anabolism = 2.308 g N \( (14.43 \text{ g protein} = 58 \text{ Calories}) \)

58 as \% of 2459 = 2.36\%

\[ \varnothing = 105.92 \times (1 - 0.019 \times 2.36) \]

\[ = 1.0 - 0.0448 = 0.9551 \]

\[ 105.92 \times 0.9551 = 101.17 \]

Theoretical N.P.U. = 101 \%

Therefore maximal N.P.U. is theoretically possible

From nitrogen balance data

\[ \Delta \text{Bal/kg}^{0.73} = I/\text{kg}^{0.73} \times \varnothing - N \quad (200 \text{ mg } N \text{ obligatory loss}) \]

\[ - 62.52 \text{ mg } N/\text{kg}^{0.73} = 126.4 \text{ mg } N/\text{kg}^{0.73} \times \varnothing - 200 \]

\[ 137.48 = 126.4 \times \varnothing \]

\[ \varnothing = 1.088 \]
TABLE 8 M.S.

Drugs taken by M.S. during the period of the balance study

Ascorbic Acid  50 mg t.d.s.
Aneurine Co. forte  2 tablets t.d.s.
Chromium sesquioxide  1.5 g daily
Aluminium hydroxide  12 tablets (Evans) 6 g daily
Commencing at the beginning of the third balance period
Valium  10 mg daily (at night)
Methyl Dopa 250 mg x 3 daily from the first day of the second balance period until last day of second balance period
Methyl Dopa 250 mg x 1 daily from the first day of the third balance period for the remainder of the balance.
Fig. 3. M.S. Nitrogen balance study on M.S. ♀ age 37
Modified Reifenstein plot

blood urea mg per 100 ml

-2.0
-1.0
0
+1.0
+2.0
+3.0

g. N. per day

-49 39 49 45 46 45
Cals Cals Cals Cals Cals Cals
/kg/day /kg /kg /kg /kg /kg
(206 kJ) (164 kJ) (206 kJ) (189 kJ) (193 kJ) (189 kJ)
Balance study on T.G. male aged 56 years

The diagnosis was a chronic pyelonephritis. The glomerular filtration rate was 3.8 ml. per minute at the time of the study.

This patient was referred to the Renal Unit in March 1970 and his complaints at that time were that he had lost 2 stones in weight within the previous three years, and his appetite had deteriorated over the preceding six months. He had experienced dryness of the mouth and extreme thirst for six months. He felt generally weak. At this time he was having nocturia twice nightly. There was no haematuria or dysuria.

His previous history showed that he had had no previous illnesses, except that on one occasion, two years prior to his referral, he had an episode of right-sided chest pain and loin pain which was severe enough to confine him to bed for a few days. This resolved spontaneously.

He was a married man and worked as a postman. His family history showed no evidence of kidney disease. Two half brothers had died of tuberculosis.

The referring hospital recorded the following serum values:

- Blood urea 142 mg per 100 ml., potassium 6.5 mEq. per litre,
calcium 9.4 mg per 100 ml., phosphate 5.6 mg per 100 ml. A chest x-ray showed right pleural calcification. Abdominal x-ray showed normal sized kidneys. Chronic renal failure was diagnosed of unknown cause.

In mid-March 1970 he was admitted to the Renal Unit for investigations the results of which were as follows:

- Serum values - blood urea 215 mg per 100 ml., chloride 122 mEq. per litre, bicarbonate 17 mEq. per litre, sodium 153 mEq. per litre,
potassium 6.3 mEq. per litre, total protein 6.6 g per 100 ml.,
albumin 4.0 g per 100 ml., calcium 7.7 mg per 100 ml., phosphate
4.7 mg per 100 ml., haemoglobin 7.9 g per 100 ml., creatinine
11.5 mg per 100 ml. A chest x-ray showed cardiac enlargement with
signs of congestive changes in the lung fields. An intravenous
pyelogram showed full concentration of the dye, and renal outlines
and emptying appeared within normal limits and the bladder a normal
shape.

A kidney biopsy was performed. This showed a tubular atrophy
which was marked throughout the biopsy. The overall appearance was
that of ischaemia, probably associated with chronic pyelonephritis.
Proliferative changes were all of the glomeruli, and suggested lobular
proliferative glomerular nephritis.

During the hospital admission T.G. had a low grade pyrexia. He
was given Ampicillin and started on a Giovannetti diet after which his
general condition improved considerably. There was a steady decrease
in blood urea and prior to discharge serum values were as follows :-
Blood urea 102 mg per 100 ml., chloride 100 mEq. per litre, bicarbonate
16 mEq. per litre, sodium 134 mEq. per litre, total protein 7.3 g per
100 ml., creatinine 9.1 mg per 100 ml. Ampicillin was stopped when his
temperature returned to normal, and he was discharged on a Giovannetti
diet.

On his next visit in May 1970 he was feeling very fit, his only
complaint being that he felt hungry and he was encouraged to take more
protein free energy. At this time his blood urea was 108 mg per 100 ml.
and serum creatinine 9.4 mg per 100 ml. Nocturia was once nightly.

In June 1970 his blood urea was 83 mg per 100 ml., serum creatinine
10.0 mg per 100 ml. and he was back to work on his rounds as a postman.

In September 1970 his blood urea was 120 mg per 100 ml., chloride
102 mEq. per litre, bicarbonate 12 mEq. per litre, sodium 139 mEq. per litre, potassium 6.2 mEq. per litre, total protein 6.8 g per 100 ml. and his serum creatinine had increased to 14.5 mg per 100 ml.

In October 1970 he was cheerful and looked reasonably well. His renal function was now deteriorating rapidly, his serum creatinine was 15.5 mg per 100 ml., total protein 7.0 g per 100 ml., calcium was 7.8 mg per 100 ml. and phosphate 10.4 mg per 100 ml. His only complaint at this time was that of diarrhoea. This was probably a symptom of his increasing uraemia.

Early in November T.G. was readmitted for peritoneal dialysis and stabilization on a strict dietary protocol.

He had had nausea and vomiting for one day just prior to this admission, otherwise he had been well at home, except for intermittent diarrhoea since September. He had nocturia once nightly but no pruritus.

The nitrogen balance commenced on the 7th November 1970.
Details from the nitrogen balance study on T.G.

As with the previous patients, a preliminary equilibration period of 4 days was allowed before urine and faecal collections commenced.

Two balance studies were attempted on this patient. The first study continued for 16 days and was divided into 4 balance periods each of 4 days duration. A second balance study was then continued for 5 days after which it was abandoned because of the high rate of food rejects and frequent vomiting.

Weight of the patient at the commencement was 63 kg and 51.75 kg at the end of the study.

Urinary protein was 2.25 g per 24 hours.

Details of this study are shown in Table 1 T.G. to Table 8 T.G. The total energy content of the diet was 40 Calories per kg body weight per 24 hours. Fat and carbohydrate supplied 46 per cent and 51 per cent of this respectively. The energy intake of 40 Calories per kg body weight was maintained over the 4 balance periods.

A large proportion of the fat was supplied as double cream, butter and small amounts in milk and egg yolk. Polysaccharides contributed 43 per cent of the total carbohydrate and were taken as wheat starch, sago, potato, Giovannetti bread, arrowroot, and Hycal. Mono and disaccharides supplied 57 per cent of the carbohydrate and were contained in grapefruit, peach, pear, marmalade, jam, carrot, glucose, lactose, sucrose and Hycal. High biological value protein was contained in egg, double cream, butter and milk. Second class protein being unessential nitrogen, was present in small amounts in the following - grapefruit, peach, pear, wheat starch, arrowroot, sago, marmalade, jam, carrot, lettuce, tomato, onion, potatoes, Giovannetti bread.

Table 3 T.G. shows the mineral content and acid-base of the diet taken by T.G. As in the other studies in the series the calcium,
magnesium, iron and copper are all supplied at low levels. The diet also shows a slight excess of base, which is common to all diets in this series of studies.

Table 4 T.G. gives the essential amino acid content of the diet. All the essential amino acids were supplied in amounts exceeding the absolute minimal requirement for a body weight of 63 kg with the exception of methionine and cystine (total sulphur containing amino acids).

Table 5 T.G. shows the serum values at the commencement of each balance period.

During the first balance period the blood urea shows a 10 per cent rise after which there is a 5 per cent drop, and a final drop of 20 per cent at the beginning of the last balance period, the chloride and sodium remain at fairly steady values. The potassium is a little high but remains reasonably steady, the tendency being to maintain a slightly reduced almost normal level whilst on the diet. This is an expected response to the low potassium diet. The bicarbonate remains at a low value, this is a feature common to all these studies.

The serum albumin remains steady around the lower limit of a normal value, the haemoglobin also remains fairly steady. The serum calcium is low, and phosphate high and this is a common feature of chronic renal failure.

Creatinine is high at 15.5 mg per 100 ml. at the commencement of the study. No record was made of the serum creatinine at the end of the study, but 24 days after this serum creatinine was 21.0 mg per 100 ml. This indicates a very rapid deterioration in renal function.
Results and discussion on data from patient T.G.

Figure 3 T.G. shows a modified Reifenstein plot of the nitrogen balance data on this patient and Table 6 T.G. shows the results in tabulated form. The total period studied extended for only 16 days, and was divided into 4 balance periods. Nitrogen balance data was not obtained for the final two balance periods (5 and 6) on this patient as the extent of vomiting and food rejection was so large that an attempt to analyse these was not considered feasible.

During the first four balance periods, the net average nitrogen intake was 2.84 g nitrogen per 24 hours. The net average faecal nitrogen was 1.233 g nitrogen per 24 hours and this represented 43 per cent of dietary nitrogen. Net average nitrogen balance (16 days) was - 1.04 g nitrogen per 24 hours. The blood urea was 135 mg per 100 ml. at the beginning of the first balance period and fell to 120 mg per 100 ml. during the fourth balance period.

The data shows that after an initial period of equilibration, improved nitrogen utilization apparently occurred during the fourth balance period. It is possible that a more prolonged period of equilibration would have been advantageous as his outpatient diet was not well controlled. The total protein intake as an outpatient was assessed at 36 g protein daily, and the Calories at 2,500 (40 Calories per kg). High biological value protein comprised 40 per cent of the total protein intake; the second class component being high. The energy intake of 40 Calories per kg body weight during this balance was the lowest in the series. Nitrogen utilization may have been more pronounced with a higher energy intake. During the fifth balance period energy intake was increased to 55 Calories per kg body weight with an increased proportion of the energy from fat.

It would seem reasonable to conclude that such a sudden increase
in the energy supply, and especially when the energy source is changed radically by an increased proportion of fat to carbohydrate then a loss of equilibrium would result.

This patient was 57 years of age and the oldest patient in the series. It may be that less flexibility in adapting to fluctuations in dietary intake is present at this age. From results of nitrogen balance studies in normal elderly people presented by Kountz, Hofstatter and Ackerman (1947) an unusually slow adjustment to dietary changes is suggested in older age groups, as their response to these changes is sluggish. After nitrogen depletion, the period of nitrogen repletion appeared to be much more prolonged and less responsive to an increase in dietary protein than in younger age groups.
TABLE 1  T.G.

Protein and energy content of diet for patient T.G., male aged 57 years

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen</td>
<td>2.84</td>
</tr>
<tr>
<td>(g per 24 hours)</td>
<td>(0.045 g N/kg body weight/24 hrs)</td>
</tr>
<tr>
<td>Total energy</td>
<td>2,490</td>
</tr>
<tr>
<td>(Cals per 24 hours)</td>
<td>(10458 kJ)</td>
</tr>
<tr>
<td>(40 Cals/kg body weight/24 hrs)</td>
<td>(168 kJ)</td>
</tr>
<tr>
<td>Average energy</td>
<td>2,490</td>
</tr>
<tr>
<td>(Cals per 24 hours)</td>
<td>(10458 kJ)</td>
</tr>
<tr>
<td>(40 Cals/kg body weight/24 hrs)</td>
<td>(168 kJ)</td>
</tr>
<tr>
<td>High biological value protein as % total protein</td>
<td>78</td>
</tr>
<tr>
<td>Fat as % total energy</td>
<td>46</td>
</tr>
<tr>
<td>Carbohydrate (CHO) as % total energy</td>
<td>57</td>
</tr>
<tr>
<td>Polysaccharides as % total CHO energy</td>
<td>43</td>
</tr>
<tr>
<td>Mono and disaccharides as % total CHO energy</td>
<td>57</td>
</tr>
<tr>
<td>Food Item</td>
<td>Amount (g)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Giovannetti bread</td>
<td>30</td>
</tr>
<tr>
<td>Butter</td>
<td>20</td>
</tr>
<tr>
<td>Homogenised milk</td>
<td>110</td>
</tr>
<tr>
<td>Double cream</td>
<td>80</td>
</tr>
<tr>
<td>Sucrose</td>
<td>65</td>
</tr>
<tr>
<td>Sweetened grapefruit</td>
<td>120</td>
</tr>
<tr>
<td>Marmalade</td>
<td>10</td>
</tr>
<tr>
<td>Jam</td>
<td>10</td>
</tr>
<tr>
<td>Pancake</td>
<td>140</td>
</tr>
<tr>
<td>Carrots</td>
<td>50</td>
</tr>
<tr>
<td>Sago pudding</td>
<td>195</td>
</tr>
<tr>
<td>Sweetened peaches</td>
<td>60</td>
</tr>
<tr>
<td>Sweetened pears</td>
<td>60</td>
</tr>
<tr>
<td>Boiled potatoes</td>
<td>80</td>
</tr>
<tr>
<td>Lettuce</td>
<td>20</td>
</tr>
<tr>
<td>Tomato</td>
<td>60</td>
</tr>
<tr>
<td>Spring onion</td>
<td>4</td>
</tr>
<tr>
<td>Hycal</td>
<td>170 ml</td>
</tr>
<tr>
<td>Strained tea (distilled water)</td>
<td>800 ml</td>
</tr>
</tbody>
</table>

(Total fluids 1,000 ml.)
TABLE 3  T.G.

Mineral content of diet eaten by T.G.

(Amounts in mg per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>339</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>376</td>
</tr>
<tr>
<td>Sodium</td>
<td>284 (+ added 20 mEq. Na; total = 32 mEq. intake)</td>
</tr>
<tr>
<td>Potassium</td>
<td>1495</td>
</tr>
<tr>
<td>Magnesium</td>
<td>81</td>
</tr>
<tr>
<td>Iron</td>
<td>7.61</td>
</tr>
<tr>
<td>Copper</td>
<td>3.36</td>
</tr>
<tr>
<td>Sulphur</td>
<td>181</td>
</tr>
<tr>
<td>Chloride</td>
<td>481</td>
</tr>
</tbody>
</table>

Acid Base Balance

Acid = 11.1 mEq.
Base = 26.4 mEq.
TABLE 4  T.G.

Essential amino acid content of diet eaten by T.G.
(mg amino acids per 24 hours)

<table>
<thead>
<tr>
<th></th>
<th>In diet</th>
<th>Recommended amino acid requirement for 62.8 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absolute minimal</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>264</td>
<td>226</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>906</td>
<td>973</td>
</tr>
<tr>
<td>Lysine</td>
<td>1197</td>
<td>722</td>
</tr>
<tr>
<td>Methionine</td>
<td>461</td>
<td>973</td>
</tr>
<tr>
<td>Threonine</td>
<td>796</td>
<td>440</td>
</tr>
<tr>
<td>Valine</td>
<td>1193</td>
<td>722</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1069</td>
<td>628</td>
</tr>
<tr>
<td>Leucine</td>
<td>1502</td>
<td>973</td>
</tr>
<tr>
<td>Cystine</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>756</td>
<td></td>
</tr>
</tbody>
</table>

Total 'S' containing amino acids in diet = 7177 mg
TABLE 5 T.G.

Blood chemistry at the commencement of each balance period for patient T.G.

(values per litre or per 100 ml. serum unless otherwise stated)

<p>| Balance period | Weight kg | Urea mg per 100 ml | Creatinine mg per 100 ml | Na mEq/l. | K mEq/l. | Cl mEq/l. | CO₂ mEq/l. | Ca mg per 100 ml | PO₄ mg per 100 ml | Alb. g per 100 ml | Hb. g per 100 ml blood |
|----------------|-----------|--------------------|--------------------------|----------|---------|---------|-----------|-------------|----------------|----------------|----------------|----------------------|
| 1.             | 62.8      | 135                | 15.5                     | 133      | 6.3     | 108     | 10        | 8.2         | 10.5          | 3.4             | 7.9                |
| 2.             | 61.7      | 145                |                          | 137      | 5.6     | 112     | 12        |             | 3.4           | 7.5             |
| 3.             | 61.75     | 140                |                          | 137      | 5.8     | 113     | 12        |             | 3.6           | 7.5             |
| 4.             | 61.9      | 140                |                          | 139      | 5.6     | 111     | 9         |             | 3.7           | 7.7             |
| 5.             | 61.75     | 120                |                          | 139      | 6.1     | 112     | 12        | 8.4         | 9.5           | 3.6             | 7.2             |</p>
<table>
<thead>
<tr>
<th>Nitrogen Balance Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>(amounts are averages in g/24 hours unless otherwise stated)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Net nitrogen intake (by analysis)</th>
<th>2.84</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.0457 g/kg body weight)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average faecal nitrogen</th>
<th>1.233</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0.0198 g/kg body weight)</td>
</tr>
</tbody>
</table>

| Faecal nitrogen as % of nitrogen intake | 43    |

<table>
<thead>
<tr>
<th>Net nitrogen balance</th>
<th>-1.04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-0.0167 g/kg body weight)</td>
</tr>
</tbody>
</table>
TABLE 7  T.G.

Prediction of theoretical N.P.U. on patient T.G. (weight = 62.8 kg)

Protein score of diet

717 mg total S containing amino acids in diet

2.84 g total dietary nitrogen

252 mg S containing amino acids per g total nitrogen

\[
\frac{252}{270} \times 100
\]

Score = 93

Net Cals in = 40 Cals/kg. Total Calories = 2,520

Average nitrogen in = 2.84 gN/24 hours (urinary leak = 2.25 g protein)

\[
(0.36 \times 0.36) = 2.48 \text{ g N net available for anabolism} = 15.5 \text{ g protein}
\]

(62 Calories)

62 as % of 2520 = 2.5

\[\Phi = 93 (1 - 0.019 \times 2.5) = 88.5825\]

N.P.U. = 89% (theoretical)

From nitrogen balance data

\[
\Delta \text{ Bal/kg}^{0.73} = I/\text{kg}^{0.73} \times \Phi - M \text{ (200 mg N)}
\]

\[
- 52.09/\text{kg}^{0.73} = 120.7 \text{ mg N/kg}^{0.73} \times \Phi - 200
\]

147.91 = 120.7 \times \Phi

\[\Phi = 1.225 = > 100\%
\]
TABLE 8 T.G.

Drugs taken by T.G. during the period of the balance study

Ascorbic acid  50 mg t.d.s.

Anurine Co. forte  2 tablets t.d.s.
Fig. 3. T.G. Nitrogen balance study on T.G. ¦ age 57
Modified Reifenstein plot

- Blood urea mg per 100 ml
- 150
- 140
- 130
- 120
- 110

- 3.0
- 2.0
- 1.0
- 0.0
+ 0.0
+ 1.0
+ 2.0
+ 3.0

- 4
- 8
- 12
- 16

days

40 Cals/kg/day
(168 kJ)
5.a. Balance study on J.D. male aged 35 years

The diagnosis was stage 3 chronic renal failure of unconfirmed aetiology. The glomerular filtration rate was 4.2 ml. per minute at the time of the study. In 1954 J.D. was examined in Dundee because of enuresis which was present for the first eight years of his life and he was known to have had a large right hydronephrosis for seven years previously. He was found to have marked proteinuria, but no urinary infection. An intravenous pyelogram done at this time was of poor quality radiographically, but gave the suspicion of a right hydronephrosis. Blood urea was then 38 mg per 100 ml. In 1956 he came to live in the Southampton area and was referred to the local hospital.

In 1965 he failed a medical examination for an Insurance proposal. At that time he was symptomless but had an increased blood pressure, proteinuria, a high serum potassium at 7.2 mEq. per litre, which when repeated was reduced to 4.5 mEq. per litre; blood urea was 73 mg per 100 ml. and haemoglobin 14.0 g per 100 ml.

In 1966 an intravenous pyelogram infused, showed a large right hydronephrosis with poor function of both kidneys. He was then seen at yearly intervals and remained symptomless until 1970 when he had low back pain, and cramp-like sensations in his calves and buttocks. He was given "Sandecal" which alleviated these symptoms. In 1970 blood urea was 125 mg per 100 ml., potassium 3.5 mEq. per litre. He was told to reduce his protein intake slightly and to avoid steaks.

In July 1971 blood urea was 134 mg per 100 ml., chloride 134 mEq. per litre, bicarbonate 16 mEq. per litre, sodium 142 mEq. per litre, potassium 3.5 mEq. per litre, calcium 5.8 mg per 100 ml., phosphate
4.5 mg per 100 ml. In August 1971 he was referred to the Renal Unit. He was complaining of pain in the buttock and cramps in the feet and wrists partially relieved with "Sandecal". He was feeling very lethargic and had been off work for two weeks. His fluid intake was approximately 5 pints daily, and he had nocturia once nightly. His appetite was good but he had lost one stone in weight within a year.

J.D. was a work study engineer. He was married and had two children. He was tall and lean although not wasted. He looked pale but was slightly pigmented. He was a pleasant, sensible and co-operative man.

His parents both died from carcinoma. There appeared to be no known history of renal disease in his family.

In early October 1971 his blood urea had reached 255 mg per 100 ml. and chloride was 102 mEq. per litre, bicarbonate 16 mEq. per litre, sodium 135 mEq. per litre, potassium 4.4 mEq. per litre, calcium 5.4 mg per 100 ml., phosphate 7.3 mEq. per litre, total proteins 6.4 g per 100 ml., albumin 4.0 g per 100 ml., haemoglobin 9.0 g per 100 ml. and serum creatinine was 12.0 mg per 100 ml.

He was instructed to take a Giovannetti diet with 20 mEq. sodium and fluid intake to be his urine volume plus 500 ml.

On 16th October his blood urea had decreased to 210 mg per 100 ml. and on 18th October was down to 200 mg per 100 ml., and it had reached 115 mg per 100 ml. on the 25th of that month. His haemoglobin was 8.6 g per 100 ml. and serum creatinine noted to be 10.0 mg per 100 ml.

He agreed at this time to come into the Metabolic Unit for balance studies and was admitted on 26th November 1971.
Details of the study on J.D.

