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DEPARTMENT OF RENAL AND METABOLIC MEDICINE

A STUDY OF NITROGEN REQUIREMENTS IN ACUTE RENAL FAILURE IN MAN

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ABSTRACT

FACULTY OF MEDICINE

RENAL AND METABOLIC MEDICINE

Master of Philosophy

A STUDY OF NITROGEN REQUIREMENTS IN ACUTE RENAL FAILURE IN MAN

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Acute renal failure (ARF) patients often show extreme muscle wasting as a result of net breakdown of body cell mass and this continues to be a major contributory factor to the high morbidity and mortality found in this condition, despite advances in treatment.

This study was designed to determine the degree of nitrogen loss found in ARF and the effects of increasing nitrogen intakes with the aim of reducing the degree of wasting and associated mortality.

Twenty-four patients with ARF of differing aetiology were studied and they received either total parenteral nutrition (TPN) or a commercial enteral feed.

Nitrogen balance, when adjusted for differences in endogenous muscle catabolism as measured by 3-Methylhistidine loss, was found to be linearly related to nitrogen intake ($P<0.02$). All patients were in negative nitrogen balance and although urea appearance correlated with nitrogen intake ($P<0.001$) only 50% of increased nitrogen intake was wasted as urea. The beneficial effect of giving upto 200 mg N kg^{-1} day^{-1} was shown.

Haemodialysis therapy was found to increase urea appearance and amino acid losses with haemodialysis and CAVH therapies were similar.

Anthropometry and plasma albumin and prealbumin concentrations were not useful in assessing changes in nutritional status in this short-term study, and TPN was required to provide an adequate nitrogen intake.

It was concluded that upto 200 mg N kg^{-1} day^{-1} as TPN should be prescribed in ARF.

DEDICATION

This thesis is dedicated to the memory of my Mother and Father.

STATEMENT OF ORIGINALITY

The author undertook all sample collections and analyses in this thesis with the exception of those routine blood measurements required for the clinical management.

Please see Acknowledgements for assistance rendered by others.

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ABBREVIATIONS USED IN THIS THESIS

°C	: degree(s) centigrade
α-KG	: alpha-ketoglutarate
μ	: micro- (10^{-6})
1-MH	: 1-methylhistidine
3-MH	: 3-methylhistidine (N-tau-methylhistidine)
AA	: amino acid(s)
Aad	: L-α-aminoadipate
Abu	: L-α-amino-n-butyrate
AC	: alternating current
ADX	: adrenalectomy
AIN	: acute interstitial nephritis
Ala	: L-alanine
approx.	: approximately
ARF	: acute renal failure
Arg	: L-arginine
Asn	: L-asparagine
Asp	: L-aspartate
ATN	: acute tubular necrosis
ATP	: Adenosine triphosphate
b	: byte
BCAA	: branched-chain amino acids
BCKA	: branched-chain ketoacid
BNX	: bilateral nephrectomy
BW	: body weight
CAVH	: continuous arterio-venous haemofiltration
CCF	: congestive cardiac failure
cf.	: confer (= compare)
Cit	: L-citrulline
CRF	: chronic renal failure
CRP	: C-reactive protein
Cys	: L-cystine
cal	: calorie
cm	: centimetre
conc	: concentration
DNA	: Deoxyribonucleic acid

ABBREVIATIONS

D & V	: diarrhoea and vomiting
EAA	: essential amino acids
ECF	: extracellular fluid
EE	: energy expenditure
e.g.	: for example (exempli gratia)
F	: female
GFR	: glomerular filtration rate
GI	: gastro-intestinal
Gln	: L-glutamine
Glu	: L-glutamate
Gly	: glycine
g	: grammes
Hcy(Ala)	: L-cystathionine
HD	: haemodialysis
HF	: haemofiltration
Hg	: mercury
His	: L-histidine
hr	: hour(s)
ICF	: intracellular fluid
IL-1	: interleukin-1
Ile	: L-isoleucine
IV	: intravenous
i.e.	: that is (id est)
I.D.	: internal diameter
J	: Joule
KIC	: α-ketoisocaproate
Km	: substrate concentration producing half-maximal velocity (Michaelis constant)
k	: kilo- (10^3)
Leu	: L-leucine
Lys	: L-lysine
l	: litre
MAC	: mid-arm circumference
MAMC	: mid-arm muscle circumference
Met	: L-methionine

ABBREVIATIONS

MLR	: multiple linear regression
MPGN	: membranoproliferative glomerulonephritis
M	: male
M	: mega- (10^6)
min	: minute(s)
mol	: moles(s)
m	: metre(s)
m	: milli- (10^{-3})
NADP	: nicotinamide-adenine dinucleotide phosphate
NAD	: nicotinamide-adenine dinucleotide
NB	: nitrogen balance
NEAA	: non-essential amino acids
NG	: naso-gastric
NI	: nitrogen intake
No. or n	: number
N	: nitrogen
N	: no
n	: nano- (10^{-9})
N.S.	: not significant
NS	: non-survivors
OAA	: oxaloacetate
Orn	: L-ornithine
Pa	: Pascal
PD	: peritoneal dialysis
PGE_2	: prostaglandin E_2
Phe	: L-phenylalanine
PIF	: proteolysis inducing factor
PP_i	: pyrophosphate (inorganic)
Pro	: L-proline
P_i	: orthophosphate (inorganic)
P	: probability of an event being due to chance alone
pH	: negative logarithm of the hydrogen ion concentration
RBF	: renal blood flow
RNA	: ribonucleic acid

ABBREVIATIONS

RQ	: respiratory quotient
rpm	: revolution(s) per minute
S	: survivors
S.D.	: standard deviation
SEM	: standard error of the mean
Ser	: L-serine
SLE	: systemic lupus erythematosus
sec	: second(s)
Tau	: L-taurine
TBPA	: thyroxine-binding pre-albumin
Thr	: L-threonine
TNF	: tumour necrosis factor
TPN	: total parenteral nutrition
Trp	: L-tryptophan
TSFT	: triceps skin-fold thickness
Tyr	: L-tyrosine
UAR	: urea appearance rate
UNA	: urea nitrogen appearance
UUN	: urine urea nitrogen
U.V.	: ultra-violet
U	: unit(s)
Val	: L-valine
Vmax	: maximal velocity
V	: volt(s)
vs	: versus
WBC	: white blood cell; leucocyte
w/v	: weight for volume
Y	: yes
yr	: year(s)
®	: registered trade mark
et al.	: and others (et alii)
%	: percent
<	: less than
>	: greater than
±	: plus or minus

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Acute renal failure (ARF) is a frequent and dramatic clinical syndrome that is associated with a wide variety of serious and potentially lethal disorders but is one of the few diseases which is completely reversible (Finn, 1983; Thibault et al., 1980).

A high morbidity and mortality rate is still associated with this syndrome and has remained at 40-50% despite improved dialysis techniques, modern drugs and other advances in clinical management. That this figure has not significantly improved since 1950 (Finn, 1983; Stott et al., 1972) cannot be entirely explained by changes in the medical population being accepted for treatment or the tendency for the mean patient age to rise (Stott et al., 1972; Kennedy et al., 1973; Kleinknecht and Ganeval, 1973).

Nutrition is one aspect of the clinical management of ARF that still allows scope for improvement and that is likely to affect the morbidity and mortality rates as a consequence of influencing the net loss of body cell mass (Condon and Asatoor, 1971; Peters et al., 1968; Acchiardo et al., 1983).

Although some nutritional studies have shown improved survival when amino acid and glucose infusions were compared with glucose alone (Abel et al., 1973; Rainford, 1981, 1987; Baek et al., 1975), others have not (Lee et al., 1967; Leonard et al., 1975; Pelosi et al., 1981; Proietti et al., 1983). Further investigations are therefore warranted to enable nutritional management to be improved, with the aim of reducing the associated mortality (Abel et al., 1973) and increasing the rate of recovery of renal function (Abel et al., 1973; Toback, 1977).

Most nutritional studies have categorised ARF patients on pathological grounds and not on a biochemical measurement of the protein catabolic rate. However, it is the protein catabolic rate which will have the greatest effect on the nutritional status. Therefore, for the purposes of a nutritional study, it is more useful

to categorise patients according to their protein catabolic rate. In addition, past nitrogen balance studies have generally relied on calculated losses of certain excretory products rather than analysing all samples. This may have introduced considerable errors, especially where haemodialysis is concerned. In this study all samples have been analysed for nitrogen, including haemodialysis effluent, the only items which have not been included are integumental and respiratory losses.

From the foregoing it can be seen that, notwithstanding advances in clinical management, ARF patients still exhibit a high morbidity and mortality, associated with a net loss of body protein. Improving their nutritional status is one of the few aspects of therapy where there is room for advancement and by reducing the net loss of body cell mass, a parallel reduction in the morbidity and mortality rates may be effected.

The remainder of this chapter will provide evidence in support of the statements made above.

1.2 Acute Renal Failure - Definition and Causes

1.2.1 Introduction

ARF can be defined as a rapid decrease in renal function causing rapidly progressive uraemia and usually oliguria (Levinsky et al., 1981). Plasma creatinine concentration rises by 45-90 $\mu mol\ l^{-1}\ day^{-1}$ and serum urea can rise by 3.5-7 $mmol\ l^{-1}\ day^{-1}$ (Relman, 1964). The serum urea may also rise because the rate of protein catabolism increases, upto 36 $mmol\ l^{-1}\ day^{-1}$ in hypercatabolic patients (Luke and Kennedy, 1967; Bricker, 1975), whilst creatinine can rise $>180\ \mu mol\ l^{-1}\ day^{-1}$ if there is significant rhabdomyolysis, (Minuth et al., 1976) or trauma (Espinel and Gregory, 1980).

Renal regulation begins with ultrafiltration of the blood, proceeds through intrarenal processing of the filtrate by the renal tubule secretory and absorptive mechanisms and ends with excretion of the urine via ureteral, vesicular and urethral conduits. Therefore the ARF syndrome may arise from :-

1) diminished renal blood flow - pre-renal ARF
2) a sudden, severe parenchymal insult - intrinsic ARF or
3) an obstruction to urine flow - post-renal ARF.

Although renal hypoperfusion, acute renal parenchymal damage and urinary tract obstruction all ultimately reduce the glomerular filtration rate, each disorder has a different and characteristic response.

1.2.2 Pre-Renal Acute Renal Failure

Prerenal acute renal failure is the most common cause of acute uraemia in hospitalised patients and is usually rapidly reversible, and is caused by some combination of hypotension, hypovolaemia and diminished renal perfusion (Bushinsky et al., 1979).

The mechanism maintaining the relative constancy of renal blood flow (RBF) and glomerular filtration rate (GFR) despite large changes in renal perfusion pressure is termed autoregulation (Thurau, 1964). This is thought to be a property intrinsic to the kidney since denervated and isolated perfused kidneys can sustain a stable blood flow and filtration during hypotension. However, in the intact animal, as the mean renal arterial blood pressure decreases progressively below 80 mm Hg (10.7 kPa), RBF and GFR fall progressively.

Disorders causing prerenal ARF can often cause intense hormonal responses which may modify the normal autoregulatory action. Severe renal vasoconstriction from both locally released and circulating renin angiotensin and catecholamines may lead to reductions in RBF and GFR (Thurau, 1964). Angiotensin II reduces its own vasoconstrictive effect by stimulating renal release of prostaglandins which cause renal vasodilatation. Heinrich et al. (1978) showed that the renal vasoconstrictive effect of a given degree of haemorrhage increases when indomethacin or other inhibitors of prostaglandin synthesis are present. The increased renal resistance seen with haemorrhage is the algebraic sum of the vasoconstricting effect of these hormones and the opposing vasodilatory effect of simultaneously released prostaglandins.

There is also evidence for the existence of an intrarenal baroreceptor. Davis (1973) reported that a decrease in renal perfusion pressure stimulates renin release. This is demonstrable in the non-filtering kidney and therefore is not dependent on solute delivery to the macula densa. This also occurs in denervated kidney and is not therefore dependent on the autonomic nervous system. This response is also present in the adrenalectomised animal and is not therefore dependent upon circulating catecholamines. In addition, plasma renin activity (Brown et al., 1964), angiotensin II (Brown

et al., 1972) and angiotensin III (des-Asparty1 AII) (Schambelan and Stockigt, 1979) are high in extracellular fluid volume depletion and cause an increased peripheral resistance via reflex vasoconstriction. The autonomic nervous system also functions in this way by interacting with the renin-angiotensin system (Zimmerman et al., 1972; Khairallah, 1972).

Prerenal stimuli causing these renal function changes may result from true intravascular volume depletion - as in haemorrhage, diarrhoea or over-diuresis - or from redistribution of the plasma volume to a 'third' space - as in rapidly developing ascites or oedema. A decrease in the effective arterial blood volume and consequent renal hypoperfusion is often seen in congestive cardiac failure (CCF) or advanced liver disease. In these conditions the decreased cardiac output and increased hepatic venous resistance cause blood to collect in systemic and portal veins with a consequently reduced arterial volume. The reduced arterial volume is detected by the kidneys and results in sodium and water retention to expand a falsely depleted vascular space (Rudnick et al., 1983). The way in which cardiac output and arterial pressure are decreased appears to influence the characteristics of the renal response. Haemorrhage causes an intense vasoconstriction, which may markedly diminish RBF and GFR, but when acute myocardial infarction reduces blood pressure and cardiac output to an equal degree RBF and GFR are much less severely reduced (Gorfinkel et al., 1972).

1.2.3 Intrinsic Acute Renal Failure

Introduction

Intrinsic ARF is caused by certain renal parenchymal insults which rapidly depress GFR, causing the blood urea and serum creatinine concentrations to rise progressively and the urine volume to decrease.

Intrinsic ARF is best classified according to the main area of damage. It has been suggested that 10-20% of cases of intrinsic ARF are caused by acute vasculitides, glomerulonephritides or acute interstitial nephritides (Suki et al., 1966), but it is useful to distinguish between hospital-acquired and non-hospital-acquired intrinsic ARF. The vast majority of hospital-acquired intrinsic ARF is caused by various forms of acute tubular necrosis (ATN) (Bushinsky et al., 1979), whilst the population acquiring ARF outside the hospital manifests a greater incidence of acute glomerular, interstitial and vascular disease. This is especially true in the paediatric population where non-ATN accounts for over 50% of cases (Anderson et al., 1977; Hodson et al., 1976).

Intrarenal Vascular Diseases and Coagulopathies

The vasculitides comprise the polyarteritis (macro- and micro-) nodosa group of disorders, hypersensitivity angiitides, Wegener's granulomatosis, scleroderma and the adult haemolytic-uraemic syndrome; Henoch-Schonlein purpura can be included in this group or with the glomerulonephritides as there can be an extra-renal vasculitis with a proliferative glomerulonephritis.

In the vasculitides the occlusion of small blood vessels by fibrin and platelet clots and progressive inflammatory constriction of vascular lumina causes glomerular ischaemia leading to diminished RBF, GFR and eventually ischaemic necrosis of glomeruli. Mesangial and endothelial cell proliferation can also develop, thereby further decreasing glomerular function. With disruption of the glomerular basement membrane, fibrin and fibrinogen pass from capillaries to Bowman's space, causing an intense proliferation of epithelial cells.

The inflammatory reaction attracts extra-renal macrophages, which, with proliferating epithelial cells leads to the formation of a characteristic crescent. This vasculitis-induced renal insufficiency is always associated with ischaemia, while more advanced and aggressive cases will show infarctive necrosis of the glomeruli and variable degrees of proliferation of cellular elements in the glomerulus.

Acute Glomerulonephritis.

These are glomerulonephritides of rapid onset and are characterised by hypertension, proteinuria, haematuria and variable extra-renal manifestations.

Although proteinuria is nearly always present in acute glomerulonephritis, the full clinical picture of nephrotic syndrome is normally only associated with membranoproliferative glomerulonephritis (MPGN) and systemic lupus erythematosus (SLE). Hypertension is commonly associated with all forms of acute glomerulonephritis, except for idiopathic, rapidly progressive glomerulonephritis and is due to sodium and water retention which leads to intravascular volume overload and consequently an elevated blood pressure (Schwartz and Kassirer, 1971).

When intrinsic ARF is caused by acute glomerulonephritis, cellular proliferation is nearly always present and the decreased glomerular capillary surface area accounts for the uraemia and oliguria.

Acute Interstitial Nephritis

Only a few diseases involving the renal interstitium abruptly diminish tubular and glomerular function, causing intrinsic ARF. Usually there is a sudden onset of uraemia, with signs of a generalised drug reaction, septicaemia or malignant infiltration of the kidney. Interstitial oedema, intense patchy or diffuse cellular infiltration and relatively well-preserved glomeruli and tubules are also typical.

Lymphocyte, monocyte and plasma cell infiltration are

characteristic of most drug reactions, of acute interstitial nephritis (AIN) complicating certain infections or of AIN without any obvious cause (idiopathic AIN).

Malignant lymphocyte or leukaemic cells are commonly found in kidneys but rarely disrupt function (Martinez-Maldonado and Ramirez-Arillano, 1966). However, infiltration can be so intense and secretions of invading cells so toxic that nephromegally and ARF result (Martinez-Maldinado and Ramirez-Arillano, 1966; Richmond, et al., 1962).

The pathogenesis of drug-induced ARF is little understood although the absence of a clear relationship to dosage suggests an immunologic mechanism. This is supported by the frequent concomitant fever, rash and eosinophilia and occasionally increased levels of IgE and antibodies to the offending drug have been found (Baldwin et al., 1968).

Tubular Disorders

The term acute tubular necrosis (ATN) is used to convey meaningfull clinical information, but it is difficult to define. Clinical recognition of the condition is largely based on the exclusion of other causes of ARF.

ATN is usually a reversible disorder which is characterised by a variable urine volume and an inability to excrete sodium and water.

The intratubular accumulation of crystals, protein or mucoid material may produce a clinical syndrome similar to ATN. The intraluminal precipitation of large amounts of uric acid can complicate tumour lysis. The large purine load created by the sudden destruction of tumours, especially lymphomas and leukaemias, initiates hepatic uric acid synthesis. The associated dehydration causes uric acid to be concentrated in the tubule lumen, whilst acidification of the glomerular filtrate transforms the soluble sodium urate into relatively insoluble uric acid, the luminal precipitate causing occlusion of the lumen and ARF.

The sudden exposure of the kidney to large loads of calcium oxalate can also cause intrinsic ARF; although crystals can be seen in the tubule lumen, the obstructive nature of the lesion has not

been proved. Calcium oxalate loading can be caused by ethanediol poisoning (Friedman et al., 1962), toxicity from the anaesthetic methoxyflurane (Mazze et al., 1971), intestinal by-pass operations (Dickstein and Frame, 1973) and ink eradicator poisoning (Friedman, 1983).

Intrinsic ARF has also been associated with high-doses of methotrexate, resulting in a crystalline precipitate of the drug in the tubule lumen (Jacobs et al., 1976). Whether the uraemia is secondary to the luminal obstruction or a direct or indirect toxic effect of this drug has not been shown.

Uraemia complicating multiple myeloma may be secondary to the associated hypercalcaemia, direct nephrotoxicity of the light-chain proteins or chemotherapy, or due to obstruction caused by intratubular precipitation of the myeloma proteins (DeFronzo et al., 1975). Dehydration and radiographic contrast agents may initiate the precipitation of the myeloma proteins.

1.2.4 Post-Renal Acute Renal Failure

Postrenal ARF is caused by some form of obstruction. The type and site of obstruction, rate of onset and degree of occlusion all determine the resulting clinical syndrome.

Oliguria and uraemia can result when a single, localised obstruction e.g. stone, is located at the level of the urinary bladder or below, whilst ureteral or renal pelvic obstruction can cause uraemia only when this is bilateral, or unilateral in a patient with a single functioning kidney. 1-15% of hospitalised patients with the recent onset of uraemia prove to have postrenal ARF and this is usually a reversible disorder (Bushinsky et al., 1979; Miller et al., 1978). In the absence of complicating infection, decompression of the urinary tract after 1-2 weeks of total obstruction can still permit complete functional recovery (Rudnick et al., 1983).

Renal prostaglandin synthesis increases shortly after the acute onset of obstruction (Olsen et al., 1976). Blockade of this hormonal response by indomethacin prevents the increase in RBF that is associated with acute hydronephrosis (Gaudio et al., 1978). The prostaglandins in turn stimulate renin secretion, which elicits local production of the potent vasoconstrictor angiotensin II (Cadnapap-Hornchai et al., 1974). As described in haemorrhage-induced prerenal uraemia, the renal vascular response to acute hydronephrosis reflects the opposing effects of prostaglandin-induced vasodilatation and angiotensin-induced vasoconstriction. The dominance of the prostaglandins in the earliest phase of obstruction accounts for the overall hyperaemia. Renal vascular resistance increases and RBF diminishes to below normal values following several hours obstruction and although the pathogenesis of late vasoconstriction may simply reflect the increasing effect of angiotensin II vasoactivity over the vasodilatory effect of prostaglandins, it has been suggested that the mechanism is more complex (Rudnick et al., 1983).

1.3 Biochemical and Metabolic Data Relevant to the Interpretation of Nutritional Studies

1.3.1 Introduction

Compared to CRF there is comparatively little information on the metabolic effects of ARF because ARF patients are critically ill and associated factors such as shock, hypoxia, infection, toxins, drugs, fever and the catabolism associated with trauma have their own biochemical effects. The metabolic results of ARF are more pronounced than in CRF where a number of adaptations have been able to take place; e.g. ARF patients are less able to control the excretion of water and electrolytes than are CRF patients with similar creatinine clearances. Thus, ARF patients easily develop oedaema and hyponatraemia, unlike CRF patients, who can normally adequately control their water and sodium excretion until terminal renal failure occurs (Black and Jones, 1979). There are also adaptive mechanisms controlling potassium homeostasis in CRF which are not apparent in ARF (Elkington et al., 1949). In addition there is a higher rate of cellular decomposition and disintegration, resulting in the liberation of intracellular products and their metabolites into the circulation which accentuates the effect of the maladjusted homeostatic mechanisms. As a result, the intensity of the clinical syndrome is closely correlated with the extent of the associated catabolism and consequently effective dialysis may produce a remarkable clinical improvement.

1.3.2 Nitrogen Balance

Protein is vital for both cell structure and biochemical and physiological function and the net loss of protein seriously compromises the organism. In starved animals death occurs when 25% to 33% body protein is lost, even though fat stores are still available for energy (Montemurro and Stevenson, 1960) and catabolic diseases such as ARF are associated with increased morbidity and mortality (Blackburn et al., 1978; Whelton, 1979).

The quantity of protein in any tissue is related to the balance

between synthesis and degradation. A net loss will occur if degradation exceeds synthesis, whether this is due to an increased degradation, reduced synthesis or a combination of the two. The term nitrogen balance is used to describe the equilibrium between nitrogen intake and nitrogen losses, via all routes, in an individual. A subject is in nitrogen balance or equilibrium when nitrogen intake equals nitrogen output. Negative nitrogen balance is used when nitrogen output exceeds intake and positive nitrogen balance when nitrogen intake exceeds output. Negative nitrogen balance indicates net protein catabolism whilst positive nitrogen balance indicates net protein synthesis (Table 1).

1. Nitrogen intake > Nitrogen loss	: Positive Nitrogen Balance
2. Nitrogen intake < Nitrogen loss	: Negative Nitrogen Balance
3. Nitrogen intake = Nitrogen loss	: Nitrogen Equilibrium

Table 1. The Three Forms of Nitrogen Balance.

Although the determination and interpretation of nitrogen balance appear straight forward, nitrogen balance measurements are difficult to conduct in a clinical setting. Previous workers (Jeejeebhoy et al., 1976; Elwyn et al., 1979) have suggested that feeding regimens need to be continued for at least 3 days and preferably 7 days, to allow for equilibrium of the large body urea pool. Because of the latter, nitrogen excretion rates should be evaluated in the context of changes in the body urea pool (Benotti and Blackburn, 1979). This becomes critical in ARF patients where the nitrogen removal is very variable and often artificially controlled.

When large changes in nitrogen intake are made, with no change in energy intake, it will take several weeks before a new steady state is reached in normal adults (Oddoye and Margen, 1979). However, in depleted patients a steady state is reached more rapidly. In malnourished patients the effect of changing intake from 5% dextrose to total parenteral nutrition (TPN) is to abruptly change nitrogen balance, which then remains fairly constant for 2 weeks or more (Elwyn et al., 1980). With changes in energy intake a steady state of nitrogen balance is achieved rapidly, even in normal subjects

(Munro, 1964).

Another difficulty is the large variability in nitrogen balance, both between patients and from day to day. Standard deviations for studies on depleted patients can be 1-2 g N day^{-1} (Elwyn, 1983). These are much higher than are found in studies on normal subjects (Oddoye and Margen, 1979). This presumably reflects that depleted patients are a non-homogeneous group of severely ill patients, with an unstable catabolic rate. This is a similar group of patients to those with ARF.

There is also the problem of accurately measuring absolute values of nitrogen balance. Intake tends to be overestimated and output underestimated. Often not all output is analysed for nitrogen, leading to the difficulty of assigning appropriate values to the un-measured losses. Blood losses for sampling, or blood gains by the infusion of blood and plasma proteins, are difficult to handle in short-term balance studies. This is because the bulk of nitrogen is in the form of blood cells or plasma proteins with long turn-over times (Bistrian, 1981). An unpredictable proportion of these are denatured and are therefore degraded much more rapidly, becoming nutritionally available.

It is clear that the technique of nitrogen balance is problematical in these patients. Even so, although other methods are available (measuring total body content of K^+, N or other elements sequentially by isotope dilution techniques, whole body counting, neutron activation analysis or the measurement of protein breakdown and synthesis by the infusion of isotopically-labelled amino acids), nitrogen balance still remains the benchmark for determining the efficacy of a given amino acid regimen.

1.3.3 Urea Production

Nitrogen from degraded amino acids is converted almost completely to urea via the urea cycle (Krebs and Henseleit, 1932), the enzymes for which are found largely in the liver. One method of assessing the rate of protein catabolism is to calculate the urea appearance rate (UAR) or net urea production. If this is significantly higher than the nitrogen intake, endogenous protein

catabolism must be occurring.

The UAR can be calculated because urea equilibrates rapidly throughout the body water, reaching the same concentration in all fluid compartments and urea excretion can be easily measured (Williams et al., 1964; Walser, 1974). (See Chapter 2, page 99 for the UAR calculation).

When using UAR to express protein catabolism, it is necessary to consider also the nitrogen intake, urea clearance and body weight. If these are constant then an increase in serum urea concentration indicates an increased urea appearance from a rise in protein catabolism. Therefore, decreased renal function, body water or increased nitrogen intake will cause the serum urea to rise independently of an increased catabolic rate.

A change in 'corrected' UAR i.e. the difference between UAR and nitrogen intake (UAR-nitrogen intake) is generally accompanied by a reciprocal change in nitrogen balance as urea is the main end product of protein degradation. When degradation of endogenous protein increases, nitrogen balance becomes more negative. UAR can be used as an estimate of nitrogen balance when nitrogen intake is known. This is possible because nitrogen in faeces and in the excretion (and changes in pool size) of other nitrogen-containing molecules is relatively independent of dietary protein and varies only slightly from day to day. There is substantial evidence that this is so in CRF (Mitch, 1981b) and although there are fewer studies of the relationship between UAR and nitrogen balance in ARF (Feinstein et al., 1981), it appears that they are closely related, at least in the absence of large gastrointestinal or wound losses.

Since urea production and excretion rates, unlike creatinine, are commonly influenced by extra-renal factors (e.g. protein catabolic rate, nitrogen intake, hormonal changes), conclusions drawn from studies of urea must be interpreted with care. Both dietary nitrogen intake and gastro-intestinal bleeding cause increased loads of nitrogen (the latter from bacterial breakdown of blood components including urea) to be delivered to the liver, via the portal circulation. These therefore enhance urea production. Serum urea concentration is also increased by tetracyclines which are anti-anabolic agents that divert amino acids from protein synthesis

to urea production. Fever, sepsis and crush injuries are all hypercatabolic states associated with increased tissue protein turnover. Since the urea cycle (Krebs and Henseleit, 1932; Fig. 1) is almost entirely restricted to the liver (Bollman et al., 1924) the urea production rate is potentially limited by liver disease, but urea cycle enzymes are so abundant that impaired urea synthesis and low serum urea concentrations are not seen until the most advanced stages of liver disease (Rudman et al., 1973; Rafoth and Onstad, 1975; Rypins et al., 1980). The decreased blood urea concentrations often found in patients with alcoholic cirrhosis are more likely to be caused by a low protein diet.

A single 'uraemic toxin' has not been identified in patients with renal insufficiency but urea loading (serum urea >54 mmol l^{-1}) in CRF patients can cause symptoms of uraemia (Johnson et al., 1972). Other putative uraemic toxins also contain nitrogen and undoubtedly arise during protein degradation and it is not surprising therefore that clinical conditions in which urea production increases are also characterised by an increased production of other nitrogen-containing waste products. Therefore UAR can be used not only as an index of protein catabolism but also of the rate of production of all uraemic toxins.

Following its production, urea has two possible fates. It may appear in urine and body water or be degraded to ammonia and carbon dioxide by intestinal bacteria (Mitch, 1981b; Johnson et al., 1972). Ammonia produced during urea degradation is transported to the liver in portal venous blood where it is used to re-synthesise urea, carbon dioxide being excreted. When protein depleted subjects are fed urea, there is some evidence that nitrogen balance improves, suggesting that nitrogen arising from the degraded urea can be used to synthesise amino acids. Since patients with renal insufficiency have a large pool of urea it was suggested that they also might be able to recycle urea nitrogen in the urea pool to synthesise amino acids and thence protein. Early experiments, designed to test the effects of low protein diets on nitrogen metabolism of uraemic patients, yielded results interpreted as showing that this did occur (Giordano, 1963), since the nitrogen requirements of chronically uraemic patients seemed to be far less than normal.

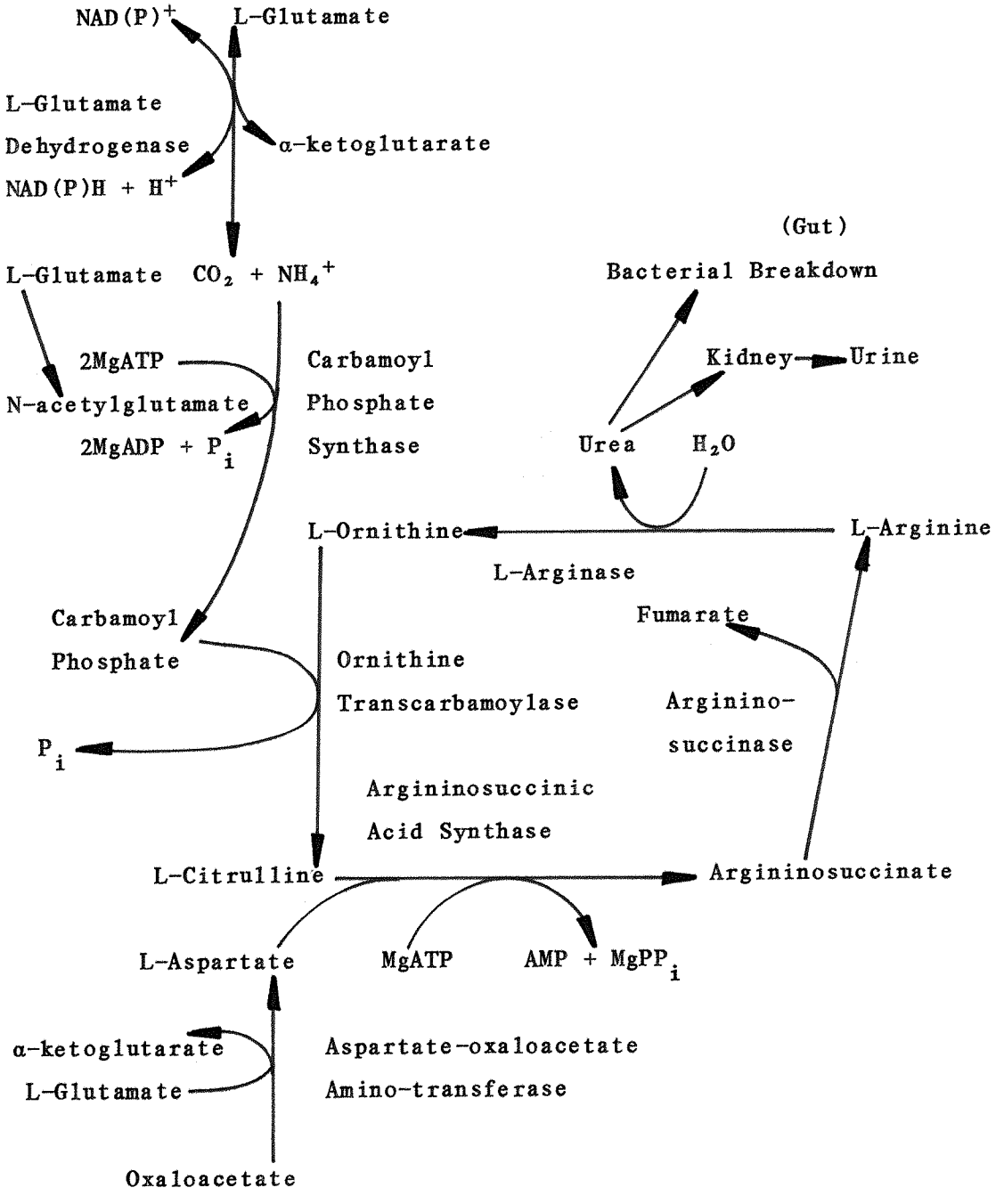


Fig. 1. Urea Cycle, Also Showing Urea Degradation by Intestinal Bacteria.

However, Varcoe et al. (1975) used isotopically labelled urea to study urea nitrogen incorporation into albumin. Less than 1.5% albumin nitrogen was formed from urea in normal subjects; this increased to 3.2% in CRF patients but was not considered to be

nutritionally significant. Nitrogen balance and urea turnover in uraemic patients has also been studied following suppression of intestinal urealysis by a non-absorbable antibiotic (Mitch et al., 1977; Mitch and Walser, 1977a). This showed that nitrogen derived from urea degradation was simply re-cycled for urea synthesis and not used for amino acid synthesis. That the nitrogen balance improved when urea degradation was suppressed further supported this conclusion.

This evidence therefore opposes the interpretation of Giordano (1963) and indicates that nitrogen derived from urea degradation is not of nutritional significance.

1.3.4 The Metabolic Response to Trauma

Trauma is followed by haemodynamic, endocrinologic, metabolic and immunological responses that are related to the severity of the injury. Cuthbertson (1942) divided the metabolic response into 'ebb' and 'flow' phases.

The ebb phase is the period immediately after the injury and lasts from a few hours after elective operation of moderate severity to 24-72 hours after more severe trauma and is characterised by 'depressed vitality' and reduced energy production, oxygen consumption and heat production. This phase is followed by the flow phase, which is characterised by metabolic changes associated with recovery. There may be a change from the flow phase back to the ebb phase with development of complications or new trauma. In the flow phase there is a resurgence of vitality and an increase in oxygen consumption, body temperature and energy production with a negative nitrogen balance. This phase usually lasts less than two weeks but may be upto several months with severe injury (Cuthbertson, 1980a, 1980b).

The metabolic response to trauma is largely regulated by the autonomic and central nervous systems and endocrinologic responses (Thorens, 1974). The balance between catabolic (catecholamines, cortisol and glucagon) and anabolic (insulin, testosterone, thyroxine and triiodothyronine) hormones is changed during trauma. Increased catecholamine secretion increases lipolysis, glycogenolysis and

gluconeogenesis and decreases the function and secretion of insulin. Thus there is an increase in blood concentrations of glucose, free fatty acids and lactate, whilst in the tissues there is a decreased glucose uptake. Cortisol secretion increases lipolysis and gluconeogenesis and inhibits glucose uptake, resulting in an increased blood glucose concentration. The main function of glucagon is to stimulate release of glycogen stores and induce gluconeogenesis (Efendic et al., 1974). As the secretion of the catabolic hormones is increased and the plasma concentrations of the anabolic hormones is decreased after trauma hyperglycaemia results.

Plasma protein concentration is reduced after surgery due mainly to an imbalance between protein breakdown and synthesis rates, redistribution of plasma proteins in the body, sequestration to the traumatised area and in some cases secretion from the wound. The decrease in protein concentration largely affects albumin (Owen, 1967; Fleck, 1976). By contrast the acute phase proteins, including C-reactive protein (CRP), increase after trauma. CRP, which is not usually measurable in plasma, appears 12-18 hours post-surgery and disappears 7-10 days later (Owen, 1967).

The total plasma amino acid concentration is decreased after major surgery and this is followed by a return to pre-operative or elevated concentrations in 2-7 days. The concentration of most individual amino acids is decreased in plasma but there are different patterns. Tyrosine, tryptophan and phenylalanine are exceptions with an increase in concentration (Aulick and Wilmore, 1979; Hoover-Plow et al., 1980; Clowes et al., 1980) and after major injury plasma branched chain amino acids (BCAA) may also increase (Wedge et al., 1976; Elia et al., 1980). The reduction in plasma concentrations is not due to fasting or anaesthesia, but to surgery (Woolf et al., 1976; Dale, 1977). There is also a reduction in most skeletal muscle free-amino acid concentrations post-operatively (Vinnars et al., 1975). However, in muscle there is an increase in BCAA, phenylalanine, tyrosine, methionine, glycine and alanine (Vinnars et al., 1975), with a marked reduction in glutamine (Vinnars et al., 1975; Milewski et al., 1982).

In more severe injury the essential amino acid (EAA) pool is decreased in both plasma and intracellular water, the extracellular

fluid (ECF) compartment is expanded, muscle phenylalanine is increased whilst lysine and glutamine are decreased (Vinnars et al., 1980). In burns, trauma and sepsis skeletal muscle releases amino acid nitrogen two to three times controls (Aulick and Wilmore, 1979; Clowes et al., 1980; O'Donnell et al., 1976) and there is a comparable outflow from plasma to visceral tissue (Wilmore et al., 1980). The increased hepatic uptake of amino acids after injury is partly due to the increase in plasma glucagon concentration known to occur (Wilmore et al., 1974). Glucagon is known to cause hypoaminoacidaemia (Liljenquist et al., 1981) and augments the splanchnic blood flow and amino acid uptake (Kibler et al., 1964). The increase in plasma phenylalanine indicates skeletal muscle catabolism because tyrosine appearance after oral loading is normal post-injury (Herndon et al., 1978) and hepatic uptake is increased (Wilmore et al., 1980).

As glucose is proportionately less available to skeletal muscle because of insulin resistance and fat mobilisation is suppressed by the high plasma insulin concentration, skeletal muscle is forced to increase protein oxidation to meet increased energy needs (O'Donnell et al., 1976). Skeletal muscle catabolises only BCAAs, releasing the other amino acids into the circulation (Adibi, 1976; Odessey et al., 1974; Snelling et al., 1982; Wedge et al., 1976). The initiating factor for these changes is thought to be release of macrophage lymphokines (Baracos et al., 1983; Clowes et al., 1983, 1976; Keenan et al., 1982). Interleukin 1 (IL-1) has been implicated in mediating the protein catabolism and acute phase response in trauma and infection and is thought to act directly on the liver to increase amino acid flux and the production of acute phase proteins (Kampschmidt et al., 1973; Powanda, 1977). IL-1 may also act directly on pancreas to increase insulin and glucagon secretion (George et al., 1975). IL-1, by stimulating PGE_2 production in muscle and hence the release of lysosomal proteases, results in an increase in protein catabolism. Clowes et al. (1983) and Loda et al. (1984) found another mediator they called Proteolysis-Inducing Factor (PIF) which caused an increase in the protein catabolic rate and was considered to be an active fragment of IL-1. Pomposelli et al. (1987) reported data suggesting that tumor necrosis factor

alpha/cachectin (TNF), not IL-1 mediates the proteolysis seen in muscle, although TNF appears to have a synergistic effect when present with IL-1 (Flores et al., 1987). See Fig. 2 for the effects of IL-1 and TNF on substrate metabolism.

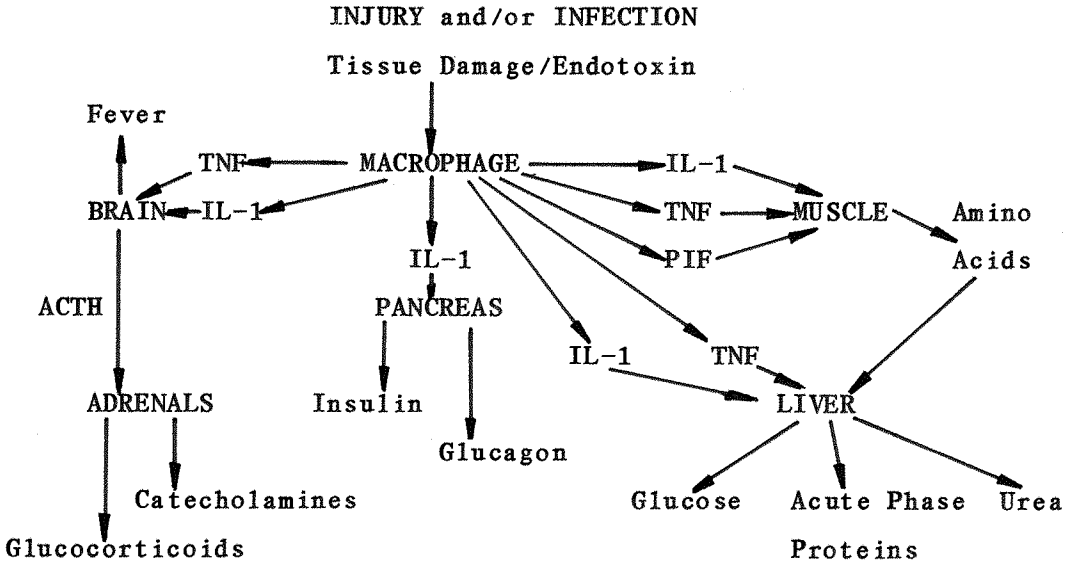


Fig. 2. Effects of IL-1 and TNF on Substrate Metabolism (After Pomposelli et al., 1988). Only the end products of stimulation are shown. All end products are released in increased amounts.

A negative nitrogen balance is typical of post-operative metabolism. The nitrogen deficit may be 16-48 g N day^{-1} in hypercatabolic states after major injuries or burns (Biebuyck, 1979). This nitrogen is largely derived from muscle tissue and the extent of the negative nitrogen balance is related to the severity of the trauma. The nitrogen loss is less in patients with pre-operative protein deprivation and the infusion of 100-150 g (400-600 kcal, 1.7-2.5 MJ) glucose day^{-1} to adults decreases the nitrogen imbalance. Although amino acid infusions show a better protein-sparing action than glucose alone, they do not entirely abolish the negative nitrogen balance (Tweedle, 1978). The protein-sparing action of insulin is important in severe trauma but is less-so in non-catabolic patients (Woolfson et al., 1979).

The catabolic phase with negative nitrogen balance lasts two to five days after general surgery and seven to ten days after major

surgery. After severe trauma and burn injuries the catabolic phase has usually disappeared after 20 days, but it can persist for weeks (Owen, 1967).

The aim of protein breakdown is to deliver amino acids for protein synthesis and provide the liver with fuel for gluconeogenesis. Duke et al. (1970) showed that the energy contribution of protein breakdown was only about 20% of total expenditure in a surgical patient, but the importance of the released amino acids for the synthesis of new proteins cannot be underestimated.

In the flow phase the negative nitrogen balance turns into a positive balance with nitrogen retention in two to five days after mild to moderate surgery and one to two weeks after major surgery. The synthetic rate of new protein is limited and the net gain of nitrogen is affected by nitrogen intake and energy supply. The simultaneous increase of intracellular potassium uptake with nitrogen retention is also clinically important and an adequate potassium intake is therefore necessary (Frost and Smith, 1953).

Both protein breakdown and synthesis increase post-trauma (Traynor and Hall, 1981; Birkhahn et al., 1981). But some studies show unchanged breakdown rates after uncomplicated elective surgery, but protein synthesis is reduced (O'Keefe et al., 1974; Crane et al., 1977). The amino acids released are used in gluconeogenesis and the synthesis of new protein, e.g. immune system and wound healing.

1.3.5 The Metabolic Response to Infection

The metabolic effects of infection are catabolic and similar to the response to trauma. Beisel et al. (1967, 1977) demonstrated that negative nitrogen balance ensues from experimentally induced infections in man as a result of a reduced intake and an increased urea production and loss.

The hormonal changes in infection are similar to trauma with an increase in catecholamines (Groves et al., 1973), cortisol (Beisel et al., 1967), glucagon (Rocha et al., 1973; Zenser et al., 1974), aldosterone (Beisel et al., 1967) and insulin (Beisel et al., 1967). The low insulin:glucagon ratio found in infection indicates the

predominance of catabolic and gluconeogenic glucagon over anabolic insulin. Three pathways have been suggested for the increased catabolism found with infection :-

1. Increase in local tissue destruction caused by the infecting organism and resulting inflammatory response. This includes an increase in vascular permeability, leukocyte migration and cell death and results in the release of lysosomal enzymes as well as histamine, kinins, prostaglandins and serotonin (Winkelman, 1971).

2. Fever. The elevation in body temperature increases catabolism. Dubois (1937) demonstrated a 13% increase in energy expenditure (EE) for each ^{o}C rise. Beisel et al. (1968) demonstrated that hyperthermia mechanically induced in normal human volunteers results in a decreased intake, increased urinary nitrogen loss and negative nitrogen balance. The increase in catabolism was probably caused by increasing the rate of all biochemical reactions. The velocity of many enzyme-catalysed reactions increase by a factor of approximately two for every 10 ^{o}C rise in temperature, the Q10 effect.

3. Lymphokines e.g. IL-1, TNF and PIF (see Metabolic Response to Trauma, section 1.3.4, page 19 and Fig. 2). IL-1 is also a pyrogen and the raised body temperature which results from IL-1 release will further increase the catabolic response (Stoner, 1987).

Infection complicating trauma increases the intensity of the catabolism and prolongs the activity of the catabolic response. Kinney (1975) demonstrated that energy expenditure (EE) after major elective surgery was increased by 10% but with complicating infection EE increased 20-50% (Duke et al., 1970; Kinney, 1975). Urinary nitrogen losses are higher post-operatively with complicating infection than without infection (Duke et al., 1970). Even if calories are given, with amino acids, in excess of metabolic expenditure, protein loss still occurs (Shaw and Wolfe, 1987; Streat et al., 1987).

After severe trauma and in sepsis there is a profound efflux of amino acids from the periphery (Duff et al., 1979; Clowes et al.,

1980, 1985; Aulick and Wilmore, 1979; Snelling et al., 1982; Greig et al., 1987). Alanine and glutamine form a major part of this efflux, but all amino acids contribute except glutamate. Intracellular glutamine is profoundly reduced in septic patients and this is proportional to the severity of the condition. In non-survivors the ICF:ECF gradient of >30 ultimately disappears (Roth et al., 1982, 1986). The concentration in ICF of BCAA and aromatic amino acids increase in muscle, but the increased BCAA concentration does not persist in non-survivors, whilst the aromatics remain elevated. Septic injured patients mobilise amino acids by muscle degradation, which is three to five times the normal rate (Clowes et al., 1980). As the septic process worsens the liver's ability to metabolise some amino acids appears to become compromised and plasma concentrations increase (Cerra et al., 1979; McMenamy et al., 1981).

1.3.6 Hypercatabolic States

It has been clearly shown that sepsis, burn injury and trauma induce a loss of cellular protein in the presence or absence of acute renal failure. In patients with sepsis or accidental injury negative nitrogen balance occurs (Moore, 1959; Beisel, 1977; Cuthbert and Tilstone, 1969) and the magnitude of the nitrogen loss indicates the size of the catabolic response.

The high nitrogen losses result from the increased catabolism of muscle protein, as the production of 3-methylhistidine (3-MH), a non-metabolisable amino acid arising primarily during degradation of skeletal muscle, is increased (Long et al., 1975, 1981). There is also a marked decrease in total body potassium suggesting a loss of lean body mass in patients with sepsis or injury.

Long et al. (1981) studied muscle breakdown in patients with skeletal trauma (not with ARF) compared to normal subjects consuming a standard hypocaloric protein-free diet. Protein degradation, measured by 3-MH loss in urine, in the trauma patients was two and a half times higher than the controls. Herrmann et al., (1980) measured protein flux using ^{15}N-labelled amino acids in catabolic patients and showed that total nitrogen turnover was increased and both degradation and synthesis were elevated but that degradation was more

affected and was dominant. Nitrogen balance was thus negative.

Wilmore et al. (1980) and Aulick and Wilmore (1979) measured the amino acid flux across the leg and splanchnic bed of trauma and sepsis patients and demonstrated that muscle protein was degraded, taken up by the liver and used to produce glucose, acute-phase proteins and urea. These measurements of muscle protein degradation and urea production confirmed the clinical evidence of decreased muscle mass and body weight in these patients and with the accelerated catabolism of acute renal failure show why the mortality rate is so high (Blackburn et al., 1978; Whelton, 1979).

1.3.7 Acute Renal Failure and Catabolism

Although it is difficult to separate the effect that renal failure may have on protein metabolism from the effects due to associated injury and/or illness, it has been shown that ARF itself is a catabolic event. In 1949, Persike and Addis showed that 24 hours after bilateral nephrectomy (BNX) the UAR of rats with ARF was higher than normal controls or rats with only 25% of normal kidney tissue. Sellers et al. (1957) demonstrated that when 15% glucose was added to the diet of rats with ARF the UAR of liver slices *in vitro* was reduced, but still exceeded the urea production of liver slices from sham-operated rats given glucose.

Lacy (1969) demonstrated that hepatic urea synthesis and amino acid uptake was increased in the perfused livers of rats with ARF relative to sham-operated controls; this worker later found (1970) that all amino acids except the BCAA were removed from the perfusate at an increased rate. Fröhlich et al. (1974) measured the relationship between hepatic glucose production and UAR in ARF and found that with BNX, compared to normal rats or sham-operated controls, there was an increased rate of gluconeogenesis and ureagenesis. This suggested that gluconeogenic amino acids were taken up by the liver and converted to glucose and urea (Fig. 3). It was thought that this higher rate of glucose production contributed to the glucose intolerance found in ARF.

In experiments using perfused liver slices it was found that endogenous nitrogen was used for the urea production. In the intact

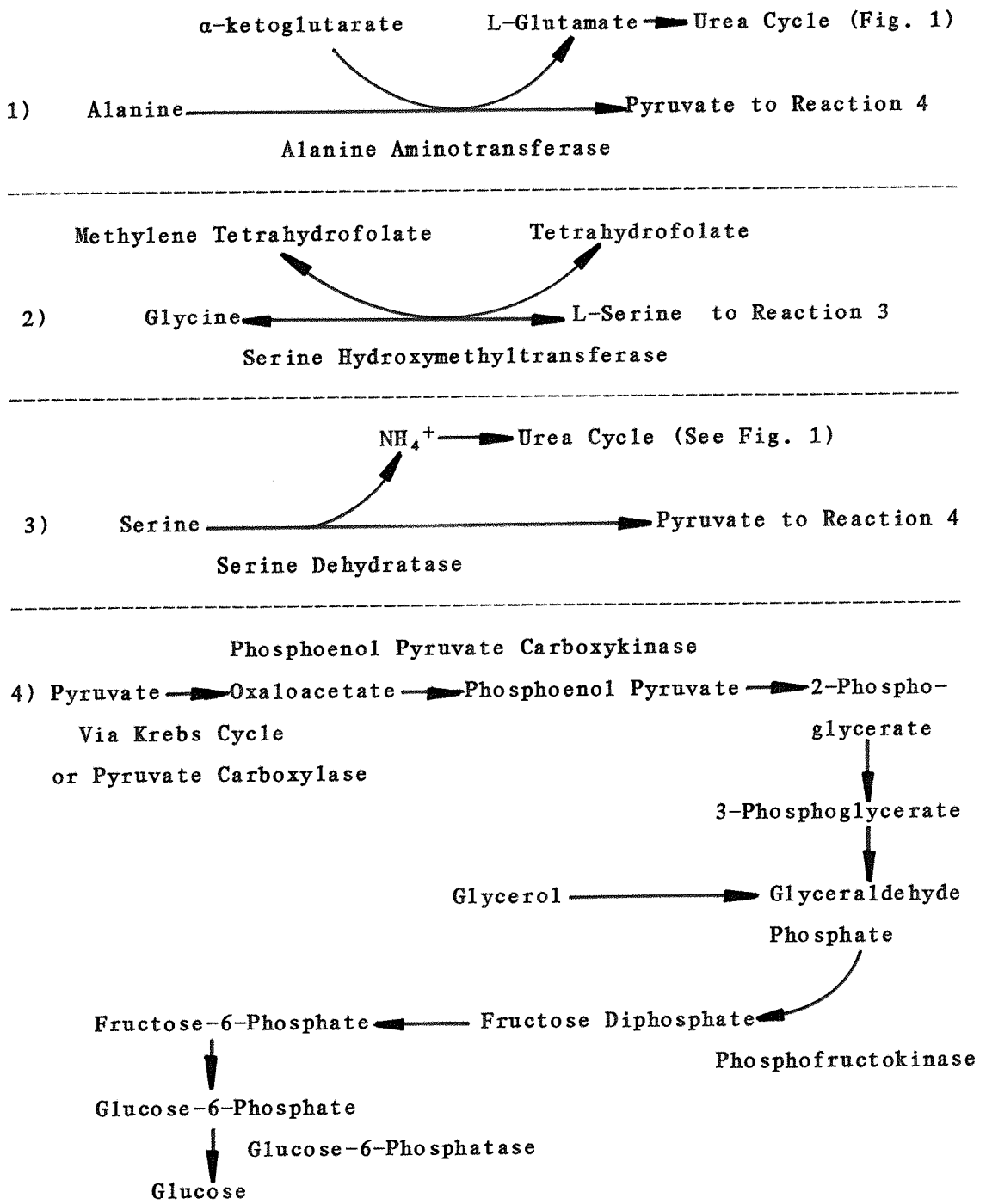


Fig. 3. The Conversion of Gluconeogenic Amino Acids to Glucose.

animal other protein sources are available. Bondy et al. (1949) compared BNX and adrenalectomised (ADX) rats with BNX rats and found indirect evidence for the increased, corticosteroid-dependent, release of amino acids from extra-hepatic tissues. They concluded

that muscle catabolism was associated with ARF, resulting in the release of amino acids which enhanced hepatic urea synthesis. Recently more direct evidence has been obtained to show an increased amino acid release from the periphery in ARF rats. In in vivo experiments and using the perfused hindquarters of rats Mitch et al. (1981a) found an increased release of amino acids which was sufficient to account for the increased urea production. Results suggested that ARF per se affects the overall nitrogen equilibrium, altering total flux as well as rates of protein synthesis and degradation. The observation that ARF stimulates amino acid release from muscle is consistent with results from CRF rats. Incubated muscle from the latter releases alanine and glutamine at a higher rate than from sham-operated and pair-fed animals (Garber, 1978; Harter et al., 1979).

Salusky et al. (1983) studied the effect of acute uraemia on protein degradation and amino acid release in the rat hemicorpus. Rats underwent BNX and were studied 30 hours later. The uraemic rats showed increased urea appearance and lower amino acid levels in plasma and intracellularly in muscle relative to controls. Muscle protein synthesis was slightly decreased.

Shear (1969) showed that the quantity of muscle per unit of deoxyribonucleic acid (DNA) was reduced 48 hours after BNX. They also calculated rates of protein synthesis following an injection of ^{14}C-leucine and concluded that protein synthesis was increased in liver and heart muscle, but decreased in skeletal muscle in ARF. Feinstein et al. (1981) showed that, in man, the catabolism in ARF could be sufficient to cause an abnormally elevated urea production with an intake of 1.4-6.5 g N day^{-1}. They estimated that the quantity of nitrogen required to offset this catabolic effect would be technically impossible to administer.

A major stimulus for excessive proteolysis in uraemia is metabolic acidosis. Metabolic acidosis induced by feeding ammonium chloride or hydrochloric acid in normal rats stimulated both glucocorticoid production and muscle protein degradation (May et al., 1986). Although glucocorticoids were required, the signal for catabolism was acidosis. CRF rats with mild metabolic acidosis excreted more nitrogen than pair-fed controls and had a high muscle

protein breakdown rate (May et al., 1987b). The defect in protein turnover is not due to glucocorticoids alone because CRF rats pair-fed a bicarbonate-supplemented diet still had an increased glucocorticoid production but protein breakdown was reduced.

Acidosis-stimulated catabolism may be due to excessive breakdown of leucine or its keto acid. As described in a later section (Essential Amino Acids, Protein Synthesis and Degradation, section 1.3.10, page 30) it has been shown that leucine stimulates muscle protein synthesis while α-ketoisocaproate (KIC) inhibits protein breakdown (Mitch and Clark, 1984). Metabolic acidosis stimulates the activity of branched-chain keto acid dehydrogenase in muscle and increases the catabolism of BCAA including leucine (May et al., 1987a). CRF rats with mild metabolic acidosis demonstrated accelerated decarboxylation of valine and leucine in muscle (Hara et al., 1987). When metabolic acidosis was corrected by feeding bicarbonate the accelerated amino acid decarboxylation was eliminated and plasma and muscle concentrations of leucine increased. The correction of acidosis also blocked the increase in muscle protein degradation. Supporting this, Jenkins et al. (1988) found a significantly reduced serum urea but no change in urea excretion (therefore, a reduced UAR, in CRF patients after 8 weeks of sodium bicarbonate supplementation.

1.3.8 Possible Mechanisms of Catabolism in Acute Renal Failure

It has been suspected for some time that some unidentified substance released from injured tissue may induce additional tissue injury in organs that were initially normal (Hörl et al., 1978). It was therefore postulated that these could be enzymes or activators of enzymes that possess the capacity to destroy normal tissue, increasing capillary permeabilty or perhaps initiating intravascular coagulation (Hörl et al., 1980b, 1982; Kuroda et al., 1981).

There is evidence that lymphokines mediate the proteolysis seen with sepsis, fever and injury (Baracos et al., 1983; Dinarello, 1984) - see Metabolic Response to Trauma and Infection, sections 1.3.4, 1.3.5 and Fig. 2, pages 19, 20 and 22. Endotoxins and endogenous chemical mediators, e.g. histamine, bradykinin, C_5A, cause monocytes

to release IL-1, which stimulates prostaglandin E_2 production in muscle. This in turn leads to the release of lysosomal proteases, which catabolise proteins in this tissue. The administration of IL-1 into healthy animals induces fever, raises serum insulin and glucose concentrations (Keenan et al., 1982), redistributes trace elements (Keenan et al., 1982), increases amino acid mobilisation, oxidation, skeletal and collagen protein degradation and hepatic protein synthesis (Yang et al., 1981, 1983; Dinarello, 1985). These responses are all seen in injury or infection and consequently in most ARF patients.

Hörl and Heidland (1980b) found enhanced proteolytic activity in sera from two out of eight patients with ARF and suggested that active proteases may play a part in the hypercatabolism of these patients. These proteases were capable of digesting bovine serum albumin. These workers also demonstrated (Hörl et al., 1982) that the protease from plasma of one poly-traumatised patient with rhabdomyolysis, sepsis and ARF could be inhibited by the addition of α_2-macroglobulin, which was only present in very low concentrations in the plasma. Neither dialysis nor the administration of aprotinin affected proteolytic digestion. In support of proteinase action in catabolism Schaefer et al. (1988a) demonstrated a 39% decrease in UNA in BNX rats treated with leupeptin, a low-molecular weight proteinase inhibitor, when compared to untreated BNX control rats.

Whilst the increased catabolism of ARF is not fully understood, glucagon (Bilbrey et al., 1974) and cortisol (Englert et al., 1958) are present in high concentrations in the blood and explain part of this effect. Experimental studies (Bilbrey et al., 1974, 1975) show a sharp increase in glucagon in ARF patient's plasma. If normal subjects are given intravenous (IV) glucagon there is an increased urea production presumably from increased glucogenic amino acids released during gluconeogenesis (Marliss et al., 1970). Raised corticosteroids increase protein degradation (Nishizawa et al., 1978; Goodlad and Munro, 1959) and stimulate IL-1 production (Dinarello, 1979), whereas serum urea is decreased after ADX in acutely uraemic BNX rats (Bondy et al., 1949; Schaefer et al., 1988c). Schaefer et al. (1988b) showed that in BNX rats the infusion of a glucocorticoid antagonist lowered myofibrillar proteinase activity,

and decreased plasma 3-MH, glucose and urea concentrations.

Clearly there are other catabolic actions occuring that could increase protein turnover and the release of cellular components into the body fluids. In addition to the accumulation of urea, additional evidence of protein destruction or muscle dissolution is reflected by the hyperkalaemia and hyperphosphataemia found in these patients.

1.3.9 The Effect of Haemodialysis on Catabolism

It is known that the daily protein requirements of CRF patients treated with haemodialysis (HD) (160 mg N kg^{-1} body weight (BW) day^{-1}) are higher than for patients with stable CRF. If their protein intake is restricted to 80 mg N kg^{-1} BW day^{-1} negative nitrogen balance results (Borah et al., 1978).

It has been demonstrated that the increased protein requirements of dialysis patients primarily depend on the frequency of dialysis, since protein nutrition can be maintained in HD patients treated with a mixed protein diet (3.2-4 g N day^{-1}) and supplemented with EAA, if the frequency of dialysis is reduced (Mitch and Sapir, 1981). Ward et al. (1979) found a 27% increase in catabolism during dialysis in CRF patients when compared to the off-dialysis state. This was not corrected by adding glucose to the dialysate and was therefore not primarily the result of gluconeogenesis.

The reasons for the increased requirements are not clear, but has recently been related to IL-1 induction and release. Monocytes can be stimulated to release IL-1 by blood/haemodialysis membrane contact, especially cuprophane membranes, (Port et al., 1987; Gutierrez et al., 1985; Bingel et al., 1988), microbial product contamination of dialysate (Favero et al., 1974; Peterson et al., 1978; Bingel et al., 1986) and sodium acetate dialysate (Bingel et al., 1987; Port et al., 1987).

Arisi et al. (1988) studied the effect of the prostaglandin inhibitor indomethacin before and during HD on UAR two to three hours after HD in 6 CRF patients. The indomethacin group had a significantly reduced UAR (116 mg N hr^{-1}) post-HD when compared with the indomethacin-free group (216 mg N hr^{-1}). This suggests that the increased catabolism on HD in CRF is at least partially stimulated by

prostaglandins and that indomethacin might reduce catabolism and improve nitrogen balance. However, Weipert et al. (1988) could find no effect of indomethacin administration on UAR of BNX rats compared to untreated BNX control rats. They concluded that prostaglandins do not play a major role in ARF hypercatabolism in the rat, unlike the hypercatabolism of septicaemia in this animal (Baracos et al., 1983). Their rat model would not therefore appear to be suitable for studying the ARF-associated catabolism with a view to the extrapolation of findings to the situation in man.

The increase in catabolism with HD is also partly related to the loss of nutrients in the dialysis bath. The HD membrane excludes large molecules but is non-selective for small molecules and this results in a loss of amino acid nitrogen of 1.4-1.8 g N HD^{-1} (Young and Parsons, 1966; Ganda et al., 1976; Ginn et al., 1968). Subjects treated by peritoneal dialysis (PD) also lose a substantial quantity of protein nitrogen, 1.9-3.2 g N, in addition to amino acids and this can lead to severe protein depletion, unless adequate amounts of dietary protein are given to replace these losses (Young and Parsons, 1969; Blumenkrantz et al., 1981; Berlyne et al., 1964; 1967b).

1.3.10 Essential Amino Acids, Protein Synthesis and Degradation

The proportion of EAA to total amino acids in dietary protein determines its quality and not all proteins have the same proportion. EAA must be provided in the diet as they cannot be synthesised in the body. Subjects fed a diet lacking in one EAA rapidly develop negative nitrogen balance because the rate of protein synthesis is reduced when any amino acid required for protein synthesis is not available (Rose, 1957). Daily requirements have been established for normal subjects (Rose, 1957) but not for patients with renal insufficiency. Three methods have been used to estimate the amino acid requirements, the determination of amino acid nitrogen required to 1) achieve nitrogen equilibrium (Rose, 1957), or 2) to achieve a normal amino acid concentration in intra- and extra-cellular amino acid pools and 3) amino acid kinetic studies (Young et al., 1985).

Histidine was included with the EAA in the following studies

because there is evidence that it is semi-essential in CRF patients (Kopple and Swendseid, 1975). When histidine and EAA in the quantities and proportions recommended by Rose are given with a low protein diet to stable CRF patients nitrogen equilibrium can be achieved, but this does not correct the amino acid concentrations in plasma and skeletal muscle to normal levels (Fürst et al., 1978; Attman et al., 1979). Although this can be done by altering the quantity and proportion of EAA ingested each day (Fürst et al., 1980), it is not clear whether this confers clinical benefit to ARF or CRF patients (Alvestrand et al., 1981), but Tizianello et al. (1985) suggested that the amino acid imbalance in CRF was potentially harmful.

Different regimens of EAA and glucose have been administered to ARF patients with conflicting results (see Mortality Studies and Nutritional Studies - Clinical, sections 1.4 and 1.5, pages 45 to 53). When diseases causing or associated with ARF do not cause marked catabolism, nitrogen equilibrium can be achieved (Mirtallo and Fabri, 1984; Blackburn et al., 1978), but when there is marked catabolism nitrogen balance is almost invariably negative (Dudrick et al., 1970; Leonard et al., 1975; Blackburn et al., 1978; Spreiter et al., 1980; Feinstein et al., 1981; Pelosi et al., 1981; Proietti et al., 1983), in spite of the infusion of EAA or EAA and non-essential amino acids (NEAA), moreover the profile of the plasma amino acid concentrations remains abnormal with both therapies (Feinstein et al., 1981).

There is evidence that infusion of EAA in ARF can increase survival and possibly hastens the recovery of renal function. Rats with nephrotoxic-induced ARF given IV amino acids were reported to have more rapid regeneration of damaged tissue and improved renal function (indicated by a lowered serum creatinine) than rats not receiving the infusion (Toback, 1977). Similarly, patients with ARF treated with EAA demonstrated a more rapid improvement in renal function, as measured by a reduction in serum creatinine (Abel et al., 1973). A subsequent study with rats however found no beneficial effect of EAA (Oken et al., 1980) and the beneficial effect of amino acid infusions remains undefined.

However, there may be a beneficial effect of EAA on protein turnover in catabolic patients. There is in vivo and in vitro

evidence that specific EAA, the BCAA and/or their metabolic products, can increase muscle protein synthesis and decrease muscle protein degradation. Following surgery (Bonau et al., 1984), the infusion of either a balanced amino acid mixture (25% BCAA) or one enriched with BCAA (45%) providing 240 mg N kg^{-1} day^{-1} and glucose (30 kcal, 126 kJ kg^{-1} day^{-1}) can improve nitrogen balance compared to glucose (600 kcal, 2550 kJ day^{-1}) alone. This occurred through an increased rate of protein synthesis and decreased protein degradation. There is also evidence that BCAA given in high concentrations with other amino acids to patients with sepsis may be beneficial and improve nitrogen retention (Lindberg and Clowes, 1981).

Preliminary data supporting such an effect in catabolic patients have been reviewed by Blackburn et al. (1981), Freund et al. (1981), Cerra (1983) and Walser (1984). At present there is no generally accepted mechanism that explains this effect and the physiological significance of the changes observed in these studies have not been established. If catabolism could be controlled a major factor (i.e. net loss of body cell mass) contributing to the excessive mortality of ARF patients would be removed (Blackburn et al., 1978; Whelton, 1979).

Only one study has been carried out in ARF patients with solutions enriched with BCAA. Proietti et al. (1983), infused a full-profile amino acid TPN solution both with and without enrichment with BCAA in hypercatabolic ARF patients. In both groups the calorie:nitrogen ratio was 350-400:1, with a nitrogen intake of 80-100 mg N kg^{-1} BW day^{-1} or 80-120 mg N kg^{-1} BW day^{-1} (BCAA-enriched TPN). The EAA:total ratio was 4.13 (TPN) or 4.17 (TPN + BCAA) with BCAA:EAA ratio of 0.424 (TPN) or 0.754 (TPN + BCAA). These workers found a significant improvement in nitrogen balance with the BCAA-enriched regimen and the mean Δ serum urea concentration remained lower in the BCAA group. There was no significant difference in mortality (20%) between the groups.

Several types of experiments in animals indicate that EAA, specifically leucine or its analogue α-ketoisocaproate (KIC) can affect protein synthesis and degradation; e.g. Morgan et al. (1971) used an isolated rat heart preparation perfused with supranormal

amounts of amino acids (AA) and found a 40% stimulation of protein synthesis. Fulks et al. (1975) added AA to the incubation fluid of skeletal muscle (diaphragm) from normal rats with a 20-30% increase in protein synthesis. They found that protein synthesis was stimulated by incubation with BCAA alone or leucine alone; protein degradation was also decreased. Buse and Reid (1975) incubated rat diaphragm with cyclohexamide to block protein synthesis and leucine incorporation. They found a decrease in protein degradation.

It is possible that some of these effects are caused by degradation products of leucine rather than leucine itself. BCAAs and their keto analogues are readily interconverted in skeletal muscle by BCAA transferases (Hutson et al., 1978; Mitch and Chan, 1979). Because leucine and its ketoanalogue are in equilibrium due to the BCAA transferase (Fig. 4), the administration of leucine or KIC must give rise to its associate. However, skeletal muscle readily degrades ketoacids and it may therefore be another degradation product which affects protein synthesis.

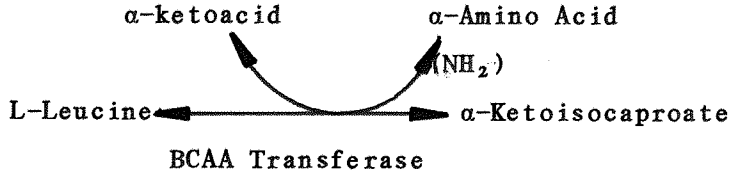


Fig. 4. The Reversible Conversion of Leucine to KIC.

Goldberg and Tischler (1981) incubated rat diaphragm with leucine and found that protein synthesis was increased, whilst degradation was decreased. When the diaphragm was incubated with KIC there was decreased protein degradation, but addition of an inhibitor of BCAA transferase to the leucine-containing incubation medium blocked the fall in protein degradation, although protein synthesis was still stimulated. Thus leucine itself had no effect on protein degradation. The addition of KIC to the mixture of leucine and BCAA transferase inhibitor reinstated the effect on protein degradation demonstrating that KIC or one of its metabolites was responsible for this effect on protein degradation in vitro (Table 2). Thus, leucine clearly had a direct effect on protein synthesis and its metabolites

Infusion Fluid	Protein Synthesis	Degradation
Leucine	↑	↓
KIC	–	↓
Leucine ⟶// KIC BCAA Transferase Inhibitor	↑	–
Leucine + BCAA Transferase Inhibitor + KIC	↑	↓

Table 2. The Effect of Leucine and KIC on Protein Synthesis and Degradation.

an effect on protein degradation. The molecular basis of this effect is not known, but is apparently not due to differential rates of charging of the specific transfer RNA for leucine (Goldberg and Tischler, 1981).

Further, the addition of ketoanalogues of BCAA to the infusion fluid of the liver of normal rats led to an increased protein synthesis. The infusion of branched-chain ketoacids in starving, obese human subjects decreased nitrogen wasting (Sapir and Walser, 1977). This was associated with a carry over effect from one week to the next. Ketoacids given daily for the first week were still effective the second week when compared with two weeks of starvation, as the control period. This effect was also found when only KIC was given to starving obese subjects (Mitch et al., 1981b). In the same study, the infusion of equimolar leucine did not change urea nitrogen excretion and therefore catabolism, but the infusion of leucine after three weeks of starvation in similar subjects was reported to decrease nitrogen excretion (Sherwin, 1978). This discrepancy may be due to the fact that the interconversion of leucine and KIC is stimulated by prolonged starvation and therefore the infusion of leucine would cause an increase in KIC.

This may be of relevance to ARF patients as Walser (1975) showed that CRF patients on a low protein diet supplemented with EAA and

ketoacids including KIC decreased net UAR to a greater extent than a similar diet supplemented with only EAA. He suggested that the keto analogues exert a nitrogen sparing effect in CRF. However this was not substantiated by a later cross-over study in CRF patients (Burns et al., 1978). Several studies have shown that the ketoacids of EAA given to CRF patients can be used to maintain nitrogen balance following transamination (Walser et al., 1973; Mitch and Walser, 1977b; Mitch et al., 1981a) despite the fact that nitrogen intake was below the minimum for nitrogen balance (Walser et al., 1973). It has also been shown that if keto acids are given with a high protein diet there is no additional beneficial effect.

Brissac et al. (1981) could show no beneficial effect on serum urea concentration or survival when an infusion of ketoacids was compared with EAA in dogs made acutely uraemic by BNX. It remains to be shown if KIC decreases protein degradation in ARF.

Rennie et al., (1986) have suggested that the improvement in nitrogen balance seen with BCAA administration is partly due to non-competetive inhibition of a sodium-dependent glutamine carrier. Glutamine efflux is consequently reduced and the improvement in nitrogen balance largely resulted from re-filling of the intra-muscular glutamine pool. Rennie et al., (1986) provided evidence that the intracellular glutamine concentration is related to the rate of protein synthesis in muscle. Supporting this, Fürst et al. (1988) and Stehle et al. (1989) showed that an improvement in nitrogen balance can result from a glutamine-peptide infusion, which was associated with an increase in the intracellular glutamine concentration in muscle.

Some studies in patients without ARF suggest that there is a limit to the amount of nitrogen that can be utilised to improve a negative nitrogen balance. Ang et al. (1983) infused three different amounts of nitrogen (7.9, 11.9 and 15.8 g N day^{-1}) as part of a TPN regimen into malnourished patients with cancer and bowel disease. They found that protein synthesis increased as nitrogen intake increased but a plateau was reached at the 11.9 and 15.8 g N day^{-1} levels. Shizgal and Forse (1980) gave TPN with different amounts of nitrogen to depleted patients and found a positive correlation between nitrogen intake and nitrogen balance at intakes

less than 204 mg N kg^{-1} BW day^{-1} but this was not evident at higher intakes in hypercatabolic patients. Similarly, Wolfe et al. (1983) gave 352 mg and 224 mg N kg^{-1} BW day^{-1} to burns patients and found that protein equilibrium was not improved with the higher diet because both protein synthesis and breakdown increased. Also in patients with 30% burns, Larsson et al. (1984) found a plateau was reached when 200 mg N kg^{-1} BW day^{-1} was infused. Finally, Greig et al. (1987) fed septic patients with 191 or 366 mg N kg^{-1} BW day^{-1}. They found that nitrogen balance became positive on days one to three with the high nitrogen diet but on days five and six there was no significant difference in nitrogen balance between the two levels of intake.

Nitrogen intake is the variable which is most effective in increasing lean body mass in depleted patients. Peters and Fischer (1980) compared calorie:nitrogen ratios of 163:1 and 204:1, whilst Smith et al. (1982) compared calorie:nitrogen ratios of 135:1 with 150:1. Both these groups found the lowest calorie:nitrogen ratio to be most effective at increasing lean body mass. In support of these findings Iapichino et al. (1984) found that nitrogen intake was the major determinant of nitrogen balance in their depleted patients. Varying the non-protein energy to nitrogen ratio had little effect in this study.

1.3.11 Insulin and Amino Acid Metabolism in Acute Renal Failure

Although insulin is known to play an important part in amino acid metabolism, there is little information available of the effects of insulin on amino acids in uraemia. In normal man insulin is an anabolic hormone that enhances amino acid transport and stimulates protein synthesis (Wool, 1969; Fulks et al., 1975).

If the peripheral insulin resistance known to occur in ARF (Arnold and Holliday, 1979; Kokot and Kuska, 1973), trauma and sepsis (Brooks et al., 1984; Wilmore, 1976) also included amino acid metabolism this could partly explain the increased net release of amino acids from muscle and the decrease in intracellular free amino acids found in CRF (Alvestrand et al., 1983). However, the action of insulin on the transport of α-amino-isobutyrate (a non-metabolisable analogue of alanine), cycloleucine (Arnold and Holliday, 1979) and

leucine (Garber, 1978) was inhibited in ARF and CRF rat muscle *in vitro*, whilst the effect on alanine was enhanced (Garber, 1978) and the inhibition of muscle tyrosine and phenylalanine release was normal (Harter *et al.*, 1979). More recently, Maroni *et al.* (1986b) found that although the system A amino acid transport system is impaired in ARF in the absence of insulin, the percentage increase in amino acid transport above the basal rate caused by insulin infusion is the same in muscle from control and ARF rats. The response of other neutral amino acid transport systems ASC and L to insulin infusion is also not affected (Maroni *et al.*, 1986a). Also, Alvestrand *et al.* (1988) found a normal inhibition of amino acid release from leg tissues and normal alanine uptake by splanchnic tissue, in response to hyperinsulinaemia in uraemic patients. It appears therefore that the phenomenon of insulin resistance does not apply to amino acid transport in uraemia in man and the rat model used by Maroni *et al.* (1986a, 1986b). This also demonstrates the difficulty of extrapolating data derived from animal studies to man.