This study lasted for 24 days and was divided into six balance periods of four days. The usual equilibration period of four days was observed.

This patient weighed 61.0 kg at the beginning of the study and 62.1 kg at the end. Urinary protein loss was 1.0 g per 24 hours. Complete details of this study can be seen in Table 1 J.D. to Table 8 J.D.

The protein and energy content of the diet are shown in Table 1 J.D. The total energy content of the diet was 49 Calories per kg body weight per 24 hours, 31 per cent was supplied as fat and 66 per cent as carbohydrate.

The actual energy intake in the six balance periods was as follows:-

Balance period 1 and 2 (8 days) 53 Calories per kg body weight.
Balance period 3 (4 days) 51 Calories per kg body weight.
Balance period 4, 5 and 6 (12 days) 48 Calories per kg body weight.

The average energy intake over the entire period of 24 days was 50 Calories per kg body weight.

The sources of the fat were double cream and butter, egg yolk and milk.

The polysaccharides were contributed by potatoes, wheat starch, cornstarch (bread, scones, custard, pancakes) and Hycal. The mono and disaccharides were contributed by jam, pineapple, sucrose and Hycal.

The sources of the high biological value protein were egg, double cream, butter and milk. The second class protein for this patient was contributed by jam, potatoes, wheat starch, carrots, grapefruit, pineapple, lettuce and cucumber.
In Table 3 J.D. are shown the mineral content and acid-base of the diet. As in the other diets used in these studies the calcium, magnesium, iron, copper intakes all appear to be lower than normal intakes. There is a slight excess of base.

Table 4 J.D. shows the essential amino acid content of the diet. It can be seen that all the essential amino acids are supplied in amounts above the absolute minimal requirement with the exception of Methionine. The amount of threonine exceeded the safe minimal requirement.

Table 5 J.D. records the serum values at the beginning of each balance period.

During the first balance period blood urea is reduced by 8 mg per 100 ml. and thereafter stays around 92 - 96 mg per 100 ml. Serum sodium and chloride remain stable throughout the balance. Potassium is also steady and tends to be slightly lower than normal values. Magnesium tends to increase very slightly during the second half of the balance to 2.1 mg per 100 ml. Haemoglobin and serum albumin show steady values. Calcium values are low, but show a slight increase during the final three balance periods. Phosphate values are high being between 10 - 13 mg per 100 ml. during the first three balance periods. The values are reduced to between 6.2 and 7.3 mg per 100 ml. during the latter half of the balance. This pattern would reflect the effect of the administration of aluminium hydroxide during the second half of the balance. Serum creatinine remains fairly stable throughout, reflecting a steady renal state. The body weight is also steady throughout.
Results and discussion on the balance data on J.D.

A modified Reifenstein plot of the balance data from patient J.D. is shown in Figure 3 J.D. and Table 6 J.D. gives the results in tabulated form. The net average nitrogen intake was 3.35 g per 24 hours. The net average faecal nitrogen was 1.46 g per 24 hours which was 43.5 per cent of the intake. The net nitrogen balance was -0.88 g per 24 hours. The blood urea fell from 104 mg per 100 ml. in the first 4 day period and then further to 91 mg per 100 ml. during the last period.

During the first two balance periods energy intake was good, and from the appearance of this data there is a possibility that improved nitrogen utilization may have occurred to a small extent. Unfortunately, during the third period the energy intake was not maintained.

A tendency to diarrhoea in this patient became exacerbated during the last three balance periods and the total faecal excretion increased, also during these last three periods faecal nitrogen increased, and as a consequence negativity of the balance.

Blood urea was maintained at around 95 mg per 100 ml. The impression gained from these results is that an apparent trend towards a more positive balance and possibly improved nitrogen utilization, appeared to be affected by two factors.

(1) A decrease in energy intake during the last four periods.
(2) A marked increase in diarrhoea and faecal nitrogen during the last three balance periods with consequent increase in the negativity of the balance.

Increased faecal nitrogen may result from:
(a) a degree of malabsorption resulting from intestinal hurry
(b) contribution from mucosal cellular debris being accelerated
(c) intestinal luminal secretions
(d) urea nitrogen
### Table 1: J.D.

Protein and energy content of the diet for patient J.D.  
**Male aged 35 years**

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen (g per 24 hours)</td>
<td>3.29 (by calculation)</td>
<td>(0.054 g N/kg body weight/24 hours)</td>
</tr>
<tr>
<td>Total energy (Cals. per 24 hours)</td>
<td>3162 (13280 kJ)</td>
<td>(51 Cals/kg body weight/24 hours)</td>
</tr>
<tr>
<td>Average energy (Cals. per 24 hours)</td>
<td>3100 (13020 kJ)</td>
<td>(50 Cals/kg body weight/24 hours)</td>
</tr>
<tr>
<td>High biological value protein</td>
<td>76.0</td>
<td></td>
</tr>
<tr>
<td>Fat as % total energy</td>
<td>31.0</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (CHO) as % total energy</td>
<td>66.0</td>
<td></td>
</tr>
<tr>
<td>Polysaccharides as % total CHO energy</td>
<td>60.0</td>
<td></td>
</tr>
<tr>
<td>Mono and disaccharides as % total CHO energy</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>Item</td>
<td>Amount (g)</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Aproten crispbread</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Hycal</td>
<td>255 ml.</td>
<td></td>
</tr>
<tr>
<td>Custard</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Scones</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td>Pancake</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Carrots</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Jam</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Homogenised milk</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Tea (distilled water)</td>
<td>950 ml.</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3 J.D.

Mineral content of diet eaten by J.D.

(Amounts in mg per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Element</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>269</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>397</td>
</tr>
<tr>
<td>Sodium</td>
<td>505</td>
</tr>
<tr>
<td>Potassium</td>
<td>1247</td>
</tr>
<tr>
<td>Magnesium</td>
<td>83</td>
</tr>
<tr>
<td>Iron</td>
<td>7.11</td>
</tr>
<tr>
<td>Copper</td>
<td>0.92</td>
</tr>
<tr>
<td>Sulphur</td>
<td>234</td>
</tr>
<tr>
<td>Chloride</td>
<td>745</td>
</tr>
</tbody>
</table>

Acid Base Balance

Acid = 16.7 mEq.

Base = 25.1 mEq.
### TABLE 4 J.D.

**Essential amino acid content of diet taken by J.D.**

(ng amino acids per 24 hours)

<table>
<thead>
<tr>
<th>In diet</th>
<th>Absolute minimal</th>
<th>Safe minimal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>325</td>
<td>223.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1049</td>
<td>961.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>1327</td>
<td>713.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>536</td>
<td>961.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>917</td>
<td>434.0</td>
</tr>
<tr>
<td>Valine</td>
<td>1375</td>
<td>713.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>1689</td>
<td>961.0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1228</td>
<td>620.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>335</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>837</td>
<td></td>
</tr>
</tbody>
</table>

Recommended amino acid requirement for 62.0 kg

- Total aromatic = 1426.0
- Total 'S' containing = 1922.0

Total 'S' containing amino acids in diet = 871 mg.
### TABLE 5: J.D.

Blood chemistry at the commencement of each balance period for patient J.D.

(values per litre or per 100 ml serum unless otherwise stated)

<table>
<thead>
<tr>
<th>Balance period</th>
<th>Weight kg</th>
<th>Urea mg per 100 ml</th>
<th>Creatinine mg per 100 ml</th>
<th>Na mEq/l</th>
<th>K mEq/l</th>
<th>Cl mEq/l</th>
<th>CO₂ mEq/l</th>
<th>Ca mg per 100 ml</th>
<th>P0₄ mg per 100 ml</th>
<th>Alb g per 100 ml</th>
<th>Hb g per 100 ml blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>61.0</td>
<td>104</td>
<td>13.2</td>
<td>141</td>
<td>4.1</td>
<td>114</td>
<td>14</td>
<td>4.7</td>
<td>10.0</td>
<td>2.1</td>
<td>8.7</td>
</tr>
<tr>
<td>2.</td>
<td>61.35</td>
<td>96</td>
<td>13.0</td>
<td>140</td>
<td>3.8</td>
<td>111</td>
<td>18</td>
<td>6.6</td>
<td>13.0</td>
<td>2.3</td>
<td>7.8</td>
</tr>
<tr>
<td>3.</td>
<td>61.55</td>
<td>96</td>
<td>140</td>
<td>3.2</td>
<td>111</td>
<td>14</td>
<td>7.8</td>
<td>11.5</td>
<td>3.4</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>62.25</td>
<td>93</td>
<td>12.3</td>
<td>134</td>
<td>3.1</td>
<td>109</td>
<td>16</td>
<td>5.4</td>
<td>8.4</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>62.1</td>
<td>96</td>
<td>11.9</td>
<td>136</td>
<td>3.2</td>
<td>109</td>
<td>17</td>
<td>6.2</td>
<td>7.2</td>
<td>3.3</td>
<td>8.4</td>
</tr>
<tr>
<td>6.</td>
<td>61.65</td>
<td>96</td>
<td>13.2</td>
<td>139</td>
<td>3.5</td>
<td>114</td>
<td>17</td>
<td>7.0</td>
<td>6.2</td>
<td>3.2</td>
<td>7.8</td>
</tr>
<tr>
<td>end</td>
<td>62.1</td>
<td>92</td>
<td>12.7</td>
<td>144</td>
<td>3.8</td>
<td>112</td>
<td>17</td>
<td>6.0</td>
<td>7.3</td>
<td>3.2</td>
<td>8.1</td>
</tr>
</tbody>
</table>
TABLE 6 J.D.

Nitrogen Balance Data

(Amounts are averages in g/24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Nitrogen intake</th>
<th>3.35</th>
<th>0.0544 gN per kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal nitrogen</td>
<td>1.46</td>
<td>0.0237 gN per kg body weight</td>
</tr>
<tr>
<td>Faecal nitrogen as % of nitrogen intake</td>
<td>43.5</td>
<td></td>
</tr>
<tr>
<td>Nitrogen balance</td>
<td>-0.883</td>
<td>-0.0143 gN per kg body weight</td>
</tr>
</tbody>
</table>
TABLE 7 J.D.

Prediction of theoretical net protein utilization on patient J.D.

Protein score of diet

871 mg - total sulphur containing amino acids

3.29 gN = total dietary nitrogen

Therefore 265 mg S containing amino acids per g total nitrogen

(270 mg = provisional pattern F.A.O.)

\[
\frac{265 \times 100}{270} = 98.1 = \text{score}
\]

\[\theta \text{ N.F.U.} = S (1-kP \text{ Cals } \% \text{)} \ (k = 0.019) \ \text{ (Miller and Payne)}\]

Net Cals in = 50 Cals/kg. Total Calories = 3100. Weight = 62.0 kg.

Average nitrogen in = 3.35 gN/24 hours (urinary leak = 1.0 g protein/24 hours = 0.16 gN).

Therefore net available for anabolism = 3.192 gN. = 19.94 g protein = 80 Calories

\[
\theta = 98.1 \ (1 - 0.019 \times 2.58)
\]

\[
= 1.0 - 0.0490 = 0.9509
\]

\[98.1 \times 0.9509 = 93.29\]

Theoretical N.F.U. = 93%

From nitrogen balance data

\[
\Delta \text{Bal/kg}^{0.73} = I/\text{kg}^{0.73} \times \theta - M \ \text{(200mgN obligatory loss)}
\]

\[-43.43 \text{ mg } N/\text{kg}^{0.73} = 156.91 \text{ mg } N/\text{kg}^{0.73} \times \theta - 200\]

\[156.57 = 156.91 \times \theta\]

\[\theta = 0.998\]

Therefore more than 93% utilization.
Drugs taken by J.D. during the period of the balance study

Ascorbic Acid 50 mg t.d.s.

Aneurine Co. forte 2 tablets t.d.s.

Chromium Sesquioxide 1.5 g daily.

Aluminium hydroxide 12 tablets (Evans) 6 g daily commencing at the beginning of the third balance period.
Fig. 3. J. D. Nitrogen balance study on J. D. age 35
Modified Reifenstein plot

blood urea mg per 100 ml

[Graph of blood urea levels]

N. per day

[Graph showing nitrogen balance over days]

53 Cals/kg per day (222 kJ)
50.5 Cals/kg (212 kJ)
47.7 Cals/kg (200 kJ)
47.9 Cals/kg (201 kJ)
The diagnosis was stage 3 chronic renal failure with a glomerular filtration rate of 3.3 ml. per minute and due to polycystic disease of the kidneys.

This patient was found to have a mass in the left abdomen in 1968 when being examined by her General Practitioner after having influenza. One month later she was referred to the hospital with a complaint of left-sided abdominal ache for one month and frequency and stress incontinence for two weeks, nocturia once nightly had occurred for some years.

Approximately one month after this visit she was considered to have an appreciable limitation of renal function. Her blood urea was 114 mg per 100 ml., total plasma proteins 6.7 g per 100 ml., haemoglobin 9.4 g per 100 ml. and serum creatinine of 5.6 mg per 100 ml. An x-ray showed a renal mass; queried as a left polycystic kidney and a right large kidney.

In August 1968 she was referred to the Renal Unit. On this occasion it was found that she had nocturia twice nightly for the previous two years and she felt tired. An examination of the abdomen showed definite polycystic kidneys - the left being larger than the right. The bladder showed no detectable abnormality. Blood urea was 140 mg per 100 ml., haemoglobin 6.3 g per 100 ml. She lived with her husband and daughter. The daughter had a urinary tract infection, otherwise there was no known kidney disease in the family.

She was next seen in February 1969. She was then feeling a little better although she looked pale. Her haemoglobin at that time was 6.3 g per 100 ml. and serum creatinine 5.6 - 6.0 mg per 100 ml.
In July 1969 her renal disease seemed to be stable and serum values were recorded at haemoglobin 9.2 g per 100 ml., urea 134 mg per 100 ml. and electrolytes within the normal range. Serum creatinine was 6.5 mg per 100 ml., this had slowly increased over the last year. She was taking iron supplements. Nocturia was once nightly and she was experiencing cramp in the legs at night. There was no oedema. The polycystic kidneys were as before, the left being larger than the right kidney. In January 1970 the serum creatinine was 7.5 mg per 100 ml., haemoglobin 9.8 g per 100 ml. and blood urea 146 mg per 100 ml. She was still taking iron supplements and feeling better.

But when next seen in September 1970, she was feeling tired and her haemoglobin was lowered to 7.5 g per 100 ml., serum creatinine was 9.1 mg per 100 ml. and blood urea 140 mg per 100 ml.

She was still feeling tired in November 1970. She had nocturia once nightly and occasional pruritus. She was also feeling thirsty and had a bad taste in the mouth coupled with a poor appetite for about four weeks with some nausea and vomiting. The haemoglobin was still 6.9 g per 100 ml., blood urea 295 mg per 100 ml., chloride 111 mEq. per litre, bicarbonate 10 mEq. per litre, sodium 138 mEq. per litre, potassium 4.5 mEq. per litre, total serum proteins 6.9 g per 100 ml., calcium 7.0 mg per 100 ml., phosphate 6.6 mg per 100 ml. but creatinine had risen to 14.0 mg per 100 ml. A uraemic odour was detectable. In view of the deterioration in renal function, protein restriction was considered necessary and M.H. was therefore admitted in November for stabilization. She was started on a Giovannetti diet with no fluid or sodium restriction, and quickly felt much better. Her blood urea gradually became reduced to 190 mg per 100 ml. at the time of her discharge.

M.H. was next seen in January 1971. Serum values were as follows:

134.
urea 104 mg per 100 ml., chloride 108 mEq. per litre, bicarbonate 14 mEq. per litre, sodium 139 mEq. per litre, potassium 4.7 mEq. per litre, total proteins 6.8 g per 100 ml., haemoglobin 6.8 g per 100 ml., creatinine 15.0 mg per 100 ml. She was feeling much better, not so tired, and she said that she found the diet not too difficult.

In April 1971 she was well but for some skin irritation. Her urea was 126 mg per 100 ml.

In July she was feeling so much better that she went to Holland on a Giovannetti diet, taking low protein bread etcetera with her. She thoroughly enjoyed her holiday. At around this time she had slight itching of the skin, and leg cramps, she was continuing in her part-time job as a school meals assistant.

At her next visit in October 1971, she had been feeling well since taking the Giovannetti diet. She had a fair appetite and her weight was steady. She agreed at this visit to come in for balance studies and was admitted early in November.
Details of the study on M.H.

This patient weighed 46.3 kg at the beginning of the study and 47.85 kg at the end. Urinary protein was 1.0 g per 24 hours. Details of this study are shown in Table 1 M.H. to Table 8 M.H. Table 1 M.H. shows the protein and energy content of the diet. The total energy content of the diet was 50 Calories per kg body weight over 24 hours and fat and carbohydrate supplied 41.5 per cent and 57.0 per cent respectively. The actual energy intake in the six balance periods was as follows:

- Balance period 1 and 2 (8 days) 45 Calories per kg body weight
- Balance period 3 (4 days) 42 Calories per kg body weight
- Balance period 4 (4 days) 50 Calories per kg body weight
- Balance period 5 and 6 (8 days) 49 Calories per kg body weight

The average energy intake over the entire period of 24 days was 47 Calories per kg body weight.

A large proportion of the fat was supplied as double cream and butter with small amounts as egg yolk, milk and synthetic cream.

The polysaccharides were contributed by potatoes, wheat-starch (bread, scones, blancmange, pancakes) and Caloreen, a glucose polymer containing five glucose units. The mono and disaccharides were supplied in marmalade, peaches and a small amount in lemon drink.

The high biological value protein was contributed by egg, double cream, butter and milk. The second class protein was contributed by marmalade, potatoes, wheat starch, carrots, peaches, lettuce, cucumber and lemon juice.

In Table 3 M.H. are shown the mineral content and acid-base of the diet. The calcium, magnesium, iron and copper content are all low. This is a constant feature of all the diets in these studies. There
is a slight excess of base.

The essential amino acid content of the diet can be seen in Table 4 M.H. With the exception of methionine, all the amino acids were supplied in amounts that were above the absolute minimal theoretical requirement for a person of M.H.'s body weight. The amounts of threonine and isoleucine exceeded the safe minimal.

Table 5 M.H. shows serum values at the commencement of each balance period. Blood urea is reduced from 134 mg per 100 ml. to 125 mg per 100 ml. during the first two balance periods. It then increased to 132 during the third balance period, thereafter slowly decreasing to 103 mg per 100 ml., an overall decline of 34 per cent. Serum sodium and chloride remained stable throughout the balance. Potassium also showed a steady value within the normal range, as also does magnesium. The haemoglobin is low, but remains steady. Albumin stays within the normal range. Serum calcium is low ranging from 5.5 to 7.0 mg per 100 ml., during the latter half. Phosphate is high ranging from 13.5 - 16.6 mg per 100 ml. during the first three balance periods, but these values are reduced during the second half of the balance, the final value being 8.2 mg per 100 ml. This is explained by the response to the administration of aluminium hydroxide at the beginning of the third balance period. Serum creatinine remains fairly stable throughout the period of the balance, commencing at 12.6 mg per 100 ml. at the commencement, rising to 14.0 mg per 100 ml. at the beginning of the third balance period and returning to 12.3 mg per 100 ml. at the end. Body weight remained steady.
Results and discussion on the balance data on M.H.

A modified Reifenstein plot of the balance data from patient M.H. is shown in Figure 3 and the data is tabulated in Table 6 M.H. During the course of the balance the net average nitrogen intake was 2.62 g per 24 hours. The net average faecal nitrogen was 0.7 g per 24 hours and this was equal to 27 per cent of the net intake. The net average nitrogen balance was -0.341 g per 24 hours. During the period that the patient was being studied her blood urea fell from 135 mg to 102 mg per 100 ml.

Using the relationships suggested by Miller and Payne (1961) and the data obtained in the study, it can be shown that the utilization of nitrogen in this patient exceeded 100 per cent of the intake (see Table 7 M.H.). The utilization in excess of that which would be predicted for normal individuals is assumed to be due to the reutilization of urea.

The general impression from the data produced on this balance study is that there is a very sensitive relationship between energy intake and nitrogen utilization and the influence of an optimal energy intake on reutilization of urea nitrogen is apparent during the last three balance periods. The implication from these studies is that in uraemic individuals there is an obligatory anabolic pathway in which the value of protein/Calorie per cent appears to arbitrate the synthetic activity.

The optimal energy intake is dependent on the intake capacity of the patient and this is often a major limiting factor in synthetic activity. Unfortunately this patient took a prolonged period and much indoctrination before she grasped the importance of eating the total diet, which she did during the second half, with, from the data presented, an apparently improved result.
Nitrogen balance in this type of patient served a very good educational purpose, and since her hospital admission her understanding of the intrinsic importance of dietary treatment and therefore following the therapeutic regime has greatly improved. It is interesting that this patient took a high proportion of the total carbohydrate as polysaccharide.
TABLE 1 M.H.

Protein and energy content of diet given to M.H.

female aged 48 years

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen (g per 24 hours)</td>
<td>2.6</td>
<td>(by calculation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.056 gN/kg body weight/24 hours)</td>
</tr>
<tr>
<td>Net average nitrogen (g per 24 hours)</td>
<td>2.62</td>
<td>(by analysis)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.0564 gN/kg body weight/24 hours)</td>
</tr>
<tr>
<td>Total energy (Cals. per 24 hours)</td>
<td>2,300</td>
<td>(9660 kJ)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(50 Cals/kg body weight/24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(210 kJ)</td>
</tr>
<tr>
<td>Average energy (Cals. per 24 hours)</td>
<td>2,180</td>
<td>(9156 kJ)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(47 Cals./kg body weight/24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(197 kJ)</td>
</tr>
<tr>
<td>High biological value protein as % total protein</td>
<td>74.8</td>
<td></td>
</tr>
<tr>
<td>Fat as % total energy</td>
<td>41.5</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (CHO) as % total energy</td>
<td>57.0</td>
<td></td>
</tr>
<tr>
<td>Polysaccharides as % total CHO energy</td>
<td>80.0</td>
<td></td>
</tr>
<tr>
<td>Mono and disaccharides as % total CHO energy</td>
<td>20.0</td>
<td></td>
</tr>
</tbody>
</table>
## Summary of diet eaten by M.H.

(Amounts in g per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aproten crispbread</td>
<td>60</td>
</tr>
<tr>
<td>Butter</td>
<td>40</td>
</tr>
<tr>
<td>Homogenised milk</td>
<td>100</td>
</tr>
<tr>
<td>Lemon drink</td>
<td>631</td>
</tr>
<tr>
<td>Custard or blancmange</td>
<td>125</td>
</tr>
<tr>
<td>Scones</td>
<td>106</td>
</tr>
<tr>
<td>Pancake</td>
<td>80</td>
</tr>
<tr>
<td>Cucumber</td>
<td>10</td>
</tr>
<tr>
<td>Lettuce</td>
<td>10</td>
</tr>
<tr>
<td>Peaches</td>
<td>60</td>
</tr>
<tr>
<td>Carrots</td>
<td>60</td>
</tr>
<tr>
<td>Potatoes</td>
<td>100</td>
</tr>
<tr>
<td>Marmalade</td>
<td>20</td>
</tr>
<tr>
<td>Tea (distilled water)</td>
<td>750 ml</td>
</tr>
</tbody>
</table>

141.
**TABLE 3 M.H.**

**Mineral content of diet eaten by M.H.**

(Amounts in mg per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>235</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>318</td>
</tr>
<tr>
<td>Sodium</td>
<td>377 (added sodium chloride not restricted)</td>
</tr>
<tr>
<td>Potassium</td>
<td>790</td>
</tr>
<tr>
<td>Magnesium</td>
<td>51</td>
</tr>
<tr>
<td>Iron</td>
<td>4</td>
</tr>
<tr>
<td>Copper</td>
<td>0.54</td>
</tr>
<tr>
<td>Sulphur</td>
<td>169</td>
</tr>
<tr>
<td>Chloride</td>
<td>560</td>
</tr>
</tbody>
</table>

**Acid Base Balance**

Acid = 11.6 mEq.

Base = 14.6 mEq.
TABLE 4

Essential amino acid content of diet eaten by M.H.

(mg amino acids per 24 hours)

<table>
<thead>
<tr>
<th>In diet</th>
<th>Absolute minimal</th>
<th>Safe minimal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>221.7</td>
<td>167</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>797.2</td>
<td>718</td>
</tr>
<tr>
<td>Lysine</td>
<td>1013.7</td>
<td>533</td>
</tr>
<tr>
<td>Methionine</td>
<td>398.2</td>
<td>718</td>
</tr>
<tr>
<td>Threonine</td>
<td>705.2</td>
<td>324</td>
</tr>
<tr>
<td>Valine</td>
<td>1048.0</td>
<td>533</td>
</tr>
<tr>
<td>Leucine</td>
<td>1290.7</td>
<td>718</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>934.8</td>
<td>463</td>
</tr>
<tr>
<td>Cystine</td>
<td>251.9</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>630.9</td>
<td></td>
</tr>
</tbody>
</table>

In diet total sulphur containing amino acids = 650 mg
### TABLE 5 M.H.

Blood chemistry at commencement of each balance period for patient M.H.

(values per litre or per 100 ml serum unless otherwise stated)

<table>
<thead>
<tr>
<th>Balance period</th>
<th>Weight kg</th>
<th>Urea mg per 100 ml</th>
<th>Creatinine mg per 100 ml</th>
<th>Na mEq/l</th>
<th>K mEq/l</th>
<th>Cl mEq/l</th>
<th>CO₂ mEq/l</th>
<th>Ca mg per 100 ml</th>
<th>P₀₄ mg per 100 ml</th>
<th>Alb. g per 100 ml</th>
<th>Hb. g per 100 ml blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>46.3</td>
<td>134</td>
<td>12.6</td>
<td>140</td>
<td>4.4</td>
<td>106</td>
<td>13</td>
<td>6.4</td>
<td>14.5</td>
<td>4.0</td>
<td>6.9</td>
</tr>
<tr>
<td>2.</td>
<td>46.1</td>
<td>135</td>
<td>13.1</td>
<td>139</td>
<td>4.2</td>
<td>107</td>
<td>18</td>
<td>5.5</td>
<td>16.6</td>
<td>3.8</td>
<td>6.4</td>
</tr>
<tr>
<td>3.</td>
<td>46.0</td>
<td>125</td>
<td>14.4</td>
<td>139</td>
<td>4.1</td>
<td>106</td>
<td>18</td>
<td>5.8</td>
<td>13.5</td>
<td>4.1</td>
<td>7.1</td>
</tr>
<tr>
<td>4.</td>
<td>46.2</td>
<td>132</td>
<td>13.4</td>
<td>139</td>
<td>3.8</td>
<td>104</td>
<td>20</td>
<td>6.4</td>
<td>10.7</td>
<td>3.9</td>
<td>7.1</td>
</tr>
<tr>
<td>5.</td>
<td>46.65</td>
<td>114</td>
<td>12.1</td>
<td>139</td>
<td>4.0</td>
<td>102</td>
<td>21</td>
<td>7.0</td>
<td>8.1</td>
<td>3.8</td>
<td>6.5</td>
</tr>
<tr>
<td>6.</td>
<td>47.4</td>
<td>112</td>
<td>12.3</td>
<td>139</td>
<td>3.9</td>
<td>101</td>
<td>22</td>
<td>7.8</td>
<td>8.2</td>
<td>3.7</td>
<td>7.0</td>
</tr>
<tr>
<td>end</td>
<td>47.85</td>
<td>103</td>
<td>13.0</td>
<td>139</td>
<td>4.4</td>
<td>101</td>
<td>23</td>
<td>7.8</td>
<td>8.2</td>
<td>3.6</td>
<td>6.5</td>
</tr>
</tbody>
</table>
### TABLE 6 M.H.

Nitrogen Balance Data

(Amounts are averages in g/24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net nitrogen intake</td>
<td>2.62</td>
<td>g N/kg body weight</td>
</tr>
<tr>
<td>Average faecal nitrogen</td>
<td>0.70</td>
<td>g N/kg body weight</td>
</tr>
<tr>
<td>Faecal nitrogen as % of nitrogen intake</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Net nitrogen balance</td>
<td>0.341</td>
<td>-0.0073 g N/kg body weight</td>
</tr>
</tbody>
</table>
TABLE 7 M.H.

Prediction of theoretical net protein utilization on patient M.H.

Protein score of diet

650 mg = total S containing amino acids

\[ 2.6 \text{ gN} = \text{total dietary nitrogen} \]

Therefore 250 mg S containing amino acids per g total nitrogen

\[ \frac{250 \times 100}{270} = 92.5\% \text{ score} \]

\[ \Theta = (N.P.U.) = S \left( 1 - k \frac{\text{Cals}}{\text{kg}} \right) \]

\[ (k = 0.019) \quad \text{(Miller and Payne)} \]

Net Cals in = 46.6 Cals/kg

Total Cals = 2,190

Weight 47 kg

Average nitrogen in = 2.62 g N/24 hours (urinary leak = 1.0 g protein/24 hours)

\[ 1 \text{ g protein} = 0.16 \text{ g N}. \]

Therefore net available for anabolism = 2.46 g N (15.37 g protein = 61 Calories)

61 as \% of 2,190 = 2.78\%

\[ \Theta = 92.59 \left( 1 - 0.019 \times 2.78 \right) \]

\[ = 1.0 - 0.0528 = 0.9471 \]

\[ 92.59 \times 0.9471 = 87.699 \]

N.P.U. = 88\% theoretical

From nitrogen balance data

\[ \Delta \text{Bal/kg}^{0.73} = I/\text{kg}^{0.73} \times \Theta - N \quad \text{(200 mg N obligatory loss)} \]

\[ 20.46 \text{ mg N/kg}^{0.73} = 146.2 \text{ mg N/kg}^{0.73} \times \Theta - 200 \]

\[ 179.4 = 146.2 \times \Theta \]

\[ \Theta - 1.228 \]

Therefore more than 100\% utilization
TABLE 8 M.H.

Drugs taken by M.H. during the period of the balance study

Ascorbic Acid 50 mg t.d.s.
Aneurine Co. forte 2 tablets t.d.s.
Chromium sesquioxide 1.5 g daily
Aluminium hydroxide 10 tablets (Evans) 6 g daily)commencing at
the beginning of the third balance period.
Fig. 3. M. H. Nitrogen balance study on M. H. ♂ age 48
Modified Reifenstein plot

blood urea mg per 100 ml

-45 Cals/kg/day
(189 kJ)

-42 Cals/kg
(176 kJ)

-50 Cals/kg
(210 kJ)

-49 Cals/kg
(206 kJ)
The diagnosis was stage 2 chronic renal failure with bilateral polycystic kidneys. The glomerular filtration rate being 6.0 ml. per minute at the time of the study.

Polycystic kidney disease was first confirmed when J.L. was seen in 1958 on account of haematuria.

Since that time he has had attacks of haematuria once or twice yearly, also episodes of intermittent abdominal pain since that date.

In 1970 he was referred to the Renal Unit. In October 1970 his blood urea was 80 mg per 100 ml., serum creatinine 6.0 mg per 100 ml., haemoglobin 16.1 g per 100 ml., calcium 9.2 mg per 100 ml., phosphate 3.3 mg per 100 ml.

J.L. lived with his father and stepmother. His own mother had died aged 52 of renal failure. He was the only child. He worked as a joiner and appeared to be quite fit except for the haematuria. He was a pleasant person although rather quiet and withdrawn.

In August 1972 he was admitted to the Renal Unit for assessment of his renal function.

At this time he was feeling very well, his appetite was good and his weight steady. He had no nausea or vomiting, no paraesthesia or numbness, no nocturia and no evidence of oedema. He was troubled with intermittent abdominal pain and heavy haematuria. He was also hypertensive and this was controlled with Methyl Dopa. His blood urea was 240 mg per 100 ml., serum chloride 111 mEq. per litre, bicarbonate 18 mEq. per litre, sodium 146 mEq. per litre, potassium 4.0 mEq. per litre, serum creatinine 12.7 mg per 100 ml., and haemoglobin 8.8 g per 100 ml. In mid-August he was initiated onto a Giovannetti, 80 mEq. sodium diet, with iron supplements. Two weeks later his urea had
decreased to 167 mg per 100 ml, his albumin was 4.2 g per 100 ml, and serum creatinine 14.2 mg per 100 ml. and he was discharged on a Giovannetti 80 mEq. sodium diet. During the following month he was seen in the Out-Patients' Clinic at regular intervals. His response to the discipline of a Giovannetti diet was very good.

By early September 1972 his urea had dropped to 135 mg per 100 ml., his serum creatinine was 13.1 mg per 100 ml., albumin was 4.0 g per 100 ml., calcium 9.7 mg per 100 ml., phosphate 6.8 mg per 100 ml. He had returned to work and was managing the diet extremely well.

Early in October his urea had decreased to 92 mg per 100 ml., chloride was 105 mEq. per litre, bicarbonate 20 mEq. per litre, sodium 140 mEq. per litre, potassium 6.1 mEq. per litre, calcium 9.6 mg per 100 ml., phosphate 7.6 mg per 100 ml. and creatinine 12.1 mg per 100 ml., and by mid-October the urea was 88 mg per 100 ml.

At this time he was admitted for balance studies. His only complaint was of some pruritus and haematuria.

His tolerance and co-operation during the metabolic balance routine were excellent.

After the balance study he continued with a Giovannetti diet at home. He managed very well, having a good understanding of the principles of the diet.

In early December his urea was controlled at 86 mg per 100 ml., when his serum creatinine was around 13 mg per 100 ml., and the following February his urea was 98 mg per 100 ml.
Details of the study on J.L.

The total balance of 24 days was divided into six balance periods each of 4 days duration. Body weight at the commencement of the study was 79.0 kg and 80.6 kg at the end.

Urinary protein was 2.7 g per 24 hours.

Details of this study can be seen in Table 1 J.L. to Table 8 J.L. The protein and energy content of the diet are shown in Table 1 J.L. The total energy content of the diet was 3,820 Calories (48 Calories per kg body weight). The average energy intake was also 48 Calories per kg, this being maintained throughout the period of the balance.

Of the 37 per cent of the total energy provided by fat, a large portion was supplied by butter and double cream, with small contributions from egg yolk and milk.

The carbohydrate which contributed 60 per cent of the total energy was composed of 74 per cent polysaccharides and 26 per cent mono and disaccharides.

The polysaccharides comprised low protein bread and crispbread, low protein flour as pancake, custard and blancmange and biscuits, sago, Caloreen as lemon drink, meringue, or custard and sago pudding.

The mono or disaccharides were present as glucose in lemon drink and custard and sago pudding, icing sugar in biscuits, grapefruit, jam, marmalade, pears and a very small quantity of lactose in milk.

The high biological value protein consisted of egg, double cream, butter and milk.

The small proportion of unessential nitrogen was contained in grapefruit, pears, low protein flour, jam, marmalade, potatoes, runner beans, sago, lettuce, cucumber, tomato, lemon juice.

Table 3 J.L. shows the mineral content and acid base of the diet. As in all the other studies the calcium, magnesium, copper and iron
are all low, and there is a slight excess of base in the diet.

Table 4 J.L. shows the essential amino acid content of the diet. All the essential amino acids were supplied in amounts above the absolute minimal theoretically required for the body weight of J.L., with the exception of the sulphur containing amino acids. Threonine was also supplied above the safe minimal theoretical requirement.

Table 5 J.L. shows the serum values at the commencement of each balance period. Blood urea remained at a fairly steady level, there was an overall 4 per cent reduction of blood urea. Serum sodium remained stable and within normal limits. Serum chloride was also steady. Serum bicarbonate commenced at 20 mEq. per litre and gradually became reduced to 16 mEq. per litre at the end of the study. This low serum bicarbonate is a common pattern in these patients with renal failure and with only a small excess of base in the diet.

Serum potassium remained steady and within normal limits. The haemoglobin showed a steady but low value. The albumin remained at the lower limit of normal throughout. The serum calcium was also at the lower level of normal. Phosphate was consistently higher than the normal range. The administration of aluminium hydroxide at the beginning of the third period does not appear to have influenced the serum phosphate. The type of aluminium hydroxide given to this patient was a different product (Wyeth) to that used in the other studies (Evans). When the faeces of J.L. were analysed it was noted that these tablets remained intact and unmixed in the faeces.

The body weight remained fairly steady, there was a weight gain of approximately 1 kg over the duration of the balance. Serum creatinine remained fairly stable throughout the balance, indicating
that renal function was reasonably constant throughout.

**Results and discussion on the balance data on J.L.**

The balance data on J.L. is shown in the modified Reifenstein plot Figure 3 J.L., and is shown in tabulated form in Table 6 J.L.

Net average nitrogen intake throughout the balance on J.L. was 4.272 g nitrogen per 24 hours (0.0537 g N per kg body weight).

The net average faecal nitrogen was 1.088 g nitrogen per 24 hours (0.0369 g N per kg) and this represented 25.5 per cent of the net intake.

The net nitrogen balance was -0.58 g N per 24 hours (-0.0073 g N per kg body weight per 24 hours).

During the period of the balance study the blood urea remained fairly steady commencing at 90 mg per 100 ml. This level was reduced to 80 mg per 100 ml. at the beginning of the fourth balance period and ending at 88 mg per 100 ml. in the final balance period. Energy intake averaged 48 Calories per kg body weight.

The results of this study would appear to indicate that the subject had achieved a steady state of equilibrium. Urea nitrogen was reduced to 80 mg per 100 ml. at the end of the third balance period, after which it increased by a very small amount during the fourth period and remained fairly steady at 85 mg per 100 ml. with a small increase during the final balance period following an intake of slightly less high biological value protein.

It appears that a reduction of blood urea nitrogen below 80 mg per 100 ml. is unlikely in these end stage azotaemic patients. In only one subject in the series (I.H.) did the blood urea become reduced to as low as 70 mg per 100 ml.

The impression from this data is that an increased anabolism may have resulted from a slightly higher protein intake and that a small reduction in high biological value protein which formed a large
proportion of the total protein intake would increase the negativity of the balance.

Giordano in 1962 has suggested from nitrogen balance studies, that a decreased blood urea nitrogen denotes utilization for anabolic purposes, when a minimal essential nitrogen and high energy intake were given. He showed that a more positive balance was enhanced by supplements of non-essential nitrogen and he found that the nitrogen balance tended to become more negative when the blood urea nitrogen fell below 80 mg per 100 ml. Giordano suggests that if the endogenous urea pool is depleted below 80 mg per 100 ml, then it is not large enough to promote the synthesis of inessential amino acids unless, as he showed, this is supplemented with ammonium citrate or urea, when the balance was improved and more nitrogen retained.

The data on J.L. may appear to support this view. The endogenous urea pool is not large enough to promote an anabolic phase, and the state of nutrition would not be likely to accelerate such a process, as J.L. was not protein depleted, serum albumin averaged 3.6 g per 100 ml.

The general impression of this study is that a steady state of nitrogen equilibrium was achieved.
TABLE 1  J.L.

Protein and energy content of diet given to J.L.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total nitrogen</strong></td>
<td>4.09 (g per 24 hours)</td>
<td>0.0517 gN/kg body weight</td>
</tr>
<tr>
<td></td>
<td>(by calculation)</td>
<td></td>
</tr>
<tr>
<td><strong>Net average nitrogen</strong></td>
<td>4.272 (g per 24 hours)</td>
<td>0.0537 gN/kg body weight</td>
</tr>
<tr>
<td></td>
<td>(by analysis)</td>
<td></td>
</tr>
<tr>
<td><strong>Total energy</strong></td>
<td>3,820 (Cals. per 24 hours)</td>
<td>48 Cals./kg body weight</td>
</tr>
<tr>
<td></td>
<td>(16044 kJ)</td>
<td>(202 kJ)</td>
</tr>
<tr>
<td><strong>Average energy</strong></td>
<td>3,807 (Cals. per 24 hours)</td>
<td>48 Cals./kg body weight</td>
</tr>
<tr>
<td></td>
<td>(15989 kJ)</td>
<td>(202 kJ)</td>
</tr>
<tr>
<td><strong>High biological value protein as % of total protein</strong></td>
<td>83</td>
<td></td>
</tr>
<tr>
<td><strong>Fat as % of total energy</strong></td>
<td>37</td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrate as % of total energy</strong></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td><strong>Polysaccharides as % of total CHO energy</strong></td>
<td>74</td>
<td></td>
</tr>
<tr>
<td><strong>Mono and disaccharides as % of total CHO energy</strong></td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Food Item</td>
<td>Amount (g)</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Aproten Crispbread</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Low protein bread</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Homogenised milk</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Lemon drink</td>
<td>975</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Runner beans</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Pears (unsweetened)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Egg (hard boiled)</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Pancake</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>Meringue</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Sago pudding</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Custard or blancmange</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>Biscuits</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Marmalade</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Jam</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Grapefruit (sweetened)</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Strained tea (distilled water)</td>
<td>960 ml</td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 3 J.L.**

Mineral content of diet taken by J.L.

(Amounts in mg per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>361</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>511</td>
</tr>
<tr>
<td>Sodium</td>
<td>592 (25.7 mEq NaCl added to increase intake to a total 110.7 mEq Na per 24 hours)</td>
</tr>
<tr>
<td>Potassium</td>
<td>1,528</td>
</tr>
<tr>
<td>Magnesium</td>
<td>108</td>
</tr>
<tr>
<td>Iron</td>
<td>11.57</td>
</tr>
<tr>
<td>Copper</td>
<td>1.26</td>
</tr>
<tr>
<td>Sulphur</td>
<td>320.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>896.0</td>
</tr>
</tbody>
</table>

**Acid Base Balance**

Acid = 19.55 mEq.

Base = 26.26 mEq.
**TABLE 4 J.L.**

**Essential amino acid content of diet taken by J.L.**

(mg amino acids per 24 hours)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>In diet</th>
<th>Absolute minimal</th>
<th>Safe minimal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>383</td>
<td>284</td>
<td>569</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1399</td>
<td>1225</td>
<td>2449 (total aromatic)</td>
</tr>
<tr>
<td>Lysine</td>
<td>1695</td>
<td>909</td>
<td>1817</td>
</tr>
<tr>
<td>Methionine</td>
<td>737</td>
<td>1225</td>
<td>2449 (total S' containing)</td>
</tr>
<tr>
<td>Threonine</td>
<td>1182</td>
<td>553</td>
<td>1106</td>
</tr>
<tr>
<td>Valine</td>
<td>1810</td>
<td>904</td>
<td>1817</td>
</tr>
<tr>
<td>Leucine</td>
<td>2203</td>
<td>1225</td>
<td>2449</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1603</td>
<td>790</td>
<td>1580</td>
</tr>
<tr>
<td>Cystine</td>
<td>448</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1091</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total 'S' containing amino acids in diet = 1185.5 mg
### TABLE 5 J.L.

Blood chemistry at the commencement of each balance period for patient J.L.

(values per litre or per 100 ml serum unless otherwise stated)

<table>
<thead>
<tr>
<th>Balance period</th>
<th>Weight kg</th>
<th>Urea mg per 100 ml</th>
<th>Creatinine mg per 100 ml</th>
<th>Na mEq/l.</th>
<th>K mEq/l.</th>
<th>Cl mEq/l.</th>
<th>CO2 mEq/l.</th>
<th>Ca mg per 100 ml</th>
<th>PO4 mg per 100 ml</th>
<th>Alb g per 100 ml</th>
<th>Hb g per 100 ml blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>79.0</td>
<td>92</td>
<td>11.7</td>
<td>140</td>
<td>4.9</td>
<td>109</td>
<td>20</td>
<td>9.4</td>
<td>6.4</td>
<td>3.4</td>
<td>9.4</td>
</tr>
<tr>
<td>2.</td>
<td>78.45</td>
<td>92</td>
<td>12.4</td>
<td>142</td>
<td>4.9</td>
<td>112</td>
<td>18</td>
<td>9.9</td>
<td>6.0</td>
<td>3.9</td>
<td>9.4</td>
</tr>
<tr>
<td>3.</td>
<td>79.9</td>
<td>92</td>
<td>142</td>
<td>142</td>
<td>4.9</td>
<td>112</td>
<td>18</td>
<td>9.0</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>79.25</td>
<td>80</td>
<td>12.6</td>
<td>140</td>
<td>4.3</td>
<td>114</td>
<td>18</td>
<td>9.5</td>
<td>5.85</td>
<td>3.55</td>
<td>9.5</td>
</tr>
<tr>
<td>5.</td>
<td>79.6</td>
<td>84</td>
<td>12.7</td>
<td>139</td>
<td>4.7</td>
<td>112</td>
<td>17</td>
<td>9.0</td>
<td>6.2</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>81.5</td>
<td>83</td>
<td>13.7</td>
<td>140</td>
<td>4.6</td>
<td>119</td>
<td>17</td>
<td>9.0</td>
<td>6.0</td>
<td>3.4</td>
<td>9.9</td>
</tr>
<tr>
<td>end</td>
<td>80.6</td>
<td>88</td>
<td>13.0</td>
<td>139</td>
<td>4.6</td>
<td>114</td>
<td>16</td>
<td>9.4</td>
<td>5.9</td>
<td>3.4</td>
<td>9.8</td>
</tr>
</tbody>
</table>
**TABLE 6 J.L.**

**Nitrogen Balance Data**

(Amounts are averages in g/24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net nitrogen intake</td>
<td>4.272</td>
<td>g/24 hours</td>
</tr>
<tr>
<td>Average faecal nitrogen</td>
<td>1.088</td>
<td>g/24 hours</td>
</tr>
<tr>
<td>Faecal nitrogen as % of nitrogen intake</td>
<td>25.5</td>
<td></td>
</tr>
<tr>
<td>Net nitrogen balance</td>
<td>-0.58</td>
<td>g/24 hours</td>
</tr>
</tbody>
</table>

(0.0538 gN/kg body weight)

(0.0013 gN/kg body weight)

(-0.0073 gN/kg body weight)
TABLE 7 J.L.