1.3.12 Energy Requirements

Energy requirements are not greatly affected by renal insufficiency per se, but are related more to associated catabolic events such as trauma, or infection (see page 22) and the age, sex and weight of the individual. These requirements can be approximated from data derived from normal subjects (Wilmore, 1977a) and increased if sepsis or trauma are present according to the degree of catabolism. In a study of post-operative and post-trauma patients with ARF the energy required to achieve nitrogen balance, 50 kcal (210 kJ) kg^{-1} BW day^{-1} (Feinstein *et al.*, 1981), was 50% greater than the recommended intake for healthy sedentary men (34 kcal, 143 kJ kg^{-1} BW day^{-1}; Committee on Dietary Allow., 1980). However, in a study of indirect calorimetry in patients with multiple organ failure both with and without ARF (Soop *et al.*, 1989), the ARF patients had a significantly lower energy expenditure compared to the patients without ARF (27.2 kcal, 114 kJ kg^{-1} BW day^{-1} compared to 31.8 kcal, 134 kJ kg^{-1} BW day^{-1}). They suggested that the reduction in aerobic metabolism in the ARF patients was greater than would be expected

from the loss of renal function. The caloric requirements for ARF patients found by Soop et al. (1989) were lower than the group of ARF patients studied by Miller et al. (1983), but were similar to the energy expenditure found by Bouffard et al. (1987) in mechanically ventilated ARF patients (26 kcal, 109kJ kg^{-1} BW day^{-1}).

There is controversy over the most suitable substrate in the critically ill. In normal subjects the provision of exogenous non-protein energy decreases nitrogen excretion and is related to the administration of both fat and carbohydrate (Wilmore, 1977b). In resting normal subjects, the provision of carbohydrate alone decreases urinary nitrogen excretion, but fat alone does not affect the rate of nitrogen loss of fasted subjects. When calories (fat and/or carbohydrate) are given to normal human or animal subjects with nitrogen both calorie sources improve nitrogen retention. In normal subjects or stable, non-catabolic patients that are unable to eat (e.g. gastrointestinal dysfunction), nitrogen equilibrium or even positive nitrogen balance can be achieved if sufficient nitrogen and calories are given, regardless of the calorie substrate. This is also the situation in sepsis patients without malnutrition (Roulet et al., 1983). In Roulet et al.'s patients in the fasting state the metabolic rate, measured by indirect calorimetry, was only 14% above a predicted normal, based on the Harris-Benedict formula (Harris and Benedict, 1919). This compared to the metabolic rate measured by Askanazi et al. (1980a) in septic-injured patients (14.2% above normal). In the patients studied by Roulet et al. (1983) with feeding the metabolic rate rose to 31% above basal, 41% including minimal activity in bed. Also, in this cross-over study when changing from amino acid plus glucose to isocaloric amino acid plus glucose plus lipid parenteral infusions nitrogen balance became significantly positive, with reduced protein degradation.

In a similar study by Baker et al. (1984) but with insulin added to both regimens, nitrogen equilibrium was achieved equally by both. However, in hypercatabolic patients Woolfson et al. (1979), found a marked decrease in nitrogen excretion when amino acids and carbohydrate or carbohydrate and insulin were given intravenously. This was not so when compared with isocaloric fat and amino acids. In burn patients Long et al. (1977) found a rapid decrease in nitrogen

excretion with carbohydrate infusion. Similar calories as fat failed to exert the same effect (Wilmore, 1977b).

These different responses may be due to the different neurohormonal signals between normo- and moderately catabolic and hypercatabolic patients. In hypercatabolic patients the neurohormonal signals are designed to accelerate gluconeogenesis and override the ability of the body to adapt the metabolism to various energy sources. However, the administration of carbohydrate in excess of the energy requirements results in excessive carbon dioxide production which may lead to respiratory problems in the critically ill (Askanazi et al., 1980a). As there is no hindrance to oxidation of fat by injured or septic patients (Stoner, 1987) and to avoid excessive carbon dioxide production, carbohydrate and fat can be used to meet the resting energy requirements.

1.3.13 Body Temperature, Sodium Transport And Metabolism

It was recognised several years ago that advanced uraemia may result in a decreased body temperature. The IV injection of urine (Herringham, 1900) or urea (Leiter, 1921) can induce hypothermia. Also, a factor isolated from uraemic plasma and cerebrospinal fluid when administered intraperitoneally reduced the body temperature of mice and rats (Feher et al., 1958). Schreiner and Maher (1961) described patients with severe uraemia who were hypothermic despite a pyogenic visceral abscess. These 'spiked' a fever during dialysis as the uraemia was partially corrected. This may have been due to the effect of haemodialysis on ion transport as upto 50% of basal energy consumption and therefore heat production, may be generated by energy transformations driving active Na^+ transport (Asano, 1976). The suppression of sodium transport by uraemia could cause hypothermia and hypometabolism.

Knochel and Seldin (1981) studied energy metabolism in 6 patients with uncomplicated but advanced uraemia before haemodialysis was instituted. After a constant diet for three days of 325 g carbohydrate, 105 g fat and 6.4 g nitrogen their respiratory quotient (RQ) was 0.78 and therefore in the hypometabolic range. The normal adult post-absorptive basal R.Q. is 0.82 (Smith, 1959; Behrendt,

1962). With a comparable diet healthy controls had an RQ of 0.83. This low RQ value (0.78) is normally only seen in untreated hypothyroid or diabetic ketotic patients and indicates that fat is the predominant fuel for basal energy metabolism; (if protein was the only fuel RQ 0.80; fat RQ 0.70; carbohydrate RQ 1.00, Peters and Van Slyke, 1946). After 6 weeks of haemodialysis therapy the RQ had increased to normal. This is compatible with a shift from fat to carbohydrate utilisation as no change in pH was observed. This suggested that haemodialysis had increased insulin sensitivity and glucose utilisation.

Later studies early in the haemodialysis course, during the first 2 weeks, suggested that the immediate response was to turn on oxygen consumption. The patient then becomes hypermetabolic, the membrane potential overshoots (Cotton et al., 1979) increases above normal (more electronegative) and then returns to normal. These workers suggested that firstly, uraemia suppresses active Na^+ transport. This is supported by evidence that suppression of Na^+ transport is the consequence of Na^+,K^+-ATPase inhibition in uraemic patients (Welt et al., 1967; Cole et al., 1968; Kramer et al., 1976). Also it is known that intracellular Na^+ concentration increases in uraemia. This could be via the passive influx of sodium from the extracellular fluid, as found in normal cells. But as the normal active efflux is inhibited in uraemia an abnormally high intracellular concentration results, with hypometabolism.

Secondly, the membrane potential overshoot and transient hypermetabolism occurring during the first two weeks of HD treatment could result from a surge of increased sodium transport. The latter results from the removal of uraemic toxins and dis-inhibition of energy production to drive the sodium active transport. As the electrogenicity of the sodium pump is predominantly a metabolic function of sodium transport (Williams et al., 1971), there is no need to postulate an increase in Na^+,K^+-ATPase. Kramer et al. (1976) have observed that the Km of ATPase is subnormal in CRF, but the ATP content of uraemic erythrocytes is either normal or elevated. This abnormal Km is corrected rapidly with HD.

These changes in sodium transport are relevant to protein metabolism as Rennie et al., (1986) have hypothesised a

sodium-dependent carrier for glutamine in skeletal muscle. This carrier would control the glutamine gradient between the intra- and extra-cellular fluid compartments as the inverse of the sodium gradient. As the intracellular glutamine concentration has a role in controlling protein synthesis (Rennie et al., 1986; Fürst et al., 1988; Stehle et al., 1989) changes in the sodium gradient could affect nitrogen balance.

1.3.14 Creatinine Metabolism

Creatinine is a poorly metabolisable internal anhydride derived from the non-enzymatic breakdown of muscle creatine phosphate (Fig. 5). The daily production is proportional to muscle mass and relatively constant for a given individual on a meat-free diet (Mayersohn et al., 1983). Normal range males: 130-180 μmol kg^{-1} BW day^{-1}; females 90-130 μmol kg^{-1} BW day^{-1}. In a steady state, when production equals excretion, 24 hr urinary excretion varies <± 10% (Page, 1960).

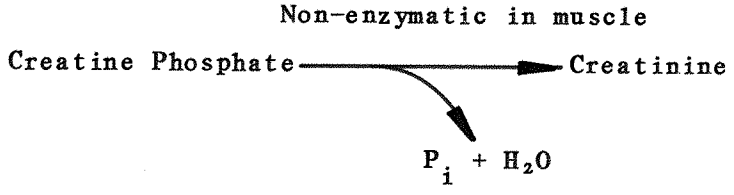


Fig. 5. The conversion of Creatine Phosphate to Creatinine.

Assuming creatinine to be evenly distributed throughout the body water (Mitch et al., 1980), it would be expected that the serum concentration would rise by 260 μmol l^{-1} day^{-1} when a 70 kg man suffers oliguric ARF. But except for ATN associated with muscle injury, it is most unusual for the serum creatinine increment to be more than 90-180 μmol l^{-1} day^{-1}. This could be explained by uraemic suppression of creatinine synthesis, recruitment of an extrarenal excretory pathway or development of a metabolic mechanism for the removal of creatinine. Subsequent to hypercreatininaemia an increased fraction of the daily load diffuses into the intestinal tract where creatinine mobilising bacteria destroy it (Mitch et al.,

1980). Therefore, the gastrointestinal tract assumes a significant excretory role for creatinine during uraemia. A daily serum creatinine increment of 130-180 μmol l^{-1} indicates an almost complete cessation of glomerular filtration, whilst bursts above 180 μmol l^{-1} suggest overproduction in addition to undersecretion, commonly seen in rhabdomyolysis (Grossman et al., 1974). The daily increment in serum creatinine is sufficiently constant to enable the dating of onset of the renal impairment by extrapolation, if the patient is anuric and has not been dialysed (Rudnick et al., 1983).

Several workers (Koffler et al., 1976; Bowden et al., 1956) have noted that creatinine may be disproportionally increased relative to the serum urea early in the course of rhabdomyolysis. Although unproven, this has been suggested to be the result of creatine release from injured skeletal muscle cells with the subsequent spontaneous dehydration to creatinine. However cell membrane damage should not release creatinine into the extracellular space (Grossman et al., 1974) because it is evenly distributed throughout body water (Mitch et al., 1980). Also, the conversion of creatine to creatinine should proceed at the same rate whether creatine is intracellular or has leaked into the extracellular space, as this occurs by a non-enzymatic process. Furthermore, a study in dogs of creatinine generation in rhabdomyolysis and ARF, does not support the creatinine release hypothesis (Swenson et al., 1979). The apparent increase in creatinine production may be due to the presence of non-creatinine chromogens (Walser, 1982).

The altered urea:creatinine ratio Seen in the early stages of ARF is transient, lasting 24-36 hrs. The catabolism of body protein increases the urea production until the usual ratio is reached by the second or third day. In CRF a 41:1 ratio of serum urea:creatinine (mmol:mmol) suggests the patient is stable, with neither excess protein intake nor excess catabolism from infection, hypermetabolism or drugs. However, most ARF patients have a ratio >41:1 for the first 7-14 days and often longer. Exceptions may include ARF from mis-matched blood transfusions or nephrotoxic antibiotics because urea production is not raised by excess catabolism in these conditions.

1.3.15 Uric Acid Metabolism

In ARF not associated with overt tissue destruction urate in plasma increases by 60–120 μmol l^{-1} day^{-1}. This usually stabilises at 890 μmol l^{-1} and if hyperuricaemia is pronounced (>890 μmol l^{-1}) skeletal muscle necrosis should be suspected. In patients with exertional rhabdomyolysis and ARF, serum urate can reach 3.3 mmol l^{-1} on day one with a serum urea of only 14 mmol l^{-1} (Suki and Eknoyan, 1976). This can also be observed in ARF after treatment for certain lymphomas. The extreme hyperuricaemia in rhabdomyolysis is probably due to two processes :-

1) Energy substrate depletion (ATP). When ATP is reduced to a critical level in the muscle cell 5'-nucleotidase is activated, adenylate is reduced, inosine synthesis is increased and uric acid production increases abruptly (Knochel et al., 1974), Fig. 6a. This probably occurs during exhaustive exercise (Fox, 1974).

2) Release of all nucleoproteins from muscle cells as a result of necrosis and the subsequent conversion to uric acid in the liver, Fig. 6b.

Hyperuricaemia is also pronounced in ARF caused by attempts to reduce body weight by a combination of fasting or caloric restriction and excessive physical exercise. Acetoacetate and β-hydroxybutyrate produced by fasting and lactate produced by exercise probably interfere with the secretion of urate by the renal tubule (Lecocq and Mc Phaul, 1965; Quick, 1935) and contribute to the hyperuricaemia before the onset of ARF. Ingestion of anorexogenic drugs such as amphetamines may also contribute to tissue injury under these conditions by means of increasing physical activity and therefore increasing the demand for energy production.

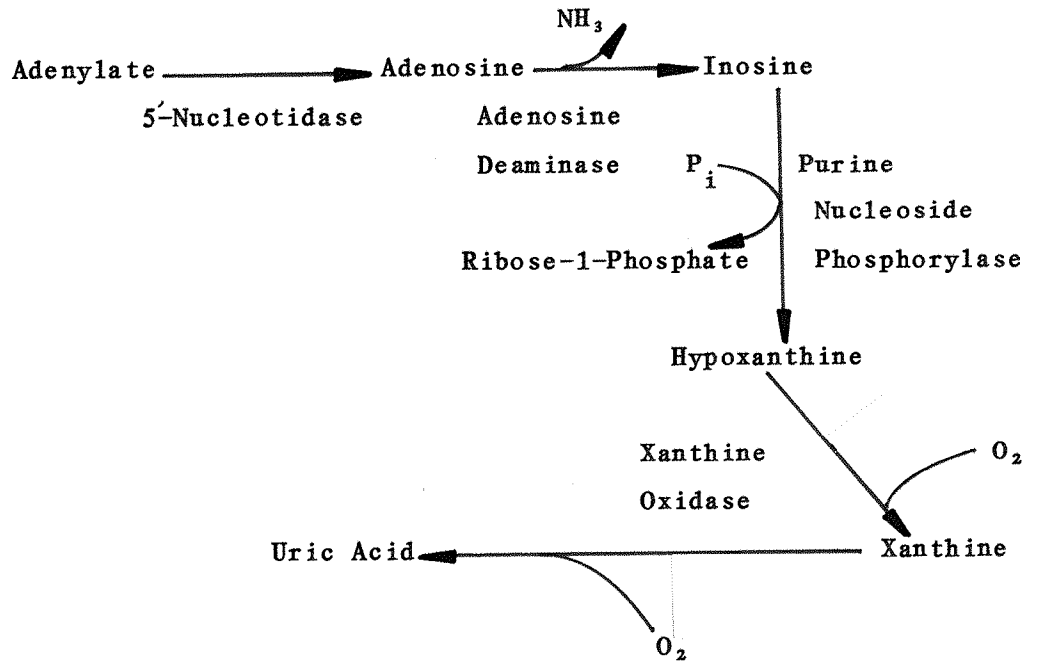


Fig. 6a. The Conversion of Adenosine to Uric Acid.

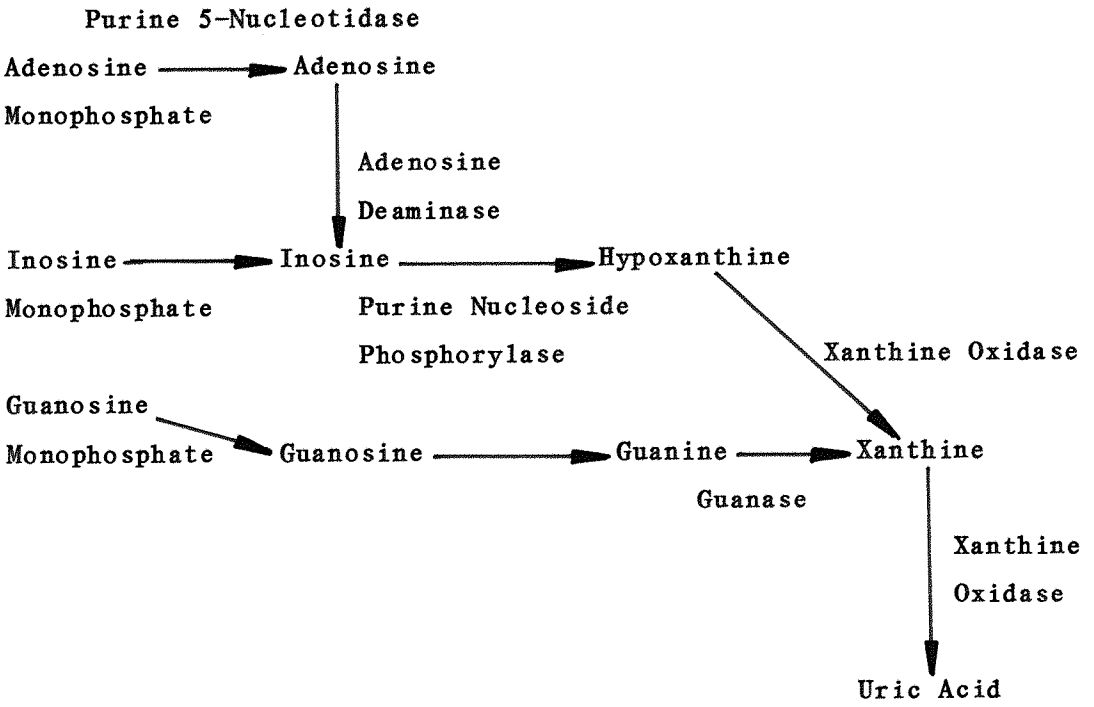


Fig. 6b. A Schematic Representation of the Breakdown of Nucleotides to Uric Acid.

1.4 Mortality Studies

There have been considerable improvements in mortality rates for patients with acute renal failure in the last 50 years, largely with the advent of haemodialysis and the introduction of early and frequent dialysis (Conger, 1975).

In the Korean war the overall mortality of 61 patients with post-trauma acute renal failure, being treated by haemodialysis was 65%, which was significantly lower than the 91% mortality of patients with oliguric ARF in World War II, without haemodialysis (Teschan et al., 1960). More recently Whelton and Donadiq (1969), looking at post-trauma ARF patients in the Vietnam war found an overall mortality rate of 65%. It was suggested that this lack of improvement in survival was due to the prevention of acute uraemia in the less severely traumatised, but was offset by improved resuscitation and evacuation techniques allowing more severely wounded patients time to develop ARF before they died from the associated illnesses.

The first comparative study of the beneficial effect of HD was by Kleinknecht et al. (1972), who divided the patients into 4 sub groups - surgical, medical, traumatic and obstetric - and found a significant reduction in morbidity and mortality in all groups between early and late dialysis. 279 patients treated before July 1968 were dialysed when the serum urea rose above 125 mmol l^{-1}, whilst a second group of 221 patients were dialysed to maintain a serum urea below 71 mmol l^{-1} which also enabled improved nutrition to be given. The respective mortality rates for these two groups were 42% and 29%. There were also improvements in mortality rates in all sub-groups between the early and late dialysis patients e.g. surgical patients 54% to 38% and medical patients 55% to 37% respectively. This improvement was largely due to a decreased incidence of septicaemia and gastro-intestinal haemorrhage in the more frequently dialysed patients. The mortality rates for the pre-1968 and post-1968 septicaemia and gastro-intestinal haemorrhage sub-groups were 24% to 12% and 55% to 27% respectively. Other workers have shown similar effects of early haemodialysis on mortality rates, e.g. Fischer et al. (1966): pre-1960 ARF patients 77% mortality whilst the post-1960 early HD patients had a 57% rate.

To exclude the possibility of time-related effects, Conger (1975) did a prospective study of 18 patients from the Vietnam war who were divided into two similar trauma groups. In Group 1 the serum urea was kept below 25 mmol l^{-1}, whilst group 2 were not dialysed until the serum urea was above 54 mmol l^{-1}. The respective mortality rates for Groups 1 and 2 were 37% and 80%. As in the earlier study, the improvement was explained by the lower incidence of septicaemia and haemorrhage.

In 1973 Abel *et al.* reported a parenteral nutritional study which demonstrated improved survival and rate of recovery of renal function. This was a double blind trial and 28 patients were given essental amino acids (1.4-3.4 g N day^{-1}) and 50% dextrose (375-900 g day^{-1}, mean 1500 kcal, 6.3 MJ day^{-1}) whilst 25 patients had isocaloric and isovolaemic 50% dextrose. The 2 groups were randomised for factors likely to affect the course of the ARF, e.g. prior serum urea and creatinine levels, age, oliguria and the interval from the injury to the intravenous therapy. The amino acids had a dramatic effect on survival, the overall mortality for the dextrose-receiving patients was 56% whilst in the amino acid group it was 25%. In the patients who were dialysed, (the ARF was not severe enough to necessitate dialysis in all patients) 65% survived in the amino acid group, whilst only 18% did so in the glucose group, also, the serum creatinine peaked after 2 days in the EAA group, whilst in the glucose group it continued to rise for 7 days. However, this was a selective study in that only 53 out of 150 patients selected for nutritional support were included and the other patients were excluded for a variety of reasons. Consequently these results may only apply to a select group of patients.

Baek *et al.* (1975) also studied the effect of an amino acid infusion on survival. They gave a fibrin hydrolysate (5.2 g N day^{-1}) and glucose (>1000 kcal, >4.2 MJ) or glucose alone intravenously to 129 post-operative ARF patients in an uncontrolled trial. There was 70% mortality in the glucose group whilst in the amino acid group it was 46%, even though there were complications, e.g. pneumonia and sepsis. There was also improved survival with the amino acids in both dialysed and non-dialysed patients.

Freund and Fischer (1980) studied 22 patients given a

full-profile amino acid solution (3.7 ± 0.2 g N day^{-1}) and glucose (2322 ± 151 kcal, 9.75 ± 0.63 MJ day^{-1}). Only 9.1% survived and they compared their data to Abel *et al.* (1973). Abel *et al.*'s study showed 61% survival in their EAA and glucose group. However, the groups were not necessarily comparable. Freund's patients had a higher mean age (yrs) (58 ± 4.2 cf. 55 ± 17); higher initial serum urea level (mmol l^{-1}) (14.2 ± 0.9 cf. 12 ± 5.8); lower pre-study 24 hr urine volume (mls) (895 ± 171 cf. 985 ± 873; and the time (days) between injury and therapy was greater (7.2 ± 0.8 cf. 4.1 ± 2.4).

Rainford (1981, 1987) compared the effect of dialysis and total parenteral nutrition on survival over a 20 year period. He divided his patients into three groups :-

1) 1958-1964 patients had a pre-dialysis serum urea of 71 mmol l^{-1} and a caloric intake of less than 1000 kcals (4.2 MJ) day^{-1}; 221 patients; 48% survival.

2) 1965-1975 pre-dialysis serum urea 33 mmol l^{-1} and a caloric intake of less than 2000 kcals (8.4 MJ) day^{-1}; 246 patients; 58% survival.

3) 1976-1980 pre-dialysis urea less than 33 mmol l^{-1}; caloric intake 3000 kcals (12.6 MJ) day^{-1}; 9 g N day^{-1}; 85 patients; 71% survival.

All groups were significantly different from each other, the major difference between groups one and two being one of early dialysis, although partial parenteral nutrition was also instigated. This would also compare with other workers results presented above, but the greatest difference was between groups two and three with the introduction of TPN. Although only significant at the 5% level, this was also the statistical significance between groups one and two with much larger numbers. Rainford felt this to be of more practical significance as the patients now accepted for treatment would not have survived long enough to develop ARF in the earlier groups.

Finally, Laville *et al.* (1985) carried out a retrospective study of 150 patients with ARF. 46% were medical patients whilst 33% were surgical. The mean daily caloric intake in 94 patients was higher in the survivors than in those who did not survive (29 kcal, 122 kJ compared with 24 kcal, 101 kJ kg^{-1} day^{-1}). These workers found that a caloric intake above 35 kcal (147 kJ) kg^{-1} day^{-1} resulted in improved

survival independently of age or non-renal complications. Also, thyroxine-binding pre-albumin (TBPA) and transferrin were significantly higher in the survivors, which indicated a higher nutritional status or a reduced acute phase response.

Other studies have not demonstrated a significant impact of nutrition on mortality.

1.5 Nutritional Studies

1.5.1 Clinical Studies

Because of the acute nature of the renal impairment the nutritional status of the patient with acute renal failure is largely determined by the previous state of health, the nature of the insult that caused the renal failure and the presence of associated illnesses and/or injuries, rather than the degree of renal insufficiency. This is because it is these associated illnesses which largely determine the degree and rate of protein catabolism.

The goals of nutritional therapy are to maintain the chemical composition of the body as close to normal as possible and to preserve the body protein stores, especially in those tissues involved in host defence and recovery, whilst minimising the accumulation of nitrogenous waste products. This priority is a result of the need by the hospitalised patient for amino acids to maintain host defence (Alexander et al., 1980), prevent bacterial colonisation and the likelihood of secondary bacterial infections (Sobrado et al., 1983). The minimisation of nitrogen accumulation can be achieved by optimising the protein intake to be just sufficient to maintain nitrogen equilibrium, whilst providing sufficient calories to minimise the catabolism of endogenous protein for energy. These goals are more difficult to achieve than in patients with chronic renal failure because of the unstable metabolic state, wider range of catabolism, complicating illnesses and injuries and the lack of metabolic adaptation because of the acute nature of the illness.

Bull et al. (1949) and Borst (1948) demonstrated that the provision of large amounts of exogenous calories could reduce urea production. Studies in the 1960's in CRF patients by Giovanetti and Maggiore (1964), Giordano (1963), Blagg et al. (1962) and Schloerb (1966), demonstrated that nitrogen balance could be improved and UAR reduced by providing a limited nitrogen intake as high quality protein or EAA. Berlyne et al. (1967a) combined these results into an ARF diet consisting of a high intake of non-protein calories and a lower protein intake than required for normal subjects (2200 kcal, 9.24 MJ day^{-1} and 2.6 g N day^{-1} as high quality protein). This

reduced the UAR and patients looked less emaciated.

Lee et al. (1967) infused 45 patients (both CRF and ARF) with a casein hydrolysate (160 mg N kg^{-1} day^{-1}), carbohydrate and lipid emulsion (25-30 kcal, 105-126 kJ kg^{-1} day^{-1}) for 6 to 31 days. They reported a decreased weight loss and more prompt recovery. Subsequently several investigators have demonstrated that the intravenous infusion of amino acids and glucose can result in a more positive nitrogen balance, better healing of wounds, maintenance of body weight, amelioration of uraemic symptoms and a reduced blood urea in post-operative patients (Wilmore and Dudrick, 1969; Dudrick et al., 1970; Abel et al., 1971; Abbott et al., 1971; Abel et al., 1972; Abel et al., 1973).

Dudrick et al. (1970) reported 10 patients (a mixture of ARF and CRF) intravenously fed with hypertonic dextrose (upto 4000 kcal, 16.8 MJ day^{-1}) and EAA (upto 4 g N day^{-1}). The intake was varied according to the serum urea concentration and the nitrogen loss on the previous day. They claimed to achieve nitrogen balance in 8 patients.

In 1971 Abel et al. and Abbott et al. reported the use of an essential amino acid solution and hypertonic dextrose in several patients. Both studies demonstrated a stabilised or reduced serum urea and reduced dialysis requirements. Abel et al., (1973), presented data from a prospective, double-blind controlled trial, the results of which have been presented earlier in Mortality Studies, Section 1.4., page 46. The main results of infusing small amounts of EAA (1.4-3.4 g N day^{-1}) and hypertonic dextrose (average 1500 kcal, 6.3 MJ day^{-1}) was a reduced or stable serum urea concentration, improved survival and recovery of renal function.

Leonard et al. (1975) demonstrated in a single-blind, controlled trial that 1.4 g N day^{-1} of EAA and 50% glucose (average 1600 kcal, 6.7 MJ day^{-1}) did reduce the rise of serum urea but made no significant impact on nitrogen balance, nor on recovery of renal function or survival. In the same year Baek et al. demonstrated improved survival in ARF patients who were given a fibrin hydrolysate solution and hypertonic glucose (EAA and non-essential amino acids (NEAA) 5.2 g N day^{-1}, average >1000 kcal, >4.2 MJ day^{-1}). The patients given amino acids demonstrated improved survival compared to

glucose alone. However, the serum urea stabilising effect of EAA found by other workers was not confirmed, possibly because of the extra non-essential nitrogen. Baek et al.'s findings demonstrated that a full-profile amino acid solution could be given to these patients without ill-effect.

Poraicu et al. (1978) gave two groups of 20 patients with toxic septic states and hypercatabolic ARF either glucose infusions or glucose and amino acids. These groups were sub-divided into four. The two glucose groups received either 13-20 or 20-27 kcal (55-84 or 84-113 kJ) kg^{-1} day^{-1}. The amino acid groups were given 50-120 mg N kg^{-1} day^{-1} and 13-20 kcal (55-84 kJ) kg^{-1} day^{-1} as glucose or 120-170 mg N kg^{-1} day^{-1} and 20-27 kcal (84-113 kJ) kg^{-1} day^{-1} glucose. Nitrogen losses were calculated using the Moore formula (Mincu and Ionescu-Tirgoviste, 1974) and the urea and nitrogen losses were adjusted for changes in serum urea concentrations. A theoretical nitrogen balance was calculated assuming 40% of nitrogen was incorporated from the infused amino acids (Hartig et al., 1975). Similar nitrogen losses occurred in comparable energy groups. Group 1, 13-20 kcal kg^{-1} day^{-1} - glucose group lost 14.4 g N day^{-1}, amino acid group lost 14.3 g N day^{-1}. Group 2, 20-27 kcal kg^{-1} day^{-1} - glucose group lost 16.8 g N day^{-1}, amino acid group lost 15.7 g N day^{-1}. The improvement in nitrogen balance in the amino acid group (3 g N day^{-1}) was not significant because of the variation in nitrogen losses. However, the serum urea concentration rose in the glucose groups, whilst it fell in the amino acid groups. This was significant ($P<0.001$) between the glucose and amino acid plus glucose groups who received the higher energy intake. They concluded that there was a decreased rate of catabolism in the patients given amino acids and glucose relative to the glucose group, although the amino acid group were more seriously ill.

Blackburn et al. (1978), divided 19 patients into two groups, those with (1) mild ARF and (2) hypercatabolic ARF with various complications. In Group 1, which was a homogeneous group, patients received either a) 1.2% EAA (Nephramine®) and 37% dextrose; b) 2.0% EAA and NEAA (Freamine®) in a 1:1 ratio with 37% dextrose or, c) 2.1% EAA and NEAA with 52% dextrose. Group 2 patients were given varied proportions of EAA and NEAA depending upon their individual status.

Fluid intake averaged 1200 ± 125 ml day^{-1}. Those patients in Group 1 only on EAA had a lower UAR. Increasing the glucose intake from 1530 to 1920 kcal (6.4-8.1 MJ) day^{-1} whilst on an isonitrogenous intake, caused a dramatic fall in the serum urea and creatinine ($P<0.05$) and nitrogen balance approached equilibrium when nitrogen intake was 4-5 g day^{-1}. Group 2 showed a reduced response to nutritional therapy.

Spreiter et al. (1980) reported 14 hypercatabolic ARF patients given varying intakes of amino acids (14-224 mg N kg^{-1} day^{-1}; mean 74 ± 13 mg N kg^{-1} day^{-1}) and hypertonic glucose (6-71 kcal, 25-298 kJ kg^{-1} day^{-1}; mean 30 ± 5 kcal, 126 ± 21 kJ kg^{-1} day^{-1}). These workers used a single compartment mathematical model (Sanfelippo et al., 1975) to derive urea nitrogen losses from the UAR. They found UAR (11.2 ± 1.8 g N day^{-1}) exceeded nitrogen intake (6.0 ± 1.2 g N day^{-1}) by 5.6 ± 1.6 g N day^{-1}. Using the same model to calculate circulating non-protein nitrogen, the estimated nitrogen balance was -9.9 ± 2.3 g N day^{-1}. Increased nutrient intake correlated significantly with improved nitrogen balance for both glucose ($R = 0.64$) and amino acids ($R = 0.5$). However, nitrogen balance only became transiently positive in four patients, when amino acid intake averaged 144 mg N kg^{-1} day^{-1} and glucose intake 50 kcal (210 kJ) kg^{-1} day^{-1}. They concluded that in hypercatabolic ARF, protein and energy requirements considerably exceed those conventionally prescribed.

Feinstein et al. (1981), in a prospective, controlled, double-blind study also showed the need for more calories and nitrogen in hypercatabolic patients. Whilst providing 1300 to 3400 kcal (5.5-14.3 MJ) and 1.4 g to 6.5 g N as amino acids many patients remained in negative nitrogen balance. There was no improvement in nitrogen balance when a larger amount of nitrogen was given as a mixture of EAA and NEAA. This group (Feinstein et al., 1983) also compared 5 patients given 2.3 ± 0.1 g N day^{-1} of EAA with 6 patients infused with 11.3 ± 1.9 g N day^{-1} of EAA and NEAA. Both groups received similar caloric intakes but no significant improvement in nitrogen balance was found with the higher intake. In both these studies they concluded that no advantage was gained by giving higher nitrogen intakes because this only resulted in a compensatory increase in UAR, with no improvement in nitrogen balance.

Mirtallo and Fabri (1984) compared patients with mild ARF given either EAA or a full-profile mixture, although the latter did not meet normal requirements for some of the EAA. Serum urea values were reduced at the same rate in both groups, no patients required dialysis and positive nitrogen balance was achieved in both groups.

Pelosi et al. (1981) studied 46 ARF patients, receiving HD at 24-48 hr intervals. All patients were in negative nitrogen balance, but the patients who received the full-profile amino acid solution (42 mg ± 14 mg EAA + NEAA N kg^{-1} day^{-1}, glucose 30-40 kcal, 126-168 kJ kg^{-1} day^{-1}) were less negative than those who were given an isocaloric EAA solution (28 mg EAA N kg^{-1} day^{-1}). The EAA:total nitrogen ratio had to be >4 before nitrogen balance improved.

Proietti et al. (1983) studied 40 patients, all of whom were dialysed at 24-48 hr intervals. Patients were assigned to one of three groups.

Group 1, 80-100 mg N kg^{-1} day^{-1}; EAA:Total 4.13; BCAA:EAA 0.424.

Group 2, 80-120 mg N kg^{-1} day^{-1}; EAA:Total 4.17; BCAA:EAA 0.754.

Group 3, 85-150 mg N kg^{-1} day^{-1}; EAA:Total 3.79; BCAA:EAA 0.6.

All groups were given a calorie:nitrogen ratio of 350-400:1. Group 3 received enteral nutrition with parenteral BCAA, whilst the other groups were given TPN. Those patients receiving a higher ratio of BCAA had a significantly less negative nitrogen balance. They concluded that the combined use of parenteral BCAA and enteral nutrition was most appropriate and that a balanced amino acid intake should be given with an EAA:total nitrogen ratio of >4 and a BCAA:EAA ratio of >0.5. These workers (1978) also studied 10 patients with ARF, 5 on a high-calorie, non-protein parenteral nutrition (2250 kcal, 9.4 MJ) and 5 given parenteral nutrition of EAA and glucose (1.8 g EAA N; 2054 kcal, 8.6 MJ). They concluded that haemodialysis was responsible for part of the diminution of plasma amino acids and that their administration reduces this effect for both EAA and NEAA.

1.5.2 Animal Studies

It is well known that ARF is a catabolic state and that the supply of metabolic substrates may be limited because of the necessary volume restriction of infusions. Since tissue repair may consequently be sub-optimal the provision of amino acids and calories may increase the rate of repair and restoration of renal function in this condition.

Since the preliminary studies of Addis *et al.* (1926), Wilson (1933) and Oliver (1944/5) it has been known that protein infusion leads to an increase in renal mass in experimental animals.

Halliburton *et al.* (1967, 1969) supplemented diets with individual amino acids for 6 days in rats after unilateral nephrectomy. Glycine caused a 21% increase in renal mass; glutamic acid 70%; proline 15% and serine 8%. The increased mass appeared to result from cellular hypertrophy rather than hyperplasia. It has also been observed that the intracellular amino acids increase during the compensatory growth that occurs after unilateral nephrectomy (Toback *et al.*, 1973). Toback *et al.* (1973) demonstrated that the concentrations of methionine, leucine and tyrosine are increased in renal cortical tissue two days after uninephrectomy, but if the animals are starved for 48 hrs after the operation these amino acids are reduced in concentration and compensatory growth does not occur. Other workers have also noted that nutrient deprivation abolishes compensatory growth.

Toback *et al.* (1979) infused amino acids (0.5 g N) and glucose (25.6 kcals, 107 kJ) or glucose (25.6 kcals, 107 kJ) alone in mercuric chloride treated rats (a model of non-oliguric, reversible, toxic ARF). They found an increased incorporation of choline into phospholipid in the amino acid treated rats, without a change in the phospholipid breakdown rate. Also the Vmax of the choline kinase and cholinephosphotransferase reactions were increased, without a change in their Km values in the amino acid infused rats. They concluded that amino acids infused after ATN can act directly on regenerating kidney cells to increase precursor availability and augment two reactions in the phospholipid biosynthetic pathway, Fig. 7. Toback *et al.* (1983) also gave amino acids (0.6 g N EAA + NEAA) and glucose

(29.2 kcal, 123 kJ) or glucose (29.2 kcal, 123 kJ) alone to rats treated with mercuric chloride in a study on renal protein metabolism. During renal regeneration in the cortical tissue of the amino acid treated rats, they found an increased rate of protein synthesis and a reduced rate of protein degradation relative to the glucose infused group. Also, the cellular deficiency of free leucine found in cortical tissue three days post mercury injection in non-infused rats ($P<0.001$), was reduced further after glucose infusion ($P<0.05$), but was corrected ($P<0.001$) in the amino acid infused rats.

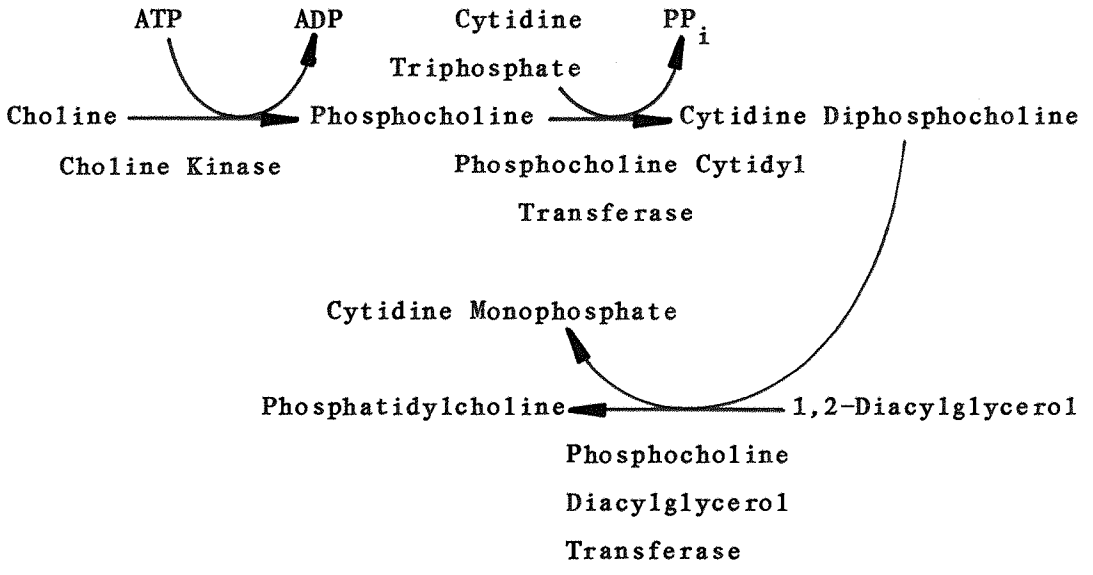


Fig. 7. The Conversion of Choline to Phosphatidylcholine.

In a similar earlier study, Toback (1977) found an enhancement of renal regeneration with amino acid infusion. This effect was associated with a reduced serum creatinine (44.5 ± 5.4 μmol l^{-1}) in the amino acid treated rats, relative to the glucose treated (93.6 ± 13.6 μmol l^{-1}) or non-infused controls (264 ± 34.5 μmol l^{-1}). This possibly indicated increased renal function and, without an increased mortality, suggested that the amino acids acted directly on the process of repair and recovery.

In contrast, Oken et al. (1980) found no beneficial effect of amino acid administration in a similar study and there was an increased mortality in the amino acid treated rats. In these animal

studies and in Abel *et al.*'s clinical study (1973) the serum creatinine was used as a measure of renal function, but the effect of improved metabolism on creatinine production was not considered and in these conditions may not be a good indicator of renal function. The experimental design and the species of rats used differed between these two studies.

In support of Oken's data, Andrews and Bates (1987) found that rats with uranyl-nitrate induced ARF had a significantly higher mortality rate if they were given a high nitrogen diet post ARF induction, after a normal diet. However, rats conditioned to protein-free or low-protein diets had significantly lower mortalities than rats maintained on normal protein diets. Rats maintained on high protein diets had better renal function than rats on normal protein diets. Changing a normal diet to a low or protein free diet after ARF induction did not improve renal function or survival, but moving high protein diet rats to a low protein diet resulted in improved survival and renal function. These workers had previously found (Andrews and Bates, 1986) that rats maintained on a low-protein diet showed significant improvement in post-ischaemic renal function and survival rates. Bevilacqua *et al.* (1983) also found an improvement in renal function measured by inulin clearance in rats with ischaemic ARF, when maintained on a normal diet compared with rats changed to a low protein diet post-ischaemia. Previously protein depleted rats also showed an increased renal function when changed to a normal diet after ARF induction.

Brezis *et al.* (1984) infused isolated rat kidney with glucose (5 mmol l^{-1}) and individual amino acids (2 and 8 mmol l^{-1}). They found a reduced renal vascular resistance with amino acids which was dose dependent although the glomerular filtration rate did not change. They concluded that amino acids have a direct vasodilating action, probably related to their role as metabolic substrates and linked to an increase in renal oxygen consumption. Amino acids therefore may have a protective effect on pre-renal ARF.

1.6 Summary

In conclusion, it is clear that mortality rates remain at an unacceptably high level in ARF, although the use of early dialysis has had some impact on this. Also, some nutritional studies have had encouraging results on the effect of nutrition on both mortality and rate of recovery of renal function, but so far these have been inconclusive and contradictory.

Therefore, more studies need to be done to clarify the role that nutrition has to play in the future management of these patients. This observation led to the design of the study which forms the basis of this thesis.

CHAPTER 2

PATIENTS, MATERIALS AND METHODS

2.1 Introduction

In this chapter the study design, patients studied, sample collection, data collection, sample and data analysis methods and validation are described.

2.2 Study Design

2.2.1 Aims :-

1) To study the protein requirements of patients with ARF of various aetiologies, categorised according to their catabolic rate and to determine whether the current dietary management of these patients is optimised.

2) To develop the methodology for undertaking accurate metabolic balance studies on patients on haemodialysis.

2.2.2 Design :-

Patients with ARF of all aetiologies were admitted to the Wessex Regional Renal Unit under the care of the Consultant Nephrologists and specialised medical and nursing staff.

All patients were put on full nitrogen balance studies continuously. All output (faeces, urine, fistula losses, drainage, dialysis and haemofiltrate effluents), except perspiration losses were collected, measured, homogenised and an aliquot analysed for total nitrogen. An accurate record was kept of all input and output and lost specimens were estimated. Samples of input were also analysed.

Fasting blood samples were taken every four days for plasma amino acids and thyroxine-binding pre-albumin, in addition to daily routine blood samples. Routine blood samples had a full Technicon SMA Profile® analysis, full blood count and haemoglobin.

Samples of plasma, urine, dialysate and ultrafiltrate effluent, were analysed for free amino acids, including 3-methylhistidine, urea, creatinine and uric acid.

A daily body weight record was kept for each patient. Triceps skin fold thickness and mid-arm circumference measurements were made every four days.

The remainder of this chapter describes in more detail the patient selection and the methods, outlined above, used in this study.

2.3 Ethical Considerations

This study was approved by the Ethical Committee of Portsmouth and South East Hampshire Health Authority. Informed consent was not obtained from either patients or relatives because this was not an invasive or interventional study. The only changes from normal management were the measurement of triceps skin-fold thickness and mid-arm circumference, removal of an extra 20 ml of blood after an 8 hour fast every four days and the collection of all excreta.

2.4 Patient Selection

All patients admitted to the Wessex Regional Renal Unit, St. Mary's Hospital, Portsmouth for treatment of ARF were considered for entry into the study. The diagnosis of ARF was determined by the consultant physician under whose care the patient was admitted.

Patients were excluded if the diagnosis was acute-on-chronic renal failure because a degree of adaptation to the CRF could have occurred which might have affected the response to nutritional management.

Patients were also excluded if the renal failure was caused by obstruction.

Initially patients able to take an oral diet were included. They were sip fed using a defined formula tube feed. However, because of the high percentage of dietary intake lost as diet rejects, selection was later restricted to patients requiring a naso-gastric (NG) tube feed or intravenous feeding.

During the latter part of the study patients were not selected if it was clear from their clinical condition that they were unlikely to require intravenous nutrition or tube feeding for more than two or three days. Prior to this much effort had been wasted in starting collections on patients who were withdrawn before sufficient data had been collected. This was because oral feeding was instituted earlier than expected or the patient died.

The maximum number of patients studied at any one time was three. This was reduced to two if HD was involved as only one dialysis machine had been modified to collect an aliquot of the dialysis effluent. The only alternative was to collect the effluent manually - see Manual Haemodialysis Effluent Collection section 2.9.2. As the author was the only person involved in the collection of HD effluent and the aliquoting and pre-treatment of all other samples, there was a limit to the number of patients it was practical to make collections from at any one time.

Patient selection for the first two years (50%) of the study was determined by a Medical Senior Registrar who was involved in the clinical and nutritional management of these patients. The clinical management was directed by a Consultant Nephrologist and was not

influenced by the admission of the patient to this study.

The observation period for each patient was terminated when an oral diet could be introduced or when the serum creatinine fell below 400 μmol l^{-1} without HD or haemofiltration or when urinary incontinence became significant.

The diagnostic and treatment data on 24 ARF patients are shown in Table 3, whilst the serum chemistries are in Table 4.

Table 3. Diagnostic and Treatment Data on Twenty-Four ARF Patients.

Patient	Died	Vent.	Age (yrs)	Sex	Wt. (kg)	Diagnosis	No. Days	Type of Nutr.	Therapy
JF	N	N	66	M	84.8	Melaena D & V	8	Oral	PD
JP	N	N	42	M	66.5	Pneumonia	8	Oral	PD
PP	N	N	36	M	72.8	Mefenamic Acid Hyper-sensitivity	4	Oral	PD
AL	Y	Y	17	M	86.7	Multiple Trauma	13	TPN	HF/HD
DH	Y	N	39	M	81.8	Carcinoid	4	Oral	HD
JA	N	Y	58	M	82.7	Thoracic Trauma	9	NG	HF
SH	N	Y	15	F	110	Appedicitis	10	TPN	HF/HD
JO'G	Y	N	54	M	63.2	TTP	9	TPN/Oral	PD/HD/HF
MY	Y	Y	62	F	74.2	Pneumonia	6	TPN/NG	HF/HD
RWo	N	N	61	M	71.7	Hyperneph-roma	3	TPN	HF
JT	Y	Y	66	M	70.1	Aortic Aneurysm	14	TPN	HF/HD
RWh	Y	Y	61	M	85	Achalasia Oesophagus	9	TPN	HF
PW	Y	N	59	F	56.1	Gastroenter-itis	3	TPN	HF

continued.

Table 3. continued.

Patient	Died	Vent.	Age	Sex	Wt.	Diagnosis	No. Days	Type of Nutr.	Therapy
AW	Y	Y	57	F	65.6	Septicaemia Gastro-ent.	9	TPN	HF
ABr	Y	Y	61	M	87.9	Small Bowel Obstr., 2nd. Adhesions	13	TPN	HF
DL	N	N	54	F	57.8	CA Colon	5	TPN	HF/HD
NG	Y	Y	71	M	91.2	Aortic Aneurysm	3	TPN	HF/HD
HH	N	N	52	M	87.4	Diverticula Disease	10	TPN	HD
MB	Y	Y	77	M	85.2	Aortic Aneurysm	11	TPN/NG	HD/HF
FT	N	N	65	F	65.8	Aortic Aneurysm	7	NG	HD
ABe	Y	Y	74	M	80.2	CA Sigmoid Perforation	4	TPN	HF
DG	N	Y	61	F	66.6	Biliary Peritonitis	5	TPN	-
BK	Y	Y	59	M	80	Aortic Aneurysm	4	TPN	HF
RS	N	Y	77	M	89	Aortic Aneurysm	12	TPN/NG	HF/HD

Mortality 54%; 58% Patients Required Ventilation.

N = no; Y = yes; M = male; F = female; TPN = total parenteral nutrition; NG = nasogastric tube feed; Oral = sip feed; HF = Continuous Arterio-Venous Haemofiltration; HD = haemodialysis; PD = peritoneal dialysis; CA = carcinoma; D & V = diarrhoea and vomiting; Nutr. = nutrition; Wt. = weight; Vent. = Artificial Ventilation; TTP = Thrombotic Thrombocytopaenic Purpura.

Table 4. Pre-Study Concentrations of Various Constituents in the Blood of Twenty-Four ARF Patients.

Patient	Urea mmol/l	Creatinine µmol/l	Urate µmol/l	K^+ mmol/l	Albumin g/l	TBPA mg/l	WBC 1000/µl	Hb g/dl
JF	29	939	410	4.6	29	156	19.7	10.5
JP	32.4	1197	986	4.8	37	196	7.1	12.0
PP	52.9	669	615	3.6	29	151	15.2	9.8
AL	26.9	490	592	5.7	32	205	13.7	9.2
DH	40.2	924	1116	5.2	30	109	13.8	12.4
JA	32.3	410	566	4.5	28	165	16.9	9.3
SH	21.6	301	705	2.9	28	189	14.2	7.5
JO'G	24.3	1066	443	4.7	28	296	6.1	6.9
MY	36.2	582	741	4.9	32	100	19.5	10.1
RWo	20.8	533	881	7.0	28	133	-	10.5
JT	58.9	841	718	4.5	34	176	17.3	10.7
RWh	31.7	447	530	4.9	23	116	27.0	9.0
PW	42.7	320	448	4.2	25	80	7.3	7.3
AW	34.8	541	779	3.3	17	33	25.7	9.4
ABr	51.3	799	1053	3.9	25	254	4.4	9.0
DL	25.6	387	664	4.6	18	112	38.4	9.8
NG	47.8	929	715	5.2	25	103	16.1	9.8
HH	81.8	1008	1191	5.6	24	333	18.7	12.6
MB	25.5	574	521	3.8	25	66	6.1	8.4
FT	26.8	347	548	5.7	29	287	7.8	9.3
ABe	83.8	616	796	5.3	28	185	13.4	6.8
DG	45.2	627	1283	3.6	32	113	25.1	11.0
BK	48.3	769	712	4.1	26	110	24.5	11.0
RS	34.0	541	417	5.9	36	223	21.9	8.8

WBC = leucocytes; TBPA = thyroxine-binding pre-albumin;
Hb = haemoglobin

2.5 Angio Access

Blood access for HD, continuous arterio-venous haemofiltration (CAVH) and intravenous feeding was obtained by the insertion of either a Quinton-Scribner arterio-venous shunt (Quinton et al., 1960) or a subclavian catheter (Vaz, 1980).

For the first three-quarters of the study all patients who required angio-access had a silicone and teflon arterio-venous shunt (Extracorporeal Inc.) inserted either into the radial artery and associated vein at the wrist or into the posterior tibial artery and long saphenous vein at the ankle. Later, a single or double lumen polyurethane subclavian catheter (Vascath Ltd.) was frequently used. This was either of the single lumen or double lumen type. With single lumen catheters a Single Needle Monitor (Gambro Ltd.) was utilised during HD or CAVH therapy to alternate the flow in the catheter.

2.6 Waste and Fluid Removal Therapies

2.6.1 Introduction

Most patients required a four hour HD on admission to the hospital, which occurred before any observations were made. During the first three-quarters of the study, after this initial HD most patients were treated by CAVH and an occasional HD if required to control serum potassium or urea concentrations. The last quarter of the study (one year) HD was often used exclusively.

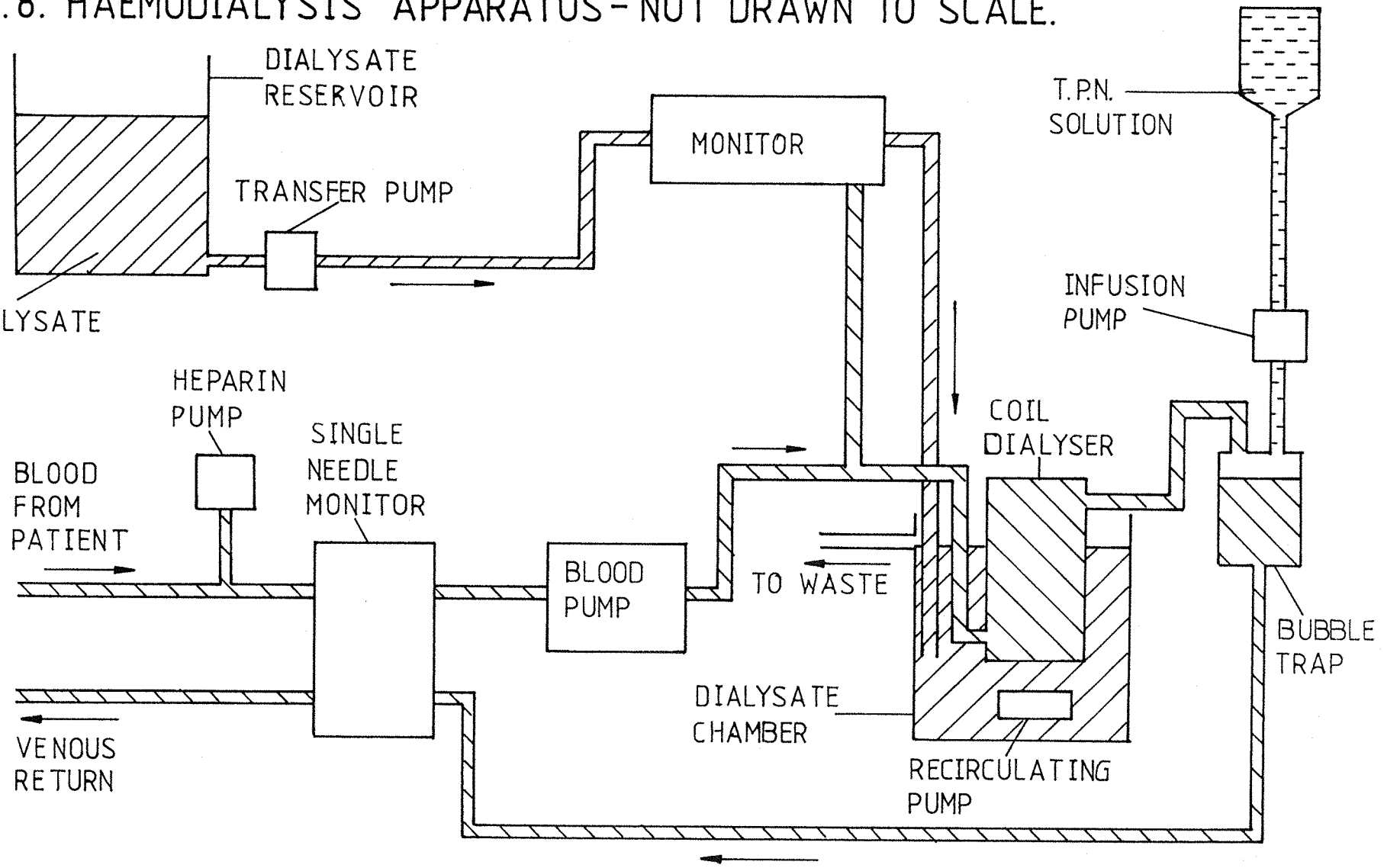
2.6.2 Haemodialysis (HD)

A locally manufactured coil dialyser was used (Merril, 1952; Hoenick et al., 1979) for all dialyses during this study. Most patients were dialysed using a standard acetate dialysate solution (Macarthy Medical Ltd., Appendix II, Table 21) and cuprophane 0.8 m^2 coil (Extracorporeal Inc.). The dialyser used a 240 l reservoir of dialysate which was pumped at a rate of 400 ml min^{-1} into a dialysis chamber housing the coil. Fluid from the dialysis chamber was recirculated through the coil and the effluent from the chamber ran to waste at the same rate as the input. Blood flow was maintained at 150–200 ml min^{-1}. No patients underwent bicarbonate dialysis. Following an initial bolus heparinisation (5000 units) a constant infusion of heparin was used to prevent the formation of blood clots. Intravenous nutrient solutions were infused into a bubble trap, on the venous side of the coil. For the arrangement of this apparatus see Fig. 8.

2.6.3 Continuous Arterio-Venous Haemofiltration (CAVH)

Initially CAVH treatment (Kramer et al., 1981, 1982) was carried out without pumps, using the patient's blood pressure to maintain the pressure differential across the filter. However, the filters (Amicon Ltd.) tended to have a short life with a rapidly reducing flow-rate and an inadequate potassium removal. These problems were overcome by the use of a pump to maintain a satisfactory blood flow rate (Fig. 9)

Fig. 8. HAEMODIALYSIS APPARATUS - NOT DRAWN TO SCALE.



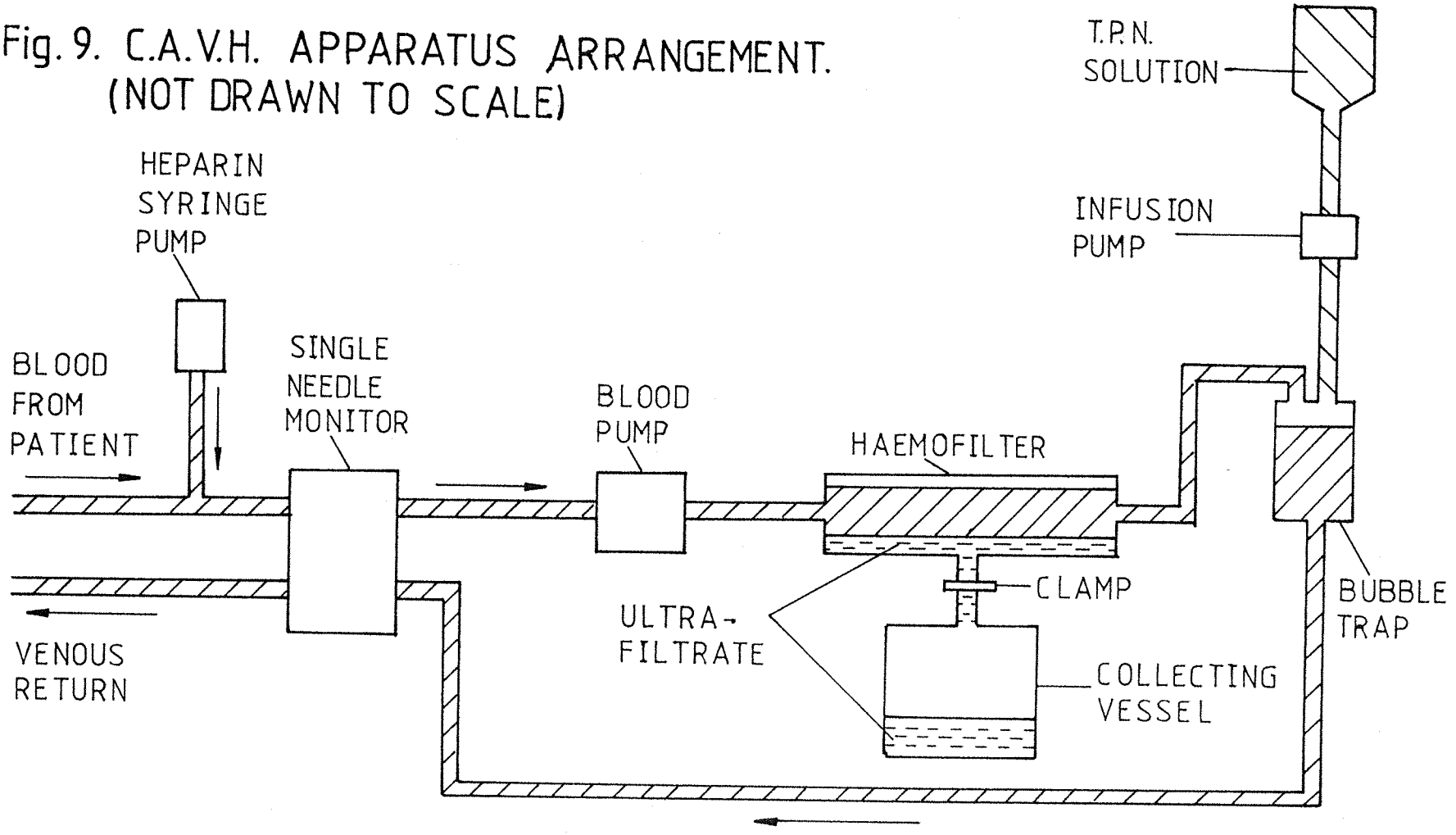
and the use of a potassium-free fluid replacement solution (Boots Ltd. - see Appendix II, Table 22 for formulation).

Blood flow rates in the range 100 to 150 ml min^{-1} were used and heparin was constantly infused into the arterial line to prevent the formation of bood clots. Ultrafiltrate flow was controlled by clamping the outlet tube, just before the collection container. Intravenous feeding and replacement solutions were infused into the bubble trap on the venous side of the filter.

2.6.4 Peritoneal Dialysis (PD)

A manual system of 2 l exchanges of dialysate infused and drained by gravity was used. Fluid removal from the patient was controlled by the use of different glucose concentrations in the dialysate solutions (Dianeal®, Travenol Ltd., Appendix II, Table 23 for formula). In one patient an addition of 10 ml Vamin Glucose® amino acid solution (Kabivitrum Ltd., Appendix II, Table 25) was used to reduce the amino acid losses in some exchanges (Jackson et al., 1979).

Fig. 9. C.A.V.H. APPARATUS ARRANGEMENT.
(NOT DRAWN TO SCALE)



2.7 Patient Nutrition

2.7.1 Introduction

The type of nutrition, method of administration and formulation was determined by the consultant physician under whose care the patient was admitted.

2.7.2 Oral

As already stated (page 61), at the start of the project patients who were well enough to take an oral diet were included. In order to accurately measure their intake without the necessity for duplicate diet analysis, to provide creatine-free and muscle-free intake and to keep the dietitian's work load within practical limits these patients were sip-fed using a commercial tube-feed - Clinifeed Iso® (Roussel Laboratories Ltd., Appendix II, Table 24). This was formulated and prepared by the Renal Unit Dietitian, who also collected any diet rejects and calculated the intake and rejects' composition. Rejects and an aliquot of the feed were analysed for total nitrogen as a check against the calculated intake.

Patients were allowed to drink tea and coffee and the volume was recorded, either by the nursing staff or the dietition, so that allowance could be made for the extra milk intake.

2.7.3 Naso-Gastric (NG)

Patients received an NG tube feed either via a fine-bore (1 mm I.D.) feeding tube (Roussel Laboratories Ltd.) or a larger bore (4.7 mm I.D.) NG tube if this was already present for the removal of NG aspirate. The commercial tube-feeds were used at half strength when given to a patient for the first time, in order to reduce the incidence of diarrhoea. This strength was given for a minimum of two days. These feeds were either pumped or drip-fed, irrespective of the strength given.

NG or sip-feeds used were Clinifeed Iso®, Clinifeed Favour® (Roussel Laboratories Ltd.) or Nutranel® (Cow and Gate Ltd.).

Clinifeed® is a whole protein feed whilst the nitrogen content of Nutranel® is derived from a mixture of amino acids and short-chain peptides - see Appendix II, Table 24 for tube feed formulae.

2.7.4 Total Parenteral Nutrition (TPN)

TPN was infused via a central line and was given :-

1) pre-mixed in a 3-litre bag (provided by the hospital pharmacy or made-up on the ward by nursing staff),

2) sequentially from individual bottles or

3) with a constant carbohydrate infusion and a simultaneous infusion of a lipid emulsion or amino acid solution.

Pre-mixed TPN solutions were exclusively used for the first three-quarters of the study when methods 2 and 3 were additionally used. All nutrition solutions were pumped using Imed® volumetric infusion pumps (Imed Ltd.). One pump was used for each bag or bottle. All patients were infused continuously when possible, including throughout HD and CAVH treatment. In one patient intravenous feeding was only given during dialysis therapy. Exceptions to continuous infusion were a) when fasting prior to phlebotomy and b) lipid emulsions were not infused during the six hours prior to the daily phlebotomy.

Daily intakes were calculated from the volumes recorded in nursing records. The amounts prescribed were also recorded.

In most cases calories were provided from both lipid and carbohydrate sources. The intended calorie:nitrogen ratio was between 200:1 to 150:1. However this inevitably varied for the supply reasons outlined in the discussion and also because there was usually an extra supply of calories from the 5% dextrose solution used as a solvent for drug administration or for priming and emptying blood lines at the start and end of HD and CAVH therapy.

Intravenous nitrogen sources used were all full-profile synthetic L-amino acid solutions, which were commercially available:

Vamin N®, Vamin Glucose®, Vamin 14® and Vamin 18® (KabiVitrum Ltd.) and Aminofusin-L-Forte® (Merck Ltd.) - see Appendix II, Table 25.

Carbohydrate energy sources used were Glucoplex 1000®, Glucoplex 1600® (Geistlich Sons Ltd.), 5% w/v dextrose and 50% w/v dextrose (Travenol Ltd.) - see Appendix II, Table 26. The Glucoplex® solutions contained glucose, electrolytes and trace elements.

Fat energy sources were Intralipid® 10% or 20% (KabiVitrum Ltd.) see Appendix II, Table 27. These were white, oil in water stabilised emulsions containing fractionated soya-bean oil, fractionated egg phospholipids and glycerol.

Addamel® (KabiVitrum Ltd., Appendix II, Table 28) was added to the amino acid solutions to provide an adequate supply of electrolytes and essential biological elements.

Vitlipid® adult (KabiVitrum Ltd., Appendix II, Table 29) was added to the Intralipid infusions to provide an adequate supply of fat-soluble vitamins.

Solivito® (Kabivitrum Ltd., Appendix II, Table 30) was added to carbohydrate or amino acid solutions to provide daily requirements of water soluble vitamins.

2.8 Anthropometry

Body weight was measured using either a weigh-bed (Sauter Ltd.) or chair scales (Avery Ltd.).

Mid-arm circumference (MAC) was measured using a fibre-glass tape measure and Holtain® skin calipers (Holtain Ltd.) were used to measure triceps skin fold thickness (TSFT). The jaws of the calipers exert a constant pressure on the skin irrespective of their distance apart. Both these measurements were taken, as far as possible, on the non-dominant arm, but the contra-lateral arm was used if a catheter was inserted into the dominant arm. These measurements were taken mid-way between the olecranon process and the tip of the acromium. It was not always possible to take the measurements in the ideal position with the arm hanging vertically, particularly if the patient was on a ventilator.

Mid-arm circumference and triceps skin-fold thickness were measured by a Medical Senior Registrar for the initial two-thirds of the study. They were then taken by the author for the remainder of the study.

Height was not measured because most patients were in a supine position throughout the 24 hrs.

2.9 Haemodialysis Effluent Collection

2.9.1 Automated Haemodialysis Effluent Collection

A coil dialyser was used for all HDs on ARF patients throughout the study. With a dialysate flow rate of 400 ml min^{-1}, a six hour dialysis produced 144 l of effluent. Therefore, because space was at a premium in the acute room in the renal unit (a large tank and pump would be required to collect this volume) and to minimise the error in accurately measuring such a volume, a dedicated effluent collection machine was designed and built. This was required to accurately sample a proportion of the effluent produced, cater for small variations in effluent flow rate and be unaffected by a temporary shut-down of the main dialysis machine (e.g. if blood lines became blocked by clot formation).

This collecting apparatus is shown in Fig. 10 and was connected to the HD machine's waste pipe via stopcocks and tubing to enable the flow to be diverted into the apparatus when required.

On entering the collection apparatus effluent was directed into a collecting tank fitted with two float-operated micro-switches, a drain tube at the base, an overflow pipe which led to a 10 l bottle and a submersible centrifugal pump. The centrifugal pump rapidly mixed in-coming effluent to ensure that the tank at all times contained an almost homogeneous solution. The overflow tank was required because the dialysis chamber in the dialyser which held the coil was rapidly drained at the end of each dialysis. The collecting tank was approximately the same volume as the subsidiary tank, and if the collecting tank was nearly full when the dialysis chamber was emptied this would lead to an overflow. Also, the pump which drained the collecting tank could not cope with the in-flow from the dialysis chamber at such times.

The drain pipe from the collecting tank was connected to the inlet port of a pump. The pump was protected by a coarse filter to prevent damage by foreign particles. The pump outlet led to a solenoid valve which diverted effluent either to a waste pipe or into a 20 l sample bottle which contained 20 ml 50% w/v thymol in isopropanol as a preservative.

Fig. 10. HAEMODIALYSIS EFFLUENT COLLECTOR.

(SCHEMATIC DIAGRAM).

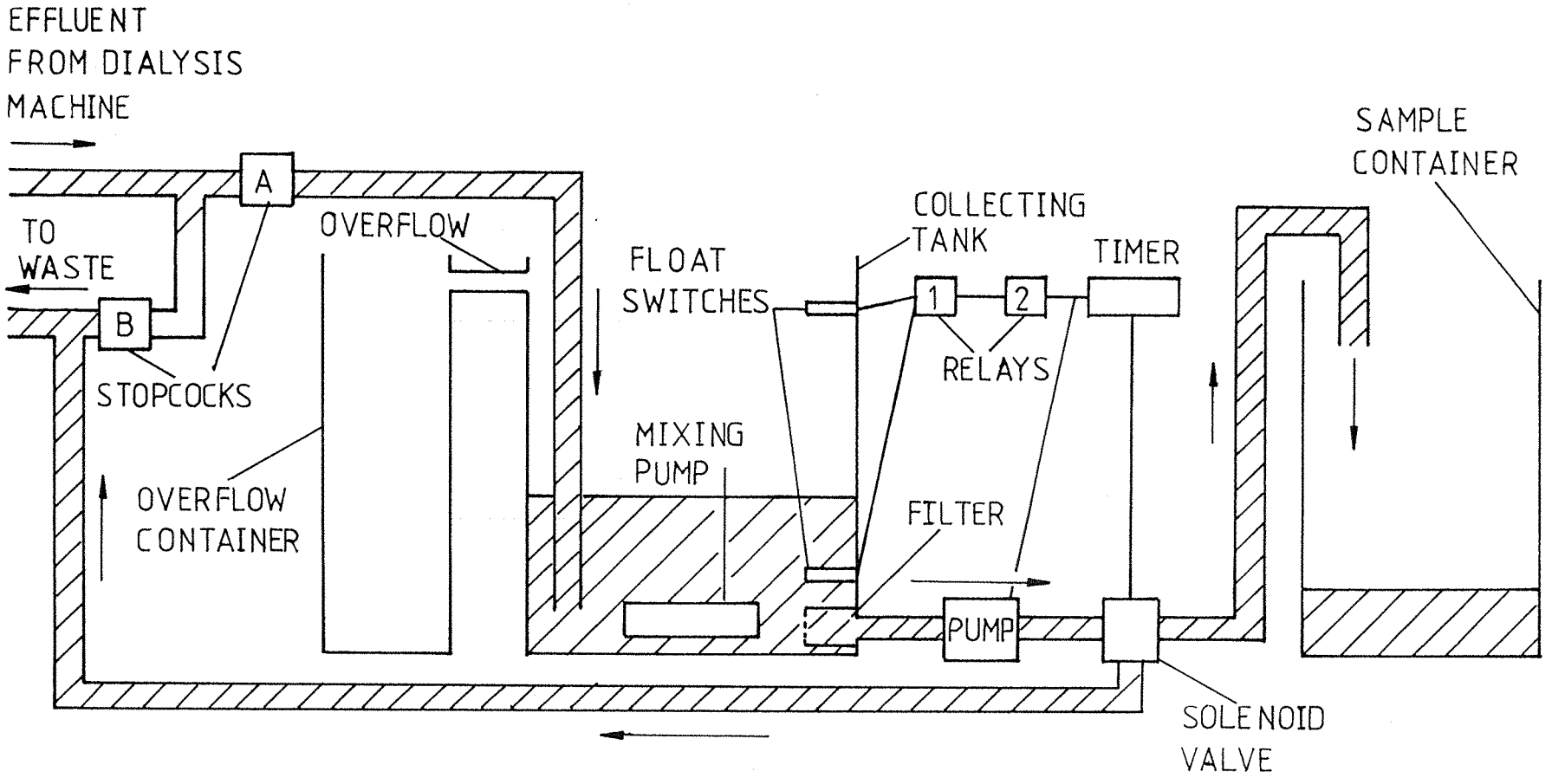
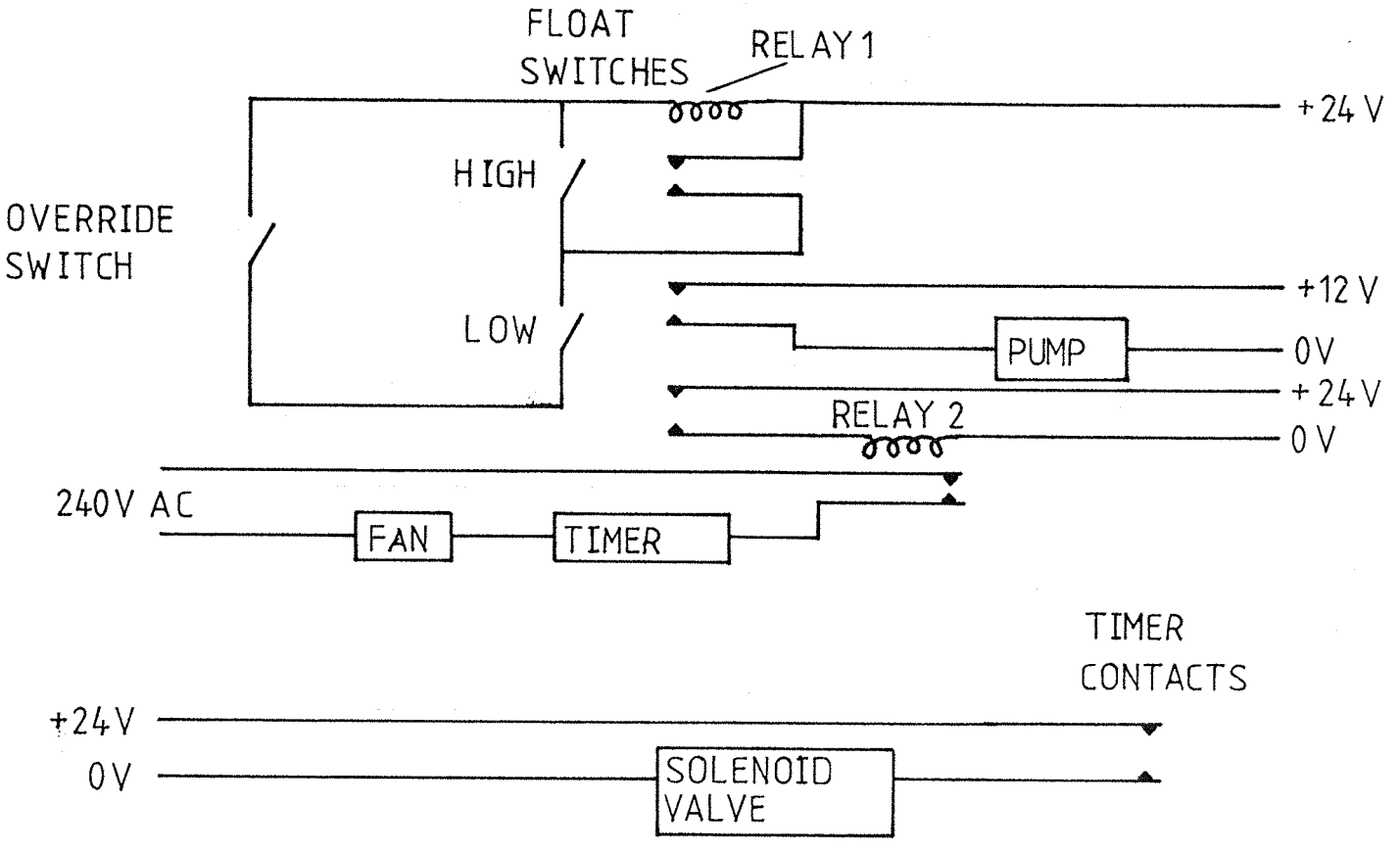


Fig. 11. HAEMODIALYSIS EFFLUENT COLLECTOR - CIRCUIT DIAGRAM.