Prediction of theoretical net protein utilization on patient J.L.

Protein score of diet

Total S containing amino acids = 1186

Total dietary nitrogen = 4.09 g

Therefore 289 mg S containing amino acids per g total nitrogen

\[
\frac{289 \times 100}{270} = 107 = \text{score}
\]

\[
\phi = S(1 - k \text{F Cals}%) \quad (k = 0.019)
\]

Net Cals in = 48 Cals/kg. Total Calories = 3,800 Weight = 79 kg

Average nitrogen = 4.27 gN/24 hours (urinary leak = 2.7 g protein/24 hours = 0.432 gN/24 hours)

Therefore net available for anabolism = 3.838 gN. (23.99 g protein = 96 Calories)

96 as % of 3,800 = 2.53%

\[
\phi = 107 \times (1 - 0.019 \times 2.53)
\]

\[
= 1.0 - 0.0480; = 0.9519
\]

\[
107 \times 0.9519 = 101.86 = \text{theoretical N.P.U.}
\]

From nitrogen balance data

\[
\Delta \text{Bal. kg}^{0.73} = \frac{I}{\text{kg}^{0.73}} \times \phi - M
\]

\[
- 23.88 \text{ mg N/kg}^{0.73} = 158.1 \text{ mg N/kg}^{0.73} \times \phi - 200
\]

\[
176.12 = 158.1 \times \phi
\]

\[
\phi = 1.114
\]
TABLE 8 J.L.

Drugs taken by J.L. during the period of the balance study

Ascorbic Acid 50 mg x 2 daily.
Aneurine Co. forte 2 tablets daily.
Chromium sesquioxide 1.5 g daily.
Aluminium hydroxide 16 tablets (Wyeth) 4 g daily, commencing at the beginning of the third balance period to the end of the sixth balance period.
Fig. 3. J. L. Nitrogen balance study on J. L. (age 36)
Modified Reifenstein plot

blood urea mg per 100 ml

-1.0
-0.5
0
+0.5
+1.0
+1.5
+2.0
+2.5
+3.0
+3.5
+4.0

48 Cals/kg/day
(202 kJ)

4 8 12 16 20 24
days

days →

+3.0
+2.0
+1.0
0
-1.0
-2.0
-3.0
-4.0

g. N. per day
The diagnosis of this patient was that of stage 3 chronic renal failure with polycystic kidneys. The glomerular filtration rate being 4.3 ml. per minute. Polycystic kidneys were first diagnosed in 1966 when the patient was experiencing recurrent right loin pain and haematuria. Prior to this in 1953 a urinary infection was treated but not investigated further.

During her pregnancy in 1956 I.H. had fairly frequent urinary infections and also toxaemia.

In 1957 a further episode of loin pain resulted in an intravenous pyelogram being made, the result of which showed no evidence of abnormality or deterioration in renal function.

Between 1966 and 1971 episodes of haematuria, renal colic and urinary tract infections recurred and were treated with urinary antiseptics. In 1966 serum electrolytes were within normal limits, blood urea was 40 mg per 100 ml. The patient was seen at regular intervals. In 1968, blood urea was 60 mg per 100 ml., haemoglobin 11.2 g per 100 ml.

In 1971, I.H. was referred to the Renal Unit. At that time she had been taking a 30 g protein diet for the previous eight weeks. Blood urea had fluctuated between 110 to 125 mg per 100 ml., serum bicarbonate between 17.5 and 22 mEq. per litre, haemoglobin 6.0 g per 100 ml., serum creatinine 7.7 mg per 100 ml. and creatinine clearance averaged 7 ml. per minute.

It was observed that she looked rather pale and sallow and was anaemic. She tired very easily and had felt unwell for the past five years.
At this stage there were frequent severe haematuric episodes lasting for several weeks. Both cystic kidneys were easily palpable.

In mid-1971 on a 30 g protein diet her blood urea was 170 mg per 100 ml., chloride 105 mEq. per litre, bicarbonate 17 mEq. per litre, sodium 171 mEq. per litre, potassium 6.1 mEq. per litre, total protein 7.0 g per 100 ml. and serum creatinine 10 mg per 100 ml. She was therefore instructed to take a Giordano Giovannetti diet. Later in the same month her only symptom was tiredness and she tolerated the diet fairly well. In the following month, July, she agreed to have a nitrogen balance study.

The family history of I.H. shows that her grandfather and mother both had polycystic kidneys.
Details of the study on I.H.

During the duration of the study which lasted for 24 days her co-operation and acceptance of the diet were excellent. The usual routine was followed and a preliminary equilibration period of four days was observed, the total balance being divided into six balance periods of four days.

Weight at the commencement of the study was 49.6 kg and 50.6 kg at the end. Urinary protein was 0.5 g protein per 24 hours. Complete details of this balance can be seen in Tables 1 I.H. to 8 I.H.

Table 1 I.H. shows that the total energy was 51 Calories per kg and was supplied as 45 per cent fat and 52 per cent carbohydrate with a theoretical 3 per cent calculated from protein.

Of the energy derived from fat, double cream, butter and a small quantity of milk and egg yolk supplied this. The carbohydrate was composed of 41 per cent polysaccharides and 59 per cent mono and disaccharides. Polysaccharides were given as potatoes, sago, Aproten crispbread, low protein bread, Caloreen and Hycal and the mono and disaccharides were supplied as sweetened fruit, marmalade and jam, Hycal and a small quantity of lactose in milk.

The high biological value protein consisted of egg, double cream, butter, milk, and the very small proportion of non-essential nitrogen was contained in fruit and vegetables, marmalade, jam, low-protein flour and sago.

Table 3 I.H. shows the mineral content and acid base of the diet. The calcium intake of this diet is seen to be low, a normal intake being around 800 mg calcium daily. The magnesium intake is also low, a normal intake would probably be in the region of 200 mg daily. Iron intake is also low, 12.0 mg of iron being the daily intake specified by the Report on recommended allowances (1969).
copper would be approximately 2.0 mg daily. This would also appear to be low. The acid base of the diet shows a slight excess of base which is very necessary in a situation where the excretion of acid is limited.

In Table 4 I.H. showing the essential amino acid content of the diet, all the essential amino acids are supplied in amounts above the absolute minimal requirement as stipulated by Rose for a weight of 50.2 kg with the exception of the sulphur containing amino acids, which were below the absolute minimal. Threonine also was supplied at a safe minimal level.

The serum values are shown at the commencement of each balance period in Table 5 I.H. There is no significant deviation in the levels of chloride, potassium, magnesium or creatinine. There is a minimal decrease in the serum albumin level from 3.1 g per 100 ml. at the commencement of the study to 2.8 g per 100 ml. at the end. The serum bicarbonate shows a very slight drop at the end of the balance, but from this there is no implication that the regime made the patient more acidic. The blood urea remained fairly stable, the overall picture showing a 20 per cent drop. During the last three balance periods when Aludrox was given, the resulting phosphate levels can be seen to be reduced, this implies diminished absorption from the gut. The serum calcium remains unchanged. A steady serum creatinine reflects a state of stable renal function during the period of the balance.

After the balance study this patient followed a very strict dietary regime (Giovannetti Diet). During the period of the balance, iron supplements were not given, but after the balance supplements of ferrous sulphate were resumed (200 mg t.d.s.) The haemoglobin one month later was increased to 10.5 g per 100 ml., the blood urea was 116 mg per 100 ml., chloride 104 mEq. per litre, plasma bicarbonate
18 mEq. per litre, plasma sodium 137 mEq. per litre, plasma potassium 4.8 mEq. per litre, serum creatinine 12.6 mg per 100 ml. and serum albumin 4.1 g per 100 ml.

The subsequent management and the dietary control on the part of the patient gave extremely good results.

Results and discussion on the balance data on I.H.

The balance data on I.H. is shown in the modified Reifenstein plot Figure 3 I.H., and Table 6 I.H. gives a summary of the balance data obtained in this study.

Net average nitrogen intake throughout the balance on I.H. was 2.73 g nitrogen (0.0544 gN per kg body weight). The net average faecal nitrogen was 0.755 g nitrogen (0.0150 gN per kg) and this represented 27.7 per cent of the net intake.

The net nitrogen balance was -0.35 g nitrogen (-0.0069 gN per kg). During the period of the balance the blood urea remained between 94 mg per 100 ml. at the commencement, decreasing steadily to 72 mg per 100 ml. at the beginning of the fourth balance period and finishing at 82 mg per 100 ml. at the end of the balance.

Energy intake was maintained at 51 Calories per kg throughout. From the results obtained during this nitrogen balance study, this patient is shown to be in a very steady state of nitrogen equilibrium. Stable equilibrium may reflect the fact that her intake was completely unchanged for the total balance of 24 days. A state of renal equilibrium is also emphasized by these results.
### TABLE 1  I.H.
Protein and energy content of diet taken by I.H.  female aged 44 years

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen (g per 24 hours)</td>
<td>2.6</td>
<td>(by calculation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.05 gN/kg body weight)</td>
</tr>
<tr>
<td>Net average nitrogen (g per 24 hours)</td>
<td>2.73</td>
<td>(by analysis)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.0544 gN/kg body weight)</td>
</tr>
<tr>
<td>Total energy (Cals. per 24 hours)</td>
<td>2,580 (1080 kJ)</td>
<td>51 Cals/kg body weight (214 kJ)</td>
</tr>
<tr>
<td>Average energy (Cals. per 24 hours)</td>
<td>2,580 (1080 kJ)</td>
<td>51 Cals/kg body weight (214 kJ)</td>
</tr>
<tr>
<td>High biological value protein as % of total protein</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Fat as % of total energy</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate as % total energy</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Polysaccharides as % of total CHO energy</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Mono and disaccharides as % of total CHO energy</td>
<td>58.7</td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 2. I.H.**

Specimen diet taken by I.H.

(Amounts in g per 24 hour unless otherwise stated)

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetened grapefruit</td>
<td>60</td>
</tr>
<tr>
<td>Marmalade</td>
<td>20</td>
</tr>
<tr>
<td>Potatoes</td>
<td>70</td>
</tr>
<tr>
<td>Fried egg</td>
<td>60</td>
</tr>
<tr>
<td>Runner beans</td>
<td>30</td>
</tr>
<tr>
<td>Sago pudding</td>
<td>230</td>
</tr>
<tr>
<td>Lettuce</td>
<td>10</td>
</tr>
<tr>
<td>Tomato</td>
<td>50</td>
</tr>
<tr>
<td>Raw carrot</td>
<td>10</td>
</tr>
<tr>
<td>Cucumber</td>
<td>10</td>
</tr>
<tr>
<td>Sweetened peaches</td>
<td>60</td>
</tr>
<tr>
<td>Double cream</td>
<td>60</td>
</tr>
<tr>
<td>Aproten crispbread</td>
<td>60</td>
</tr>
<tr>
<td>Jam</td>
<td>20</td>
</tr>
<tr>
<td>Low protein bread</td>
<td>60</td>
</tr>
<tr>
<td>Butter</td>
<td>70</td>
</tr>
<tr>
<td>Caloreen</td>
<td>15</td>
</tr>
<tr>
<td>Homogenised milk</td>
<td>100</td>
</tr>
<tr>
<td>Hycal</td>
<td>170 ml</td>
</tr>
<tr>
<td>Strained tea (distilled water)</td>
<td>750 ml</td>
</tr>
</tbody>
</table>
TABLE 3 I.H.

Mineral content of diet taken by I.H.

(Amounts in mg per 24 hours unless otherwise stated)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>275.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>328.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>387.0</td>
</tr>
<tr>
<td></td>
<td>added sodium chloride was unrestricted</td>
</tr>
<tr>
<td>Potassium</td>
<td>1071.0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>65.0</td>
</tr>
<tr>
<td>Iron</td>
<td>4.74</td>
</tr>
<tr>
<td>Copper</td>
<td>1.07</td>
</tr>
<tr>
<td>Sulphur</td>
<td>176.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>662.0</td>
</tr>
</tbody>
</table>

Acid Base Balance

Acid = 11.5 mEq.
Base = 18.7 mEq.
**TABLE 4**

**Essential amino acid content of diet taken by I.H.**

(mg amino acids per 24 hours)

<table>
<thead>
<tr>
<th>In diet</th>
<th>Absolute minimal</th>
<th>Safe minimal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>226</td>
<td>180.7</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>807</td>
<td>778.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>1044</td>
<td>577.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>414</td>
<td>778.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>709</td>
<td>351.4</td>
</tr>
<tr>
<td>Valine</td>
<td>1061</td>
<td>577.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>1328</td>
<td>778.1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>953.5</td>
<td>502.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>234</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>663</td>
<td></td>
</tr>
</tbody>
</table>

Total 'S' containing amino acids in diet = 648 mg
<table>
<thead>
<tr>
<th>Balance period</th>
<th>Weight (kg)</th>
<th>Urea (mg per 100 ml)</th>
<th>Creatinine (mg per 100 ml)</th>
<th>Na (mEq/l.)</th>
<th>K (mEq/l.)</th>
<th>Cl (mEq/l.)</th>
<th>CO₂ (mEq/l.)</th>
<th>Ca (mg per 100 ml)</th>
<th>P0₄ (mg per 100 ml)</th>
<th>Alb. (g per 100 ml)</th>
<th>Hb. (g per 100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>49.6</td>
<td>94</td>
<td>9.7</td>
<td>139</td>
<td>5.0</td>
<td>107</td>
<td>21</td>
<td>8.5</td>
<td>6.1</td>
<td>3.1</td>
<td>8.9</td>
</tr>
<tr>
<td>2.</td>
<td>49.8</td>
<td>78</td>
<td>8.8</td>
<td>138</td>
<td>4.7</td>
<td>107</td>
<td>19</td>
<td>8.2</td>
<td>5.6</td>
<td>3.2</td>
<td>8.9</td>
</tr>
<tr>
<td>3.</td>
<td>50.1</td>
<td>74</td>
<td>8.7</td>
<td>138</td>
<td>4.7</td>
<td>107</td>
<td>19</td>
<td>8.5</td>
<td>5.7</td>
<td>2.8</td>
<td>9.1</td>
</tr>
<tr>
<td>4.</td>
<td>50.35</td>
<td>72</td>
<td>8.7</td>
<td>138</td>
<td>4.4</td>
<td>105</td>
<td>18</td>
<td>8.8</td>
<td>5.6</td>
<td>3.1</td>
<td>8.9</td>
</tr>
<tr>
<td>5.</td>
<td>50.654</td>
<td>81</td>
<td>9.5</td>
<td>135</td>
<td>4.6</td>
<td>107</td>
<td>18</td>
<td>9.2</td>
<td>4.8</td>
<td>3.0</td>
<td>8.6</td>
</tr>
<tr>
<td>6.</td>
<td>50.6</td>
<td>84</td>
<td>8.7</td>
<td>139</td>
<td>4.5</td>
<td>107</td>
<td>19</td>
<td>9.1</td>
<td>4.7</td>
<td>2.9</td>
<td>8.7</td>
</tr>
<tr>
<td>end</td>
<td>50.6</td>
<td>82</td>
<td>9.0</td>
<td>136</td>
<td>4.0</td>
<td>105</td>
<td>17</td>
<td>9.0</td>
<td>4.4</td>
<td>2.8</td>
<td>8.9</td>
</tr>
</tbody>
</table>

TABLE 5: I.H.

Blood chemistry at the commencement of each balance period for patient I.H.

(values per litre or per 100 ml. serum unless otherwise stated)
<table>
<thead>
<tr>
<th>Nitrogen Balance Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Amounts are averages in g/24 hours unless otherwise stated)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net nitrogen intake</td>
<td>2.73</td>
<td>gN/kg body weight</td>
</tr>
<tr>
<td>Average faecal nitrogen</td>
<td>0.755</td>
<td>gN/kg body weight</td>
</tr>
<tr>
<td>Faecal nitrogen as % of nitrogen intake</td>
<td>27.7</td>
<td></td>
</tr>
<tr>
<td>Net nitrogen balance</td>
<td>-0.35 gN</td>
<td>-0.0069 gN/kg body weight</td>
</tr>
</tbody>
</table>
TABLE 7 I.H.

Prediction of theoretical net protein utilization on patient I.H.

Protein score of diet

648 mg = total S containing amino acids

2.6 gN = total dietary nitrogen

Therefore 249 mg S containing amino acids per g total nitrogen

(270 mg = provisional pattern F.A.O.)

\[ \frac{249 \times 100}{270} = 92.22 \text{ = score} \]

where

\[ \Phi = S \left(1 - k \times \text{Cals} \%\right) \]

\[ k = 0.019 \]  \text{(Miller and Payne)}

Net Cals in = 51.5 Cals/kg. Total Calories = 2,590.

Nitrogen in = 2.73 gN (urinary leak = 0.5 g Protein = 0.08 gN)

Therefore net available for anabolism = 2.65 gN (16.56 g Protein) = 66 Calories

66 as % of 2,590 = 2.54%

\[ \Phi = 92.22 \left(1 - 0.019 \times 2.54\right) \]

\[ 92.22 \left(1 - 0.04826\right) \]

\[ 92.22 \times 0.95174 = 87.769 \]

N.P.U. = 88% theoretically.

From nitrogen balance data

\[ \Delta \text{Beh/kg}^{0.73} = I/\text{kg}^{0.73} \times \Phi - M \text{ (200 mg N)} \]

\[ -20.08 \text{ mg N/kg}^{0.73} = 152 \text{ mg/kg}^{0.73} \times \Phi = 200 \]

\[ 179.92 = 152 \times \Phi \]

\[ \Phi = 1.18 \]
Drugs taken by I.H. during the period of the balance study.

Ascorbic Acid 50 mg t.d.s.
Aneurine Co. forte 2 tablets t.d.s.
(Aludrox) Aluminium hydroxide 6 tablets (Evans) 3 g daily commencing at the beginning of the fourth balance period.
Chromium sesquioxide 1.5 g daily.
Fig. 3. I. H. Nitrogen balance study on I. H. Ψ age 44
Modified Reifenstein plot

- Blood urea mg per 100 ml

- G. N. per day

- 51 Cals/kg/day
  (214 kJ)
<table>
<thead>
<tr>
<th>Subject</th>
<th>B mg N/kg per 24 hrs</th>
<th>FN x 100 IN</th>
<th>Net N mg N/kg per 24 hrs</th>
<th>HBWF as % total protein</th>
<th>N.P.U.(#) from balance data</th>
<th>Average energy intake Cals/kg per 24 hrs</th>
<th>Fat as % of total energy</th>
<th>CHO as % of total energy</th>
<th>Polysaccharides as % of CHO energy</th>
<th>Mono/di saccharides as % of total CHO energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.H.</td>
<td>-6.97</td>
<td>27</td>
<td>54.4</td>
<td>78</td>
<td>1.18</td>
<td>51 (214 kJ)</td>
<td>45</td>
<td>52</td>
<td>41</td>
<td>58.7</td>
</tr>
<tr>
<td>J.L.</td>
<td>-7.303</td>
<td>26</td>
<td>53.789</td>
<td>83</td>
<td>1.114</td>
<td>48 (202 kJ)</td>
<td>37</td>
<td>60</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>M.H.</td>
<td>-7.34</td>
<td>27</td>
<td>56.0</td>
<td>75</td>
<td>1.228</td>
<td>47 (197 kJ)</td>
<td>41.5</td>
<td>57</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>R.M.II</td>
<td>-8.7</td>
<td>30</td>
<td>43.735</td>
<td>75</td>
<td>1.549</td>
<td>53 (223 kJ)</td>
<td>41</td>
<td>57</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>J.D.</td>
<td>-14.32</td>
<td>43.5</td>
<td>54.4</td>
<td>76</td>
<td>0.998</td>
<td>50 (210 kJ)</td>
<td>31</td>
<td>66</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>T.G.</td>
<td>-16.763</td>
<td>43</td>
<td>45.77</td>
<td>78</td>
<td>1.225</td>
<td>40 (168 kJ)</td>
<td>46</td>
<td>57</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>M.S.</td>
<td>-21.04</td>
<td>26</td>
<td>55.7</td>
<td>83</td>
<td>1.088</td>
<td>46 (193 kJ)</td>
<td>36</td>
<td>61</td>
<td>78</td>
<td>21</td>
</tr>
<tr>
<td>R.M.I</td>
<td>-25.5</td>
<td>52</td>
<td>36.38</td>
<td>78</td>
<td>1.16</td>
<td>38 (160 kJ)</td>
<td>50</td>
<td>47</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>S.Y.A</td>
<td>-28.5</td>
<td>56.5</td>
<td>56.7</td>
<td>72</td>
<td>0.78</td>
<td>50 (210 kJ)</td>
<td>37</td>
<td>61</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>S.Y.B</td>
<td>-37.3</td>
<td>60</td>
<td>55.94</td>
<td>72</td>
<td>0.619</td>
<td>59 (248 kJ)</td>
<td>38</td>
<td>60</td>
<td>33</td>
<td>67</td>
</tr>
</tbody>
</table>
These studies were prompted by recurring reports and observations that a high energy intake seems to be a most decisive factor in the success or otherwise of the Giordano-Giovannetti dietary regime in the treatment of chronic renal failure. The basis of this treatment is to reduce protein metabolism to a minimum by supplying a diet low in total nitrogen, contained primarily in high biological value protein, and including minimal but adequate amounts of essential amino acids. Emphasis is made upon the maintenance of a sufficiently high energy intake to promote maximal protein utilization.