The switches in the collecting tank, via relays and an electronic circuit (Fig. 11), switched on and off the pump, an electro-mechanical timer that operated the solenoid valve and a cooling fan. The top switch turned the pump on when the tank was full of fluid and the lower switch turned the pump off when the fluid had reached that level. The lower switch was required to prevent damage to both the mixing pump and the sampling pump if they should run dry. The sampling pump flow rate was 500 to 600 ml min^{-1}, to ensure that the collecting tank was gradually emptied. The bottom switch could be overridden by a manual switch to enable the tank to be drained.

The solenoid timer was adjusted to provide a sampling time of 8.4 seconds (sec) in a total cycle time of 120 sec (7%). The sample proportion of the total effluent was verified by gravimetric analysis - see Method Modification and Validation section 2.15.

At the start of each dialysis the effluent was diverted from the waste pipe to the collecting tank by closing stopcock B and opening stopcock A and the power switched on once blood began to flow through the coil. Once there was sufficient fluid in the base of the tank the mixing pump was switched on.

The apparatus was observed for one filling and emptying of the collecting tank to ensure that the system was functioning correctly. The apparatus was then left unattended by the author until the end of the dialysis - nursing staff were not trained in the operation of this machine.

At the end of the dialysis the stopcocks were operated in reverse order, to re-direct any effluent to waste if the machine was to be used for a patient who was not part of the study.

Any fluid which had overflowed as a result of draining the coil compartment, was emptied into the collecting tank and the tank drained by use of the override switch - sampling was not overriden.

The apparatus was then rinsed with tap water, and sterilised with either formalin solution or hypochlorous acid (Amuchina Ltd., Appendx II, Table 31). The HD machine sterilisation method was changed from formalin solution to hypochlorous acid during the period covered by this study. The outside of the apparatus was sterilised using a sodium hypochlorite solution (Appendix II, Table 31) and the pump tube removed from the peristaltic sampling pump, when used (see

Method Modification and Validation, section 2.15.2).

By measuring the sample volume collected and multiplying by 14.3 the total volume of effluent could be calculated (7% of the total volume was sampled). Regular verification of this factor was performed.

2.9.2 Manual Haemodialysate Effluent Collection

If more than one patient being studied required simultaneous HD or when the collection apparatus was temporarily out of action manual collections were made.

Effluent from the dialysis machine was directed into a 40 l tank equipped with a stopcock. 5 l volumes were measured in a volumetric flask. This was then thoroughly mixed by inversion and a 5 ml aliquot removed, using an Oxford® macro pipettor (BCL Ltd.) and added to a sample bottle containing thymol in isopropyl alcohol as a preservative. The remainder of the 5 l of effluent was discarded and another 5 l volume measured. This process was repeated until the end of dialysis and all the effluent had been sampled. Subsequent samples taken during the same dialysis were collected in the one container. By measuring the total sample volume at the end of dialysis, and multiplying by 1000 the total volume of effluent was calculated.

2.10 Excreta Sample Collection

All sample collections were made in sequential 24 hr periods and timed from 0800 hrs to 0800 hrs.

After measurement in a 1 litre calibrated plastic jug, nursing staff collected all urine, haemofiltration effluent, peritoneal dialysis effluent, suction (tracheal aspirate mixed with sterilised water), NG aspirate, wound drainage and some colostomy fluid (depending on consistency) in 2.5 or 5 l plastic bottles to which 2 ml or 4 ml 50% w/v solution of thymol in isopropanol had previously been added. The thymol was used to acidify the sample and reduce the effect of bacterial action (Alper, 1974). A 50% solution was used to reduce the volume of preservative added to the container.

Faecal samples were emptied from the bedpan into a 1700 ml plastic, squat, cylindrical container with the lid covering an opening the full diameter of the container. If the patient was incontinent of faeces soiled bed linen was saved in polythene bags, subsequently scraped clean and, if necessary, rinsed with distilled water. Patients who were given a continuous tube or sip-feed were given 400 mg carmine red dye in a capsule to mark the faeces. A capsule was given at 0800 hrs at the start and end of each four day collection period. Faeces marked with the earlier capsule were included in the period, whilst faeces marked by the next marker were excluded from that period, but included in the next.

All liquid samples were weighed in the containers, which were pre-weighed and marked with this weight prior to sample collection. The sample weight was obtained by subtracting the empty weight from the full weight. If a single substance was collected in more than one container over a 24 hr period, the contents were pooled if they were sufficiently liquid not to require homogenisation. After mixing with a stirring rod an aliquot was taken. Alternatively, samples were homogenised using a suitably sized Waring® Commercial stainless steel blender (Waring Ltd.). An appropriate fluidity of sample was obtained by the addition of distilled water. As before, the final sample weight was obtained by subtraction and was recorded. An Avery® 10 kg balance (Avery Ltd.) was used to weigh samples. If the total 24 hr volume was too large to be measured by pooling e.g. peritoneal

dialysate effluent, aliquots from each container were mixed proportionately.

Single 10 ml and 20 ml aliquots of NG aspirate, suction, drainage, colostomy, faeces, sputum, vomitus and diet reject homogenates were stored at -20 °C until analysis.

HD, CAVH, PD effluents and urine were each separated into 20 ml, 10 ml, 3 ml and 2 ml aliquots.

10 ml samples had 100 µl concentrated hydrochloric acid added to further lower the sample pH to prevent loss of ammonia and add to the anti-bacterial effect of the thymol.

3 ml samples had 30 µl 5% (or 50% w/v to urine only) sodium hydroxide solution added to prevent uric acid precipitation.

2 ml aliquots of the CAVH, PD effluent and urine samples were added to an equal volume of a precipitating solution which contained sulphosalicylic acid, L-nor-leucine as an internal standard and a lithium buffer (Appendix II, Table 32) for subsequent amino acid analysis. This mixture was thoroughly shaken and centrifuged at 2400 rpm for 15 min at 4 °C in a Mistral® 6L centrifuge (MSE Ltd.). The supernatant was separated and stored at -20 °C until analysed.

A 3 ml aliquot of the HD effluent was added to 150 µl of a second precipitating solution containing the same ingredients as above, but at different concentrations (Appendix II, Table 32). These samples were mixed, centrifuged and stored in the same way.

2.11 Blood Samples

Blood samples were routinely taken each day between 0800 hrs and 0900 hrs by medical or nursing staff either by peripheral venepuncture or by a subclavian catheter. When the subclavian catheter was used, 10 ml of blood was removed prior to sampling to avoid contamination with heparin or intravenous fluids. All patients were supine when blood samples were taken and no blood was taken during or immediately after a dialysis. These conditions were required to avoid variation in blood constituents caused by circadian rhythms and pooling caused by recent fluid removal.

The intention was to take fasting blood samples every four days. This was not possible when intravenous infusions were not stopped. The patient was considered to be fasting if TPN infusions had been stopped for at least 8 hrs, 20 mls extra blood were taken on these fasting days, 10 ml in a plain tube and allowed to clot and 10 ml in a tube which contained lithium heparin as an anticoagulant. These were centrifuged at 2400 rpm at 4 °C for 15 min, within one hour of collection. 2 ml plasma was then added to 2 ml of a protein precipitating solution containing sulphosalicylic acid 6% w/v and norleucine as an internal standard in a lithium buffer (Appendix II, Table 32. This was stoppered, shaken to thoroughly mix the solutions and then centrifuged under the same conditions as before, for a further 15 mins. The supernatant was then decanted into a 4 ml tube, stoppered and stored at -20 °C. The remaining serum and plasma were also decanted and stored at -20 °C until analysis.

2.12 Record Data Collected

From nursing records infusion volumes, body weight, body temperature and pulse were recorded.

Routine biochemical and haematological data, patient data, diagnosis, complications and progress were all obtained from the patient's notes.

2.13 Biochemical Methods

2.13.1 Urea

A commercial kit (Diagnostic Kit No. 124 7700, Boehringer Corporation (London) Ltd.) that utilised an enzymatic colourimetric manual method (Boehringer Corporation (London) Ltd., 1983) was used to measure the concentrations of urea in samples of CAVH, HD, and PD effluent and urine.

This method utilised the cleavage of urea by the enzyme urease in a buffered medium to form ammonium carbonate. Berthelot's reaction (Berthelot, 1859) was then used to measure the ammonium ions present by the reaction of ammonium ions on phenol and hypochlorite to give a coloured complex, indophenol.

Initially ammonia and hypochlorite react to give chloramine. The chloramine then reacts with phenol to give quinone chloramine. This further couples with phenol to form the yellow associated indophenol, which dissociates in alkali to give a blue chromogen (Bolleter et al., 1961). Sodium nitroprusside was included as a catalyst to speed up the reaction (Horn and Squire, 1966).

To optimise the colour production, each sample was diluted with 0.9% w/v sodium chloride solution in the following ratios: urine 1:100, haemofiltrate 1:40, PD effluent and plasma (including plasmapheresis effluent) 1:10 and HD effluent 1:5. These dilution ratios were determined by trial and error. Representative samples were analysed at different dilutions until the absorbance of the resulting chromogen was between 0.10 and 1.0.

Aliquots of sample and phosphate buffer/urease suspension were mixed in a stoppered tube and incubated at 37 °C for 10 min in a water bath (Grant Instruments Ltd.). Aliquots of a phenol/sodium nitroprusside solution followed by a sodium hydroxide/sodium hypochlorite solution were added, mixed and incubated for a further 15 mins.

The absorbance of the sample, standard or quality control sample were read against a reagent blank in a Pye-Unicam SP6-500® U.V. spectrophotometer (Pye-Unicam, Ltd.) set to 550 nm wavelength and 1 cm light path. Urea concentrations were calculated from the

absorbance values of the samples and standard. Over the range of concentrations measured the absorbance of indophenol is directly proportional to the ammonium ion (and hence urea) concentration.

2.13.2 Total Nitrogen

Nitrogen compounds in all samples, but excluding blood samples, were oxidised to ammonium sulphate by Kjeldahl digestion (Kjeldahl, 1883a, 1883b). The concentration of ammonium ions was then measured on a Technicon AAII® continuous flow analyser (Technicon Instruments Corp.) fitted with an ammoniacal nitrogen manifold and utilised a colourimetric method.

1 g of homogenate, 2 ml HD, PD or CAVH effluent, or 500 µl urine was added to 7 g potassium sulphate and 0.8 g copper sulphate in a 250 ml digestion tube. 12 ml concentrated, nitrogen-free sulphuric acid was added followed by 6 ml 60% w/v hydrogen peroxide. This mixture was then heated in a block heater set to 420 °C for 30 min and after cooling was diluted with distilled water to a volume of 250 ml. (Technicon Industrial Method No. 369-75A/A, 1975). Sulphuric acid was required for the conversion of nitrogen compounds to ammonium ions, the speed of the reaction was increased by the use of copper as a catalyst (Wilfarth, 1885) and the addition of a salt to raise the boiling point of the mixture; potassium sulphate was preferred to reduce glass etching (Giorgio, 1974). Hydrogen peroxide was added to accelerate the conversion of carbon compounds to carbon dioxide. Hydrogen peroxide was preferred to other oxidising agents because it can shorten digestion time with no loss of ammonia, even in the presence of chloride (Giorgio, 1974).

Diluted digests were analysed at a rate of 40 samples per hour on a continuous flow analyser. Ammonium ions were dialysed into an acid diluent and sequentially mixed with a buffer (pH 12.8 to 13.0), sodium salicylate/sodium nitroprusside reagent and sodium hypochlorite solutions. This mixture was incubated at 37 °C and the absorbance of the chromogen formed was measured in a colourimeter at 660 nm wavelength with a 15 mm flow cell.

In this method the ammonium ions form an emerald-green coloured complex wth sodium salicylate at an alkaline pH, chloride ions being

required and sodium nitroprusside was used as a catalyst to speed up the reaction (Technicon Industrial Method No. 334-74A/A, 1976; Reardon et al., 1966; Wall and Gehrke, 1974; Searcy et al., 1967; Crooke and Simpson, 1971).

2.13.3 Creatinine

A Beckman Creatinine 2® analyser (Beckman Ltd.) was used to measure creatinine concentrations in samples of urine, HD, PD and CAVH effluents.

This analyser uses a kinetic Jaffé (Jaffé, 1886) reaction with a single alkaline-picrate reagent.

All samples were analysed undiluted except for urine which was diluted 1:10 with 0.9% w/v sodium chloride.

Each sample was added to the alkaline-picrate reagent in a reaction cell with a 1 cm light path, the absorbance was measured at 520 nm wavelength 25.6 sec after the sample addition.

Creatinine combines with picric acid in the presence of hydroxyl ions to produce a red coloured reagent (Jaffé, 1886).

It is likely that the reaction has two steps, first the formation of an activated picrate ion, then the production of a creatinine-picrate complex (probably a Janovsky complex), with the loss of the hydroxyl ion. A yellow bis complex may then be formed (Butler, 1975a, 1975b; Vasiliades, 1976).

The use of a kinetic technique minimises the interference from both fast and slow pseudocreatinine substances (Bartels and Bohmer, 1971; Fabiny and Ertinghausen, 1971; Heinegard and Tiderstrom, 1973; Cook, 1971; Larsen, 1972).

2.13.4 Uric Acid

A commercial kit (No. 242616, Boehringer Corporation (London) Ltd.) that utilised an enzymatic colourimetric method was used to measure uric acid in samples of urine, HD, CAVH and PD effluents. The method used was a kinetic ALDH-UV method (Ziegenhorn et al., 1979; Haeckel, 1976; Boehringer Corporation (London) Ltd., 1985).

Uric acid in the presence of oxygen undergoes hydrolysis to

allantoin, carbon dioxide and hydrogen peroxide through the action of the enzyme uricase.

Ethanol is then converted to acetaldehyde by hydrogen peroxide via the peroxidase activity of the enzyme catalase.

Acetaldehyde is then oxidised by the enzyme aldehyde dehydrogenase to acetate, hydrogen being transferred to NADP to give $NADPH_2$. The absorbance of the $NADPH_2$ is measured at 340 nm wavelength.

The method was adapted for use on a Coulter Kem-O-Mat II® clinical chemistry analyser (Coulter Ltd.), the reaction cuvettes were incubated at 30 °C and the change in absorbance of nicotinamide dinucleotide phosphate measured using a 340 nm filter.

Most samples were analysed undiluted, however, some urine needed to be re-analysed after diluting 1:10 with 0.9% w/v sodium chloride if the inititial analysis indicated that the concentration was outside the range of the method.

2.13.5 Thyroxine-Binding Pre-Albumin (TBPA)

A single radial immunodiffusion method (Mancini et al., 1963; Behring Diagnostics Ltd., 1985) was used utilising commercially prepared plates to measure TBPA in fasting plasma samples.

M-Partigen® immunodiffusion plates were used (Behring Diagnostics Ltd.) and contained monospecific antiserum to human prealbumin in an agar gel.

An aliquot of a sample, quality control or one of three standard solutions was placed into each well using a Partigen® 5 µl glass capillary dispenser. The plate was then covered and left to stand at room temperature for a minimum of two days.

The TBPA in the solution (antigen) diffuses out into the antibody-containing gel. When the antibody/antigen concentrations are optimal a circular precipitin line is formed.

A Behringwerke® ruler was then used to measure the square of the diameters of the precipitin rings. Two diameters at right angles to one another were measured for each well and the mean of these calculated.

The squared diameter for each standard was plotted against concentration and the unknowns calculated from this reference curve,

using a Basic program written for a Hewlett-Packard HP85® computer. The diameter of the precipitin ring is proportional to the antigen concentration.

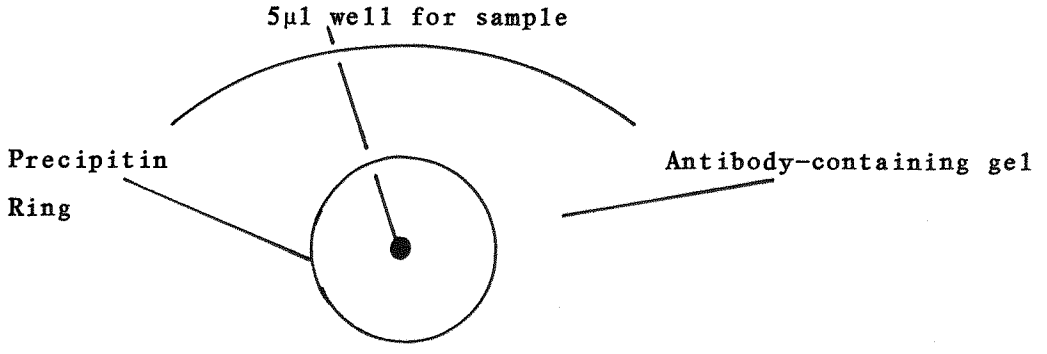


Fig. 12. Showing the Precipitin Ring Formed in the Single Radial Immuno-Diffusion (SRID) Technique.

2.13.6 Free Amino Acids

After pre-treatment of the sample as described in section 2.10, to remove the proteins and add an internal standard (nor-leucine), an aliquot was filtered through a 0.2 µm cellulose nitrate membrane, prior to analysis on a Chromaspek J-180® (Hilger Analytical Ltd.) amino acid analyser.

This analyser uses a single ion-exchange column filled with spherical beads of a sulphonated polystyrene resin, with cross-linkages (Hamilton, 1960). It utilises electronic control for the ratio mixing of two lithium-citrate elution buffers (Thomas et al., 1970) to produce a hydrogen ion and lithium molarity elution gradient (Piez and Morris, 1960). Following reaction with ninhydrin utilising ascorbic acid, dissolved in the eluting buffers, as a reducing agent (Niece, 1975), the eluted amino acids are detected by continuous effluent colourimetry originally developed by Spackman et al., (1958). This analyser was developed from a design by Thomas et al. (1970).

Ninhydrin, a powerful oxidising agent, causes oxidative decarboxylation of the α-amino acids, with the production of carbon

dioxide, ammonia and an aldehyde with one carbon less than the parent amino acid. Reduced ninhydrin (hydrindantin) then reacts with the liberated ammonia to form a blue complex - Ruhemann's purple - which maximally absorbs light of 570 nm wavelength. The intensity of this blue complex is a linear function of the concentration of the α-amino groups present. The imino acids, proline and hydroxyproline, react with ninhydrin to form a yellow colour, which is measured at 440 nm wavelength.

Samples were automatically loaded onto the top of the column and eluted with ratio mixing of two lithium-citrate-borate buffers to separate the amino acids. The eluate was then mixed with 1.5% w/v ninhydrin reagent in segments separated by bubbles of nitrogen gas and was pumped through a reaction coil in an oil bath at 96 °C. The absorbance was measured at 570 nm and 440 nm and recorded on a Vitatron 2001® chart recorder (Vitatron, Ltd.). A Supergrator 3® computing integrator (Columbia Scientific Industries Ltd.) was used on-line for the identification and calculation of the individual amino acid concentrations using the area under each peak. Amino acids wrongly identified were measured and calculated manually using peak heights from the chart recording.

2.14 Pathology Laboratory Methods

2.14.1 Introduction

The following methods were used by the local pathology laboratory service on routine bloods sampled daily. These analytes were required for the day to day clinical management of these patients.

2.14.2 Biochemistry

A Technicon SMA 12/60 Continuous Flow Auto Analyser® (Technicon Ltd) was used for the following :-

Serum Urea - a colourimetric method using diacetyl monoxime reagent.

Serum Creatinine - a colourimetric method using the Jaffé reaction with an alkaline-picrate reagent.

Serum Sodium and Potassium - flame photometry with lithium as a reference was used and aspiration into a propane/air flame.

Serum Albumin - the bromo-cresol green dye binding method.

2.14.3 Haematology

Haemoglobin and Leucocytes (WBC) - were measured using a Coulter Counter S+1® analyser (Coulter Ltd).

2.15 Method Modification and Validation

2.15.1 Introduction

In this section the modifications made to the recommended methods and the verification of the use of these methods are described.

In all analytical methods the linearity and range of each method were checked using standard dilutions. To ensure that other components in the samples did not interfere in the assays recovery experiments were carried out. Quality control samples were analysed in each batch of analyses for all methods to determine the precision and accuracy of each method. If single quality control samples fell outside of defined limits that batch of analyses was repeated.

The results of these experiments are in Appendix III.

2.15.2 Haemodialysis Effluent Collector

This apparatus was initially constructed with a magnetic stirrer to mix the contents of the collecting tank and a diaphragm pump to remove dialysate from this tank.

However, it was soon noticed that the stirring bar was easily dislodged, causing poor mixing. Consequently a submersible centrifugal pump was fitted, which was much more effective and reliable.

The ratio of sample to waste volumes was determined gravimetrically using water instead of dialysate. The outputs from the sample and waste lines were collected 12 times and weighed. The timing mechanism that controlled the solenoid valve was adjusted to optimise sampling time and volume.

Mixing adequacy was determined visually by observing the distribution of dye added to the collecting tank whilst water flowed in at 400 ml min^{-1} (during a dialysis, effluent would flow into the collecting tank at this flow rate). It was also assessed by the measurement of the ammonium concentration in the sample and waste lines (Fig. 10, page 75) following the addition of 50 g ammonium chloride to the collecting tank with a water inflow of 400 ml min^{-1}.

The concentration of ammonia in the solutions was measured using the Technicon AAII® salicylate method - see total nitrogen method (page 84) and Appendix III, Table 37 for the results.

When first used it was clear that the noise level was too high. The cabinet, including the pump base, was manufactured from steel plate on a metal frame. In addition space was at a premium in the Acute Room and this apparatus was physically separate from the HD machine, which gave rise to flow problems. Consequently the apparatus was built into the base of a dialysis machine, still on a metal frame but with wooden doors and base, which reduced the noise.

The solenoid timer was not adjusted throughout the four years of study, but the apparatus was checked periodically for sampling ratio, as described above.

The pump had to be replaced twice. The first breakdown occurred at the end of a dialysis. On the second occasion increased noise and poor performance allowed replacement before actual failure. The diaphragm pump was then replaced with a peristaltic pump, of the same type used in the HD machine and gave no further trouble.

2.15.3 Urea

The manual method described in the reagent manufacturer's instructions was followed (Boehringer Corporation (London) Ltd., 1983). All volumes were proportionately reduced to economise on the use of reagents for convenience.

A range of standards were analysed to determine the linearity and range of the method, replicates of a sample for in-run precision and two quality control samples were analysed in each analysis run for between run precision and accuracy - an in-house urine sample preserved with thymol and a Precipath U® (Boehringer Corporation (London) Ltd.) quality control serum.

Recovery experiments were done to determine the accuracy of the method with the different sample types, in order to exclude the possibility of interfering substances significantly affecting the results.

2.15.4 Total Nitrogen

This method has been in routine use in this department since 1976. Over 10,000 samples have been analysed since then.

Acid Digestion

The Technicon® digestion method (Technicon Ltd., 1975) provided wth the digestion apparatus recommended a sample size of 1 to 2 g to be digested for 30 min at 420 °C by a digestion mixture of 20 ml concentrated nitrogen-free sulphuric acid, 15 g potassium sulphate, 0.5 g mercuric oxide and 5 to 20 ml hydrogen peroxide. An adequate volume of the hydrogen peroxide was indicated by clearing of the tube contents.

Tecator Ltd. who also market a similar digestion system recommend the same digestion temperature and time. However, they recommend commercially available digestion tablets providing 7 g potassium sulphate and 0.35 g mercuric oxide to be used with 10 ml conc. sulphuric acid with or without hydrogen peroxide (Mossberg, 1978). The Tecator® method was found to be adequate for our samples. However, because of the toxicity of mercuric oxide, studies were carried out using copper sulphate as the catalyst with various amounts of hydrogen peroxide. Recovery experiments showed that 1 g or 1 ml of sample, 12 ml conc. sulphuric acid, 6 ml 60% hydrogen peroxide and two catalyst tablets providing 7 g potassium sulphate, 0.8 g copper sulphate (tablets type CM, Thompson and Capper Ltd.) digested for 30 min at 420 °C was optimal for complete digestion.

Colourimetric Analysis

The diluted digests were then analysed on a Technicon AAII® continuous flow analyser fitted with an ammoniacal nitrogen manifold (Technicon Ltd., 1976) as previously described (page 84). The only modifications to this system were the removal of the initial dilution stage between the sampler and the manifold - to increase the sensitivity of the method - and the use of 0.1 ml min^{-1} sample tube and 0.6 ml min^{-1} pump tube for the sample diluent. All reagents and

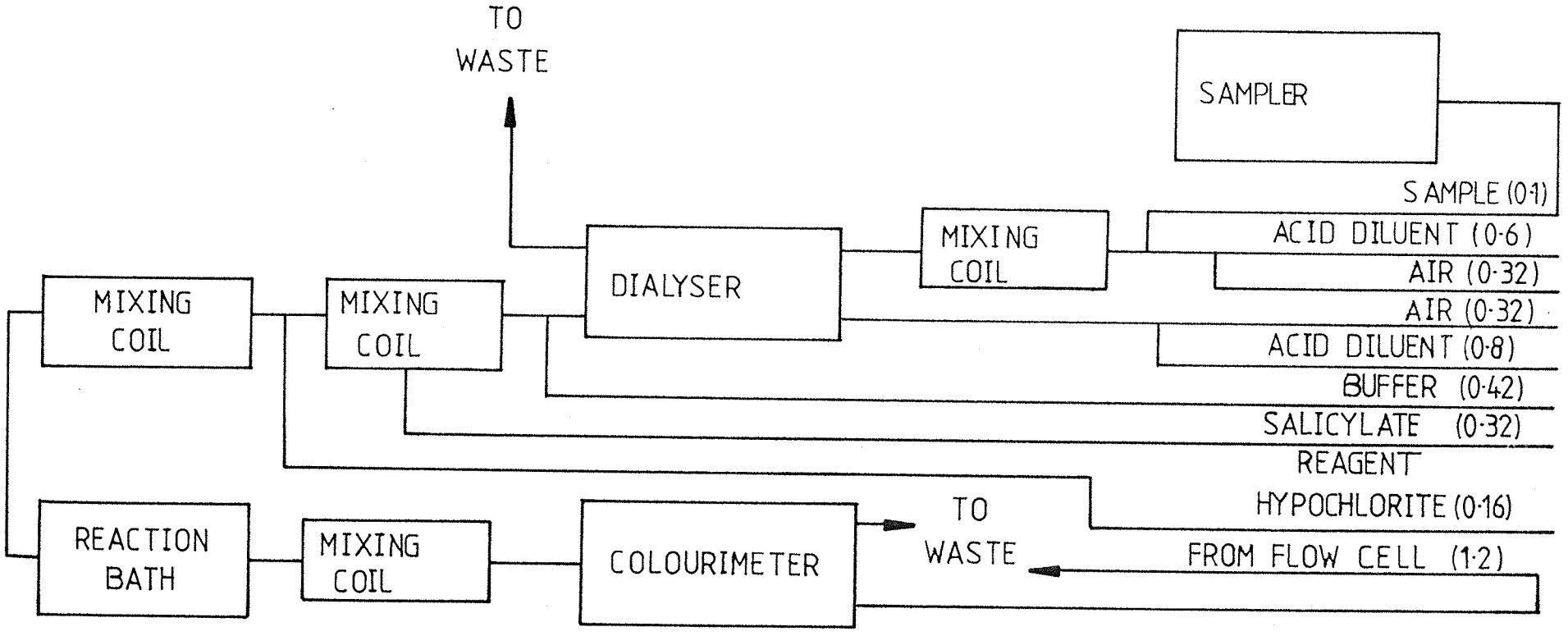


Fig. 13. TOTAL NITROGEN ANALYSER FLOW DIAGRAM.

NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ml/min.

other pump tubes were as recommended - see Fig. 13 for flow diagram.

To cater for the wide concentration range measured a urea standard containing 5 g N ml^{-1} was used. For most analyses this was a mid-range standard (peak height 50% recorder full-scale). However, by adjusting the calibration of the photometer, the peak height of the standard was adjusted from 25% to 100% recorder full-scale, thus accomodating a wide range of sample concentrations.

Within run reproducibility was determined by repeated analysis of an ammonium chloride standard, linearity by standard dilution and between run precision by the inclusion of two quality control samples in each digestion. The aqueous standard used was manufactured from Analar grade urea (BDH Ltd), preserved with thymol solution and stored at -20 °C. This standard was checked against a commercial standard (Sigma Ltd.).

2.15.5 Creatinine

The method was not modified for use with the samples obtained in this study.

A quality control sample was run with each batch of samples to determine the between run precision.

Recoveries were carried out on each sample type to detect any effect that interfering substances may have on the results. The linearity and range of the method was checked using a range of standards.

2.15.6 Uric Acid

The reagent manufacturer's instructions prescribed the mixing of 100 µl sample with 2000 µl of mixed buffer/substrate/catalase solution in a cuvette and reading the absorbance at 340 nm after 3 min. 5 µl uricase suspension was then added, mixed and the final absorbance read after a further 15 to 20 min incubation at 20 to 25 °C.

For use on the KEM-O-Mat® analyser (Coulter Ltd.) the uricase suspension was diluted 1:40 with 0.9% w/v sodium chloride, whilst the buffer/substrate/catalase solution was used at the same concentration as the manual method.

In use, 40 µl sample was added to 300 µl reagent in a cuvette. An absorbance reading at 340 nm was then taken after 4 mins and 30 µl uricase suspension was added 5 mins after the sample and reagent were originally dispensed. The final absorbance reading was taken after a further 15 mins incubation at 30 °C. A calibration standard (Sigma Ltd.) and quality control sample (Boehringer Corporation (London) Ltd.) were run with each batch of samples.

The within run precision was determined by repeatedly analysing a single sample during one analysis run. Between run precision was calculated from the results obtained for a Precipath U® (Boehringer Corporation (London) Ltd.) quality control sample analysed in each batch of samples.

The linearity and range of the method was determined by analysing a range of dilutions of a standard, using 0.9% w/v sodium chloride as the diluent.

A range of standard additions to samples were made to each sample type and recoveries calculated to ensure that other components in the samples were not interfering in the assay.

2.15.7 Thyroxine-Binding Pre-Albumin

The manufacturer's instructions were followed.

Within run (1 plate) precision was determined by repeated analysis of a single sample and between run precision from the quality control data.

The addition of standard to samples was used to determine recovery for this method.

2.15.8 Free Amino Acids

As previously described (page 87) samples were analysed on a Chromaspek J-180® dedicated amino acid analyser (Hilger Analytical Ltd.). Since this analyser was installed in this department in October, 1980 over 3600 analysis runs have been carried out.

Soon after installation (January, 1981) the recommended ninhydrin reagent which used 2-methoxyethanol as solvent was replaced by the ninhydrin reagent recipe previously used in this department

with an LKB amino acid analyser (LKB Biochrom Ltd., 1977) as a less toxic alternative. This was later (1983) changed in formulation as a result of development work by LKB Biochrom Ltd. (Baldesten and Dilley), see Appendix II, Table 33. As a result of this change in solvent, Solvaflex® pump tubes could be replaced by standard flow rated tubes.

Also in 1983 the reducing agent for the air stable ninhydrin was changed from a cyanide solution as recommended by Hilger Ltd (Thomas et al., 1966) to ascorbic acid (Niece, 1975) which was dissolved in the lithium-citrate buffer solutions used to elute the amino acids from the column, see Appendix II, Table 34. Ascorbic acid was added in sufficient quantity to maintain the maximum ninhydrin response over a period of five days.

By varying the oil bath temperature and ninhydrin concentration the colour production was optimised (Appendix III, Figs. 30 and 31). The use of these modifications increased the sensitivity of the analysis and this was further improved when analysing HD samples by changing the ninhydrin pump tube from a rating of 0.2 ml min^{-1} to 0.15 ml min^{-1}.

The reproducibility was determined by repeated analysis of a standard, linearity and analysis range by analysing dilutions of a standard solution. Between run precision and accuracy were determined by analysis of quality control plasma and/or standard.

Prior to this study only plasma free amino acids were analysed. Consequently recovery experiments were carried out on the other sample types.

Three precipitating solutions were used for pre-treatment of the samples. These were derived from an application note published by Dionex Ltd. (Pickering and Soto, 1978) and a loading buffer recommended by LKB Biochrom Ltd. (1977).

The purpose of these solutions is to remove interfering proteins from the sample by precipitation, to control the cation concentration (eluant species), to lower the sample pH to approx. 2.2 to ensure that the application band at the top of the column was narrow, the addition of a bactericide as a preservative (phenol), thiodiglycol to prevent the oxidation of methionine and the addition of nor-leucine as an internal standard. By the use of an internal standard, any

volume changes caused by the precipitation of proteins, would be automatically adjusted for.

One solution was designed for use with samples containing little or no protein i.e. standards, CAVH effluent and urines without significant proteinuria. Another solution was for use with samples containing protein and relatively high concentrations of amino acids - plasma, PD effluent and urine with significant proteinuria and a third solution for samples with low amino acid content - i.e. HD effluent (see Appendix II, Table 32 for formulae).

The recommended analyser programme was modified. With a temperature change before nor-leucine elution it was not possible to separate this amino acid from the neighbouring peaks. This problem was also found when running an LKB 4101® amino acid analyser, reason unknown and was resolved by delaying the column temperature change until after this amino acid had been washed off the column.

Initially a column change from 42 °C to 60 °C was used to shorten the analysis time, but a lack of reproducibility of separation of methyl-histidines and histidine peaks resulted in a change to a constant 42 °C and consequently much improved elution time reproducibility and separation.

The formula of the two elution buffers was altered when ascorbic acid reductant was used. By reducing the amount of citric acid in each buffer, it was found to be unnecessary to adjust the pH. However, new batches of citric acid had to be checked and the formula adjusted accordingly.

Finally, whilst it was recommended that a lithium-free acid buffer be used when loading samples onto the column, as no difference in separation could be found when this was used, the practice was discontinued.

During the period of study this department joined a scheme operated by Dr. J. Rattenbury for the External Quality Assessment of Quantitative Amino Acid Analyses. The results of our participation in this scheme are given in Appendix III, Table 38.

2.16 Calculations and Statistical Methods

2.16.1 General Calculations

A Sinclair QL® computer (640 kb, double disc-drive; Amstrad Ltd.) running a commercial spreadsheet program (Abacus®, Psion Ltd.) was used to calculate mean daily concentrations for most parameters measured for each patient divided into periods of 3 to 8 days. As far as possible 8 day periods were used.

Daily and meaned data were then transferred to a compatible database program (Archive®, Psion Ltd.) to facilitate selection of data.

Statistical calculations on this data were carried out using an Oxstat® program (Oxford Logic Ltd.) on an IBM PC® computer (256 kb, double disc-drive, IBM Ltd.). Meaned data were manually entered and then saved on disc.

Serum albumin, TBPA and plasma free amino acid concentrations, MAC and TSFT measurements taken at the end of each 3 or 4 day period were meaned in each patient prior to analysis. For all other data an average of all daily measurements per observation period were used.

All amino acid data was calculated and analysed statistically using locally written dedicated Basic programs on an IBM PC® computer.

2.16.2 Amino Acid Nitrogen

Amino acid nitrogen was calculated from the individual amino acid losses. The losses for each amino acid (μmol/day) were converted to mg N day^{-1} by multiplying by a factor which depended on the number of molecules of nitrogen per mol of that amino acid. The individual amino acid nitrogen losses were then totalled.

2.16.3 Body Urea Pool Adjustment

UAR and nitrogen balance data were adjusted for changes in the body urea pool.

Urea was assumed to be evenly distributed in body water

(Williams et al., 1964) and body water was considered to be 60% body weight (Walser, 1974) at the start of the study.

Change in body weight was assumed to be a change in body water.

Therefore :-

Urea nitrogen pool adjustment (mg N kg^{-1} day^{-1}) = [change serum urea (mmol l^{-1}) × change body weight (kg) × 28.0134] ÷ [number days × mean body weight (kg)].

2.16.4 Urea Nitrogen Appearance (UAR)

Urea Nitrogen Appearance (UNA) :-

Urea N Loss (mg N kg^{-1} day^{-1}) + urea nitrogen pool adjustment (mg N kg^{-1} day^{-1}).

2.16.5 Nitrogen Balance

Nitrogen Balance :-

Nitrogen Intake (mg N kg^{-1} day^{-1}) - [Nitrogen loss (mg N kg^{-1} day^{-1}) + urea nitrogen pool adjustment (mg N kg^{-1} day^{-1})].

N.B. In patients with melaena the faecal losses were excluded from the nitrogen loss calculation.

2.16.6 Creatinine Appearance

Creatinine was considered to have the same volume of distribution as urea (Mitch et al., 1980; Williams et al., 1964), therefore :-

Creatinine Appearance (μmol kg^{-1} day^{-1}) = [change in serum creatinine (μmol l^{-1}) × change body weight (kg) + creatinine loss (μmol)] ÷ [number days × mean body weight (kg)].

2.16.7 Uric Acid Appearance

Uric acid was assumed to have a volume of distribution half that of urea (Wyngaarden, 1955), therefore :-

Urate appearance ($\mu mol\ kg^{-1}\ day^{-1}$) = [change in serum uric acid ($\mu mol\ l^{-1}$) × change in body weight (kg) × 0.5 + urate loss (μmol)] ÷ [number days × mean body weight (kg)].

2.16.8 Catabolic Rate Index

A modification of the Bistrian formula (Bistrian, 1979) was used to calculate a catabolic rate index (CRI) as a measure of catabolism.

Bistrian formula :-

$UUN - [(0.5 \times NI) + 3]\ g\ N\ day^{-1}$.

UUN = urine urea nitrogen (g N)
NI = nitrogen intake (g N)
3 = obligatory nitrogen losses (g N day^{-1} - Rutten *et al.*, 1975).

As this formula was to be used in renal patients, urea nitrogen appearance was used in place of UUN, and as other data was expressed as mg N $kg^{-1}\ day^{-1}$ and the obligatory nitrogen loss was assumed to be in a 70 kg individual, the formula became :-

$UNA - [(0.5 \times NI + 43)]\ mg\ N\ kg^{-1}\ day^{-1}$.

UNA = urea nitrogen appearance (mg N $kg^{-1}\ day^{-1}$)

2.16.9 Mid-Arm Muscle Circumference (MAMC)

MAMC was calculated from the MAC and TSFT data using the following formula :-

$$\text{MAMC (mm)} = \text{MAC (mm)} - [\pi \times \text{TSFT (mm)}]$$

2.16.10 Statistical Methods

The statistical methods used were: multiple linear regression analysis (MLR), least square linear regression analysis, Spearman's rank sum test, Wilcoxon's non-parametric rank sum correlation test and Student's t test (paired or un-paired). Rank sum methods were used if the data was not normally distributed in any one group.