An insufficient energy intake has an immediate and direct effect on the biochemical equilibrium, and as most patients are unable to tolerate a large proportion of fat in their diet, and much repetition of very sweet foods is generally disliked, the dietary energy requires very careful management if this regime is to be accepted. In addition an impaired utilization of dietary carbohydrate, shown as an abnormal insulin response pattern is well recognized in a proportion of patients with chronic renal failure (Luke, Briggs, McKiddie and Kennedy, 1968). The type of carbohydrate could therefore be of importance.

The studies described in this thesis have been carried out on eight uraemic patients.

1. Nitrogen Balance

The present work expands the data already presented by Hyne et al. (1972) and supports the conclusions. Figure 4 shows that with an increase in energy intake, nitrogen equilibrium is enhanced. As shown in the previous studies, the improvement in nitrogen balance which can be achieved in subjects with chronic renal failure on an intake of approximately 3 g nitrogen, as high biological value protein, is greater than would be expected from data obtained on normal subjects on
Fig. 4 Improvement in $\Delta B'$ with increasing energy intake

Correlation coefficient $r = 0.763$ \( n = 8 \) and $P$ is less than $0.05$
the same nitrogen and energy intakes, and this confirms the
observation made first by Giordano (1963).

Therefore when the minimum essential amino acids are supplied,
the normal requirement for an additional non-essential nitrogen intake
of approximately 2.55 g daily, demonstrated by Rose and Wixom (1955)
is shown to be lower in uraemic patients, presumably because there
is an enlarged urea pool which can serve as a non-essential nitrogen
source.

2. Net Protein Utilization

In these studies the net protein utilization (NHU derived) has
been assessed from the nitrogen balance data, and the results
demonstrate additional nitrogen utilization, compared with that shown
when calculated from the nitrogen intake (of high biological value
protein) and the energy supplied (NHU theoretical).

Occasionally net protein utilization would seem to imply more
than an external nitrogen balance would suggest. As an example of
this Oomen (1970) describes a situation in which a population of
sweet-potato eaters showed an external, persistently negative nitrogen
balance, and yet there was no evidence of hypercatabolism, but every
indication of an exceptionally good state of nutrition. He suggests
a mechanism of nitrogen fixation from the very high carbohydrate, low
protein diet, by micro-organisms in the colon. It appears that an
additional nitrogen utilization is operating as an adaptive process.

In the present studies the net protein utilization assessed from
balance data implies an additional protein utilization into the in vivo
synthesis of amino acids, and this would reflect the reutilization
of the available urea pool.

The NHU represents the ratio of nitrogen utilization to the
total available, assuming that dietary nitrogen is utilized maximally.
Fig. 5. Showing relationship between \( \frac{FN}{DN} \) and \( \Delta B' \)

Correlation coefficient \( r = 0.682 \)  \( n = 8 \) and \( P \) is greater than 0.05
If \( \Box \) is neglected \( r = 0.968 \)  \( n = 7 \) and \( P \) is less than 0.01
All the NFUs derived from balance data gave a higher value compared with the NFU value assessed theoretically from the dietary intake, with the exception of S.Y., and also with one other exception (J.D.) all these values indicate greater than 100 per cent utilization.

3. Faecal Nitrogen

The present studies show a clear relationship between the faecal nitrogen expressed as a percentage of the dietary nitrogen and the $\Delta B'$ if the point $\Psi$ (results on M.S.) is excluded (Figure 5).

Normally there are two components to faecal nitrogen, the inevitable endogenous or metabolic nitrogen, which is contributed by luminal secretions, mucosal cellular debris and intestinal microflora, and the exogenous or dietary component. In normal individuals the metabolic or endogenous component of faecal nitrogen is assessed as 53 mg nitrogen per kg$^{0.73}$ (Miller and Payne, 1964). Although this cannot be applicable to uraemic individuals, in the present studies if the inevitable metabolic faecal nitrogen is calculated from these normal figures, it is found to be slightly higher than the average total faecal nitrogen from the balance data. This lower faecal nitrogen would indicate an increased nitrogen utilization. For example, the calculated inevitable faecal nitrogen on I.H. would be 0.94 g, but her average faecal nitrogen from balance data is 0.75 g (28 per cent of dietary nitrogen). As the body urea pool in this patient was also depleted, an increased dietary nitrogen intake may have enhanced nitrogen utilization still further and given a more positive nitrogen balance. Other patients in this study were shown to have a lower faecal nitrogen than would be expected in normal individuals. This pattern, however, was not shown by J.D., S.Y.,
R.M.(I) and T.G. who had a higher faecal nitrogen and this increased faecal nitrogen is shown to be a reflection of the nitrogen balance; as nitrogen utilization diminishes, faecal nitrogen is shown to increase. Therefore in uraemic individuals faecal nitrogen must constitute an important adaptive excretory facility, when urea nitrogen is apparently diverted into faecal nitrogen.

The resulting $\Delta B$ on M.S. was more negative than the ratio of faecal nitrogen to dietary nitrogen would suggest, faecal nitrogen being 28 per cent of the nitrogen intake. But it is noted that the urinary nitrogen in this patient was higher than all other studies with the exception of S.Y. M.S. was also menstruating during the balance study. These two factors would contribute to a more negative nitrogen balance.

4. The mechanism of utilization of additional nitrogen

From studies on normal and uraemic subjects, Walser and Bodenlos (1959) and Richards (1972) have suggested that in normal subjects 44 per cent of the total body urea pool can be recycled continuously and that 10 per cent of this total pool is potentially available for incorporation into body tissues. This reutilization is made possible by the activities of indigenous urease producing micro-organisms in the colon. The urease production depends on the size of the urea pool and, as Richards (1972) points out, the urease production increases with increasing body urea concentration. Presumably there must be a limit to the proliferation of these micro-organisms, which would probably be reached at very high blood urea concentrations. Also with such an increase of substrate, there must be a limiting velocity at which the enzyme urease becomes saturated with substrate, and therefore control of urea pool size beyond certain values becomes impossible.
Giordano (1963) made the point that reutilization of urea pool nitrogen is also apparently restricted at low values of blood urea (80 mg per 100 ml.) and no further improvement in nitrogen balance is possible. At this level presumably, substrate concentration would not increase sufficiently for the activity of the enzyme urease to proceed. In the present studies for example, J.L. appeared to be unable to reutilize urea nitrogen when a blood urea level of 80 mg per 100 ml. was recorded, and as urea is equally distributed throughout the body pool, a decreased blood urea must be a reflection of a depleted total body urea pool, not large enough to permit urealysis and recycling.

It seems, however, that control of the size of an expanding urea pool can be achieved and a steady equilibrium appears possible, when the increment to the urea pool matches the rate of hydrolysis and subsequent synthesis of non-essential amino acids.

The factors arbitrating the proportion of nitrogen from the body urea pool entering into either the urea cycle or into the synthetic pathway would be:

The amount of energy supplied, and probably the source of this energy.

The size of the body urea pool, which is the substrate for urease hydrolysis and therefore can influence the supply of urea nitrogen to the entero-hepatic circulation.

The provision in the diet of the minimal essential amino acids for nitrogen equilibrium, and the reduction of dietary unessential nitrogen.

It is noteworthy that Richards et al., (1967) observed that the greater the protein depletion of body tissues, the greater was the nitrogen incorporation and also protein synthesis was enhanced when
there was less unessential nitrogen in the diet.

The factors determining the proportion of nitrogen entering into either the urea cycle or alternatively to be incorporated into body tissues, appear therefore to be dependent on the combined synthetic potential of the urea pool and the energy supplied. The greater the proportion of urea recycled the smaller the proportion of nitrogen incorporated unless the energy supply is liberal.

With a lack of non-essential nitrogen in the diet, and a blood urea greater than 80 mg per 100 ml., the dietary energy supplied must be the arbiter between these two alternative pathways.

5. Energy

The critical influence of an insufficient energy intake on nitrogen retention in uraemia can be examined in the studies of Herndon, Freeman and Cleveland (1957). Energy intakes of around 30 Calories per kg body weight resulted in a less efficient utilization of the protein supplied than that obtained in normal subjects on similar intakes. In normal individuals, Allison and Anderson (1945) have shown that when energy intake is held constant and nitrogen intake increased a curvilinear relationship is apparent and energy is shown to be limiting. The patients in Herndon et al.'s study reached equilibrium on 1.0 g protein and approximately 30 Calories per kg body weight. The efficiency of utilization cannot be maximal on this energy intake. In normal individuals Munro (1951) has shown that on a fixed protein intake, utilization of protein increases with an increase in dietary energy.

The NPU can be assessed on patient 3, diet d, of the Herndon study, in which the protein source is whole egg, which has a protein score of 100. If whole egg is assumed to be completely absorbed
the NHJ should be 100 per cent assuming that sufficient energy is supplied.

In patient 3, weight = 58.6 kg, intake = 27 to 30 Calories per kg, 0.43 g protein per kg, theoretical NHJ (θ) of egg on the energy intake given is as follows. Energy intake = 1,758 Calories, nitrogen intake = 4.07 g N = 102 Calories which is 5.8 per cent of the energy intake \( θ = S \left(1 - k \times \text{protein as percentage of total Calories}\right) \) (k = 0.019) (Miller and Payne, 1963, see Appendix). 
\[ θ = 100 \left(1 - 0.019 \times 5.8\right) = 88.98 \text{ per cent (theoretical NHJ).} \]
This shows that the energy did not permit maximum utilization of the protein supplied, implying that the protein was used for energy purposes.

In the present study, and in that of Hyne et al. (1972) it has been shown that the improvement in \( \Delta B' \) continues with additional increments to the energy intake up to 55 Calories per kg body weight, which is the limit in our series of studies thus far. There are, however, human limitations but the possibility of still further increasing the energy intake by dietary modifications, in terms of palatability, flavouring, etcetera remains to be investigated. A total of 2,500 Calories (40 Calories per kg body weight) would normally be required for maintenance by a man of 63 kg. If this is compared with a total of 3,460 Calories (55 Calories per kg) it can be seen that an additional 15 Calories per kg were given above the normal maintenance requirement.

The question of any further improvement in \( \Delta B' \) with an increase in energy intake above this level must be entirely speculative. From our experience, most patients found difficulty in taking much more than 50 Calories per kg.
It is also noted that Robson, Kerr and Ashcroft (1967) observed that with a decrease in energy intake a more negative nitrogen balance and also a more rapid rate of urea pool expansion occurred in uraemic patients.

The results of the present studies clearly suggest that there is an additional demand for energy over and above that required in normal individuals, and the reason for this would be the necessity for a greater utilization of nitrogen, with consequently an increased demand for tricarboxylic acid cycle intermediates.

In normal individuals on low energy diets, an increasing catabolism of endogenous fat and protein, results in tissue protein being used for energy purposes, with increasing urinary nitrogen. In uraemic subjects in a similar situation of an energy deficit, an even more marked negative nitrogen balance would occur because there would be less energy supplied from endogenous fat. It is noteworthy that in the uraemic situation where there is a high energy requirement, these patients rarely deposit fat, and the total energy reserve (carbohydrate and fat) would always appear to be very low. McCracken (1968) found the energy cost of fat formation to be 15.9 Calories per gram of fat. A resulting accelerated tissue catabolism, would result in an increased nitrogen retention and hence in an expansion of the urea pool.

Miller (1968) considers that the enzymic reactions of oxidative phosphorylation are not promoted by substrate concentration, but by an increased rise in inorganic phosphate and a decreased level of adenosine triphosphate (ATP). In the uraemic subject the rate at which the urea pool can be used to synthesize protein depends on the availability of ATP.

6. Amino acid synthesis

For the utilization of urea nitrogen and for the subsequent
Fig. 6  Outline of suggested urea recycling in uraemic individuals showing the key position of pyruvate oxaloacetate and \( \alpha \) ketoglutarate in the production of amino acids from non-protein sources (After H. A. Lee)
synthesis of amino acids, urea must be hydrolysed by intestinal micro flora with the production of ammonia, which is subsequently incorporated into amino nitrogen. A suggested scheme for the recycling of urea in uraemic patients is shown in Figure 6: (after H.A. Lee).

Ammonia combines with α-ketoglutarate from the tricarboxylic acid cycle to form glutamic acid, which then serves as a general amino group donor, reacting with various keto acids to form the corresponding non-essential amino acids by transamination. Thus carbon derived from glucose is incorporated into glutamic acid and subsequently into other amino acids. Availability of the keto acid precursors of amino acids is a necessary preliminary to their transamination from glutamate. A continuous supply of dietary energy, especially carbohydrate is an essential requirement for this process. Catabolic reactions lead to the combustion of most metabolites as CO₂ and H₂O and NH₃, with the exception of several intermediates of the tricarboxylic acid cycle, for example, oxaloacetate, α-ketoglutarate and succinyl CoA, which can be diverted into anabolic or gluconeogenic pathways.

There are some reactions which can replace these intermediates. Thus, malic enzyme catalyses the formation of malic acid from pyruvate, and pyruvic acid carboxylase catalyses the formation of oxaloacetate from pyruvate. Therefore an adequate supply of pyruvate from carbohydrate would ensure these replenishing reactions.

7. The provision of ATP with relation to urea recycling

In uraemic individuals the metabolic demand for ATP is increased when there is a prior demand for energy for urea recycling. In this situation the supply of the TCA cycle intermediates could be less than
the demand, and the cycle would become drained, and the mechanism of incorporation of urea nitrogen and synthesis of amino acids impeded. This is an essential process for these subjects when taking minimal protein diets. If the energy supply is not sufficient then this facultative anabolic function cannot succeed, and the situation could in certain aspects be compared with that operating in semi starvation. In 1971 Gallina and Dominguez showed that obese subjects on low energy diets, with partial replacement of the high biological value protein by urea nitrogen, can only remain in nitrogen balance when sufficient carbohydrate is taken. Also Geiger and Himmi (1959) found that normal rats were in negative nitrogen balance when they were given high fat, low carbohydrate diets with adequate essential amino acids supplemented with ammonium salts. They concluded that non-essential amino acids were not being synthesized efficiently, presumably due to lack of TCA intermediates.

In the uraemic subject the demand for ATP and a continuous supply of pyruvate and TCA cycle intermediates is high and obligatory. In conditions of energy scarcity the amount of carbohydrate supplied would be critical. It would therefore seem to be desirable to supply a significant proportion of the energy as carbohydrate to these patients.

8. A suggested partitioning of the energy supplied to the uraemic patient

Assuming that normal energy requirements for maintenance are met, two energy demands predominate, without which catabolism would occur.

Firstly, energy is required for urea recycling via the Krebs Henseleit cycle, and a total of four high energy phosphate equivalents are required for each urea molecule produced (Krebs, 1964). Thus the larger the body urea pool the greater the energy required for recycling.

Secondly, energy is required for protein synthesis, and
approximately 150 Calories are needed for each gram of nitrogen converted into protein (Miller and Payne, 1963); a continuous supply of TCA cycle intermediates would be a prerequisite for this anabolism.

The present studies can demonstrate this additional requirement and a partitioning of the energy supplied has been suggested (Tables 10 - 17). The energy expenditure was estimated approximately by doubling the basal requirement to give the energy required for daytime (12 hours) activities (Brody, 1945). It must be borne in mind however that there is a tendency to underestimate requirements for activity in subjects of heavy body weights and to overestimate these in subjects of lighter body weight. Miller and Payne (1962) considered this method to be a reasonable approximation, and when applied to these patients it is considered to be more realistic than attempting to reconstruct individual activities from the data of Durnin and Passmore (1967) as no record was made of these. All patients in this study were allowed out of hospital each afternoon when they were fairly active. A recorded energy expenditure on such patients would form a valuable study in the future.

Table 10 shows the theoretical assessment of the partitioning of the energy supplied to M.H. As 44 per cent of the total body urea pool is considered by Walser and Bodenlos (1959) to form part of the nitrogen source available for synthetic purposes, the energy requirement for synthesis of this portion of the urea pool was assessed at 166 Calories (7.6 per cent of the total energy). A theoretical assessment of that portion of the energy required for the synthesis into body protein of 1.46g nitrogen amounts to 10.0 per cent of the total energy or 219 Calories, making the total additional energy requirement 385 Calories. Thus of the total energy supplied at the rate of 47 Calories per kg, 1,805 Calories remained for basal and energetic function. The assessed
total energy requirement is 1,744 Calories. Thus there is a surplus of 60 Calories supplied. The apparent total energy requirement for M.H. is therefore estimated at approximately 45 Calories per kg. It is possible that the energy requirement for synthetic purposes has been underestimated. The amount of nitrogen retained has been calculated from the Θ (NHU) value, only when this value exceeded 1.0. A Θ value in excess of 1.0 is assumed to be conclusive evidence of additional nitrogen retained. But with a Θ value no greater than 1.0 nitrogen retention would still be probable in patients where nitrogen equilibrium was maintained and urea pool size controlled.

Similar calculations have been made for other patients. In the assessment on I.H. (Table 11) there is a surplus of 490 Calories supplied. This patient may have used more energy for activities as she was probably more active than some of the others studied, also it will be noted that as the body urea pool was depleted, any improvement in NHU would be limited by this. This patient commented many times that she felt extremely well on the regime. The apparent energy requirement of I.H. would be 41 Calories per kg. In the partitioning of the energy assessment on T.G. Table 12 a deficit of approximately 340 Calories supplied is shown. The body urea pool in this patient was enlarged (55 g urea) at the commencement of the study. These calculations would imply that the energy required for recycling an enlarged urea pool would be limiting and the NHU could have been improved with an increased energy intake. At an intake of 40 Calories per kg body weight the estimated nitrogen retained is only 2.59 g from an available 24.2 g urea (11.3 g urea nitrogen). An energy intake of around 45 Calories per kg would be desirable and the apparent requirement was 45 Calories per kg body weight.

The assessed energy partitioning on R.M.I Table 13a shows that with a total urea pool of 43.0 g urea only 1.4 g nitrogen was retained,
and there was a slightly higher energy requirement for urea recycling than for anabolism. From this assessment a deficit of 353 Calories is shown. The apparent requirement is 45 Calories per kg whereas the actual Calories supplied were 37.7 Calories per kg. In this patient an energy deficit is apparently limiting any further nitrogen utilization.

In the assessment on R.M. II Table 13b. the urea pool is enlarged to 50 g urea but as the energy intake was 53.7 Calories per kg, nitrogen utilization was improved and 5.7 g nitrogen were retained in this assessment. A deficit of 215 Calories is shown, and the apparent requirement is 58 Calories per kg, but nitrogen utilization showed an improvement on the actual intake of 54 Calories per kg.

The assessed energy partitioning on J.D. (Table 14) allows no energy for protein synthesis as the Θ value was 0.998, although the energy intake was 50.4 Calories per kg and the body urea pool 41.2 g. In this assessment the additional energy was used only for urea recycling. As the urea pool was moderately enlarged, and as nitrogen equilibrium showed no rapid deterioration, it is considered that nitrogen retention must have occurred. At the same time however nitrogen excretion via the faeces was higher than in some of the other studies and this patient also had some diarrhoea. There was no definite evidence of nitrogen retention in this patient and so the apparent energy requirement was assessed to be only 38.2 Calories per kg which implies a surplus of 752 in the total Calories supplied (50.4 Calories per kg.)

The energy partitioning on M.S. Table 15 shows that more energy was required for urea recycling than for nitrogen incorporation and an excess of 345 Calories of the total supplied is indicated. The apparent requirement is 40 Calories per kg. The total body urea pool was not greatly enlarged at 25.0 g urea.
The relationship between available urea pool (44% of total body urea) and the apparent energy requirement.

Correlation coefficient $r = 0.774$, $n = 8$ and $P$ is less than 0.05.
The assessed energy partitioning on J.L. Table 16 shows a greater energy requirement for urea recycling than for nitrogen incorporation. The urea pool was only moderately enlarged and at an energy intake of 48 Calories per kg nitrogen retention was only 1.06 g. From this assessment an excess of 857 Calories of the total supplied, is found and the apparent requirement was only 37.2 Calories per kg. A nitrogen retention higher than the $Q$ value, probably occurred in this instance for the faecal nitrogen was low and the urea pool was controlled at a fairly low total value.

Table 17 shows the apparent energy requirement and the available urea pool when calculated on a per kg body weight basis, and Figure 7 shows a clear relationship between the assessed apparent energy requirement and the available urea pool per kg body weight. It must be emphasized that these derivations are entirely theoretical and entail several assumptions, for example that 44 per cent of the total body urea pool is available for reutilization, and that the NHU assessed from the balance data is a reasonable estimate.

9. Conclusions from studies. Facultatory mechanisms

These studies demonstrate unmistakeably that a decreasing faecal nitrogen indicates an increasing nitrogen utilization and that nitrogen utilization is improved by an increasing energy intake.

Man is well adapted to prolonged periods of curtailed energy supplies and in all instances energy requirements take precedence over all other requirements, and strict priorities for its consumption are evident (Keys, Brozek, Henschel, Mickelson and Taylor, 1950). Likewise in the uraemic individual a strict pattern of priorities is seen. The utilization of an enlarged urea pool whether by recycling, incorporation into body tissues or excretion, is dependent on the

196.
energy supplied. The prior energy demand must be for urea synthesis, thereafter the obligatory adaptive mechanisms of either excretion, and the gut seems to be an important pathway for this, or synthesis and retention are primarily dependent on the amount of available energy.

These studies also emphasize the importance of the maintenance of a state of equilibrium. The precarious balance of these patients can be influenced by a variety of factors such as mineral imbalance as in the study on R.M. when the influence of sodium balance was seen, and also on M.S. when a state of hypotension showed a direct effect on nitrogen balance, and it is probable that malabsorption may have influenced the equilibrium of S.Y.

It is therefore apparent that a state of equilibrium can be jeopardised by the interrelationship of numerous factors, one of the most important of which is control of the size of the urea pool. A pattern of precise regulation and a dynamic balance appears feasible in end state renal failure, and a prolonged and constant energy intake with a liberal proportion of carbohydrate is crucial to the achievement of a steady state.

The ability of the patient to continue on such a dietary regime is decisive; the type of energy source therefore could be of the utmost importance from the point of view of palatability and acceptability by the patient. The merits or otherwise of providing a greater proportion of polysaccharides to monosaccharides and disaccharides as the carbohydrate source, and the proportion of the total energy given as fat have been examined.