MLR was used to analyse data which was known to be affected by more than one variable.

CHAPTER 3

RESULTS

3.1 Introduction

This chapter reports the statistical analysis of the effects of nitrogen intake and catabolic rate on the nutritional indices of ARF patients. The loss of 3-MH, uncorrected for changes in plasma concentration, was used as a measure of catabolism. Since the parameters used to measure nutritional status are affected by both the nutritional intake and the consequences of catabolism it was necessary to attempt to resolve these effects into separate components.

There was a wide variation in both catabolic rates and intake between patients which allowed the use of multiple linear regression analysis (MLR).

Most parameters measured are expressed in units kg^{-1} body weight day^{-1} which was a mean of the daily results from a minimum of the first 3 days and a maximum of the first 8 days of observation in each patient. Only one such observation period for each patient was used in the statistical analysis of the data. Appendix I contains the mean and standard deviations of all parameters calculated in this way. Unless otherwise stated all results are reported as mean ± S.D.

Where adjustments were made for changes in body pool the tissue pool was assumed to be constant i.e. changes in body weight were assumed to be changes in body water.

3.2 3-Methylhistidine Loss

The loss of 3-methylhistidine (3-MH) was 4.43 ± 1.32 μmol kg^{-1} BW day^{-1} and the daily change in plasma 3-MH concentration was -0.35 ± 2.9 μmol l^{-1} day^{-1}.

3-MH loss was negatively correlated with the daily change in plasma 3-MH, $R = 0.507$, $P<0.01$.

3-MH loss and plasma 3-MH concentration were not significantly correlated.

Patients were divided into two groups on the basis of whether or not they had received surgical intervention or any other trauma prior to study.

No difference in the degree of catabolism (3-MH loss) could be distinguished between these two groups by student's t test.

3.3 Nitrogen Intake

The nitrogen intake was 113.1 ± 41.9 mg N kg^{-1} BW day^{-1}.

Nitrogen Intake was not significantly correlated with 3-MH loss, P>0.1, the parameter used as a measure of catabolism. This allowed the use of these parameters as independent factors in the MLR analyses.

The percentage of the prescribed nitrogen intake received daily by patients with different feeding methods is presented in Table 5.

There was a significant difference between the enteral and parenteral groups by student's t test.

Table 5. Percentage Nitrogen Intake Received Daily With Different Feeding Methods.

		Mean %	S.E.M.	n	
ENTERAL					
a	ORAL	52.4	6.1	5	*
b	NG	53.7	15.6	5	
c	ALL ENTERAL	53.0	7.9	10	**
PARENTERAL					
d	TPN ONLY	84.0	3.0	19	***
e	TPN and NG	97.1	18.2	3	
f	ALL TPN	85.8	3.5	22	

* Group a vs. Group b N.S.

** Group c vs. Group f P<0.001

*** Group d vs. Group e N.S.

3.4 Urea Nitrogen Appearance

The mean and standard deviation of the Urea nitrogen appearance rates in the 24 patients were 161.8 ± 40.4 mg N kg^{-1} day^{-1}. MLR analysis of urea nitrogen appearance with 3-MH loss and nitrogen intake as independent variables gave the results shown in Table 6.

Thus :-

$$UAR = (0.5 \times NI) + (17.19 \times 3\text{-MH}) + 29.34$$

Where: UAR = urea appearance rate (mg N kg^{-1} day^{-1})

NI = nitrogen intake (mg N kg^{-1} day^{-1})

3-MH = 3-methylhistidine loss (μmol kg^{-1} day^{-1})

This data shows a significant positive effect on UAR of both nitrogen intake ($P<0.001$) and catabolism, as measured by 3-MH loss, ($P<0.001$) with 50% of an increase in nitrogen intake contributing to the UAR.

To provide a visual representation of this data UAR was plotted first against nitrogen intake after correcting for a standard 3-MH loss (4.43 μmol kg^{-1} day^{-1}, Fig. 14 and against 3-MH loss after correcting for a standard nitrogen intake (113.1 mg N kg^{-1} day^{-1}, Fig. 15).

A comparison was made between UAR calculated on days immediately prior to HD (pre-HD) and on HD days. Similarly, UAR was calculated in patients who had CAVH on the day prior to HD and CAVH combined therapy. This data is shown in Table 7 and Fig. 16.

The nitrogen intake for each patient on the HD day and the pre-HD day did not differ by more than 32%. The intake on HD days was not significantly different from pre-HD days in the HD alone group, whilst the HD day intake was significantly lower than pre-HD days in the CAVH + HD group.

Only data from adjacent days with similar intakes was used.

Differences between treatments were analysed by Student's paired

Table 6. Multiple Linear Regression Analysis of UAR Against Nitrogen Intake and 3-MH Loss.

Dependent Variable = UAR

No. Complete cases = 24

Overall Correlation Coefficient F = 14.97 R^2 = 0.588

Variable	Slope	T	P<
N Intake	0.50	3.695	0.001
3-MH loss	17.19	3.999	0.001
Intercept	29.34		

Table. 7. UAR Compared Between Pre-HD and HD Days.

HD Alone	UAR (mg N kg^{-1} BW day^{-1})		
	Mean	S.E.M.	n
Pre-HD	159.5	19.3	6
HD	288.7	48.7	6
CAVH + HD			
Pre-HD CAVH	196.6	39.3	5
HD and CAVH	310.6	65.7	5

t test.

UAR was significantly higher on HD days when compared to days pre-HD ($P<0.03$) or pre-HD with CAVH treatment ($P<0.03$).

There was no statistical difference in the increase in urea appearance on HD between the CAVH/HD and HD alone groups.

Fig. 14. UREA APPEARANCE RATE vs. NITROGEN INTAKE.

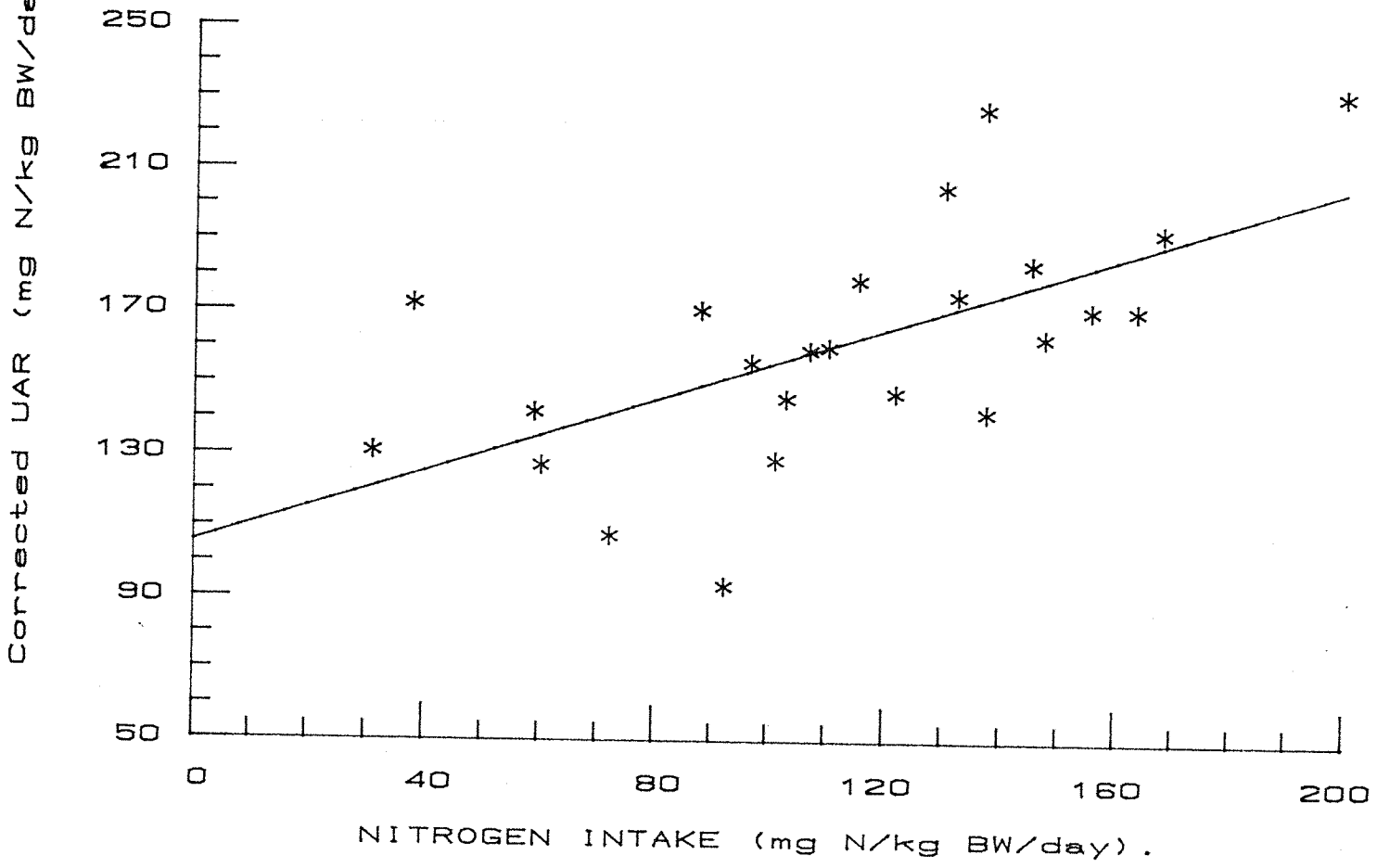


Fig. 15. UREA APPEARANCE vs. 3-METHYLHISTIDINE LOSS.

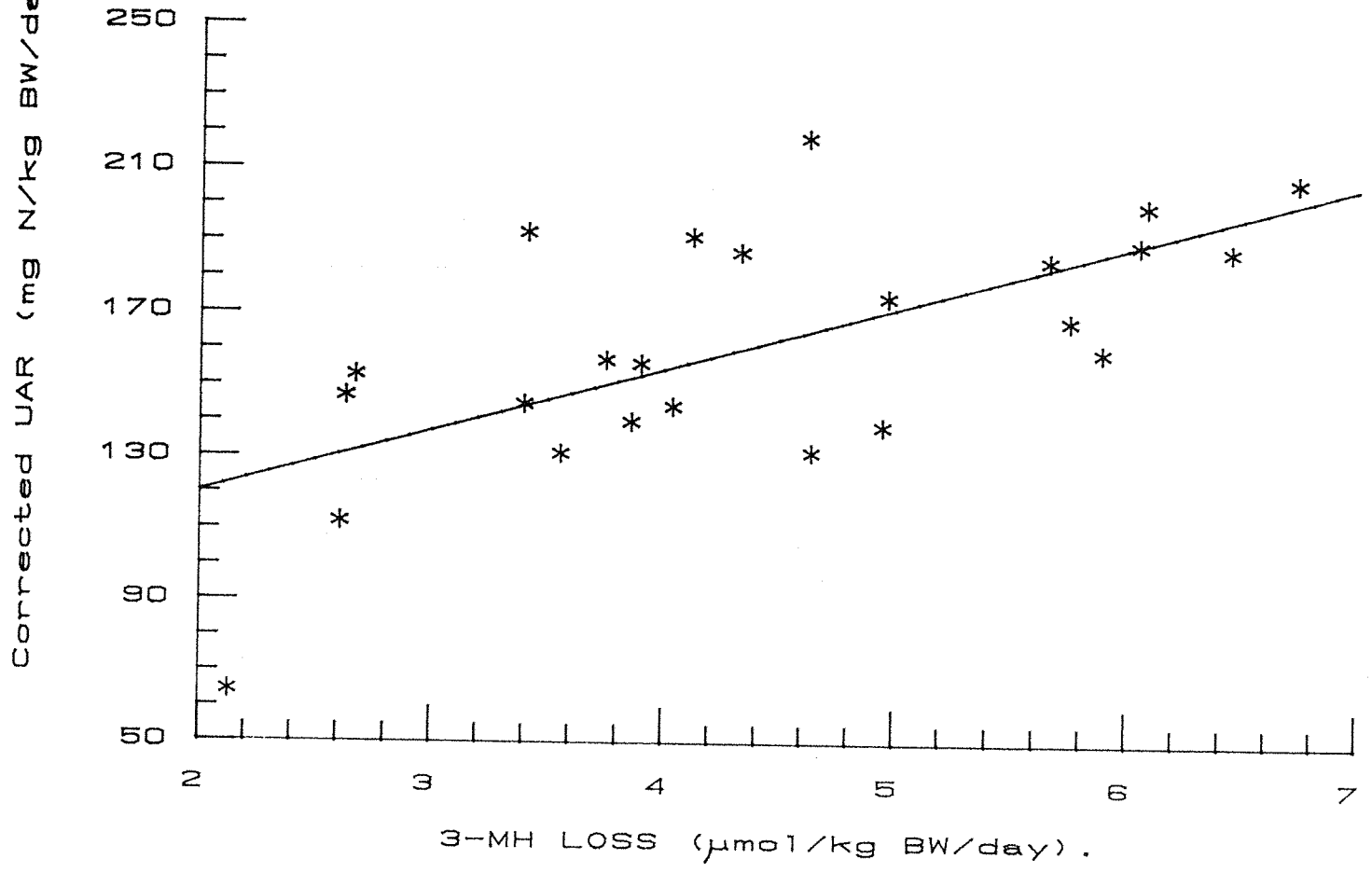
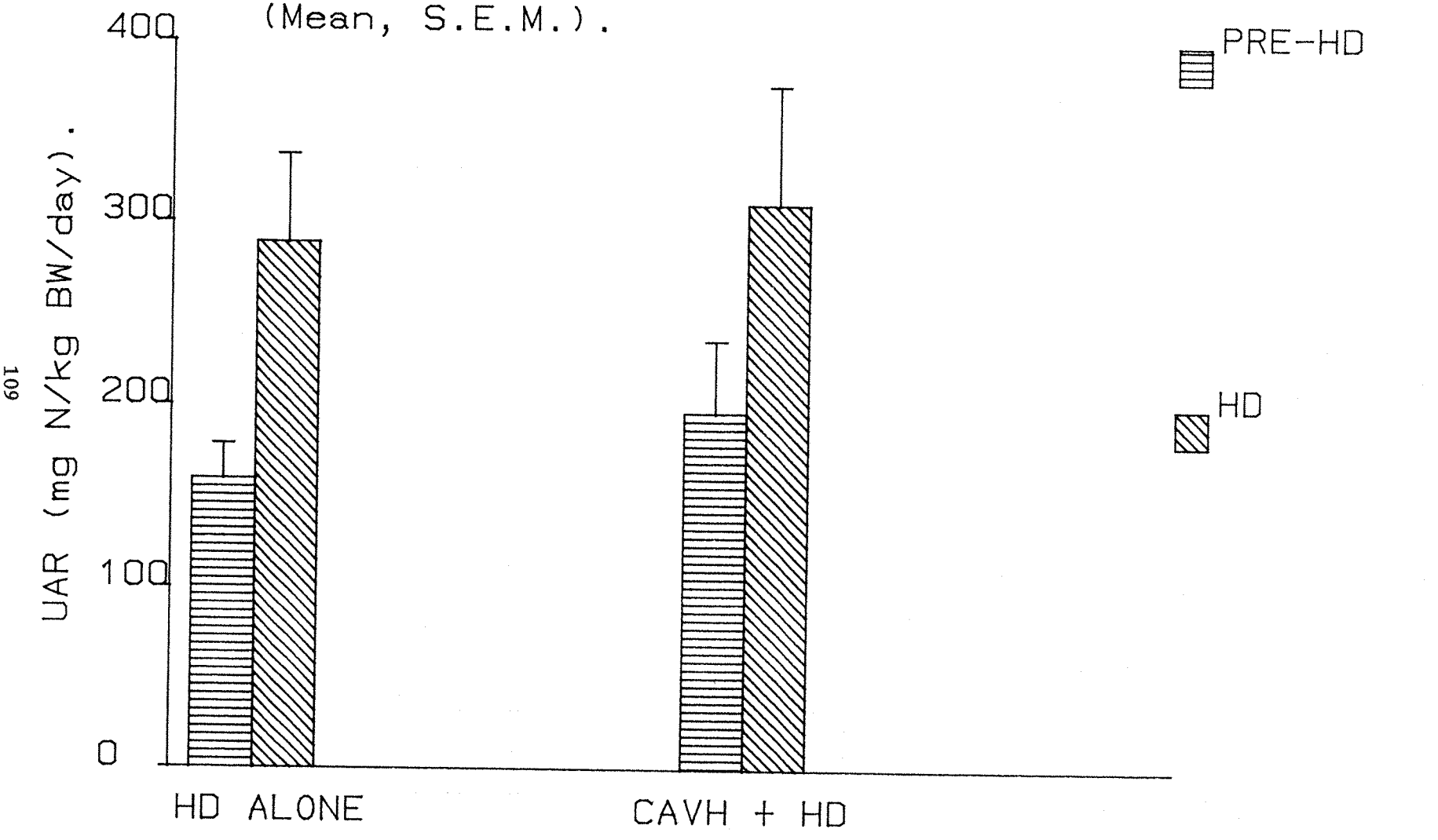


Fig. 16. UREA APPEARANCE RATE (UAR) ON PRE-HD AND HD DAYS. (Mean, S.E.M.).



3.5 Nitrogen Balance

The mean corrected nitrogen balances (see Chapter 2, section 2.16.5 for calculation) in 24 patients were -108.9 ± 54.1 mg N kg^{-1} day^{-1}. Fig. 17 shows a bar graph of this data, including nitrogen intakes and losses.

MLR analysis of the nitrogen balance data with nitrogen intake and 3-MH loss as independent variables gave the results shown in Table 8. This data demonstrates a significant positive effect of nitrogen intake on nitrogen balance ($P < 0.02$) and a negative effect of catabolic rate on nitrogen balance ($P < 0.001$), with 50% of an increase in nitrogen intake contributing to the improvement in nitrogen balance.

Thus :-

$$NB = (0.5 \times NI) - (24.89 \times 3\text{-MH}) - 55.42$$

where NB = nitrogen balance (mg N kg^{-1} day^{-1})

NI = nitrogen intake (mg N kg^{-1} day^{-1})

3-MH = 3-methylhistidine loss (μmol kg^{-1} day^{-1})

To provide a visual representation of this data the nitrogen balance was plotted first against 3-MH loss after correcting for a standard nitrogen intake (113.1 mg N kg^{-1} day^{-1}, Fig. 18) and against nitrogen intake after correcting for a standard 3-MH loss (4.43 μmol kg^{-1} day^{-1}, Fig.19).

The nitrogen balance of survivors (S) and non-survivors (NS) was compared by Wilcoxon rank sum test. Non-survivors were patients whose death terminated the observation period. The median values were S: -130 mg N kg^{-1} day^{-1} n = 11, NS: -166 mg N kg^{-1} day^{-1} n = 15.

There was no significant difference between these two groups.

There was also no significant difference between these two groups when non-protein energy intakes were compared.

Table 8. MLR Analysis of Nitrogen Balance Against Nitrogen Intake and 3-MH Loss in 24 ARF Patients.

Dependent Variable = Nitrogen Balance

No. Complete cases = 24

Overall Correlation Coefficient F = 11.09 R^2 = 0.514

Variable	Slope	T	P<
N Intake	0.50	2.552	0.020
3-MH loss	-24.89	-3.983	0.001
Intercept	-55.42		

Fig. 17. NITROGEN INTAKE, LOSS AND BALANCE OVER ALL SUBJECTS (Mean, S.D.).

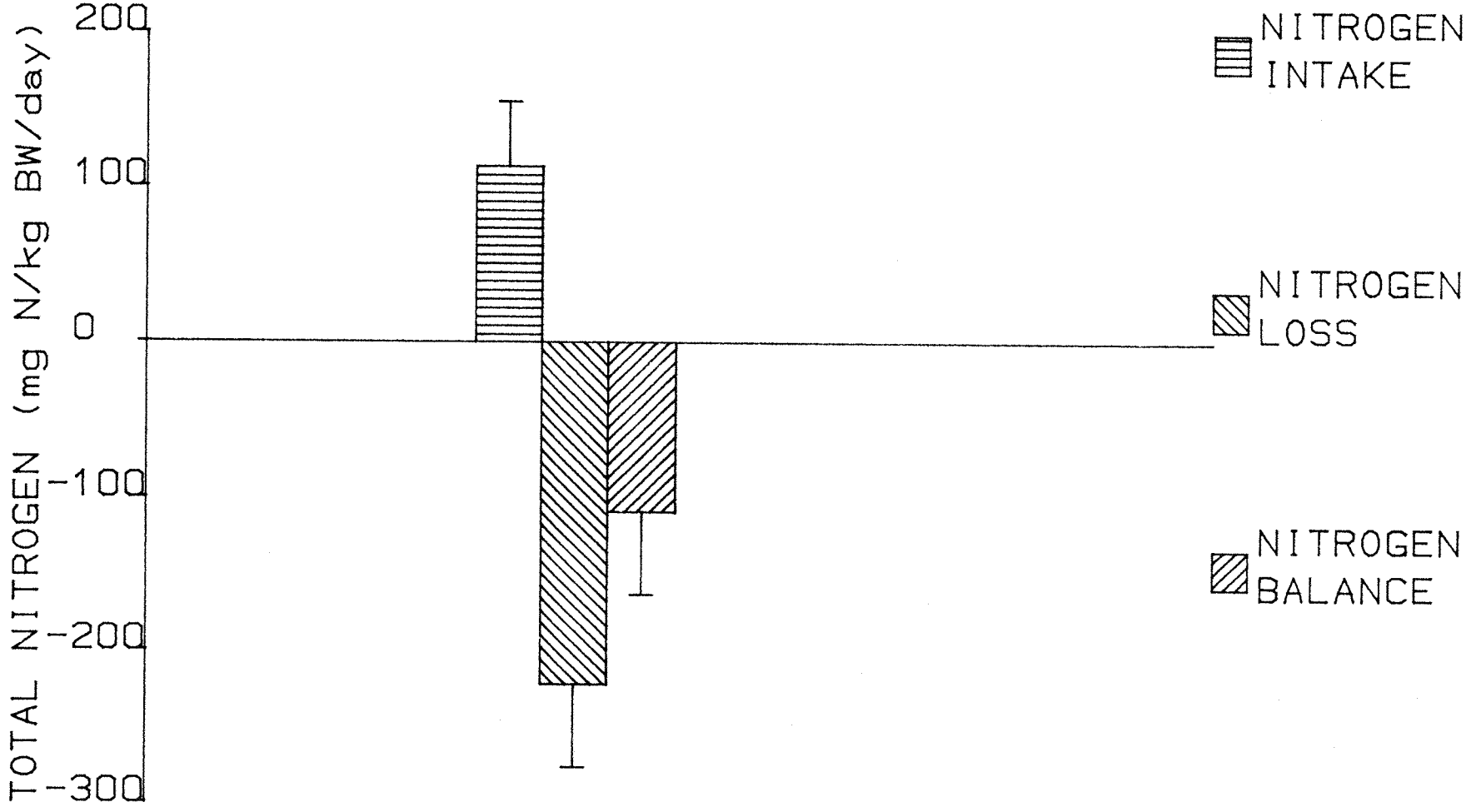


Fig. 18. NITROGEN BALANCE vs.
3-METHYLHISTIDINE LOSS.

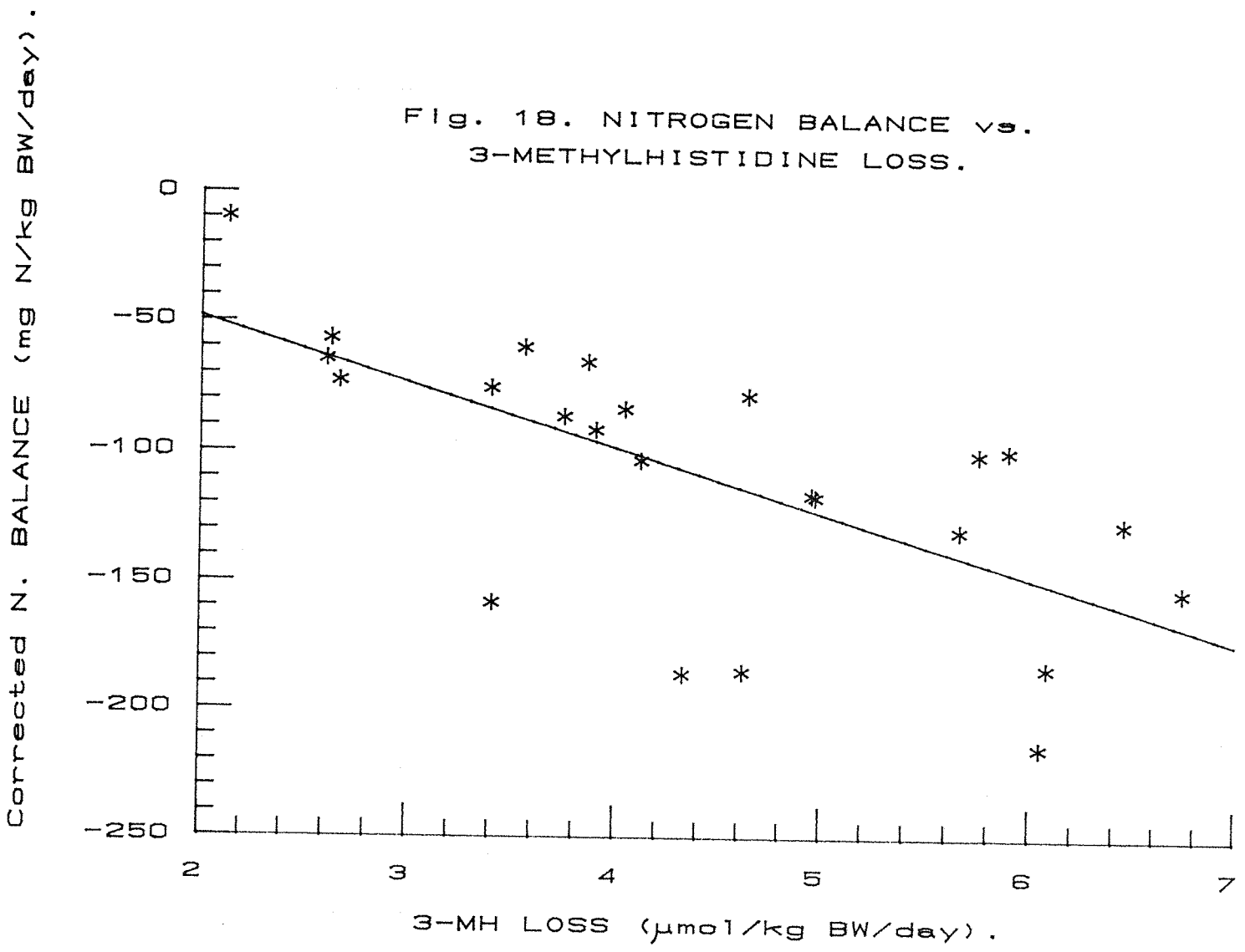
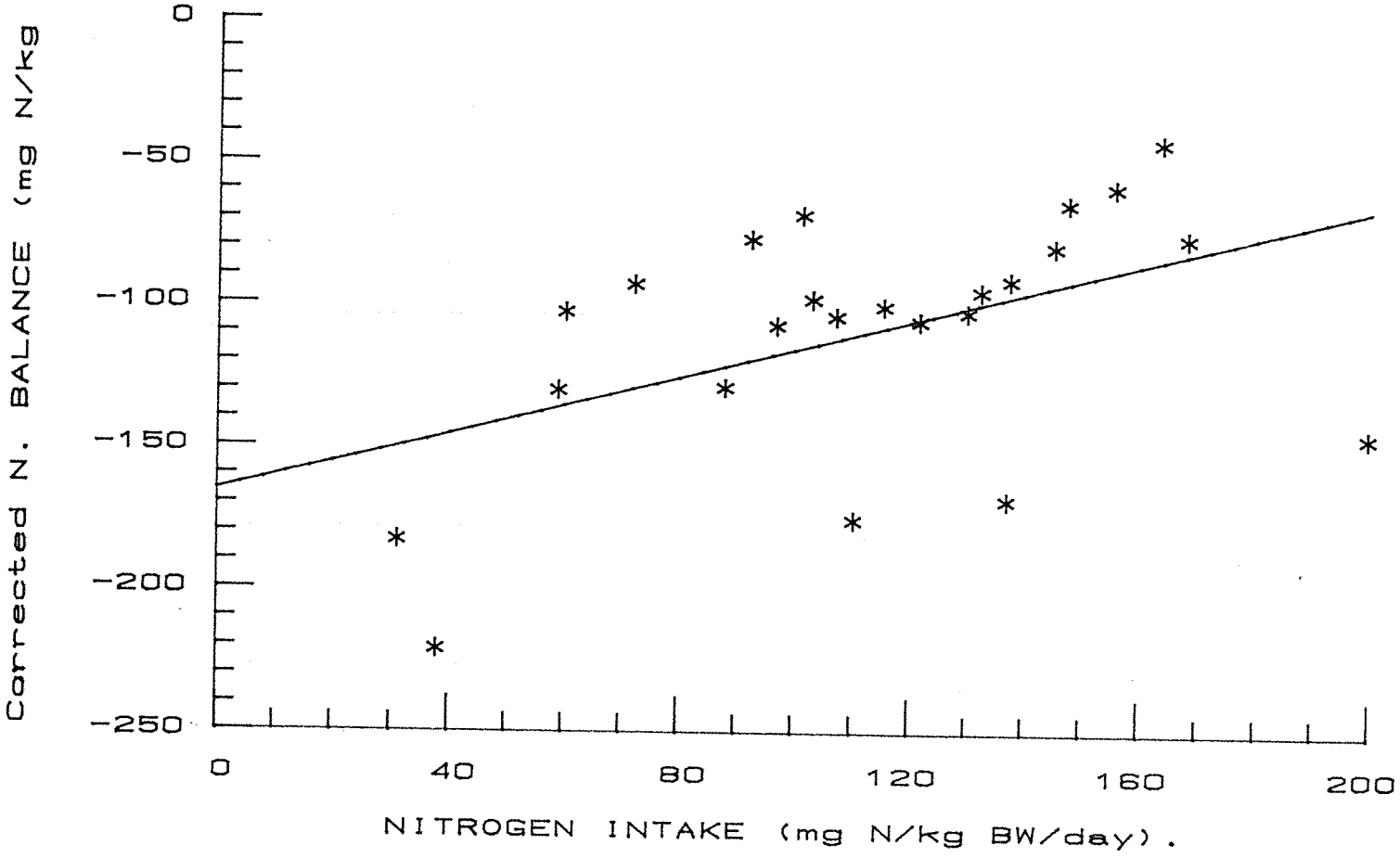


Fig. 19. NITROGEN BALANCE vs. NITROGEN INTAKE.



3.6 Non-Urea Nitrogen Loss

The non-urea nitrogen loss was 61.3 ± 26.1 mg N kg^{-1} day^{-1} and was not related to either nitrogen intake or catabolism by MLR analysis.

3.7 Creatinine Appearance

Creatinine appearance was 86.8 ± 29.9 μmol kg^{-1} day^{-1} and was not found to be correlated with nitrogen intake or catabolism when analysed by MLR analysis, as for non-urea nitrogen.

3.8 Uric Acid Appearance

Uric acid appearance rate was 50.5 ± 19.9 μmol kg^{-1} day^{-1} and the MLR analysis of this data is shown in Table 9.

Uric acid appearance was not found to be correlated with nitrogen intake, but was positively correlated with 3-MH loss ($P<0.02$).

To provide a visual representation of this data uric acid appearance was plotted against 3-MH loss after correcting to a standard nitrogen intake (113.1 mg N kg^{-1} day^{-1}) Fig. 20.

Table 9. Relationship of Uric Acid Appearance to Nitrogen Intake and 3-Methylhistidine Loss.

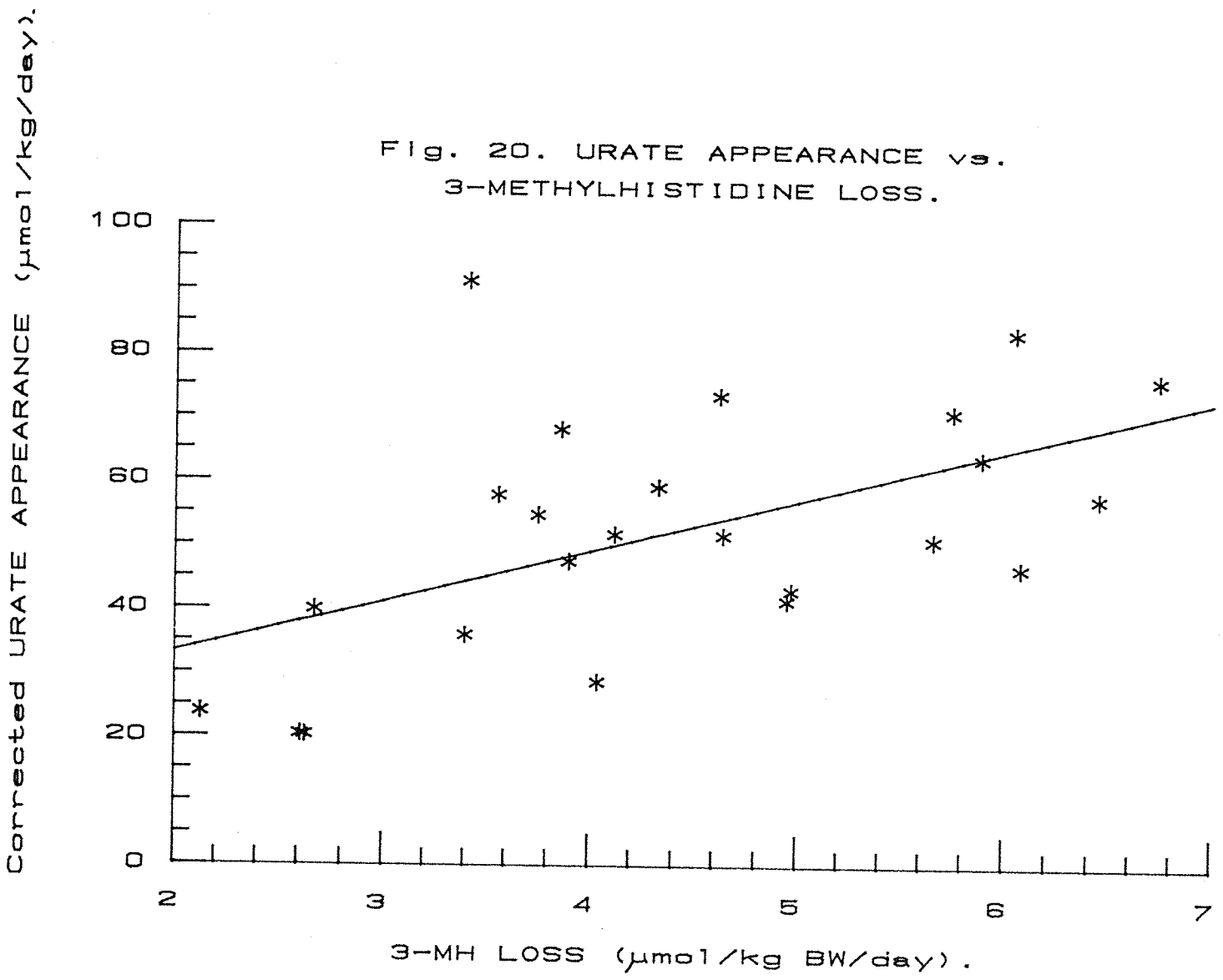
Dependent Variable = Uric Acid Appearance

No. Complete cases = 24

Overall Correlation Coefficient F = 3.61 R^2 = 0.265

Variable	Slope	T	P
N Intake	0.02	0.260	0.797
3-MH loss	7.56	2.672	0.015
Intercept	14.53		

Fig. 20. URATE APPEARANCE vs. 3-METHYLHISTIDINE LOSS.



3.9 Urea:Creatinine Ratio

The plasma urea:creatinine ratio (μmol:μmol) and UAR (μmol kg^{-1} day^{-1}):creatinine appearance (μmol kg^{-1} day^{-1}) ratio were 70.8 ± 26.6 and 75.6 ± 27.7 respectively.

Neither ratio was found to be related to either nitrogen intake or 3-MH loss by MLR analysis.

3.10 Plasma Proteins

3.10.1 Thyroxine-Binding Pre-Albumin

The fasting plasma TBPA concentrations in those patients who had not been infused with blood or plasma proteins were 218.9 ± 96.9 mg l^{-1}, $n = 9$.

No significant correlation was found between mean daily change in TBPA concentrations and nitrogen intake or 3-MH loss by MLR analysis. This was the case when measurements were included from all patients or only those patients who did not receive blood or plasma protein infusions.

3.10.2 Serum Albumin

The serum albumin concentrations were 28.4 ± 3.4 g l^{-1}, $n = 9$.

No relationship could be found between mean daily change in serum albumin concentrations and nitrogen intake or 3-MH loss when analysed by MLR analysis. Only Serum albumins measured in patients not receiving blood or plasma protein infusions were included in this analysis.

3.11 Anthropometric Measurements

3.11.1 Mid-Arm Circumference (MAC)

The mid-arm circumference measurements (mm) were a) males, 291 ± 25.2, n = 10; b) females, 289.6 ± 30.5, n = 5.

No significant correlation was found between nitrogen intake or 3-MH loss and daily change in MAC in either males or females when analysed by MLR analysis.

3.11.2 Triceps Skin-Fold Thickness (TSFT)

The mean and standard deviation of the TSFT measurement (mm) were a) males 10.5 ± 4.0, n = 10; females 15.3 ± 4.6, n = 5.

No significant correlation was found between nitrogen intake or 3-MH loss and daily change in TSFT in either males or females when analysed by MLR analysis.

3.11.3 Mid-Arm Muscle Circumference (MAMC)

The mean and standard deviation of the MAMC measurement (mm) were a) males 257.9 ± 19.1, n = 10; b) females 241.6 ± 35.8, n = 5.

When analysed by MLR analysis no correlation could be demonstrated between daily change in MAMC and nitrogen intake or 3-MH loss in either male or female patients.

3.11.4 Body Weight

The mean change in body weight day^{-1} was −0.32 ± 0.6 kg.

When the change in body weight day^{-1} was analysed using MLR, no correlation could be found with nitrogen intake or 3-MH loss.

3.12 Free Amino Acids

3.12.1 Amino Acid Losses

Because 3-MH loss may also be affected by factors which may be common to other amino acids, e.g. sharing the same transport mechanisms, patients were categorised into four groups according to their protein catabolic rate (Table 10), using a modified form of the Bistrian formula as described in the Methods chapter, section 2.16.8.

By analysing data within a restricted catabolic rate range the consequences of varying the nitrogen intake could be determined, whilst the results of catabolism could be shown by comparing between group differences or combining all the groups and using linear regression analysis.

In the analysis of the amino acid data some patients provided data for more than one CRI group, but only one observation period was used from each patient within each CRI group.

Table 10. The Distribution of the Catabolic Rate Index in Each Group.

Group	CRI	Mean	SD	n
1	0-40	31.3	8.5	8
2	40-80	57.6	11.3	14
3	80-120	98.5	10.2	12
4*	>120			

* Patients too few for analysis.

In Table 11 the mean and standard deviation of nitrogen intake in each catabolic rate group is shown.

Nitrogen intake and CRI were shown to be independent of each other within each CRI group by the Spearman rank correlation method which eliminates the effect of skewness.

No significant difference between the nitrogen intakes in the catabolic rate groups was found by one way analysis of variance.

Table 11. Nitrogen Intake (mg N kg^{-1} day^{-1}) in the Four Catabolic Rate Groups.

Group	CRI	Mean	SD	n
1	0 - 40	112.1	37.6	8
2	40 - 80	111.2	43.1	14
3	80 -120	108.9	42.4	12

Total amino acid nitrogen loss (mg N kg^{-1} day^{-1}) in each catabolic rate group and calculated as described in the Methods chapter, section 2.16.2 is presented in Table 12.

Total amino acid nitrogen loss was significantly higher in the 80-120 CRI group when compared to either of the lower CRI groups using Wilcoxon non-parametric rank sum test.

Total amino acid nitrogen loss was not correlated with nitrogen intake either within each catabolic group or over all groups when analysed by the Spearman rank correlation test.

Table 12. Amino Acid Nitrogen Loss (mg N kg^{-1} day^{-1}) in Each CRI Group.

CRI Group	Mean	n	Groups Compared	Wilcoxon quotient	P<
0-40	5.9	8	1,2	1.02	N.S.
40-80	7.6	14	2,3	2.2	0.05
80-120	10.9	12	1,3	2.0	0.05

Total and Essential Amino acid nitrogen loss as a percentage of nitrogen intake in each catabolic group are presented in Tables 13 and 14. There was no difference between the groups for total amino acid nitrogen loss, but essential amino acid nitrogen loss in the 80-120 CRI group was significantly higher than the 40-80 CRI group.

Total and essential amino acid nitrogen loss as a percentage of total nitrogen loss in each catabolic rate group are presented in Table 15 and 16.

Amino acid nitrogen as a percentage of the total nitrogen loss was not significantly different between the CRI groups by Wilcoxon rank sum test. This was also true of the essential amino acid nitrogen expressed as a percentage of the total nitrogen.

Table 13. Amino Nitrogen Loss as a Percentage of Nitrogen Intake in Each CRI Group.

CRI Group	Mean	SD	n	Groups Compared	Wilcoxon quotient
0-40	6.68	6.05	8	1,2	0.82
40-80	8.15	4.9	14	2,3	1.8
80-120	13.31	14.11	12	3,4	0.53

Table 14. Essential Amino Acid Nitrogen Loss As a Percentage of Nitrogen Intake in Each CRI Group.

CRI Group	Mean	SD	n	Groups Compared	Wilcoxon quotient	P<
0-40	2.61	2.73	8	1,2	0.95	N.S.
40-80	2.93	1.69	14	2,3	2.16	0.05
80-120	5.82	7.32	12	1,3	1.93	N.S.

Combined haemofiltrate and urine amino acid nitrogen losses were 8.56 ± 2.4 mg N kg^{-1} day^{-1}, n = 15 and haemofiltrate amino acid nitrogen loss alone was 8.31 ± 2.74 mg N kg^{-1} day^{-1}, n = 15.

When the amino acid nitrogen losses in patients treated by CAVH were compared between the catabolic rate groups there were no significant differences in either CAVH and urine or CAVH effluent losses only.

Table 15. Amino Acid Nitrogen Loss As a Percentage of Total Nitrogen Loss in Each CRI Group.

CRI Group	Mean	SD	n
0-40	3.46	2.15	8
40-80	3.75	1.17	14
80-120	3.89	1.12	12

Table 16. Essential Amino Acid Nitrogen Loss As a Percentage of Total Nitrogen Loss in Each CRI Group.

CRI Group	Mean	SD	n
0-40	1.3	0.97	8
40-80	1.35	0.41	14
80-120	1.59	0.61	12

Haemodialysis effluent and urine amino acid nitrogen losses were 7.24 ± 2.4 mg N kg^{-1} day^{-1}, n = 8 and HD effluent amino acid nitrogen loss alone was 7.13 ± 2.37 mg N kg^{-1} day^{-1}, n = 8.

When the amino acid nitrogen losses in patients treated by HD were compared between the catabolic rate groups no significant difference was found, whether total amino acid losses (including urine) or only amino acid nitrogen losses in the HD effluent were compared.

There was also no significant difference between amino acid losses in the CAVH treated patients when compared to the HD patients, either within catabolic rate groups or over all groups. In addition, the proportion of the amino acid nitrogen to total nitrogen loss in the CAVH group was not significantly different from the HD group.

Table 17. Total Individual Amino Acid Loss ($\mu mol\ kg^{-1}\ day^{-1}$) Related to Catabolic Rate Index.

Amino acid	Spearman ρ	P<	n = 24
* Valine	0.675	0.001	
* Isoleucine	0.584	0.01	
* Leucine	0.698	0.001	
* Lysine	0.456	0.05	
Arginine	0.506	0.02	
Tyrosine	0.642	0.01	
3-Methylhistidine	0.456	0.05	
* Phenylalanine	0.414	0.05	

* Essential Amino Acids

Table 17. contains the results of Spearman rank sum analysis for those individual amino acid losses which correlated significantly with CRI.

3.12.2 Plasma Amino Acids

Fasting individual plasma amino acid concentrations were not correlated with nitrogen intake within each CRI group.

Fasting plasma amino acids which correlated with catabolic rate index are presented in Table 18.

Significant negative correlations between plasma amino acids and CRI were found only for threonine ($P<0.02$), serine ($P<0.05$) and glutamine ($P<0.05$) by Spearman's rank correlation test. No other correlations were found.

Table 18. Fasting Plasma Amino Acid Concentrations Related to Catabolic Rate Index.

Amino acid	Spearman ρ	P<	n = 21
Threonine	-0.551	0.02	
Serine	-0.441	0.05	
Glutamine	-0.465	0.05	

Table 19 and Fig. 21. shows fasting plasma amino acid concentrations in normal subjects and 24 ARF patients.

Table 19. Fasting Plasma Amino Acid Concentrations (μmol/l) in Normal Subjects and ARF Patients.

	Normal Subjects (n = 10)		ARF Patients (n = 19)			
Amino Acid	Median	Range	Median	Range	Wilcox	P<
L-Taurine	70.0	52- 79	73.0	22-787	0.53	NS
L-Aspartate	3.7	1- 6	4.8	1- 57	1.22	NS
L-Threonine	156.0	10-192	76.1	36-393	-3.53	0.05
L-Serine	131.5	77-150	75.3	36-284	-2.78	0.05
L-Asparagine	52.5	45- 67	43.0	28-121	-2.34	0.05
L-Glutamate	33.0	19- 48	40.8	14-100	2.0	0.05
L-Glutamine	639.5	495-775	417.0	263-739	-3.76	0.05
L-α-Amino-adipate	6.6	3- 13	3.0	1- 6	-3.58	0.05
L-Proline	191.0	140-254	165.7	83-713	-0.62	NS
L-Glycine	286.5	211-360	158.0	88-617	-2.94	0.05
L-Alanine	356.7	271-443	215.5	116-880	-2.55	0.05
L-Citrulline	46.3	38- 56	23.9	14- 50	-3.49	0.05
L-α-Amino-n-butyrate	23.2	21- 42	17.0	6- 67	-1.28	NS
L-Valine	212.0	182-311	177.5	126-294	-1.97	0.05
L-Cystine	54.5	24- 63	99.4	41-196	2.66	0.05
L-Methionine	30.0	26- 42	26.5	12-361	-0.66	NS
L-Cystathionine	0.0	0- 0	8.7	1- 61	4.36	0.05
L-Isoleucine	65.5	32- 96	55.1	31- 93	-1.10	NS
L-Leucine	120.0	107-184	115.0	71-182	-1.15	NS
L-Tyrosine	52.2	45- 78	54.6	33- 97	0.32	NS
L-Phenylalanine	56.5	51- 70	89.3	54-242	-3.69	0.05
L-Histidine	107.5	57-163	58.0	39-178	3.58	0.05
L-3-Methyl-Histidine	12.7	4- 17	34.3	14- 61	-4.20	0.05
L-1-Methyl-Histidine	1.7	0- 22	3.6	0- 9	-1.28	NS
L-Tryptophan	21.5	9- 32	26.8	13- 43	-1.22	NS
L-Ornithine	101.0	72-120	65.5	26-201	2.55	0.05
L-Lysine	198.0	159-271	147.3	81-315	2.80	0.05
L-Arginine	98.5	45-111	45.7	30- 90	4.08	0.05

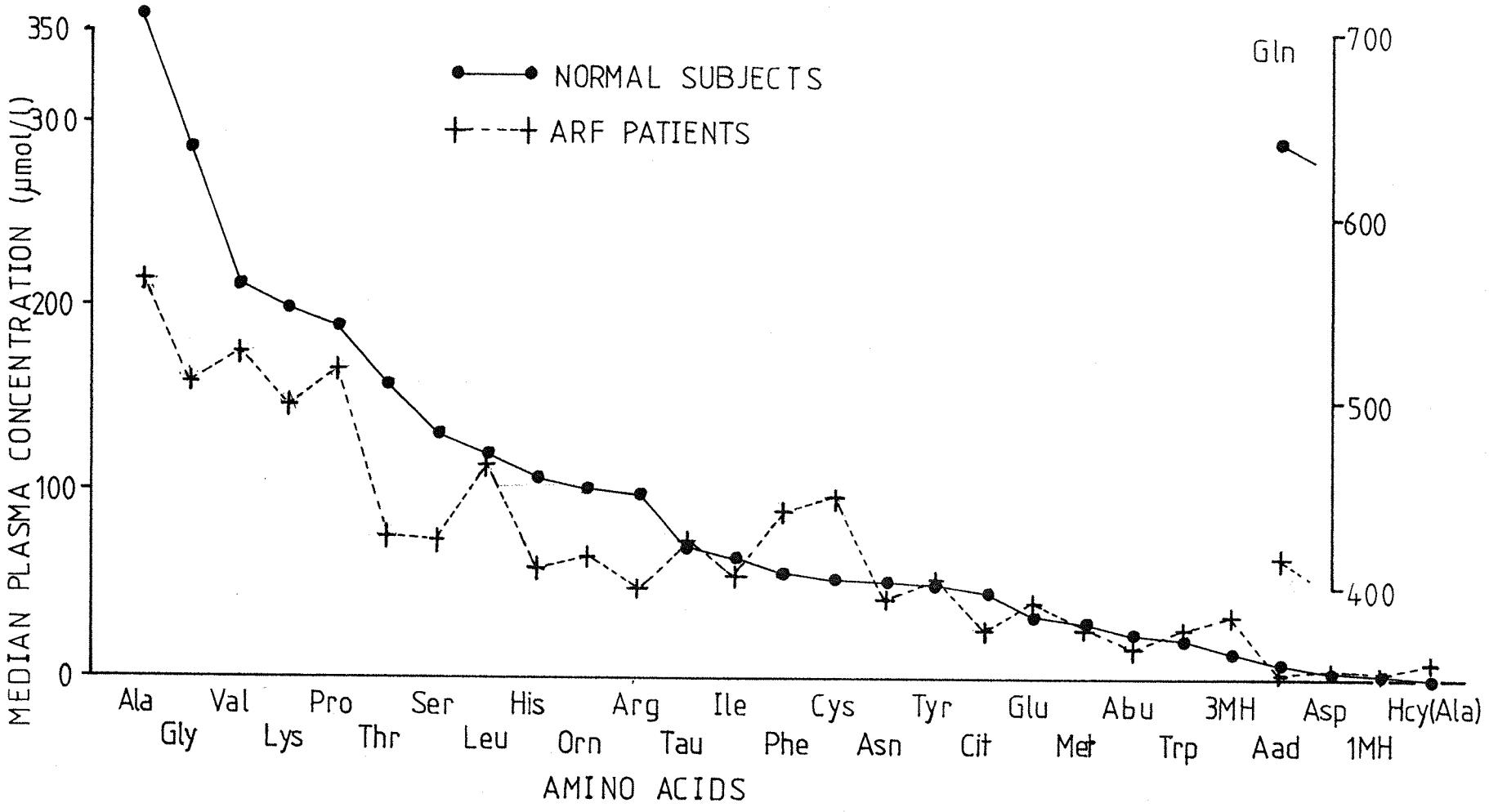


Fig. 21. PLASMA AMINOACID PROFILE IN NORMAL SUBJECTS AND 19 ACUTE RENAL FAILURE PATIENTS.

CHAPTER 4

DISCUSSION

4.1 Introduction

Throughout this discussion it is necessary to keep in mind that the initial metabolic response to injury or trauma, the ebb period, occurred before any observation periods were started because of the delay in referring patients to the renal unit. This avoided the initial period of inevitable negative nitrogen balance associated with the ebb period known not to be reversible by nutritional means. The metabolic changes are therefore a result of the combined effects of continuing trauma, infection and ARF to varying degrees.

Studies were started on 41 patients but sufficient data (three or more days of observation) was only obtained from 27 patients. Three of these 27 patients were excluded from the final analyses because they had severe gastro-intestinal (GI) haemorrhage high in the GI tract with only small amounts of melaena. This would have resulted in a UAR which was unrelated to the degree of protein catabolism due to the breakdown of blood cells by gut bacteria, with the consequent ammonia production leading to excess urea production (Cohn et al., 1956). One patient with a colostomy and high blood loss from a low GI haemorrhage which gave large amounts of melaena was included in the analyses because in this case the rapid excretion of blood cells from the gut was expected to prevent bacterial breakdown from influencing the measured urea appearance.

4.2 Waste Collection

The main problem in making complete 24 hr collections of all waste material was human error and was largely a result of the pressure of work on nursing staff. The patients were nursed in two separate situations.

1. Those patients who did not require special nursing care (i.e. 1:1 patient:nurse ratio with the patient usually requiring artificial ventilation), were therefore only one of several patients a nurse was required to care for. Thus it was more likely that mistakes would be made, bearing in mind that studies were carried out in a busy renal unit and not a special metabolic unit.

2. With a 1:1 patient:nurse ratio mistakes were less likely, probably as a result of reduced distractions from other patients and higher motivation for accuracy because of the condition of the patient and the effect that inaccuracies could have on the patient's progress. However, emergencies arising during record keeping and sample collection were also potential problem areas.

It was important to measure and aliquot samples accurately, as mistakes would affect a minimum of three days of collections and falsely low waste collection volumes would result in an artificially more positive nitrogen balance.

Incontinence was not usually a problem as faecal material was only produced in small amounts because of the low oral intakes and low residue feeds used and melaena was only present in a minority of patients (n = 3). Urinary incontinence was a deciding factor in the termination of a study only once.

To keep up to date with the patient's condition, treatment changes and to correct any collection problems at least two visits were made to the ward each day.

The time spent on the ward each day varied throughout the four years of the study. During the observation of those patients who did not require HD therapy, it was only necessary to make two or three visits to the ward of relatively short duration to :-

a) remove 24 hr collections and blood samples

b) collect data from patient's notes and charts

c) make sure that HD was not likely (from results of morning's

blood samples) and to re-stock the ward with empty sample containers.

For the last 12 months of the study much more time was needed on the ward as a result of a large increase in the proportion of patients treated by HD. As previously stated, (Chapter 2, section 2.9.1) each HD required my setting up of the collection apparatus prior to the start of dialysis. Also, there was occasionally a delay in starting, depending on avalability of staff, poor blood flow (partially blocked access), the disposal and replacement of dialysate if the conductivity (i.e. concentration) was outside recommended limits, or a coil needed replacing if leakage occurred on pressure testing.

Once started I observed the apparatus for at least one filling and emptying of the collecting tank - which could take upto one hour - to ensure that the switches and pumps functioned correctly. The apparatus was then left until the end of the dialysis, when another three-quarters to one hour was required to finish sampling, clean and sterilise the machine.

The sampling pump flow rate in the HD collection apparatus was 500-600 ml min^{-1}, to ensure that the collecting tank was gradually emptied. Wth a faster flow rate any mixing errors would have been accentuated because of inadequate mixing of the in-coming fluid with the static pool.

4.3 3-Methylhistidine Loss as a Measure of Muscle Catabolism

In this study the loss of 3-MH has been used as a measure of whole-body muscle catabolism. This amino acid is found in actin in all cells (Asatoor and Armstrong, 1967) and the myosin heavy chain from white muscle fibres (Johnson et al., 1967; Young and Munro, 1978). It is formed by post-translational modification of histidine residues of polypeptide chains (Young et al., 1972) and is released on degradation of these proteins. It is not a substrate for amino-acyl transfer RNA charging in vitro (Young et al., 1972) and therefore is not re-utilised for protein synthesis, nor metabolised (Long et al., 1975) but is rapidly excreted in urine (Long et al., 1975). It has been used as a marker of skeletal myofibrillar protein catabolism in studies of trauma (Iapichino et al., 1985; Grecos et al., 1984; Henneberg et al., 1985; Long et al., 1981), infection (Long et al., 1981; Sjolin et al., 1989; Wannemacher et al., 1975; Leverve et al., 1984), malnutrition and starvation (Long et al., 1981; Lowry et al., 1985; Young et al., 1973).

The use of urinary 3-MH as an indicator of skeletal muscle catabolism has been supported by some (Ballard and Tomas, 1983; Long et al., 1988; Sjolin et al., 1989) and criticised by others (Rennie and Millward, 1983; Millward et al., 1980; Rennie et al., 1984). Such criticism is based on the existence of major endogenous non-skeletal muscle sources of 3-MH (e.g. the fast protein turnover of splanchnic tissue (Wassner and Li, 1982).

However, in animals protein deficiency results in a fall in 3-MH excretion (Haverberg et al., 1975) and also in skeletal muscle degradation (Millward et al., 1975). Also, in rats treated with large doses of corticosterone there is a transient increase in 3-MH production (Tomas et al., 1979) with a simultaneous increase in skeletal muscle protein breakdown (Odedra et al., 1983). In normal volunteers undergoing a period of starvation and parenteral re-feeding a correlation was found between whole-body protein breakdown and 3-MH excretion (Tracey et al., 1988). Also in support of the use of 3-MH loss as a marker of whole-body protein catabolism Iapichino et al. (1985) found a decreased 3-MH excretion with an improved nitrogen balance in post-trauma patients treated with TPN

and Sjolin et al. (1989), in direct measurements in infected man of 3-MH release from splanchnic tissue and leg, found that only a minor proportion of urinary 3-MH originated in gut tissues and the major part came from muscle protein breakdown. In patients undergoing small-bowel resection Long et al. (1988) found that the contribution of intestinal to urinary 3MH loss was negligible.

The 3-MH:creatinine ratio, as used by others as a measure of the fractional degradation rate of myofibrillar protein (Young and Munro, 1978; McKeran et al., 1978) was not used in this study because of potential inaccuracy of the creatinine measurement caused by the presence of interfering substances (see Chapter 4, sections 4.8 and 4.10). There was also the potential problem of differential removal rates of 3-MH and creatinine, as a result of the different filtration and dialysis coefficients in CAVH and HD therapies. This is known to occur between urea and creatinine. It is well known that during dialysis the depletion of the plasma pool of urea is rapidly supplemented from the body pool. The replenishment of the plasma creatinine pool is a much slower process, so that these substances are not removed at equivalent rates. This problem could not be circumvented by an adjustment for the 3-MH body pool change, as 3-MH is not evenly distributed in body water because plasma and intracellular concentrations are not equivalent (Rennie et al., 1981; Bergström et al., 1972, 1978; Lee et al., 1987).

As there was no significant correlation between plasma 3-MH concentration and 3-MH loss, any loss of 3-MH via the GI tract did not bias the results. Any GI loss would have made only a small contribution to the total loss if this resulted largely from bacterial breakdown because antibiotics were often prescribed.

There was a negative correlation between 3-MH loss and change in plasma concentration. This probably resulted from reduced HD and CAVH treatment in the patients with lower UARs and therefore reduced 3-MH removal.

In this study the loss of 3-MH was not corrected for changes in plasma concentrations as no significant contribution from this factor was found when initially included in the MLR analysis of the UAR data.

4.4 Nitrogen Intake

Nitrogen intake was not related to the degree of protein catabolism in the patients studied. The intake of each patient was prescribed by the consultant in charge. The amount administered was often less than prescribed (see Table 5, Chapter 3, page 104) because of problems of access and supply. Blocked access lines and/or infection round the entry site of a subclavian catheter necessitated the removal and insertion of a new catheter in the contra-lateral subclavian. Occasionally insufficient supplies of TPN solutions were available on the ward when demand was exceptionally high. Reduced intakes were also caused by the need to lower raised blood sugar and lipid concentrations. Nutritional support, when in competition with other therapeutic manoeuvres, never ranked high. This is also indicated by the similarity between the mean nitrogen intakes between the CRI groups (See Table 11, Chapter 3. page 123).

Patients allowed to feed themselves orally received the lowest percentage of the prescribed intake because of anorexia and nausea associated with ARF and the lack of palatability of sip-feeding a commercial tube feed. Although the patients fed naso-gastrically had a higher mean percentage intake received than those fed orally the difference was not significant.

TPN was the most successful of the single feeding methods employed, with a mean of 84% of the prescribed intake received. With 19 patients fed by this technique TPN was the most commonly used method, although this was due in part to the selection of patients for study. After the initial experience of oral feeding, which had a high degree of diet rejection, only patients fed naso-gastrically or intravenously were included in the study. This data supports Rainford's view (1981) that in ARF TPN is essential to ensure an adequate supply of nutrients.

In the further analysis of the data no distinction was made between the types of feeding as studies on other groups of patients have found no difference in the effects on nitrogen balance. This was so whether a comparison was made between an amino acid solution infused intra-gastrically or parenterally in obese (Vernet *et al.*, 1986) or normal subjects (Bennegard *et al.*, 1984), an intravenous and

a jejunal, elemental feed compared in post-operative patients (Muggia-Sullam et al., 1985), parenteral and enteral nutrition compared after accidental injury or major abdominal surgery (Grote et al., 1987) or in malnourished primates (Dempsey and Mullen, 1987).

4.5 Urea Appearance

UAR was significantly correlated with both nitrogen intake and 3-MH loss. 50% of an increase in nitrogen intake contributed to the UAR.

Urea is the main end product of nitrogen metabolism in man and is therefore a major component of nitrogen balance. It is mainly formed in the liver and the rate of ureagenesis is subject to wide fluctuations in response to physiological and pathological stimuli. However, it is not urea production but UAR which is more closely related to nitrogen balance and UAR is the difference between urea production and urea hydrolysis by gut bacteria (Walser et al., 1973). It can be measured as the sum of urea excretion (loss) and change in the body pool and is closely correlated with nitrogen balance (Grodstein et al., 1980).

The two nitrogen atoms used in urea synthesis come directly from ammonia and aspartate, (see Fig. 1, Chapter 1, page 16) but both these derive nitrogen from glutamate. Mitochondrial glutamate dehydrogenase converts glutamate to ammonia (Krebs et al., 1978) while aspartate-oxaloacetate aminotransferase transfers glutamate nitrogen to aspartate (Meijer et al., 1975).

The main extrahepatic sources of nitrogen for urea biosynthesis are ammonia in the portal vein from bacterial activity in the gut, alanine and glutamine. In muscle BCAAs transaminate with α-ketoglutarate to form glutamate which then transfers its amino-N to pyruvate in the reaction catalysed by alanine aminotransferase, (Felig, 1973), see Fig. 22. Glutamine is formed by the glutamine synthase reaction which incorporates the amide-N into glutamate to form glutamine (Fig. 23). Ammonia required as the source of the amide-N can be derived from the circulation or through the metabolism of other amino acids, e.g. BCAAs. Alanine is transported to the liver (Felig, 1973) whilst glutamine is taken up by the gut and largely converted to alanine, less to citrulline, ammonia and proline which are then released into the portal vein (Weber et al., 1977). All are urea precursors. Ammonia from urea breakdown in the gut is also transported via the portal system to the liver (Walser, 1980), see Fig. 24. Other amino acids are also metabolised in the liver yielding

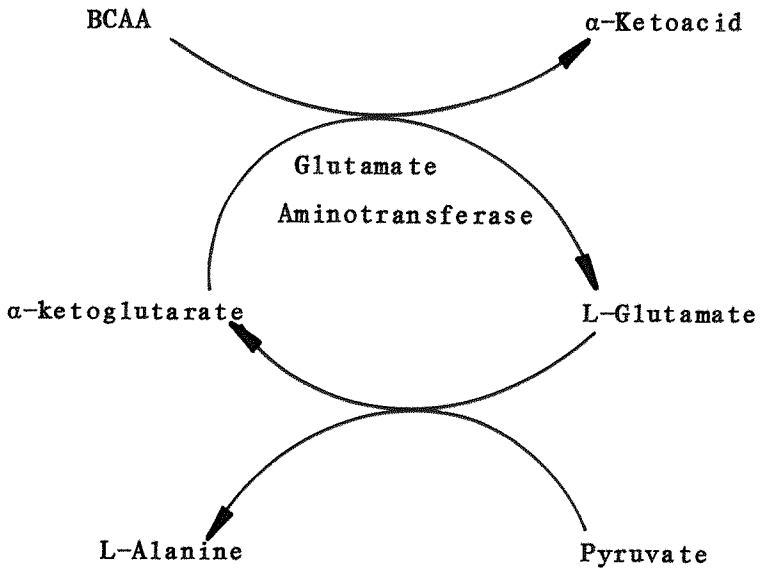


Fig. 22. The Transfer of BCAA Nitrogen to Alanine.

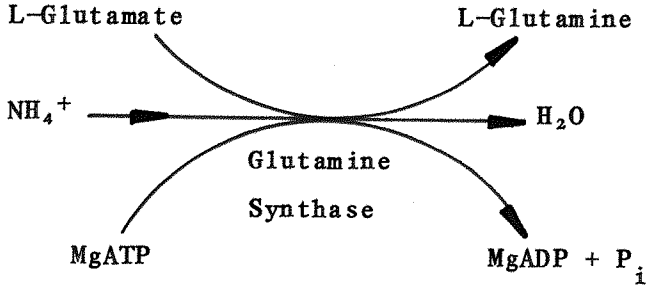


Fig. 23. The Glutamine Synthase Reaction.

ammonia which is channelled into urea synthesis (Krebs et al., 1978). Most BCAA transamination occurs in skeletal muscle, although BCAA are also metabolised in the liver to a limited extent (Hagenfeldt et al., 1980).

The most important factors determining the UAR rate are the circulating concentrations of amino acids and ammonia, whether from exogenous sources or from endogenous protein catabolism with the release of amino acids into the body pool. Rafoth and Onstad (1975) and Vilstrup (1980) observed a linear relationship between plasma amino acid concentration and urea production. This is not a result of

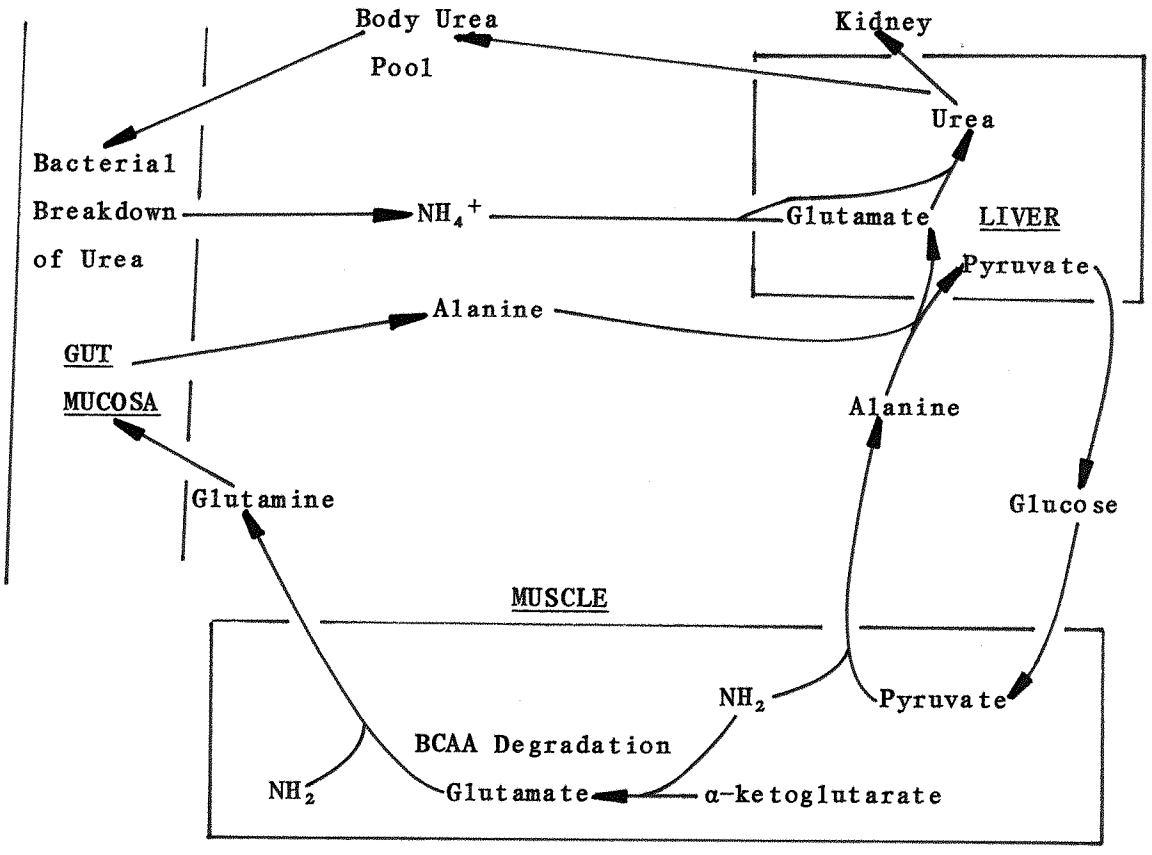


Fig. 24. The Main Inter-Organ Exchange of Amino Acids.

enzyme induction because turnover times of urea cycle enzymes are too slow (Das and Waterlow, 1974; Nicolletti et al., 1977).

The formation of carbamoyl phosphate from the condensation of ammonia, carbon dioxide and phosphate (from ATP) in the mitochondrion is required for the entry of ammonia into the urea cycle. The activity of carbamoyl phosphate synthetase, the enzyme governing this reaction, is rapidly affected by changes in the concentration of its required co-factor, N-acetylglutamate (Shigesada et al., 1978). Stewart and Walser (1980) found that mitochondrial N-acetyl glutamate concentration increased with plasma amino acid concentration causing carbamoyl phosphate synthetase activation. This was apparently a substrate effect on N-acetyl glutamate synthetase caused by an increase in mitochondrial glutamate. Therefore, glutamate is not only the precursor of nitrogen atoms incorporated into urea, but also activates the urea cycle (Fig. 1, Chapter 1, page 16).

The hormonal milieu will also affect urea appearance. Glucagon is known to stimulate hepatic uptake of α-amino nitrogen (Kibler

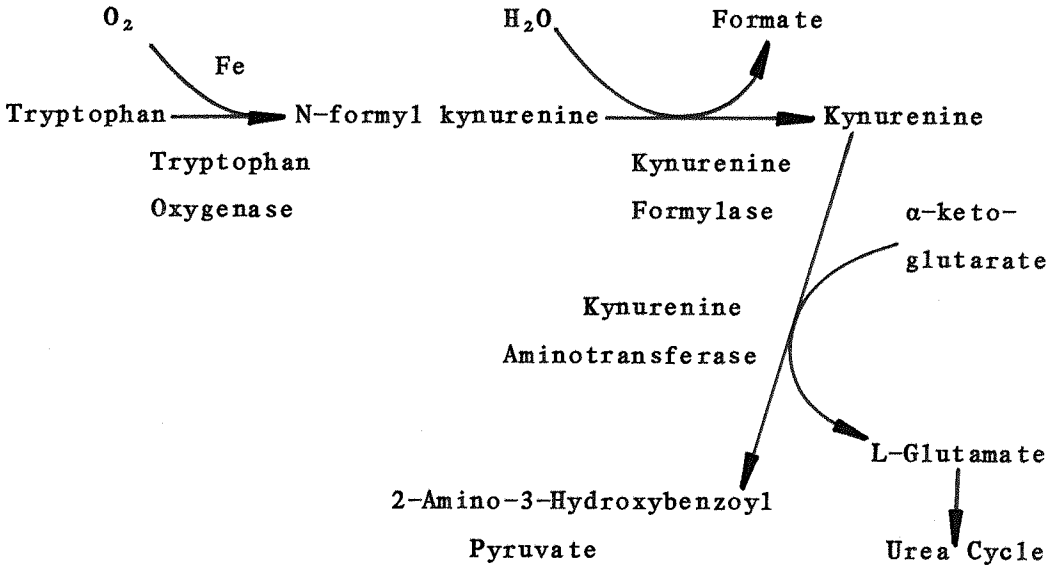


Fig. 25. Several Steps in the Catabolism of Tryptophan.

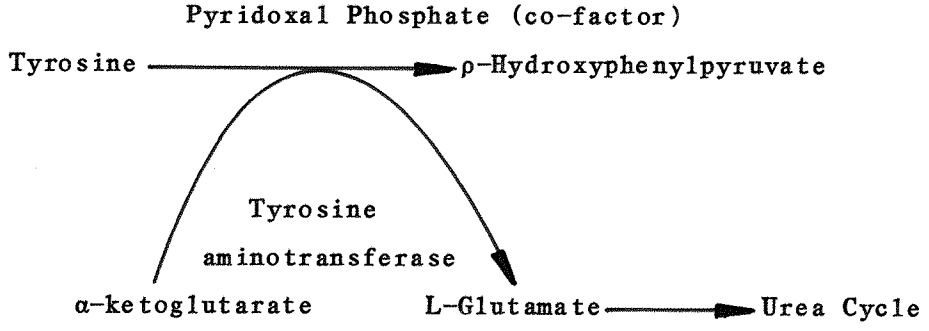


Fig. 26. The Tyrosine Aminotransferase Reaction.

et al., 1964) and alanine (Cherrington et al., 1983), increase gluconeogenesis in the liver (Shoemaker et al., 1959) and increase ureagenesis (Yamazaki and Graetz, 1977). Insulin inhibits gluconeogenesis in the liver (Chiasson et al., 1976) whilst cortisol enhances gluconeogenesis by the induction of pyruvate carboxylase and phosphoenol pyruvate carboxykinase (Baxter and Forsham, 1972), (see Fig. 3, Chapter 1, page 25) whilst also inducing tryptophan oxygenase (Fig. 25) and tyrosine aminotransferase, see Fig. 26 (Nakamura et al., 1980). The increased oxidation losses of tryptophan and tyrosine causes these EAA to be rate limiting for protein synthesis and consequently there is increased ureagenesis from amino acids

which cannot be used for protein synthesis.

The utilisation of dietary nitrogen would not be expected to be 100%, even with a negative nitrogen balance, as amino acid requirements vary according to the type of protein being synthesised and some wastage through deamination will occur with each passage of blood through the liver. In this study, the slope of the regression line of UAR with nitrogen intake demonstrates that 50% of an increased nitrogen intake was wasted as urea (Fig. 14, Chapter 3, page 107). This value is similar to those obtained in other studies. 47% wastage was shown in five oliguric ARF patients by Mirtallo and Fabri (1984) given TPN when UAR was measured on days between dialyses. Radrizzani et al. (1986) gave TPN to malnourished patients and found an improvement in nitrogen balance of 56% (approx. 44% urea wastage). These patients had impaired GI function and there was no renal or hepatic failure. 45% nitrogen wasting was also found in critically injured patients given intravenous nutrition for six days post-trauma (Iapichino et al., 1984).

The urea generated from nitrogen intake in this study was thus similar to other studies on patients with or without ARF and would not be expected to be sufficient to increase dialysis requirements, confirming Mirtallo and Fabri's (1984) data. However, this data does not support Feinstein et al. (1981, 1983) who carried out a double-blind controlled study with two different nitrogen intakes. They found that patients given the higher nitrogen intake exhibited a higher UAR. The difference in nitrogen intakes equalled the difference in UARs. Therefore, they concluded that there was no advantage to be gained by giving higher nitrogen intakes. However, although the initial UAR was comparable between these two groups, an endogenous measure of protein catabolism was not made and ARF patients are known to have an unstable catabolic rate. Also only a small number of patients were studied, five in the low-nitrogen group and six in the high-nitrogen group.

The regression slope of UAR against nitrogen intake confirms the validity in ARF of the calculation of the Catabolic Index as used by Bistrian (1979) which includes a factor of 0.5 for the amount of urea derived from nitrogen intake and was derived from data on normal subjects (see Chapter 2, section 2.16.8).

When UAR was compared between adjacent days of combined CAVH and HD treatment and CAVH therapy, the UAR on CAVH plus HD was found to be significantly increased. Increased UAR was also found on the days treated with HD alone when compared to adjacent days off dialysis (Table 7 and Fig. 16, Chapter 3, pages 106 and 109). This could not be accounted for by differences in nitrogen intake. This increase in UAR demonstrates the increased protein catabolism with HD therapy reported by others (Gotch et al., 1977; Borah et al., 1978; Ward et al., 1979).

This increase in catabolism has been related to the activation of complement and subsequent IL-1 induction (Goodman et al., 1982) by :-

1. HD membranes, especially cuprophane (used in this study), (Port et al., 1987; Gutierrez et al., 1985),

2. microbial contamination of dialysate (Favero et al., 1974; Peterson et al., 1978) and

3. sodium acetate dialysate (Bingel et al., 1987; Port et al., 1987).

The increased catabolism with combined CAVH/HD therapy relative to CAVH therapy may just be the superimposition of the increased catabolism with HD on the catabolic rate with CAVH since the CAVH filter membrane (Diafilter®, Amicon Ltd.) was made from a more biocompatible material than the dialysis cuprophane membrane. However, the more biocompatible CAVH membrane may have led to a reduced activation of complement (Levett et al., 1986) and consequently a lower catabolic rate relative to the periods of HD. Alternatively it is likely that the absence of potentially contaminated dialysis fluid and/or the absence of potentially IL-1 inducing sodium acetate from the ultrafiltrate side of the CAVH membrane could have the same effect.