In the present studies the percentage of the total energy given as fat ranged from 31 per cent to 50 per cent and the total carbohydrate from 47 per cent to 66 per cent. The percentage of the
Fig. 8 Variation of $\Delta B'$ with increased CH$_2$O as % of total energy
Fig. 9 Variation of ΔB with increased fat as % of total energy.
Fig. 10 Variation of $\Delta B'$ with increased mono and disaccharides as % of total CH$_2$O energy

$\Delta B'$ (mg N/kg per day)

Mono/disaccharides as % of total CH$_2$O energy
Fig. 11 Variation of $\Delta B'$ with increased polysaccharides as % of total CH$_2$O energy
total carbohydrate energy given as polysaccharides ranged from 33 per cent to 80 per cent and the proportion of the total carbohydrate given as mono or disaccharides ranged from 20 per cent to 67 per cent (Table 9).

From the results of these studies, $\Delta B'$ is shown to be independent of the type of carbohydrate, and of the proportion of fat to total carbohydrate within the range studied (Figures 8 - 11).

It therefore seems that there is some latitude in the selection of the energy providing foods in the Giordano-Giovannetti diet. As the provision of energy forms the major part of the diet, patients can be encouraged to introduce some flexibility according to individual preference into what is probably the most difficult obstacle in their dietary therapy, that is, the ingestion of sufficient energy.

It is perhaps worthy of comment that M.H. who was Scottish, took the highest proportion of polysaccharides in her diet. She preferred and was able to eat with comparative ease, large quantities of starch containing foods such as bread and scones, whereas English patients found this impossible.

10. Factors influencing the acceptance of the diet

Some difficulties were encountered in inducing patients to take their high energy intakes. A feeling of satiety was always very prevalent, especially during the first few days on the regime.

Food acceptance, and the size of meal tolerated are influenced by the energy content and sensory appeal, taste, volume, texture and appearance. The size of meal which can be taken, and the pattern of meal distribution, are determined by very complex interactions of various behavioural factors. The previous meal pattern has a direct influence on the patient's ability to follow the experimental regime, also many people like to take the bulk of their daily intake in the
evening. Patients often show strong preferences, and a preference for meat and meat flavours is common, and also as the appetite is stimulated by increasing the protein content, high energy low protein diets are difficult to maintain.

It was found that the subjects in these studies usually required four days to adjust to the feeding routine. The length of intervals possible between meals was determined largely by the total energy content of the foregoing meal, and it was therefore considered to be extremely important to divide the energy load into numerous smaller meals as far as possible.

Various factors can militate against acceptance of a diet. It is known that a strange environment is not conducive to the promotion of a good appetite, even in fit individuals.

Acidosis can be a problem in the maintenance of these patients. It may be advantageous to use a greater proportion of egg white which is less acid producing than the yolk, the phospholipid content of which contributes more to the hydrogen ion load. Acidosis might also be controlled to a certain degree by including as much base in the diet as possible, within the confines of the total nitrogen and potassium intake, in the form of vegetables or fruit.

11. Future developments

A possible future development of the Giordano-Giovannetti diet might be the determination of an ideal amino acid pattern, not at present defined for uraemic individuals. Dietary requirements for sulphur containing amino acids for example, may be less than those stipulated by Rose et al., (1949) for normal individuals. Using this criterion it is of note that in all the present studies, the sulphur containing amino acids are contained in amounts less than that suggested.

It is also noteworthy that Hughes (1955) has shown that diets
consisting mainly of vegetable protein supported rapid growth in children. In Hughes' investigation the essential amino acid requirements for nitrogen equilibrium in the adult (Rose et al., 1949) were all fulfilled with the exception of methionine.

It is conceivable that the amino acid patterns of some vegetables for example, the potato (Hughes, 1958) could when combined with other vegetable sources form a suitable pattern, fulfilling the minimal requirements of uremic patients. It may be possible to control the potassium content by a process of leaching or dialysing although the effect of this on other intra or extracellular ions is not known, and some replacement may be necessary perhaps in the form of potassium-low vegetables. A potato based starch could then be used as a vehicle for combining the energy source. This would make a very good basis for incorporating carbohydrate and fat. Savoury foods are generally more acceptable and the potato could form a popular ingredient for almost any meal.

In nitrogen balance studies on normal subjects using minimal protein intakes Kofranyi and Jekat (1964) showed that the inclusion of potato with egg protein gave an improved biological value. The successful use of a potato diet in terminal renal failure was reported by Orlowski and Polec (1966). This diet contained only potato, butter, sugar, vegetables and fruit. The disadvantage of the diet was a very high potassium content, and it could therefore not be used when potassium retention was a problem.

In instances when the urea pool becomes depleted, more nitrogen could possibly be given in the form of potassium-low vegetables thus adding more variety and palatability to the diet.

The prolonged maintenance of high energy intakes even if largely synthetic, would be facilitated if the energy source could be disguised
and presented in familiar forms, textures and flavours.

This concept should not present an insurmountable problem to progressive food manufacturers, whose many resources include a large range of realistic flavourings embodied in familiar textures. Indeed the era of the synthetic steak is almost a reality.

Richards (1972) visualises the formulation of a diet which would be almost completely synthetic and contain practically no natural form of protein.

A synthetic and varied energy source retaining the familiar landmarks of texture, flavour and smell would certainly be of great value to a patient attempting to achieve the very high energy intake inherent in the Giovannetti regime.

Many developments and refinements to such a regime can be envisaged and some valuable experience has been gained in a series of studies using essential amino acids in tablet form combined with largely synthetic high energy diets. For example, such a diet was given in the form of three menus rotating over a three day period as shown in Table 18. In addition to these menus 15 Kidnamin tablets containing the minimum safe requirements of amino acids and a total nitrogen of 1.05 g were taken daily. A photograph of these diets is shown in Figure 12.

The **pancake or waffles** contained a mixture of double cream and Prosparol (Duncan Flockart) or Farma Classic Cream (Unigate), water and protein free flour or cornstarch, and colouring. They were used either as a savoury pancake or sweet waffle with ice cream. Each pancake or waffle supplied 100 Calories and contained 0.1 g protein.

The **sago pudding** contained sago, arrowroot, glucose, double cream and Prosparol or Mazola oil, water and lemon flavouring. This pudding supplied 460 Calories and 0.75 g protein.
Figure 12.
Three alternating daily menus containing from 1.66 to 2.07 g nitrogen, which have been used with essential amino acid tablets.
### TABLE 18

**Basic diet daily**

(plus 15 Kidnamin tablets 1.05 g N)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight g</th>
<th>Protein g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancake or waffle</td>
<td>140</td>
<td>0.42</td>
</tr>
<tr>
<td>Lettuce</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td>Sago pudding</td>
<td>210</td>
<td>0.75</td>
</tr>
<tr>
<td>Raspberry ice cream</td>
<td>85</td>
<td>0.3</td>
</tr>
<tr>
<td>Pineapple fudge</td>
<td>115</td>
<td>0.33</td>
</tr>
<tr>
<td>Protein free bread</td>
<td>120</td>
<td>0.516</td>
</tr>
<tr>
<td>Unsalted butter</td>
<td>40</td>
<td>0.3</td>
</tr>
<tr>
<td>Orange marmalade</td>
<td>100</td>
<td>0.1</td>
</tr>
<tr>
<td>Double cream and water (as milk)</td>
<td>30</td>
<td>0.45</td>
</tr>
<tr>
<td>Sugar</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Caloreen</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Tea</td>
<td>600 ml</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>200 ml</td>
<td>3.27</td>
</tr>
</tbody>
</table>

In addition to the basic diet

**Menu A**

<table>
<thead>
<tr>
<th>Food</th>
<th>Weight g</th>
<th>Protein g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>60</td>
<td>7.14</td>
</tr>
<tr>
<td>Calories = 3,950</td>
<td></td>
<td>(1.66 gN) 10.41 total</td>
</tr>
<tr>
<td>Na = 7 mEq., K = 7 mEq.</td>
<td></td>
<td>including 9.39 g H.B.V.P.</td>
</tr>
<tr>
<td>Dietary Acid = 11.9 mEq. Dietary Base 4.52 mEq.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Menu B**

<table>
<thead>
<tr>
<th>Food</th>
<th>Weight g</th>
<th>Protein g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>30</td>
<td>8.88</td>
</tr>
<tr>
<td>Beetroot</td>
<td>60</td>
<td>0.78</td>
</tr>
<tr>
<td>Boiled sweets</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Calories = 3,000</td>
<td></td>
<td>(2.07 gN) 12.93 total</td>
</tr>
<tr>
<td>Na = 6 mEq., K = 13 mEq.</td>
<td></td>
<td>including 11.13 g H.B.V.P.</td>
</tr>
<tr>
<td>Dietary Acid = 9.83 mEq. Dietary Base 9.86 mEq.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Menu C**

<table>
<thead>
<tr>
<th>Food</th>
<th>Weight g</th>
<th>Protein g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirloin steak</td>
<td>30</td>
<td>8.04</td>
</tr>
<tr>
<td>Potatoes</td>
<td>100</td>
<td>1.40</td>
</tr>
<tr>
<td>Calories = 3,000</td>
<td></td>
<td>(2.03 gN) 12.71 total</td>
</tr>
<tr>
<td>Na = 4 mEq., K = 16 mEq.</td>
<td></td>
<td>including 10.29 g H.B.V.P.</td>
</tr>
<tr>
<td>Dietary Acid = 7.4 mEq. Dietary Base = 9.82 mEq.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The raspberry ice-cream was made from a mixture of double cream and 'Farma classic cream' or 'Prosparol', glucose and Caloreen, raspberry or other flavouring and colouring, and then frozen. 360 Calories were supplied, and the protein content was 0.3 g protein.

Pineapple fudge was made from Caloreen and icing sugar double cream or 'Farma classic cream' and water, pineapple, colouring and flavouring and was served after freezing. The Caloric content was 265 and the protein content 0.33 g.

The possibility of developing a synthetic diet is very promising, and it was found that the following items were well tolerated. A mixture of starches used as a basis includes potato starch, combined with cornstarch, wheat starch and ground sago or arrowroot. This mixture has been used as potato cakes, combining a small quantity of Mazola oil or double cream or 'Farma classic cream'. This basic mixture can include added vegetables such as chopped fried onions or parsley which will give added base. The potato cakes can be grilled or baked and were found to be very popular. Each potato cake contains 225 Calories and not more than 0.2 g protein. A similar mixture has been used for producing savoury biscuits with a content of 38 Calories and not more than 0.05 g protein per biscuit. Various savoury flavourings (International Flavourings and Fragrances Limited) were used including chicken, cheese and tomato. These biscuits were easily tolerated, and ten biscuits or more can be taken daily. Waffles or pancakes can be made from the same basic ingredients and taken as a savoury dish suitably flavoured, or alternatively flavoured with vanilla and served with honey or a syrup made from caramelised sugar and Caloreen, flavoured with a variety of fruit flavours, or the waffles are served with various frozen creams. Iced caramel cream is made from
a mixture of Caloreen and water which is whipped and added to a corn starch mixture made with 'Farma classic cream' or double cream, and fruit flavourings or shisky added. Calories per portion are 330 and protein content less than 0.1 g.

Caramel or honey bar was very popular and is made from Caloreen, low protein flour and ground sago or protein free semolina, butter, honey or caramelised sugar with suitable flavourings and colouring. Each bar contains 488 Calories with a protein content of less than 0.2 g. From this recipe a frozen 'coconut' ice was evolved in which chopped carrot or pineapple were used, thus adding base to the diet. These frozen bars can be served with a hot 'chocolate' sauce made from custard powder or corn starch and 'Farma classic cream' or double cream and water, with a chocolate flavouring, the Caloric content of which is 148 and 0.42 g of protein. It was found that sweet foods are taken more easily in frozen form, but the savoury foods are generally preferred and the chicken flavour was very popular.

The possibility of the maintenance of a high energy intake for any length of time is improbable unless the maximum variation and latitude can be given in the choice of palatable foods and in the type of energy source. The formulation of a synthetic type of energy source which would resemble familiar and desirable foods, would constitute a very great advance in assisting these patients to discard the habits of a lifetime and adopt novel eating patterns.
TABLE 10

Theoretical assessment of the partitioning of the energy supplied to M.H.

Age 48 years. Weight = 47.0 kg.
Net energy intake = 2,190 Calories (46.4 Cals/kg).
Basal energy requirement 70 Cals/day/kg$^{0.73}$ (Brody, 1945)
Weight$^{0.73} = 16.62$  $16.62 \times 70 = 1,163$ basal Calories per 24 hours (0.01718 Cals/kg/minute).
Requirement for daytime (12 hours) = 1,163 Calories approximately
Requirement for night-time (12 hours) = 581 Calories approximately
Therefore total energy requirement = 1,744 (basal and activity) (37 Cals/kg)

Blood urea at commencement = 134 mg per 100 ml.
Total body water at commencement = 24.08 litres.
Total body urea pool at commencement = 32.27 g urea.
Urea theoretically available for reutilization per 24 hours (Richards, 1972) 44% of total urea pool (potential source of nitrogen for recycling).
44% of 32.3 = 14.2 g urea = 6.617 g urea nitrogen = 41.4 g protein = 166 P/Cals.
166 as % of 2190 = 7.6% of total energy required for urea recycling.
From balance data $\Theta = 1.22$ representing the ratio of net nitrogen utilization, assuming that dietary nitrogen is utilized maximally.
Then an additional 22% is retained.
22% of 6.617 g of urea nitrogen (nitrogen available for synthesis) = 1.46 g N.
1.46 g nitrogen incorporated into body protein = 9.1 g protein

150 Calories required per g N retained (Miller and Payne, 1964).

1.46 x 150 = 219 Calories for anabolism, as % of 2190 = 10%.

Therefore 7.6 plus 10.0 = 17.6% of total energy required for
reutilization and incorporation of urea nitrogen = 385 additional
Calories = 8 Cals/kg.

Basal energy required = 1,163 Cals/24 hours plus 385 = 1,548 leaving
642 Calories for activities (derived from the energy supplied).

581 Calories calculated (approximately) for activities.

Therefore there is a surplus of 60 Calories in the total supplied
(1.28 Cals/kg).

Therefore 45 Cals/kg (189 kJ) is the apparent requirement.
TABLE 11

Theoretical assessment of the partitioning of the energy supplied to I.H.

Age 44 years. Weight = 50.2 kg.

Net energy intake = 2,580 Calories (51 Cals/kg).

Basal energy requirement 70 Cals/24 hours/kg (Brody, 1945)

Weight \(0.73\) = 17.43 \(x\) 70 = 1,220 basal Calories per 24 hours.

Requirement for daytime (12 hours) = 1,220 Calories approximately.

Requirement for night-time (12 hours) = 610 Calories approximately.

Therefore total energy requirement = 1,830 Calories (basal and activity)

\(36.5\) Cals/kg

Blood urea at commencement = 94 mg per 100 ml.

Total body water at commencement = 25.79 litres.

Total body urea pool at commencement = 24.2 g urea.

Urea theoretically available for recycling = 44% of total urea pool.

44% of 24.2 = 10.65 g urea = 4.96 g urea nitrogen = 31.0 g protein = 124 P/Cals.

124 as % of 2580 = 4.8% of total energy required for urea recycling.

From balance data \(\Phi = 1.18\).

Then an additional 18% of nitrogen is retained.

18% of 4.96 g urea nitrogen = 0.8928 g nitrogen incorporated = 5.58 g protein.

150 Calories required per g of retained nitrogen

0.8928 \(x\) 150 = 134 Calories.

As % of 2580 = 5.2%

Therefore 4.8 plus 5.2 = 10.0% of total energy required for reutilization and incorporation of urea nitrogen = 258 additional Calories = 5.6 Cals/kg

212.
basal energy required = 1,220 Cals per 24 hours plus 258 = 1478
leaving 1102 Calories for activity (derived from the energy supplied)
610 Calories (approximately) calculated for activity.
Therefore a surplus of 492 Calories supplied (9.8 Cals/kg).
Therefore 41 Cals/kg (172 kJ) is the apparent requirement.
TABLE 12

Theoretical assessment of the partitioning of the energy supplied to T.G.

Age 57 years. Weight = 62.8 kg.
Net energy intake = 2,490 Calories (40 Cals/kg).
Basal energy requirement 70 Cals/day/kg \^0.73 (Brody, 1945)
Weight \^0.73 = 20.54 \times 70 = 1,438 basal Calories per 24 hours.
Requirement for daytime (12 hours) = 1,438 Calories approximately
Requirement for night-time (12 hours) = 719 Calories approximately
Therefore total energy requirement = 2,157 (basal and activity)

(34 Cals/kg)

Blood urea at commencement = 135 mg per 100 ml.
Total body water at commencement = 40.8 litres.
Total body urea pool at commencement = 55.1 g urea.
44% of total urea pool = 24.24 g urea = 11.3 g urea nitrogen =
70.63 g protein = 283 P/Cals.
283 as % of 2,490 = 11.4% of total energy required for urea recycling.
From balance data \( \Omega = 1.225. \)
23% of 11.3 g urea nitrogen = 2.599 g nitrogen incorporated =
16.24 g protein.
150 Calories required per g nitrogen retained.
2.599 \times 150 = 390 Calories.
390 as % of 2490 = 15%.
11.4 plus 15.0 = 26% of total energy required.
283 plus 390 = 673 additional Calories required for reutilization and incorporation.

214.
Basal Calories required = 1438 plus 673 = 2,111 Calories leaving only 379 Calories for activity derived from energy supplied. 719 Calories calculated for activities. Therefore a deficit of 340 Calories for activities (5.4 Cals/kg). Therefore apparent requirement = 45 Cals/kg (189 kJ).
TABLE 13a

Theoretical assessment of the partitioning of the energy supplied to R.M.I

Age 46 years. Weight = 51.7 kg.

Net energy intake = 1,950 Calories (37.7 Cals/kg).

Basal energy requirement 70 Cals/day/kg⁰.73 (Brody, 1945)

Weight ⁰.73 = 17.81 x 70 = 1,247 basal Calories per 24 hours.

Requirement for daytime (12 hours) = 1,247 Calories approximately.

Requirement for night-time (12 hours) = 623 Calories approximately.

Therefore total energy requirement = 1,870 (basal and activity)

(36 Cals/kg).

Blood urea at commencement = 160 mg per 100 ml.

Total body water at commencement = 26.36 litres.

Total body urea pool at commencement = 43.0 g urea.

Urea theoretically available for reutilization per 24 hours =

44% of total pool.

44% of 43.0 = 18.92 g urea = 8.82 g urea nitrogen = 55.13 g protein = 221 P/Cals.

221 as % of 1,950 = 11% of total energy required for urea recycling.

From balance data Θ = 1.16.

Then an additional 16% is retained.

16% of 8.82 g urea nitrogen = 1.4112 g nitrogen.

1.4112 g nitrogen incorporated into body protein = 8.82 g protein.

150 Calories required per g nitrogen retained.

1.4112 x 150 = 212 Calories for anabolism.

As % of 1950 = 10.9%.
Therefore 11.0 plus 10.9 = 22% of total energy required for reutilization and incorporation of urea nitrogen = 430 additional Calories required (8.4 Cals/kg).

Basal energy required = 1,247 Cals/24 hours plus 430 = 1,677

leaving 270 Calories for activities (derived from energy supplied)

623 Calories calculated for activities.

Therefore deficit of 353 in total Calories supplied (6.8 Cals/kg).

Therefore 45 Calories/kg (189 kJ) is the apparent requirement.
TABLE 13b

Theoretical assessment of the partitioning of the energy supplied to R.M. II

Aged 46 years. Weight = 50.8 kg.

Net energy intake = 2,726 Calories (53.7 Cals/kg).

Basal energy requirement 70 Cals/day/kg$^0.73$ (Brody, 1945).

Weight$^{0.73} = 17.59 \times 70 = 1,231$ basal Calories per 24 hours.

Requirement for daytime (12 hours) = 1,231 Calories approximately.

Requirement for night-time (12 hours) = 615 Calories approximately.

Therefore total energy requirement = 1,846 (basal and activity)

(36.5 Cals/kg)

Blood urea at commencement = 190 mg per 100 ml.

Total body water at commencement = 26.42 litres.

Total body urea pool at commencement = 50.2 g urea.

Urea theoretically available for reutilization per 24 hours =

44% of total pool.

44% of 50.2 = 22.09 g urea = 10.29 g urea nitrogen = 64.31 g protein

= 257 P/Cals.

257 as % of 2726 = % of total energy required for urea recycling.

From balance data $\Theta = 1.549$.

Then an additional 5% is retained.

55% of 10.29 g urea nitrogen = 5.6595 g nitrogen = 35.37 g protein

incorporated.

150 Calories required per g nitrogen retained.

5.66 x 150 = 849 Calories for anabolism.

As % of 2,726 = 31%.
Therefore 9.0 plus 31.0 = 40% of total energy required for reutilization and incorporation of urea nitrogen = 1090 additional Calories (21.5 Cals/kg).

Basal energy required = 1,231 Cals/24 hours plus 1090 = 2,321.

Leaving 400 Calories for activities (derived from energy supplied)

615 Calories calculated for activities.

Therefore a deficit of 215 Calories in the total supplied (4.3 Cals/kg).

Therefore 58 Cals/kg (244 kJ) is the apparent requirement.
TABLE 14

Theoretical assessment of the partitioning of the energy supplied to J.D.

Age 35 years. Weight = 61.6.5 kg.
Net energy intake = 3,100 (50.4 Cals/kg).
Basal energy requirement 70/Cals/day/kg 0.73 (Brody, 1945)
Weight 0.73 = 20.3 x 70 = 1,423 basal Calories per 24 hours.
Requirement for daytime (12 hours) = 1,423 Calories approximately.
Requirement for night-time (12 hours) = 711 Calories approximately.
Therefore total energy requirement = 2,134 (basal and activity)
(34.5 Cals/kg).

Blood urea at commencement = 104 mg per 100 ml.
Total body water at commencement = 39.65 litres.
Total body urea pool at commencement = 41.2 g urea.
Urea theoretically available for reutilization per 24 hours = 44% of total pool.
44% of 41.2 = 18.13 g urea = 8.45 g urea nitrogen = 52.8 g protein
= 211 P/Cals.
211 as % of 3,100 = 6.9% of total energy required for urea recycling.
From balance data Θ = 0.998.
Therefore no concrete evidence of nitrogen retained.
Therefore 6.9% of total energy required for reutilization of urea
nitrogen = 214 additional Calories required (3.5 Cals/kg).
Basal energy required = 1,423 Calories per 24 hours plus 214 = 1,637,
leaving 1,463 Calories for activities (derived from energy supplied).
711 Calories calculated for activities.
Therefore a surplus of 752 in the total Calories supplied (12.2 Cals/kg).

Therefore the apparent requirement = 38.2 Cals/kg (160 kJ)
### TABLE 15

Theoretical assessment of the partitioning of the energy supplied to M.S.

**Age 37 years. Weight 53.5 kg.**

Net energy intake = 2,459 Calories (46 Cals/kg).

Basal energy requirement 70 Cals/day/kg \(^{0.73}\) (Brody, 1945)

Weight \(^{0.73}\) = 18.25 x 70 = 1,278 basal Calories per 24 hours

Requirement for daytime (12 hours) = 1,278 Calories approximately

Requirement for night-time (12 hours) = 639 Calories approximately

Therefore total energy requirement = 1,917 (basal and activity) (35.8 Cals/kg)

Blood urea at commencement = 90 mg per 100 ml.

Total body water at commencement = 27.79 litres.

Total body urea pool at commencement = 25.0 g urea.

44% of total urea pool (urea pool available for recycling)

44% of 25.0 = 11.0 g urea = 5.13 g urea nitrogen = 32.1 g protein = 128 P/Cals.

128 as % of 2,459 = 5.2% of total energy required for urea recycling.

From balance data \(\Theta = 1.088\)

Then an additional % is retained

% of 5.13 g urea nitrogen = 0.4617 g nitrogen incorporated

= 2.9 g protein.

0.46 x 150 = 69 Calories for anabolism.

As % of 2,459 = 2.8%.

Therefore 5.2 plus 2.8 = 8% of total energy required for reutilization and incorporation of urea nitrogen = 197 additional Calories (3.7 Cals/kg).

222.
Basal energy required = 1,278 Cals/24 hours plus 197 = 1,475 Calories
leaving 984 Calories for activities (derived from the energy supplied)
639 Calories calculated for activities
Therefore a surplus of 345 in the total Calories supplied (6.35 Cals/kg).
Therefore 40 Cals/kg (168 kJ) is the apparent requirement.
TABLE 16
Theoretical assessment of the partitioning of the energy supplied to J.L.

Age 36 years. Weight = 79.0 kg.
Net energy intake = 3,807 Calories (48 Cals/kg).
Basal energy requirement 70 Cals/day/kg^{0.73} (Brody, 1945).
Weight^{0.73} = 24.29 x 70 = 1,700 basal Calories per 24 hours
Requirement for daytime (12 hours) = 1,700 Calories approximately
Requirement for night-time (12 hours) = 850 Calories approximately
Therefore total energy requirement = 2,550 (basal and activity)

(37 Cals/kg)

Blood urea at commencement = 92 mg per 100 ml.
Total body water at commencement = 51.16 litres.
Total body urea pool at commencement = 47.1 g urea.
44% of 47.1 (urea pool available for recycling)
= 20.7 g urea = 9.65 g urea nitrogen = 60.3 g protein = 241 P/Cals.
241 as % of 3,807 = 6.3% of total energy required for urea recycling
From balance data θ = 1.114
Then an additional 11% is retained.
11% of 9.65 = 1.06 g nitrogen incorporated
1.06 x 150 = 159 Calories for anabolism.
As % of 3,807 = 4.2%
Therefore 6.3 plus 4.2 = 10.5% of total energy required for reutilization
and incorporation of urea nitrogen = 400 additional Calories
(5.0 Cals/kg).
Basal energy required = 1700 Cals/24 hours plus 400 = 2,100 Calories

224.
leaving 1,707 Calories for activities (derived from energy supplied).

850 Calories calculated for activities.

Therefore a surplus of 857 in the total Calories supplied (10.8 Cals/kg).

Therefore 37.2 Cals/kg (155 kJ) is the apparent requirement.
<table>
<thead>
<tr>
<th>Subject</th>
<th>$\theta$</th>
<th>Actual energy supplied Cals/kg</th>
<th>Apparent energy requirement Cals/kg</th>
<th>Total body urea pool g urea</th>
<th>44% of total urea pool g urea</th>
<th>44% total urea pool g urea/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.M.II</td>
<td>1.549</td>
<td>53.7 (223 kJ)</td>
<td>58 (244 kJ)</td>
<td>50.2</td>
<td>22.09</td>
<td>0.4348</td>
</tr>
<tr>
<td>R.M.I</td>
<td>1.16</td>
<td>37.7 (160 kJ)</td>
<td>45 (189 kJ)</td>
<td>43.0</td>
<td>18.9</td>
<td>0.3656</td>
</tr>
<tr>
<td>M.H.</td>
<td>1.22</td>
<td>46.4 (197 kJ)</td>
<td>45 (189 kJ)</td>
<td>32.27</td>
<td>14.2</td>
<td>0.3234</td>
</tr>
<tr>
<td>T.G.</td>
<td>1.225</td>
<td>40.0 (168 kJ)</td>
<td>45 (189 kJ)</td>
<td>55.1</td>
<td>24.24</td>
<td>0.3859</td>
</tr>
<tr>
<td>I.H.</td>
<td>1.18</td>
<td>51.0 (214 kJ)</td>
<td>41 (172 kJ)</td>
<td>24.2</td>
<td>10.65</td>
<td>0.2121</td>
</tr>
<tr>
<td>M.S.</td>
<td>1.088</td>
<td>46.0 (193 kJ)</td>
<td>40 (168 kJ)</td>
<td>25.0</td>
<td>11.0</td>
<td>0.2056</td>
</tr>
<tr>
<td>J.D.</td>
<td>0.993</td>
<td>50.0 (210 kJ)</td>
<td>38 (160 kJ)</td>
<td>41.2</td>
<td>18.13</td>
<td>0.2941</td>
</tr>
<tr>
<td>J.L.</td>
<td>1.114</td>
<td>48.0 (202 kJ)</td>
<td>37 (155 kJ)</td>
<td>47.1</td>
<td>20.7</td>
<td>0.2620</td>
</tr>
</tbody>
</table>

The apparent energy requirement and available urea pool.


APPENDIX

1. Glomerular filtration rate

Determination of glomerular filtration rate gives an assessment of glomerular function and renal blood flow. A measure of the volume of glomerular filtrate which is produced each minute (G.F.R.) can be assessed by finding the clearance of a substance which is neither reabsorbed nor secreted by the renal tubular cells. The polysaccharide Inulin is a substance used in human individuals and is given intravenously (or injected subcutaneously).

The amount of Inulin excreted each minute $U_{IN} \times V$ is equal to that amount filtered at the glomerulus where $U_{IN}$ = concentration Inulin in urine.

$V$ = rate urine flow in ml/min.

The factor $\frac{U_{IN} \times V}{P_{IN}} = C_{IN}$ (gives volume of filtrate per minute) $\Rightarrow$ G.F.R

$P_{IN}$ as the concentration of Inulin in glomerular filtrate equals that in plasma $P_{IN}$.

Inulin clearance in normal subjects is $C_{IN}$ (G.F.R.) = 125 ml/min.

Clearance of substances in human plasma which are reabsorbed or secreted by renal tubules.

Most substances occurring in human plasma are reabsorbed by tubular cells and the clearance value is lower than inulin clearance, an example of such is glucose. Clearance of substances reabsorbed is increased with increasing plasma levels.

A few other substances such as creatinine are secreted and therefore they have a higher clearance value than Inulin and the clearance is decreased with increasing plasma levels.
Assessment of renal tubular function

Para-amino-hippuric acid (P.A.H.) or Diodrast, which are actively secreted by the tubular cell and have higher clearance values than inulin, are used to assess renal function. Tubular maximal reabsorption or secretory capacity can be measured by assessing the maximal reabsorption for glucose ($T_{\text{NG}}$) and maximal secretion for P.A.H. ($T_{\text{MPA.H.}}$).

In practice a quick determination of the glomerular filtration rate can be found by estimating the creatinine clearance.

\[
\text{Creatinine clearance} = \frac{\text{Urine creatinine}}{\text{Urine volume/24 hours}} \times \frac{\text{Serum creatinine}}{1440 \text{ minutes}}
\]

2. Prediction of Net Protein Utilization

Biologically, the value of a diet is affected by the amounts of its various components, for instance the nutritive value of the protein content is directly affected by the energy yielding constituents.

Platt, Miller and Payne (1961) have formulated a system whereby the nutritive values of the protein in various diets can be predicted. Thus:

\[
\Theta = S (1 - kP)
\]

where $\Theta = \text{net Protein utilization (N.P.U.)}$ or the efficiency with which the protein is utilized, and denotes combined digestibility and the biological value of the protein absorbed. So that N.P.U. is the product of biological value and digestibility and is a function of the amino acid composition and also of protein Calories.

Biological value represents the proportion of absorbed nitrogen which is retained. Net protein utilization represents the proportion of the intake (food) nitrogen retained. Therefore if dietary protein is completely absorbed and fully utilized then the biological value and the
net protein utilization = 100. In practice this level of efficiency is not achieved.

*S* = Protein score based on chemical data (F.A.O., 1957) from which proteins are given a score, depending on the essential amino acid pattern and based on the estimated amino acid requirements of man.

Sulphur containing amino acids are found to be the most limiting; therefore the values for methionine and cystine are considered to be critical.

\[ P = \text{Percentage of Protein Calories in the Diet (F. Cals %)} \]

K = a constant 0.019, the value for which has been determined experimentally by several workers, using various species and a wide range of protein and protein concentrations.

Miller and Payne (1961) in predicting the protein value of a diet assumed that the fraction of food protein which is diverted for energy is directly proportional to the concentration of protein calories in the diet.

\[ I_{e} = K P \quad \text{where} \quad I = \text{intake N (total)} \]

\[ I_{e} = \text{intake nitrogen used for energy (catabolic).} \]

This concept is based on a consideration of mass law, the rates of oxidation of amino acids and glucose are proportional to their concentration at the cell. It is shown that net protein utilization decreases at high protein concentrations.

N.P.U. represents the proportion of intake (food) nitrogen retained and can be measured by total body analysis or nitrogen balance procedure.

Thus \[ \Delta \quad \text{Balance depends on} \]

Nitrogen intake I

the efficiency of utilization of this intake N \[ \Theta \]
and obligatory nitrogen loss (M)

Therefore \( \Delta \text{Bal.} = I - \Theta - M \)

The factor M is the obligatory endogenous nitrogen losses, and this amount is supplied by a minimal protein intake which satisfies maintenance requirements.

M is composed of

- Urinary nitrogen (endogenous)
- Faecal nitrogen (metabolic)
- Integumental nitrogen (dermal and hair)

and sweat (50 mg N/day/kg\(^{0.73}\)) total = 250 mg N/day/kg\(^{0.73}\)

Integumental and dermal losses are not known in uraemic patients, and may differ from those of normal individuals. Therefore in all calculations M is taken as 200 instead of 250 mg N/day/kg\(^{0.73}\).

3. a. Diets used by Borst (1948) in the treatment of uraemic patients

\( e.g. \) butter soup

- sugar 150 g
- salt free butter 150 g
- flour 20 g
- water 300 g
- coffee extract
- Vitamins parenterally

supplying 1,775 Calories
2 g protein divided into six aliquots

OR

Frozen butter pills

- 200 g sugar
- 200 g butter

supplying frozen 2,330 Calories
0.8 g protein

230.
b. Intra-gastric drip feed used by Bull and Joekes (1949)

Glucose 400 g 
peanut oil 100 g
Acacia to emulsify
water up to 1 litre
contains 2,500 Calories and is dripped at a steady rate through 24 hours. Vomit is collected; sieved to return through the tube.

c. Kolff (1952) used various alternatives based on Borst's butter soup for treatment uraemic patients.

e.g. cooked rice and butter
baked potato and butter
jelly, biscuit and butter
pureed tomato, sugar and butter
cooked celery, butter and cream
or frozen butter pills x 30 to provide 150 g butter and 1,150 Calories.
ACKNOWLEDGEMENTS

This work has only been possible through the co-operation of the eight patients who participated in the study.

I am especially grateful to Professor J.W.T. Dickerson and to Dr. H.A. Lee for the time they have given, and for their most valuable help and encouragement.

I am also very indebted to Professor A. Polak for the facilities which were made available for me during the course of these investigations.

I must also thank Miss Pamela Andrews for her expert assistance with the typescript, and I would like to record how much I appreciate what she has done.
REFERENCES


EDELMAN, I.S., HALEY, H.B., SCHLOSSER, F.R., SHALDON, D.B.,
FRIIS-HANSEN, B.J., STOLL, G. and MOORE, F.D. (1952). Surgery,
Gynaecology and Obstetrics, 95, 1.
Butterworth, London.
FISHERBERG, A.H. (1944). In Hypertension and Nephritis, 127, 228, 229.
Lea and Febiger, Philadelphia.
FOOD AND AGRICULTURE ORGANISATION (1957). Protein requirements.
F.A.O. Rome.
FORD, J., PHILLIPS, M.E., TOTIE, F.E., LUCK, V.A. and DEWARDENER, H.E.


J. Biol. Chem., 91, 373.
Biochem., 40, 1663.
Economics Research Report No. 4. United States Department of
Agriculture.
Perkoff, G.T., Thomas, C.L., Newton, J.D., Selman, J.C. and Tyler, F.H.
Platt, B.S., Miller, D.S. and Payne, P.R. (1961). In recent advances
Endocrinology, 5, 367.
Her Majesty's stationery office, London.
Richards, P.; Metcalfe-Gibson, A., Ward, E.E., Wrigng, O.H. and

THE EFFECT OF CALORIC INTAKE ON NITROGEN BALANCE IN CHRONIC RENAL FAILURE

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(Received 24 July 1972)

SUMMARY

1. Nitrogen balance in uraemic patients on similar nitrogen intakes improves with increasing caloric intake in the range of 36–55 cal/kg body weight.
2. The degree of improvement in nitrogen balance is compatible with increased utilization of endogenous nitrogen probably as a result of increased dietary calories.

Key words: calories, nitrogen balance, chronic renal failure.

Giordano (1963) demonstrated that nitrogen equilibrium could be achieved in chronic renal failure on a nitrogen intake which was less than the minimal nitrogen requirement for normal subjects. His diet was mainly synthetic, and a practical diet with low-protein foods was first devised by Giovannetti & Maggiore (1964), later modified to suit British palates by Shaw, Bazzard, Booth, Nilwarangkur & Berlyne (1965). This diet contained a large proportion of high-biological-value protein providing all the essential amino acids except methionine in the amounts recommended by Rose, Wixom, Lockhart & Lambert (1955), a methionine supplement and an adequate supply of calories.

Since that time there has been considerable controversy about the minimal nitrogen requirement for equilibrium in chronic renal failure. Shaw et al. (1965) recommended 0.26 g of protein per kg/body weight, Giovannetti & Maggiore (1964) 0.3 g and Ford, Phillips, Toye, Luck & de Wardener (1969) 0.5 g. Giovannetti (1966) stated that for the diet to be successful a high caloric intake was necessary. Ford et al. (1969) concluded that caloric intake had no effect on nitrogen metabolism in chronic renal failure. However, since their experimental protocol did not compare the effects of changing caloric intakes on nitrogen balance while on isonitrogenous diets, their nitrogen intake changing over a fourfold range, no firm conclusions can be drawn. We report here the results of an investigation into the effect of caloric intake on nitrogen metabolism in chronic renal failure.

MATERIALS AND METHODS

Patients

Our observations are based on studies carried out on seven patients with different degrees of stable chronic renal failure, all of whom remained uninfected throughout the balance.

Correspondence: Dr H. A. Lee, Wessex Regional Unit, St Mary's Hospital, Milton Road, Portsmouth.
Table 1. Clinical details of seven patients with stable chronic renal failure on whom nitrogen balance studies were carried out

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Creatinine (mg/100 ml)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Blood urea (mg/100 ml)</th>
<th>B.P.</th>
<th>Haemoglobin (g/100 ml)</th>
<th>Albumin (g/100 ml)</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.T.</td>
<td>42</td>
<td>M</td>
<td>88.9 85.3</td>
<td>8.5 8.0</td>
<td>&lt;5 18.6</td>
<td>74 92</td>
<td>130 90</td>
<td>11.2 3.3</td>
<td>3.4 Glomerulonephritis</td>
<td></td>
</tr>
<tr>
<td>C.B.</td>
<td>36</td>
<td>M</td>
<td>50 50.6</td>
<td>6.0 5.1</td>
<td>18.6 68</td>
<td>38</td>
<td>160 100</td>
<td>14.4 5.0</td>
<td>5.0 Analgesic nephropathy</td>
<td></td>
</tr>
<tr>
<td>J.W.</td>
<td>31</td>
<td>M</td>
<td>66.2 68.1</td>
<td>17.5 18.0</td>
<td>&lt;5 96</td>
<td>80</td>
<td>170 80</td>
<td>11.3 3.6</td>
<td>3.6 ? Chronic pyelonephritis</td>
<td></td>
</tr>
<tr>
<td>T.G.</td>
<td>57</td>
<td>M</td>
<td>62.8 61.9</td>
<td>15.5 3.8</td>
<td>135 120</td>
<td></td>
<td>180 100</td>
<td>7.6 3.4</td>
<td>3.6 ? Chronic pyelonephritis</td>
<td></td>
</tr>
<tr>
<td>M.H.</td>
<td>49</td>
<td>F</td>
<td>46.3 47.8</td>
<td>12.6 13.0</td>
<td>3.4 134</td>
<td>103</td>
<td>160 60</td>
<td>6.9 4.0</td>
<td>3.6 Polycystic kidneys</td>
<td></td>
</tr>
<tr>
<td>R.M.</td>
<td>46</td>
<td>F</td>
<td>50.8 51.5</td>
<td>13.5 14.0</td>
<td>1.5 190</td>
<td>150</td>
<td>140 85</td>
<td>5.8 3.1</td>
<td>3.1 ? Gouty nephropathy</td>
<td></td>
</tr>
<tr>
<td>I.H.</td>
<td>44</td>
<td>F</td>
<td>49.6 50.5</td>
<td>9.7 9.0</td>
<td>6.0 94</td>
<td>82</td>
<td>130 80</td>
<td>8.8 3.1</td>
<td>2.8 Polycystic kidneys</td>
<td></td>
</tr>
</tbody>
</table>
Calorie intake and nitrogen balance in uraemia

studies. None of these patients required dialysis during the observation period or had required dialysis before starting these balance studies. All were volunteers who gave informed consent to the studies after a full explanation of the protocol. The clinical details of the patients before balance are shown in Table 1.

Methods

Seven nitrogen balances were carried out on seven patients in a Metabolic Ward according to the principles of Reifenstein, Albright & Wells (1945). Balances were carried out with a constant dietary intake of known nitrogen and caloric content, over 4-day periods totalling 8 days in two studies, 12 days in two studies, 16 days in one study and 24 days in two studies.

Before balance studies urinary excretion of protein, urea and electrolytes were measured on two 24 h urine specimens, with a diet closely approximating to the experimental one.

Urine was collected over 48 h periods and kept at 4°C. Faecal collections were made over 4 days by using carmine markers to delineate balance periods. Blood for urea, electrolytes, total protein, albumin and haemoglobin was taken before breakfast on day 1 and at 4-day intervals, the final specimen being taken on the morning after the end of the last balance period.

Urea was measured by the diacetyl monoxine method (A.C.P. Technical Bulletin Number 9) and creatinine by automatic colorimetry (Technicon AutoAnalyzer, method N.116). Sodium and potassium were measured by flame photometry (Technicon AutoAnalyzer, method N.21A). Serum bicarbonate was measured by the phenolphthalein method (Technicon AutoAnalyzer, method N.8B) and total serum protein by the biuret method. Albumin was measured by the Bromocresol Green method (A.C.P. Technical Bulletin Number 11). Haemoglobin was measured on a Coulter S automatic counter. Urine, faeces, and diet were analysed for total nitrogen by a micro-Kjeldahl method (Ingram, 1962).

Since urea may be utilized as a nitrogen source in chronic renal failure the net body-protein nitrogen change \( B' \) was estimated from the external balance \( B \) by allowing for changes in body urea nitrogen. Urea was assumed to be distributed equally throughout total body water at the same concentration as in plasma. Total body water was taken as 65% of body weight in men and 52% in women (Edelman, Haley, Schloerb, Shalton, Friis-Hansen, Stoll & Moore, 1952) and changes in plasma urea between the beginning and end of each 4-day period were converted into changes in total body-water urea nitrogen in g per day \( \Delta \text{UN} \). Thus \( B' = B - \Delta \text{UN} \).

Diet

A diet as close as possible to the experimental diet was taken for 1–2 weeks as an outpatient, and the experimental diet was started as an in-patient 4 days before the balance studies began except in one patient R.M. A detailed dietary history was taken from each patient and the experimental diet tailored to suit individual tastes.

The diet was designed to provide a net intake of 40–45 mg of nitrogen/kg body wt. per day in five studies and 50–54 mg/kg per day in two studies. The total required dietary nitrogen was calculated by adding the amount of urinary protein nitrogen excreted per day to the net nitrogen intake. The main source of high-biological-value protein was whole egg which was served fried (three studies) or made into a pancake with low-protein flour (two studies) or made into a waffle (one study). Milk, double cream and butter supplied the rest of the high-
Table 2. Nitrogen balance data on seven patients with stable chronic renal failure. The results shown are the mean values per day for the whole balance study.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Balance duration (days)</th>
<th>Total protein nitrogen (g/day)</th>
<th>Urinary protein nitrogen (g/day)</th>
<th>Net protein nitrogen (mg/kg)</th>
<th>Calories (total) (cal/kg)</th>
<th>Urinary nitrogen (g/day)</th>
<th>Faecal nitrogen (g/day)</th>
<th>Rejects nitrogen (g/day)</th>
<th>External balance (B)</th>
<th>Δ UN (g/day) (mg/kg per day)</th>
<th>B' = B − Δ UN Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.T.</td>
<td>8</td>
<td>3.68</td>
<td>1.17</td>
<td>2.51</td>
<td>29.0</td>
<td>3200</td>
<td>3.47</td>
<td>1.44</td>
<td>−1.23</td>
<td>0.6</td>
<td>−1.83</td>
</tr>
<tr>
<td>C.B.</td>
<td>12</td>
<td>2.72</td>
<td>0.384</td>
<td>2.34</td>
<td>46.0</td>
<td>2150</td>
<td>2.84</td>
<td>1.11</td>
<td>−1.23</td>
<td>−0.38</td>
<td>−0.91</td>
</tr>
<tr>
<td>J.W.</td>
<td>8</td>
<td>2.80</td>
<td>0.256</td>
<td>2.54</td>
<td>39.0</td>
<td>3700</td>
<td>2.55</td>
<td>0.964</td>
<td>−0.71</td>
<td>−0.41</td>
<td>−0.30</td>
</tr>
<tr>
<td>T.G.</td>
<td>16</td>
<td>2.84</td>
<td>0.36</td>
<td>2.48</td>
<td>39.4</td>
<td>2490</td>
<td>2.85</td>
<td>1.23</td>
<td>−1.24</td>
<td>−0.18</td>
<td>−1.06</td>
</tr>
<tr>
<td>M.H.</td>
<td>24</td>
<td>2.71</td>
<td>0.16</td>
<td>2.55</td>
<td>54.0</td>
<td>2170</td>
<td>2.51</td>
<td>0.81</td>
<td>0.05</td>
<td>0.66</td>
<td>−0.5</td>
</tr>
<tr>
<td>R.M.</td>
<td>12</td>
<td>2.10</td>
<td>0.112</td>
<td>1.99</td>
<td>38.7</td>
<td>2760</td>
<td>2.14</td>
<td>0.673</td>
<td>0.06</td>
<td>0.77</td>
<td>−0.36</td>
</tr>
<tr>
<td>I.H.</td>
<td>24</td>
<td>2.79</td>
<td>0.31</td>
<td>2.48</td>
<td>50.0</td>
<td>2600</td>
<td>2.36</td>
<td>0.763</td>
<td>−0.34</td>
<td>0.06</td>
<td>−0.28</td>
</tr>
</tbody>
</table>
biological-value protein which was kept between 70% and 80% of the total protein intake. Second-class protein was contained in vegetables and fruit, low-protein bread (wheatstarch flour, Energen, Rite Diet) and crispbread (Aproten crispbread, Carlo Erba), jam, marmalade, sugar and cornflour.

The caloric intake was kept constant in any one study and ranged between 36 and 55 cal/kg per day. The percentage carbohydrate calories varied between 50% and 70%. The carbohydrate calorie sources were Hycal (Beecham's Limited), Caloreen (Scientific Hospital Supplies Limited), wheatstarch, glucose, jam, sugar, cornflour and sucrose. The fat calories were derived from butter and double cream.

A duplicate 24 h diet was analysed for total nitrogen in each 4-day period. All rejects were collected and analysed in 4-day pools. The essential amino acid content of each diet was estimated from tables prepared by Orr & Watt (1957) and met the minimal requirements as set out by Rose et al. (1955) except for methionine. No methionine supplement was given. The sodium content of the diet was based on known 24 h urine sodium excretions measured before balance. All subjects took vitamin C (75 mg/day) and compound vitamin B supplements (four tablets daily).

RESULTS

Balance data on all patients are summarized in Table 2. Balance technique was considered satisfactory in all seven studies and all patients were in a steady state throughout the balance studies. The results of a typical nitrogen balance study are shown in Fig. 1.

The nitrogen intake achieved in these patients was very close to that desired with a variation of 39–54 mg/kg body weight except in one case J.T. (29 mg/kg body weight) where proteinuria had been underestimated.
Caloric intake and nitrogen balance

Fig. 2 shows the relationship between B' expressed in mg of nitrogen/kg body weight and caloric intake in cal/kg body weight. Although the net nitrogen intakes are not exactly the same a regression line shows a relationship with caloric intake. Fig. 3 shows that over the narrow range of net nitrogen intake obtained in our experiments there was no correlation between net intake and B'.

In Fig. 2 two points have been added from the data of Ford et al. (1969) and two from Herndon, Freeman & Cleveland (1958). These four results have been selected from these workers' data because the nitrogen intake corresponded closely to that used in the present study.

Urinary nitrogen, B' and dietary nitrogen

Table 3 shows no correlation between urinary nitrogen and B'. It is of interest that urinary nitrogen is similar to total dietary intake at all values of B', the urinary nitrogen/dietary nitrogen ratio approaching close to 100% (98.4 ± 11.1% SD) irrespective of B'.

Caloric intake, blood urea and B'

No relationship was found between the amount of blood urea and B' nor was blood urea correlated with faecal nitrogen (B. E. B. Hyne, unpublished observations).
Calorie intake and nitrogen balance in uraemia

Fig. 3. Variation of B' (corrected nitrogen balance) with increased net dietary nitrogen intake within the range obtained in these patients, showing no significant improvement.

Table 3. Urinary nitrogen, B' and dietary nitrogen. Note how urinary nitrogen closely approximates total nitrogen intake at all values.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Urinary nitrogen (mg/kg)</th>
<th>B' (mg/kg)</th>
<th>Total nitrogen intake (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.T.</td>
<td>40.1</td>
<td>-21.17</td>
<td>42.5</td>
</tr>
<tr>
<td>C.B.</td>
<td>56.3</td>
<td>-16.86</td>
<td>53.9</td>
</tr>
<tr>
<td>J.W.</td>
<td>37.9</td>
<td>-4.5</td>
<td>41.6</td>
</tr>
<tr>
<td>T.G.</td>
<td>45.9</td>
<td>-17.1</td>
<td>45.8</td>
</tr>
<tr>
<td>M.H.</td>
<td>54.0</td>
<td>-10.9</td>
<td>54.0</td>
</tr>
<tr>
<td>R.M.</td>
<td>41.6</td>
<td>-7.1</td>
<td>40.8</td>
</tr>
<tr>
<td>I.H.</td>
<td>47.2</td>
<td>-5.6</td>
<td>55.5</td>
</tr>
</tbody>
</table>

Discussion

Considerable experimental work has shown an inter-relation between nitrogen balance, dietary nitrogen intake and caloric intake. Calloway & Spector (1954) in a survey of the literature concluded that 'at each fixed adequate protein intake there is an individual limiting energy level beyond which increasing calories without protein or protein without calories is without
benefit. For normal men on a mixed diet with a dietary nitrogen intake of 3-4 g daily no further improvement in nitrogen balance can be achieved by increasing the daily caloric intake above 800. The nitrogen balance in such individuals was about — 4 g of nitrogen daily. This implies that no further improvement in nitrogen retention can be achieved by increasing the caloric intake above 800 calories if the protein intake is not also increased. In contrast, considerably better nitrogen balance can be achieved in chronic renal failure on nitrogen intakes of about 3 g/day provided that the diet contains a high proportion of high-biological-value protein. Giordano (1963) suggested that this improvement might be due to reutilization of ammonia derived from urea by hydrolysis by gut bacteria. Work by Rose & Dekker (1956) in the rat showed that ammonia and urea could be utilized as a 'non-essential' nitrogen source.

![Graph](attachment:image.png)

**Fig. 4.** Variation of $B'$ with the caloric intake. The solid line, calculated from the data of Miller & Payne (1963), represents the relationship between $B'$ and caloric intake in normal individuals on a low-nitrogen intake of 3-4 g per day. The single points show the improvement in $B'$ with increasing caloric intake in patients with chronic renal failure on similar low-nitrogen intakes. ○, Results from the present study; △, results from Ford et al. (1969); □, results from Herndon et al. (1958).

Walser & Bodenlos (1959) demonstrated recirculation of ammonia nitrogen derived from urea in normal subjects and Richards, Metcalfe-Gibson, Ward, Wrong & Houghton (1967) showed that uraemic subjects incorporated more ammonia nitrogen than normal subjects on a comparable diet. The total incorporation of ammonia nitrogen increased if a low-protein high-biological-value-protein diet was given.

Our work has confirmed the value of low-protein diets containing a high proportion of high-biological-value protein in maintaining nitrogen equilibrium in uraemic subjects. In addition we have shown that a further improvement can be obtained by increasing the caloric intake to 55 cal/kg body weight, i.e. a total daily caloric intake of 3850 cal for a 70 kg patient. This degree of improvement cannot be explained by a greater utilization of dietary nitrogen.
Calorie intake and nitrogen balance in uraemia

which should be maximal at a much lower caloric intake. This is shown in Fig. 4. The solid line represents a theoretical maximum B' in normal subjects on a nitrogen intake of 3 g derived from the equations of Miller & Payne (1963) and assuming a protein source of maximum biological value. It can be seen that little improvement in B' can be expected above 25 cal/kg. Additional points from studies by Ford et al. (1969) and Herndon et al. (1958) suggest that below caloric intakes of 30 cal/kg body weight a uraemic subject may utilize protein less efficiently than normal subjects. This is supported by the work of Robson, Kerr & Ashcroft (1967), who found an increased urea-production rate in patients on low-calorie low-mixed-protein diets which decreased if the caloric intake was increased or high-biological-value-protein diets used. They did not assess high-caloric intakes with high-biological-value-protein diets.

At present there is no evidence to show whether improvement continues with caloric intakes of above 55 cal/kg or if a plateau is reached in the region of 50-55 cal/kg. In any event most patients will be unable to tolerate a caloric intake of more than 60 cal/kg body weight.

It has been suggested (Shaw et al., 1965) that the main reason for the superiority of high-biological-value-protein diets over a mixed-protein diet is that the essential amino-acid requirement as defined by Rose et al. (1955) is met in this situation. However, work by Fisher, Brush & Grininger (1969) has shown that the requirement for phenylalanine and valine may be considerably decreased on a low-protein diet. Although our diets were deficient in methionine according to the requirements as set out by Rose et al. (1955) this did not prevent an improvement in B' with increasing caloric intake.

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REFERENCES


The recurrent haematuria syndrome. R. P. Burden, L. J. Booth, W. N. Boyd, B. G. Ockenden and G. M. Aber, Renal Research Unit, North Staffordshire Hospital Centre. Clinical features, renal function and histology have been correlated with selective renal angiographic appearances in twenty patients with either recurrent painless haematuria or recurrent loin pain with haematuria, in whom there was an absence of urinary infection. Renal function changes were noted in ten of the patients, and a structural abnormality of the urinary tract was found in all patients. The association between the relapsing nephrotic syndrome and specific allergy has only been recorded in a few cases (Hardwick et al, 1959; Wittig and Goldman, 1970) and is of great interest. We know of at least five patients with seasonal exacerbations of their nephrotic syndrome. We have investigated three of these in detail. In each, the nephrotic syndrome was associated with allergy to grass pollen. We have sought using two different antisera, and both direct and indirect techniques, but again none was detected. Early in 1972 a preliminary rise in IgA, and a positive Clq test for circulating IgG; a rise in total and antigen-specific IgE; a fall in C3; a rather diffuse increase in thickening of the basement membrane. A renal biopsy was performed at the time of the 1971 relapse and showed a "minimal change" pattern on light microscopy, the only feature of note being a focal increase in thickening of arteriolar walls was also seen. Renal angiography was normal in these individuals with painless haematuria but all those with loin pain and haematuria showed evidence of focal ischaemia and sometimes cortical infarcts. The differences between the two groups of patients and possible aetiological factors are discussed.

Seasonal nephrotic syndrome. W. G. Reeves, J. S. Cameron, C. S. Ogg, Guy's Hospital and the Royal Postgraduate Medical School, London. The association between the relapsing nephrotic syndrome and specific allergy has only been recorded in a few cases (Hardwick et al, 1959; Wittig and Goldman, 1970) but is of great interest. We know of at least five patients with seasonal exacerbations of their nephrotic syndrome; we have investigated three of these in detail. In each, the nephrotic syndrome was associated with allergy to grass pollen. We concentrate here on detailed findings in one of these patients—a boy aged 11 years when he first had the nephrotic syndrome in 1968. Various parameters were measured regularly from the autumn of 1969 to July 1972, and the seasons 1970 and 1971 have been analysed in detail. The main relapse of the syndrome occurred in June and July of each year, and the seasonal features of note in the plasma were: a fall in total and antigen-specific IgG; a rise in total and antigen-specific IgE; a fall in C3; a preliminary rise in IgA, and a positive Clq test for circulating antibody-antigen complexes. A renal biopsy was performed at the time of the 1971 relapse and showed a "minimal change" pattern on light microscopy, the only feature of note being a rather diffuse increase in thickening of the basement membrane on electron microscopy, most areas examined being 500 to 600 nm. Immunofluorescent staining failed to reveal any IgG, IgM, IgA, IgD and C3, fibrinogen or grass pollen antigen. IgE was sought using two different antisera, and both direct and indirect techniques, but again none was detected. Early in 1972 a course of desensitisation was given. During the following pollen season the patient had mild rhinitis but his urine remains protein-free. Another boy was treated with cyclophosphamide 3 mg/kg for 8 weeks in 1970; although he had only mild proteinuria for the past two grass pollen seasons he still suffers from severe hay fever. The total and antigen-specific IgE still show sharp rises. The third man first suffered a seasonal nephrotic syndrome in 1959, and now has persistent proteinuria, worse in June: his proteinuria also increases after corticosteroids are withdrawn. Possible mechanisms involved in the pathogenesis of this syndrome will be described, and their relevance for atopic diseases and the "minimal change" relapsing nephrotic syndrome discussed.

In vitro synthesis of rat glomerular basement membrane in nephrotoxic glomerulonephritis (NTN). M. R. Daha, J. de Graeff and A. A. H. Kassenaar, Departments of Nephrology and Chemical Pathology, University Hospital, Leiden, The Netherlands. The purpose of this study was to investigate the glomerular basement membrane (GBM) synthesis of normal and nephritic rats. Glomerulonephritis was induced by intravenous injection of rabbit-anti-rat-GBM-antiserum. Glomeruli for in vitro studies were isolated in the cold using the method of Krakower and Greenspon as modified by Spiro (1967). As an index of GBM synthesis the proline incorporation in the collagen of the GBM was used. The isolated glomeruli were incubated for seven hours in buffer containing 14C-proline. Glucose oxidation was linear up to 12 hr for normal glomeruli and up to 7 to 8 hr for NTN glomeruli. Two different incubation media were used: medium I, consisting of a Krebs-Ringer phosphate buffer; medium II, a Krebs-Ringer buffer with HEPES but without phosphate. Normal glomeruli thus incubated showed only minor changes by light and electron microscopy. The GBM synthesis, as measured by 14C-proline incorporation was 722 ± 60 dpm/100 μg DNA for normal glomeruli using medium I. This value was 1750 ± 92 dpm 24 hr after induction of NTN. In medium II these values were respectively 6671 ± 309 and 10257 ± 257. The GBM synthesis, in medium II was maximal between 6 and 16 hr after induction of NTN. A correlation was found between the amount of kidney-fixing antibodies administered and the maximal stimulation of GBM synthesis. We conclude that after induction of NTN an initial stimulation up to 100% of GBM synthesis occurs. A correlation between the immunological damage to the GBM and GBM synthesis seems to exist.

Immunoglobulin classes and complement components in glomerular deposits. Professor Ag, Jean Berger, Hôpital Necker, Paris. Complex-type nephritis is a broad concept in which are embraced several forms of glomerulonephritis (GN) which are quite different from a clinicopathological point of view. The composition of the deposits seems to define more precisely each type of GN. Some examples are as follows: 1) Chronic focal GN with haematuria is characterized by diffuse mesangial deposition of IgA, IgG, and C3. 2) The deposits in Henoch-Schönlein purpura nephritis contain IgA, IgG, C3 and fibrinogen. 3) IgM is the main component of the deposits in focal glomerulosclerosis. 4) The humps of acute GN contain IgG and much C3. On the other hand, the deposits in membranous GN contain IgG and little C3. 5) Deposition of C3 without immunoglobulins or early complement components appears to define a special form of membranoproliferative GN. 6) Clq is often prominent in lupus nephritis. It must be stressed that the composition of the deposits remains identical throughout the whole course of the disease and that the first indication of recurrence in kidney transplants is the deposition of complexes with the same composition as in the patient's own kidneys.
Renal haemodynamic studies and haemodynamic studies in terminal uraemics before and after diuretic therapy. Ugur Ulku and Kemai Onen, Cerrahpaşa Medical Faculty, Istanbul University, Turkey. The renal excretory patterns of solutes, renal functional and haemodynamic changes were investigated in 9 patients with end-stage kidney failure before and after 200 mg of furosemide i.v. during renal vein catheterisation. In six of the patients cardiac output was also determined. After furosemide, the values for V, UNaV, UKV, UGluV and GFR (8.68 ml/min) increased significantly. A slight rise of C(H2O) (from 0.1 to 0.35 ml/min) was observed and may be explained by the increased solute load to the counter-current multiplier and by a slight increase in its already depressed activity. The high values for C(Na)/GFR ratios (0.02) values suggest that the glomerulotubular balance is significantly altered and that the tubular fractional absorption of sodium is depressed. The high C(H2O) and cardiac output values (mean 7.6 L/min) suggest that the volume dependent mechanism for natriuresis is active. C(PAH) (52.3 ml/min) and RPF (184 ml/min) as calculated by the determination of EP A H (CP A H /EP A H ) were increased significantly. A slight rise of 0. H 2 O (from 0.1 to 0.35 ml/min) was observed and may be explained by the increased solute load to the counter-current multiplier and by a slight increase in its already depressed activity. The high values for C(Na)/GFR ratios (0.02) values suggest that the glomerulotubular balance is significantly altered and that the tubular fractional absorption of sodium is depressed. The high C(H2O) and cardiac output values (mean 7.6 L/min) suggest that the volume dependent mechanism for natriuresis is active. The renal excretory patterns of solutes, renal functional and haemodynamic changes were investigated in 9 patients with end-stage kidney failure before and after 200 mg of furosemide i.v. during renal vein catheterisation. In six of the patients cardiac output was also determined. After furosemide, the values for V, UNaV, UKV, UGluV and GFR (8.68 ml/min) increased significantly. A slight rise of C(H2O) (from 0.1 to 0.35 ml/min) was observed and may be explained by the increased solute load to the counter-current multiplier and by a slight increase in its already depressed activity. The high values for C(Na)/GFR ratios (0.02) values suggest that the glomerulotubular balance is significantly altered and that the tubular fractional absorption of sodium is depressed. The high C(H2O) and cardiac output values (mean 7.6 L/min) suggest that the volume dependent mechanism for natriuresis is active. C(PAH) (52.3 ml/min) and RPF (184 ml/min) as calculated by the determination of EP A H (CP A H /EP A H ) were different and TM(PAH) were found to be 5.88 and 11.8 mg/100 ml before and after furosemide respectively. The filtration fraction (FF) was extremely low (0.045). This suggests very low oncotic pressure in post glomerular peritubular capillaries which might be a factor working against the reabsorption of sodium. Arterial and renal venous O2 differences were 2.1 and 1.7 before and after furosemide (normal 1.5—1.7). Total O2 uptake by the kidney before and after furosemide was 5.02 and 4.05 ml/min. The differences were not statistically significant. TNa/O2 values were found 19.1 and 22.4 before and after furosemide. This suggests that the effect of furosemide is on the membrane transport of Na.

The renal clearance of a new amino acid, homoarginine, in normal subjects and patients with cystinuria. B. D. Cox, D. Calame and J. S. Cameron, Guy's Hospital, London, England. Cystinuria is characterised by the hyperexcretion of four dibasic amino acids: cystine, ornithine, arginine and lysine. Frimpter (1965) showed that in addition to these four aminoacids, patients with cystinuria also excreted the mixed disulphide of cystine and homocystine. Ion exchange chromatography of urinary guanidine compounds and aminoacids in 7 stone-forming cystinuric patients showed that all were excreting an unidentified Sakaguchi-negative amino acid in addition to the five compounds just discussed. Comparison with standard compounds suggested that this was homoarginine, previously reported in leguminous plants. Examination of urine from five normal subjects showed that homoarginine is also excreted in health, but in much smaller quantities. After concentration, plasmas from both normal subjects and the cystinuric patients were shown to contain homoarginine in quantities of about one hundredth that of arginine, the levels being somewhat lower in the cystinuric patients' plasma. The renal clearance of homoarginine was higher than that of arginine in normal subjects, but although the clearance was many times greater for both compounds in the cystinuric patients, in all those studied the clearance of arginine exceeded that of homoarginine. These findings support the suggestion of Dest and Rose (1951) that the tubular reabsorptive site (or sites) which are defective in cystinuria require an \( \alpha \)-amino acid group at one end of the molecule and another amino acid at the other with a chain of 4-6 carbon atoms between; and that yet other compounds which satisfy these requirements might be excreted in excess in cystinuric patients.

The effect of caloric intake on nitrogen balance in chronic renal failure. B. E. B. Hyne, Edna Powell, and H. A. Lee. Nitrogen balances were carried out on 7 adult patients in stable chronic renal failure. Diets were designed to give patients a nitrogen intake of between 40 and 54 mg/kg body weight. High biological value protein accounted for more than 70% of the protein allowance. The caloric intake was kept constant at any one study and ranged between 36 and 55 calories/kg body wt. Carbohydrate provided between 50 and 70% of the total caloric intake. No correlation was found between net nitrogen intake and nitrogen balance over the range studied. A highly significant correlation was obtained between caloric intake and nitrogen balance. Nitrogen balance in uraemic patients on similar nitrogen intakes improves with increasing caloric intake in the range 36-55 calories/kg body wt. Patients in advanced chronic renal failure, unlike normal individuals, show improvement in nitrogen balance on low nitrogen intakes (3-4 g/day) when the caloric intake is increased above 25 calories/kg body wt. The degree of the improvement in nitrogen balance is compatible with increased utilization of endogenous nitrogen probably as a result of increased dietary calories.

Plasma renin, exchangeable sodium and vascular reactivity in patients with terminal renal failure. S. M. Rosen and P. J. A. Robinson, Department of Renal Medicine, Leeds University Hospital and the General Infirmary at Leeds, Leeds, England. Plasma renin and exchangeable sodium were measured in two groups of patients with terminal renal failure treated by maintenance dialysis therapy. Group A consisted of seven patients with a mean blood pressure less than 110 mm Hg, and group B consisted of five hypertensive patients whose mean blood pressure was higher than 116 mm Hg. Mean plasma renin was higher in the hypertensive group. The higher levels of plasma renin in group B could not be the sole reason for the hypertension, since plasma renin is known to rise during dialysis in spite of the decrease in blood pressure. Although blood pressure could be reduced by the reduction of sodium in individuals during dialysis, the mean value for exchangeable sodium was marginally lower in the hypertensive group. In group A, there was an inverse correlation between plasma renin and exchangeable sodium, suggesting that the 'end-stage' kidney still responds to removal of sodium by release of renin. This correlation did not exist in group B, in which there was a higher concentration of plasma renin for a given level of exchangeable sodium. Vascular reactivity of forearm blood vessels in these patients was measured by infusion of noradrenaline into the brachial artery and determining the threshold dose required to produce an alteration in blood flow in the ipsilateral forearm. The threshold dose increased as the level of renin decreased for a given value of exchangeable sodium. These results support the hypothesis that the effect of renin on blood pressure is dependent on levels of exchangeable sodium and hypertension ensues when there is an inappropriately high concentration of plasma renin.