The increased catabolism of HD has also been related to the loss of amino acids (Ward et al., 1979) and glucose (Wathen et al., 1977) during HD. Wathen et al. (1977) found evidence of increased gluconeogenesis (a decrease in blood pyruvate concentration and increases in ketone bodies) during HD. However, these findings would only provide a partial explanation for the difference between therapies. Glucose was present in both HD dialysate and CAVH

replacement solutions and Ward et al. (1979) found no effect on the catabolism during HD by increasing the glucose concentration in the dialysate. The increased catabolism was therefore not related to the increased gluconeogenesis. The difference in amino acid nitrogen losses found in this study accounted for only a small fraction of the difference in urea nitrogen appearance between the CAVH and CAVH/HD groups, although representing over 9% of the nitrogen intake. Whilst the replacement of amino acid losses should be considered, it is more practical to consider nitrogen requirements in terms of the nitrogen balance.

4.6 Nitrogen Balance

Nitrogen balance was significantly improved by increased nitrogen intake ($P<0.02$) and was worsened by increased catabolism (increased 3-MH loss) - Table 8, Figs. 18 and 19, Chapter 3, pages 111, 113 and 114. Mean nitrogen loss was twice the mean nitrogen intake (nitrogen loss 223 mg N kg^{-1} day^{-1}; nitrogen intake 113 mg N kg^{-1} day^{-1}). Consequently, most patients had a markedly negative nitrogen balance and no patient was in positive balance.

There was a close relationship between the regression slopes of nitrogen intake with both UAR and nitrogen balance (both slopes were 0.5) - Tables 6 and 8, Figs. 14 and 19, Chapter 3, pages 106, 111, 107, 114). This is understandable as UAR is the main determinant of nitrogen balance (Walser, 1980; Grodstein *et al.*, 1980). The variation in nitrogen losses other than urea did not therefore have a significant effect on the regression slope since non-urea nitrogen was not related to either nitrogen intake or catabolism.

The significant improvement in nitrogen balance ($P<0.02$) with an increase in nitrogen intake supports other clinical studies in ARF patients (Spreiter *et al.*, 1980; Mirtallo and Fabri, 1984).

If all patients had received a nitrogen intake of 200 mg N kg^{-1} BW day^{-1} and allowing for 50% nitrogen wastage, only one patient would have been in positive nitrogen balance. This demonstrates how catabolic this group of patients were and the importance of measuring all nitrogenous losses. If non-urea nitrogen had not been measured the nitrogen balance would have been 27% more positive. In addition, the nitrogen balance would have appeared to be more positive if measurements had only been made between dialyses, as in other studies (Blackburn *et al.*, 1978; Mirtallo and Fabri, 1984).

The nitrogen balance data was divided into two groups, those whose death terminated the observation period and those who survived. No difference was found between the patients who died and those who did not, although the median nitrogen balance for the non-survivors was more negative than the survivors. There was also no significant difference between these two groups for nitrogen intake or caloric intake. This data does not support Abel *et al.* (1973) who found a significant improvement in survival with the infusion of dextrose and

EAA, results which have not been repeated by other investigators (Feinstein et al., 1981; Freund and Fischer, 1980). However, Abel et al. (1973) only studied their least catabolic patients (some did not require dialysis) and consequently this study is not comparable because of the range of aetiologies, complications and catabolic rates and these two groups were not matched for trauma, age, ARF aetiology or time period between the date of the initial insult and the start of the observation period.

Mault et al. (1983) found a difference in mortality between patients with a positive energy balance and those with a negative energy balance. Also, Laville et al. (1985) found an improved survival in patients who received more than 35 kcal (147 kJ) kg^{-1} day^{-1}. As the nitrogen intake in this study was intimately associated with the caloric intake there was no significant difference between the caloric intakes in these two groups either. In this study all patients received less than 35 kcal (147 kJ) kg^{-1} day^{-1}. Considering the large negative nitrogen balances, it is likely that the intakes provided were too low and too few patients were studied to show a significant effect of nutrition on survival.

4.7 Non-Urea Nitrogen

No significant correlation between nitrogen intake or 3-MH loss (catabolism) and non-urea nitrogen generation could be determined in these patients, even though a correlation was found between urate appearance and catabolic rate. The other components of the non-urea nitrogen - creatinine, amino acids, protein, wound drainage, aspirate, faeces - thus obscured the effect of the urate appearance as the creatinine and amino acid nitrogen did not show a relationship with 3-MH loss.

This data confirms the observation in CRF patients that non-urea nitrogen is relatively independent of dietary protein (Mitch, 1981b). This has been used in support of the calculation of nitrogen balance by measuring UAR, adding 2 g N day^{-1} as the non-urea nitrogen and subtracting this value from nitrogen intake (Walser, 1981). A similar non-urea nitrogen factor can be calculated from the present study for ARF patients, 4.27 g N day^{-1} for a 70 kg man, which is over twice the CRF value. However, the wide variability of non-urea nitrogen losses ($\pm$ 1.82 g N day^{-1}) caused by variations in wound drainage, aspirate, faeces and GI losses, makes a calculated nitrogen balance insufficiently precise for accurate assessment and cannot replace direct measurement of total nitrogen losses. In this study the mean non-urea nitrogen was 27% of the mean nitrogen losses.

4.8 Creatinine Appearance

No correlation was found between creatinine appearance and nitrogen intake or 3-MH loss in this group of patients.

Creatinine is formed non-enzymatically from creatine which is present in muscle both as creatine and creatine phosphate. Several organs are involved in creatinine synthesis. In kidney there is transamidination between arginine and glycine forming glycocyamine (guanidinoacetic acid) and ornithine. Guanidinoacetic acid is then methylated in liver by S-adenosylmethionine to form creatine, which is transported to muscle for active uptake, Fig. 27. The synthesis of guanidinoacetic acid is regulated by feedback control from the concentration of creatine and creatine synthesis is controlled to match the muscle mass in patients with wasting diseases (Van Pilsum and Wolin, 1958). 98% of creatine is found in muscle (Hunter, 1928) and this is continuously, irreversibly dehydrated to creatinine by a non-enzymatic reaction (Hahn and Meyer, 1928). The fraction of body creatine converted to creatinine averages 1.7% day^{-1}, but varies with the relative proportions of creatine and phosphocreatine in muscle. Creatine is converted to creatinine at a rate of 1.1% day^{-1} and phosphocreatine at 2.64% day^{-1} (Vignos and Warner, 1963). The body pool of creatine can be increased by dietary creatine (Crim *et al.*, 1975). In addition to urine, creatinine is also excreted with faeces (Wixom *et al.*, 1979) and creatinine is degraded in gut in CRF patients (Jones and Burnett, 1974; Mitch *et al.*, 1980).

Some studies have shown that urinary creatinine (and therefore creatinine production in patients with normal kidneys) increases in trauma patients (Long *et al.*, 1981; Ballard *et al.*, 1981) and a clinical dictum holds that creatinine production increases in ARF associated with rhabdomyolysis. In both cases this would be unexpected as the conversion of creatine to creatinine is by a non-enzymatic reaction and cell membrane damage should not release creatinine into the extracellular space (Grossman *et al.*, 1974) because creatinine is evenly distributed throughout body water (Mitch *et al.*, 1980). These studies were made using the Jaffé reaction to measure creatinine but this is subject to interference by a number of non-creatinine chromogens (see Chapter 4,

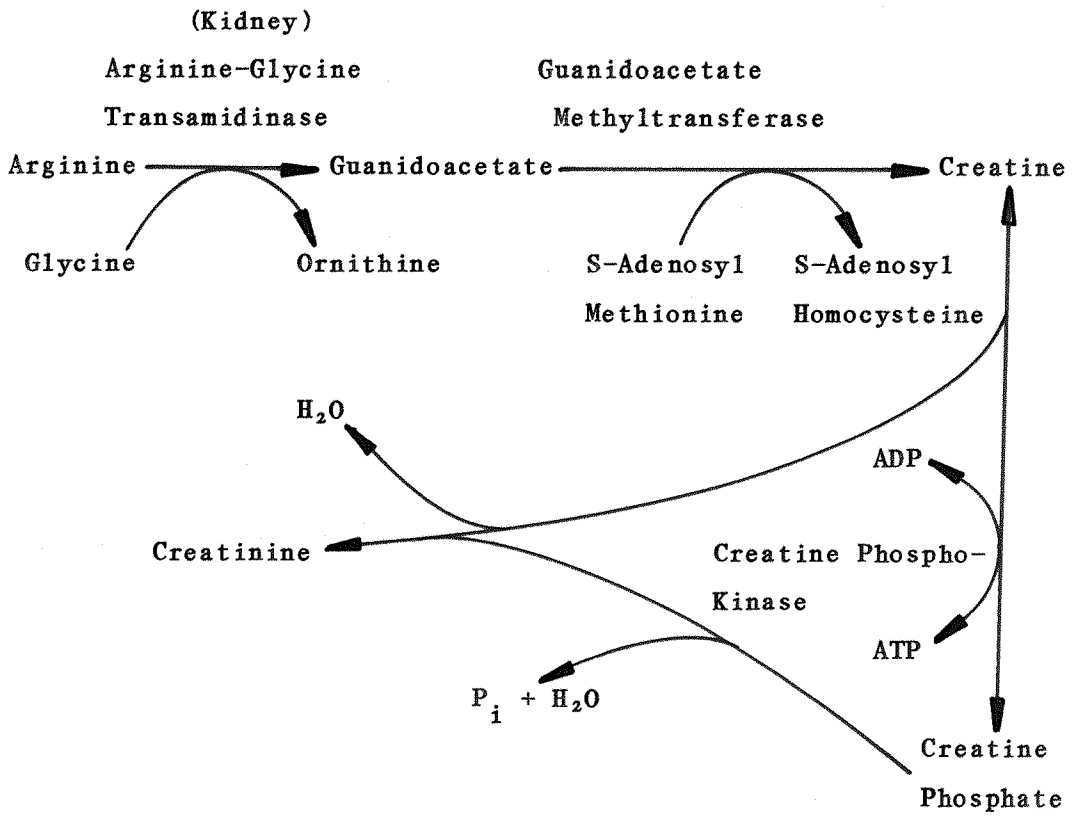


Fig. 27. Creatinine Synthesis.

section 4.10). Conversely, no increase in creatinine production could be found in dogs with muscle injury and ARF (Swenson et al., 1979).

The lack of any correlation between creatinine appearance and 3-MH loss or nitrogen intake in this study appears to support Swenson et al. (1979). But although problems associated with dietary intake of creatine and creatinine were avoided (most patients were fed by TPN or were otherwise given a commercial liquid feed, both of which did not contain creatine or creatinine) a Jaffé method was used to measure creatinine and and this can be affected by interfering substances known to be present in some patient's samples e.g. bilirubin, biliverdin (Knapp and Hadid, 1987).

4.9 Uric Acid Appearance

Urate appearance was found to correlate significantly with 3-MH loss but not with nitrogen intake.

Uric acid is the final degradation product in the metabolism of dietary or endogenous purines in man. The formation of purine nucleotides occurs by two routes, de novo synthesis and salvage pathways (Davidson, 1972). All living cells can synthesise purines de novo but the most active tissue is the liver. Also, the hydrolysis of nucleoproteins to nucleosides (purine base and ribose sugar) or free purines provides another source. The free purine bases, from dietary origin or the metabolism of endogenous nucleotides, may be reutilised in the formation of their respective mononucleotides (Rastegar and Thier, 1972; Kelley et al., 1973; Sperling et al., 1973; Cartier and Hamet, 1974). The purine degradation pathway is shown in Fig. 6b, Chapter 1, page 44.

The concentration of uric acid in the extracellular fluid should be referred to as urate, as in man 98% is in the form of monohydrogen sodium urate (Wilcox et al., 1972; Kippen et al., 1974). Four to five percent of plasma urate is bound to plasma proteins (Kovarsky et al., 1976) but this binding is reduced in uraemia (Postlethwaite et al., 1974).

The concentration of urate in body fluids is determined by the difference between the rates of production and elimination. In normal man the body pool of urate is 5.9 to 7.1 mmol and turnover is 3.6 to 4.2 mmol urate day^{-1}. Two-thirds of this turnover is excreted in urine and one-third in biliary, gastric and intestinal secretions is broken down by colonic bacteria i.e. uricolysis (Sorenson, 1960; Rastegar and Thier, 1972; Kelley et al., 1973; Sperling et al., 1973; Cartier and Hamet, 1974). Urate is metabolised in the gut at an increased rate in uraemia (Sorenson, 1965). There is normally a significant exogenous contribution to the body urate pool and urinary urate excretion (Griebsch and Zollner, 1974). Coe et al. (1976) found that normal men on a purine-free diet excreted 33 μmol urate kg^{-1} day^{-1}. The mean urate appearance in this study was 50.5 μmol kg^{-1} day^{-1}.

Twenty-four hour urine urate excretion correlates with urate turnover in patients with normal renal function (Scott et al., 1969;

Bianchi et al., 1979) and in uncomplicated malaria without renal failure urine urate excretion has been found to be elevated (Sitprija, 1985). Tungsanga et al. (1984) considered that the increased urate production seen in their hypercatabolic, infected, ARF patients was partly due to the protein catabolism, but also may have been due to ischaemia (Wooliscroft et al., 1982), secondary to impaired microcirculation which is seen in severe infection (Sitprija, 1985). This ischaemia may result in energy substrate depletion (ATP) which can increase urate appearance (Knochel et al., 1974) - see Chapter 1, section 1.3.15 and Fig. 6a, Chapter 1, page 44.

In the current study a significant correlation was found between urate appearance and 3-MH loss but not between urate appearance and nitrogen intake. The lack of correlation with intake was expected as all patients were given a purine-free diet.

The correlation with 3-MH demonstrates the increased catabolism of nucleoproteins released from tissue breakdown and resulted in an increased urate synthesis in the liver, with consequent release into the body pool. This has been shown in patients with rhabdomyolysis (Zachau-Christiansen, 1959) and in the hypercatabolic ARF patients with infection studied by Tungsanga et al. (1984).

4.10 Urea:Creatinine Ratio

Blood sampling for urea and creatinine analyses was timed to allow the body pools to equilibrate after HD treatments.

No relationship was found between 3-MH loss or nitrogen intake and the urea:creatinine ratio whether expressed as the plasma concentration ratio or the 24 hr appearance ratio (mol kg^{-1} day^{-1}). This was contrary to expectations considering the significant relationship of urea appearance with 3-MH loss and nitrogen intake and the lack of any relationship between these two parameters and creatinine appearance. The lack of correlation with the plasma ratio could be interpreted as being due to a wide variation in the ratios caused by the different filtration and dialysis coefficients for urea and creatinine in CAVH and HD therapies. It is well known that during dialysis the depletion of the plasma pool of urea is rapidly supplemented from the body pool. The replenishment of the plasma creatinine pool is a much slower process, so that these substances are not removed at equivalent rates. The ratio of urea to creatinine will therefore change as a result of dialysis independently of catabolism or intake. However, this would not be applicable to the appearance ratio as total losses were adjusted for body pool change.

Another factor that could result in a larger variability in the ratio would be non-specificity of the creatinine analysis method. Some patients were known to have raised bilirubin levels - known to interfere in some Jaffé methods (Knapp and Hadid, 1987) - and the presence of bilirubin and other interfering metabolites e.g. ketones (Kroll et al., 1987) could have resulted in a much larger variation in creatinine appearance than was actually the case, especially if these substances did not correlate with nitrogen intake or catabolism.

This data shows that in this group of patients and using the analysis methods described in Chapter 2, the use of the urea:creatinine ratio to determine a change in the urea appearance is not valid.

4.11 Plasma Proteins

4.11.1 Thyroxine-Binding Pre-Albumin

No correlation was found between TBPA concentrations measured in blood plasma and nitrogen intake or catabolism as measured by 3-MH loss.

The measurement of TBPA as an index of protein and energy malnutrition was proposed by Ingenbleek et al., (1972). Because of its short half-life (two days - Socolow et al., 1965) TBPA responds rapidly to dietary change, (Smith et al., 1975; Shetty et al., 1979). In man plasma concentrations fall shortly after the start of a fast (Gofferje and Kozlic, 1977).

TBPA contains a high concentration of tryptophan, an EAA known to play a key role in the control of protein synthesis (Ingenbleek et al., 1972; Nakamura et al., 1980) and plasma concentrations correlate with BCAA (Young and Hill, 1981) which are major precursors for protein synthesis and fatty acid synthesis (Adibi, 1976).

A transient decrease in the plasma concentration is found in inflammatory conditions e.g. after injury (Ramsden et al., 1978) and sepsis (Cooper and Ward, 1979). The acute phase response accompanying trauma, inflammation, infection and malignancy (Koj, 1975) results in a decrease in plasma TBPA concentrations, which is therefore considered to be a negative acute phase reactant. TBPA is produced in the liver which alters the synthetic rate of acute phase reactants in response to an increase in the concentration of IL-1. IL-1 is released from mononuclear phagocytic cells in the extra-hepatic disease focus (Sztein et al., 1980; Kushner et al., 1980). Prostaglandin E_2 will also affect acute phase reactant synthesis, possibly via monocyte production of IL-1 (Smith, 1976). Studies using radio-labelled TBPA have shown that the half-life is unchanged in both the normal state and the inflammatory response and an increase or decrease in concentration is due to an altered synthetic rate (Oppenheimer et al., 1965).

Plasma TBPA concentrations were measured on day one of each observation period and at four day intervals thereafter. No significant correlation was found between mean daily change in TBPA

concentrations and nitrogen intake or catabolism. This result was to be anticipated as there was a variable inflammatory response both within and between patients, as measured by the variation in daily leucocyte count and several patients were diagnosed as having septicaemia. Also, HD treatment has been shown to induce IL-1 generation (Bingel et al., 1986). Consequently an acute phase response would be expected to partially mask dietary effects on TBPA in these patients.

As the stress-induced post-operative responses (acute phase response) on plasma TBPA concentrations did not appear to be influenced by nitrogen intake in the patients studied by Bleiberg-Daniel et al. (1985), this is also likely to be the case in this group of patients. The acute phase response thus masked any nutritional effect. In addition, the low molecular weight of TBPA may facilitate movement between the intra- and extra-vascular compartments (Bleiberg-Daniel et al., 1985) as with albumin (Fleck et al., 1981) and lead to low plasma concentrations. Plasma concentrations are probably also affected by the different states of hydration of these patients, but it was not possible to make an adjustment according to the packed cell volume because of blood losses occuring during CAVH and HD treatments in addition to any GI bleeding which is likely in this type of patient (Kleinknecht et al., 1972; Maher and Schreiner, 1962; Milutinovich et al., 1977) and because blood transfusions were given.

Carpentier et al. (1982) studied ARF patients who either required dialysis or had preserved an adequate urine volume. They found a significant increase in plasma TBPA concentrations after three and six days respectively after re-feeding. Their patients did not have a significant inflammatory response as measured by C-reactive protein (CRP). Although we did not measure CRP concentrations in plasma several patients did exhibit an inflammatory response as indicated by raised blood leucocyte counts.

Therefore, in the group of patients currently under study, the use of plasma TBPA concentration as a useful marker of adequate nutrition is not supported.

4.11.2 Albumin

Serum albumin concentrations did not correlate with either 3-MH loss or nitrogen intake.

This major plasma protein serves to maintain the colloid osmotic pressure and transports metals, ions, drugs, hormones and metabolites. Albumin is synthesised in the liver (normal rate 5% body pool day^{-1}) and is not stored but directly enters the vascular system.

Albumin synthesis (Rothschild et al., 1984) depends on :-

1. Oncotic pressure in the hepatic extracellular fluid (synthesis is reduced if the concentrations of other proteins are increased, as found in inflammatory situations where synthesis of acute phase proteins is increased).

2. Amino acid supply, if this is decreased albumin synthesis is decreased.

3. Hormones e.g. anabolic steroids and thyroxine increase albumin synthesis with a simultaneous increase in catabolism or turnover.

4. Lymphokines e.g. IL-1 induce an increase in the hepatic synthesis of acute phase proteins, with a consequent decrease in albumin synthesis (Merriman et al., 1975).

The mean half-life of albumin is 19 days (Peters, 1975). Albumin catabolism occurs in the cells of many tissues, especially capillary endothelial cells and is broken down during pinocytotic transport. The fractional degradation rate is fairly constant over a wide range of absolute degradation rates. The absolute degradation rate falls if the serum concentration falls, possibly to compensate for this (Rothschild et al., 1984).

Albumin moves between the extra and intra-vascular compartments and 35 to 40% of this exchangeable pool is intravascular. Albumin is 'lost' through the capillary walls at ten times the synthetic rate and most is returned via the lymphatic system (Fleck et al., 1981). Changes in vascular permeability, e.g. as found with inflammation, cause rapid falls in the serum concentration as a result of loss into the extracellular fluid (Fleck et al., 1981). Serum concentration is also affected by short-term changes in the vascular volume e.g. hydration changes found in renal failure patients. Serum

concentrations can be reduced 15% after 30 minutes of recumbency (Aull and McCord, 1957) due to expansion of the vascular compartment as a result of the reduced hydrostatic pressure. Therefore, one might expect lower serum albumin concentrations in patients confined to bed, as in this study.

Serum albumin concentrations were measured on day one of each observation period and at four day intervals thereafter. No correlation was found between daily change in serum albumin concentrations and nitrogen intake or 3-MH loss. This was expected as the period of observation (three to eight days) is short relative to the half-life of albumin.

Serum albumin concentrations are probably also affected by the different states of hydration of these patients, but it was not possible to make an adjustment according to the packed cell volume (see previous discussion of TBPA, Chapter 4, section 4.11.1).

Albumin is therefore not a good marker of nutritional status in short term studies on ARF patients.

4.12 Anthropometry

No significant correlation with nitrogen intake or 3-MH loss could be demonstrated with mean daily change of MAC, TSFT or MAMC calculated from measurements made at four day intervals. This is not surprising considering the large errors known to exist with such techniques and the small changes which would be expected to occur as each study was of a relatively short duration (three to eight days). The presence of any oedema, which was common, would additionally have interfered with the measurements. Other investigators have been unable to show a correlation with other nutritional indices (Bencich et al., 1986; Bishop and Ritchey, 1984; Gray and Gray, 1979; Burgert and Anderson, 1979).

Mean daily body weight change also did not correlate with nitrogen intake or 3-MH loss. This was no doubt due to the variable fluid balance of these patients resulting from dialysis and various intravenous infusions.

4.13 Free Amino Acids

4.13.1 Introduction

Amino acids are the monomer units of biopolymers and are linked by peptide bonds to form polypeptides, or proteins. There are twenty common protein amino acids which are classified nutritionally into EAA and NEAA. EAA cannot be synthesised in the body and must be provided in the diet for nitrogen equilibrium to occur, whilst NEAA can be manufactured from carbon and nitrogen precursors.

4.13.2 Amino Acid Losses

Amino acid losses in ARF patients are largely related to the dialysis techniques used. With their relatively low molecular weights amino acids have a similar clearance to urea (Paganini et al., 1982).

The significant increase in the total amino acid nitrogen loss in the highest CRI group (Table 12, Chapter 3, page 123) is probably related to the increased solute removal required because of the higher urea appearance in this group. It was not an effect of an increased nitrogen intake and thence loss in this group as there was no correlation of amino acid nitrogen loss with nitrogen intake and the mean nitrogen intakes for each CRI group were not different.

Expressed as a percentage of nitrogen intake, only EAA nitrogen loss was significantly increased in CRI Group 3 (Table 14, Chapter 3, page 124). This was to be expected as nitrogen intake was similar in each group and most of the amino acids whose loss correlated with CRI were EAA (Table 17, Chapter 3, page 126).

When amino acid nitrogen loss was expressed as a percentage of the total nitrogen loss there were no significant differences between the CRI groups, whether EAA nitrogen or total amino acid nitrogen percentages were used. Thus, the changes in total nitrogen loss between groups was paralleled by the changes in amino acid loss. With increased nitrogen losses and hence solute removal, amino acid losses will have also increased, demonstrating that the plasma pool was not sufficiently depleted by dialysis or CAVH treatment to alter this relationship.

There was no significant difference between the CRI groups for amino acid nitrogen lost in the CAVH ultrafiltrate, whether urinary losses were included or not. This was also the case for the HD losses and there was also no difference between amino acid nitrogen lost via HD or CAVH treatment.

The CAVH amino acid losses in these patients (Chapter 3, section 3.12.1, page 124) were similar to losses reported by other investigators (Paganini et al., 1982; Davenport and Roberts, 1986; Roberts and Davenport, 1987) and HD amino acid losses (Chapter 3, section 3.12.1, page 125) were also similar to published data (Young and Parsons, 1966; Ginn et al., 1968; Ganda et al., 1976; Kopple et al., 1973; Wolfson et al., 1982) in both CRF and ARF patients. Amino acid nitrogen loss as a percentage of the nitrogen intake (9.4% in this study) is similar to calculations made by other workers during CAVH treatment (Davenport and Roberts, 1986; Paganini et al., 1982).

Paganinini et al. (1982) claimed that less amino acids were lost via CAVH than HD treatment, when they compared 39 hr of CAVH losses (EAA) in two ARF patients with 5 hr of HD losses (total amino acids - NEAA + EAA) in CRF patients with NEAA and EAA infusions. Their claim was apparently supported when amino acid losses on adjacent days of CAVH and CAVH/HD therapies were compared in this study. However, no difference in amino acid loss was found between CAVH and HD treatments when losses were averaged over a minimum of three days and expressed in mg N kg^{-1} day^{-1}. As there was no significant difference in UAR between the CAVH and HD therapies, similar amounts of urea would need to be removed and therefore similar amounts of amino acids, if dialysing to a set serum urea concentration. It is more realistic to compare losses over several days as in this study, than single days as CAVH is a continuous treatment whilst HD is often used intermittently. However, if CAVH is a less catabolic treatment than HD, the reduced requirement for urea removal could result in lower amino acid losses when expressed as an average daily loss over several days.

The individual amino acid losses are related to the mean plasma concentrations. The interpretation of changes in plasma amino acid concentrations is difficult as these are affected by nutritional

supply, changes in the rates of uptake and release from tissues and amino acid catabolism and synthesis, which are affected by hormonal changes.

The amino acid losses which differed between the CRI groups (Table 17, Chapter 3, page 126) were those known to be affected by trauma and infection (Dale, 1977; Askanazi et al., 1980b, 1980c; Aulick and Wilmore, 1979; Hoover-Plow et al., 1980; Clowes et al., 1980; Wedge et al., 1976; Greig et al., 1987). The significant relationship between BCAA loss and CRI found in this study is in agreement with the increased plasma concentrations found after major injury (Wedge et al., 1976; Birkhan et al., 1980) and starvation (Felig et al., 1969). Dale (1977) found increases in valine, leucine and isoleucine (BCAA) as well as phenylalanine, tyrosine and lysine in post-surgical patients. The BCAA tend not to be increased in sepsis (Greig et al., 1987; Woolf et al., 1976; Freund et al., 1978). Therefore the changes found in this current study could be interpreted as indicating the presence of continuing 'stress' in these patients. However, the increases would also be influenced by the inadequate intake as determined by the large negative nitrogen balances.

Although the oxidative use of BCAA is enhanced under acute catabolic conditions (Tischler and Fagan, 1983) free BCAA are released from muscle and this is thought to occur by the release of BCAA from protein breakdown (Lindsay, 1980) a portion escaping to the extracellular compartment. Most BCAA aminotransferases are present in skeletal muscle (Shinnick and Harper, 1976) but not liver. Therefore, unlike most amino acids, the liver has a restricted capacity to control the plasma concentration of BCAAs and increased plasma concentrations and losses can occur. The increased losses of BCAAs therefore result from the release of BCAA into the circulation from protein catabolism in muscle.

Lysine, an EAA, is found in relatively high concentrations in the intracellular muscle pool (Bergström et al., 1974) and therefore with increased catabolism there would be a loss from muscle cells into the extracellular compartment. The increased loss of lysine with an increase in CRI demonstrates release from protein breakdown in muscle.

Plasma phenylalanine concentrations are consistently elevated in injured patients and this amino acid is only catabolised in the liver. However, in uncomplicated trauma tyrosine appearance was normal after a phenylalanine load (Herndon et al., 1978) and hepatic uptake was increased following injury (Wilmore et al., 1980). Thus, in this study the increase in phenylalanine loss is unlikely to reflect a decreased hepatic uptake since tyrosine loss was also increased with catabolism (tyrosine is formed from the breakdown of phenylalanine via phenylalanine hydroxylase) and this increased tyrosine loss was not due to a high tyrosine content in the amino acid solutions infused.

4.13.3 Plasma Amino Acids

Introduction

The plasma amino acid concentrations (Table 19 and Fig. 21, Chapter 3, pages 128 and 129) were measured following an 8 hr period when no amino acids were given either by mouth or by infusion. They were therefore dependent on the rates of protein synthesis and catabolism, amino acid catabolism and synthesis, rates of transport between intra- and extracellular fluid compartments and between organs and changes in the hormonal milieu.

Only ten amino acids were present in normal concentrations: taurine, aspartate, proline, α-aminobutyrate, methionine, isoleucine, leucine, tyrosine, 1-methylhistidine and tryptophan; five were raised compared to normal subjects: glutamate, cystine, cystathionine, phenylalanine, 3-MH and the remaining thirteen were reduced in concentration: threonine, serine, asparagine, glutamine, α-aminoadipate, glycine, alanine, citrulline, valine, histidine, ornithine, lysine and arginine. These findings are similar to those of other workers (Feinstein et al., 1981; Novarini et al., 1987; Proietti et al., 1978; Druml et al., 1986).

Most amino acids were low or normal in concentration, indicating that they did not accumulate and were removed by the various treatments. The low concentrations of NEAA suggests that these, as well as EAA, should be provided. The low concentrations of most EAA were expected as amino acid supply was insufficient to meet requirements, a negative nitrogen balance was found in all patients.

In acute catabolic conditions several amino acids are high in plasma, whilst in ARF they are reduced and this suggests that mechanisms other than acute protein catabolism affect the pool size. These may include the effect of uraemic toxins on enzyme systems, the type of insult, degree of kidney damage (which affects the amount of amino acid synthesis in kidney), removal of waste products by dialysis methods, ensuing complications (sepsis, trauma), type and amount of feeding, and drug treatment (especially infusion with hormones, e.g. insulin and glucocorticoids).

Major Gluconeogenic Amino Acids

Serine, alanine and glycine are the main amino acids used for gluconeogenesis (Cherrington, 1981). Serine and glycine are interconvertible via the serine hydroxymethyltransferase reaction (Fig. 3, Chapter 1, page 25).

Serine has a unique stimulatory effect on glycogen synthesis in liver and muscle in ARF (Tizianello *et al.*, 1980; Stepinski *et al.*, 1982). Serine supplementation in ARF rats causes a significant increase in glycogen synthetase activity, with improvement in glycogen stores (Hörl, 1980a) and there may therefore be an increased requirement for serine in ARF.

From liver perfusion experiments in rats with ARF, serine was found to have a particularly strong stimulatory effect on hepatic gluconeogenesis (Fröhlich *et al.*, 1977, 1978; Riegel *et al.*, 1983) and serine appears to be synthesised primarily from glycine in kidney (Pitts *et al.*, 1970). Serine is also the only NEAA shown to stimulate protein synthesis *in vitro* (Galbraith and Buse, 1981).

The kidney is the only organ to generate serine into the systemic circulation in the postabsorptive state and there is a direct correlation between renal serine output and arterial concentration (Tizianello *et al.*, 1980, 1987). The reduced plasma concentrations of serine could therefore be explained by impaired renal production and the synthesis of serine by kidney is impaired in uraemia (Tizianello *et al.*, 1980, Kopple and Fukuda, 1980).

The low alanine concentrations may also relate to reduced renal metabolism, but the liver plays a major role in alanine metabolism where it is the major substrate for gluconeogenesis being involved in the glucose-alanine cycle (Felig, 1973) and is also formed in gut by transamination of glutamine, which also had a low concentration in plasma. The low concentrations of all three gluconeogenic amino acids could also indicate the inadequate caloric intake (21.5 ± 7.1 kcal, 90 ± 30 kJ kg^{-1} day^{-1}) relative to the energy requirements, with a consequent depletion of glycogen stores and an increased demand for gluconeogenic substrates. Expected changes in the hormonal milieu would also increase gluconeogenesis.

Urea Cycle Amino Acids

The liver is the main site of urea production and ornithine, citrulline and arginine are the three main intermediates in the urea cycle. At the start of the cycle ornithine reacts with carbamoyl phosphate to form citrulline. Argininosuccinate formed from citrulline and aspartate then undergoes cleavage to arginine and fumarate and arginine is then cleaved to form ornithine and urea (Fig. 1, Chapter 1, page 16).

Reduced renal metabolism may account for the low concentrations of arginine and ornithine found in CRF patients and the ARF patients under study, (Tizianello et al., 1980) but these changes may also derive from accelerated ureagenesis (Flügel-Link et al., 1983). In addition, hepatic arginase activity and ornithine aminotransferase activity are enhanced in experimental ARF (Kopple and Cianciaruso, 1984). A reduced renal use of citrulline may contribute to the increased concentrations found in both CRF and ARF, but this has been questioned (Walser, 1980). The decreased renal uptake of citrulline is responsible for the decreased renal production of arginine in CRF (Tizianello et al., 1980). The kidney requires citrulline for the synthesis of arginine, which in turn is required for the first stage of creatine synthesis. A decreased renal use of citrulline is not evident from plasma concentrations in ARF as plasma citrulline and arginine concentrations are normal or low compared to healthy subjects (Druml et al., 1986; Feinstein et al., 1981). The low plasma citrulline and arginine concentrations found in this study confirms Feinstein et al.'s (1981) data, and may result from a depletion of the body pool by kidney replacement therapies or from the accelerated ureagenesis, present in this group of catabolic patients, especially as they were fed. The correlation of arginine loss with CRI (Table 17, Chapter 3, page 126) adds support to the kidney replacement therapy argument.

Branched Chain Amino Acids

BCAA, valine, isoleucine and leucine, are distinguished from the other amino acids by the fact that they are largely transaminated and degraded in peripheral tissues, most BCAA absorbed after a meal pass through the liver unchanged (Wahren et al., 1976). BCAA are therefore major carriers of amino nitrogen from viscera to peripheral tissues and are transaminated in muscle for the synthesis of alanine and glutamine, prior to gluconeogenesis in the liver (Felig, 1973, 1981; Elia and Livesey, 1981) - see Fig. 3, Chapter 1, page 25 and Figs. 22, 23, and 24, Chapter 4, pages 138 and 139.

The low valine and normal leucine and isoleucine plasma concentrations demonstrate that the metabolism of BCAA in ARF is not altered to the same degree as in CRF patients, who exhibit low plasma leucine and isoleucine concentrations in addition to a low valine concentration.

The abnormal BCAA plasma profile found in this study and in CRF partly resembles that induced by nutritional antagonism in rats given a low protein diet and excess leucine (Shinnic and Harper, 1977). Consequently, the relative proportions of these amino acids provided by the infusion solutions may be important and ARF patients may require different proportions compared to normal subjects. The Rose formula for EAA used in TPN infusions in this study may have caused antagonism between these amino acids, since increasing valine and decreasing leucine and isoleucine in the EAA preparation given to CRF patients can normalise plasma amino acid profiles (Alvestrand et al., 1983). In support of this, the provision of EAA two to three times the normal requirements according to the Rose formula with a low protein diet to CRF patients, does not normalise the valine pools in plasma and muscle, both pools remaining 40 to 45% lower than control subjects (Alvestrand et al., 1983).

Carbohydrate intolerance is a frequent finding in uraemia (DeFronzo et al., 1973) and is primarily due to peripheral tissue insensitivity to insulin (DeFronzo et al., 1981). Insulin secretion is usually enhanced as an adaptive response but BCAA exhibit normal sensitivity to insulin in uraemia (Alvestrand et al., 1988).

The effect of insulin on branched chain keto acids (BCKA) in

normal subjects and uraemics is different. After oral glucose all BCKAs are decreased in normals but only α-ketoisovalerate (keto acid of valine) in uraemics is decreased, therefore suggesting that the metabolism of valine is different to the other BCAA in uraemia (Schauder et al., 1981). Hyperinsulinaemia may therefore contribute to the low plasma valine concentrations found in this group of ARF patients. Other evidence also suggests that the metabolism of valine is different from isoleucine and leucine in uraemia. Alvestrand et al. (1988) found in CRF patients that the fractional splanchnic uptake of valine was signifcantly greater in uraemic patients than healthy control subjects, whilst the uptakes of leucine and isoleucine were the same as control subjects. They also found that intracellular valine concentration in muscle was significantly lower than the healthy subjects, whilst the muscle leucine and isoleucine concentrations were not different from the control values.

Glutamine

Gut mucosal cells play an important role in amino acid metabolism in both normal and disease states (Windmueller 1982; Souba and Wilmore, 1983; Souba et al., 1983). In many species glutamine is removed by gut epithelial cells and serves as the principal respiratory fuel. In rats glutamine is the principal fuel utilised by enterocytes, 60% of glutamine is oxidised to carbon dioxide (CO_2) and glutamine is responsible for 35 to 40% of total CO_2 production. Glutamine is metabolised by post-absorptive dog gut and this rate increases three fold with a glutamine infusion sufficient to increase arterial plasma concentration two fold (Souba et al., 1985). Glutamine can be removed from the intestinal tract lumen (after a meal) or from the bloodstream via the capillary bed. The uptake of circulating glutamine can be reduced by the administration of glutamine via the bowel lumen. Gut glutamine consumption may therefore be a balance between these two routes (Windmueller, 1982). Glutamine is readily synthesised in skeletal muscle where most glutamine is produced and releases glutamine to provide fuel for the intestinal tract.

Chang and Goldberg (1978) and Odessey et al. (1974) have proposed reactions that participate in glutamine synthesis. Using

isolated diaphragm incubated with different amino acids the BCAA were found to be the principal amino donors, synthesising glutamate from α-ketoglutarate. Glutamine can then be readily formed from glutamate and ammonia. These reactions generate glutamine which with alanine accounts for 60% amino acid nitrogen released by muscle (Felig, 1981).

Following surgery (Kapadia et al., 1982), injury (Askanazi et al., 1980b), sepsis (Roth et al., 1982) and glucocorticoid administration (Muhlbacher et al., 1984), intracellular glutamine in skeletal muscle is decreased by 50%, associated with a three fold increase in glutamine efflux from muscle. There is a simultaneous fall in circulating glutamine concentration (as found in this study) and an accelerated uptake of glutamine by the intestinal tract (Souba and Wilmore, 1983; Souba et al., 1983). Thus, catabolic depleted patients have diminished stores of glutamine and increased visceral demands. The low intracellular concentration of glutamine would appear to be partly due to a deficiency of substrate limiting the rate of glutamine synthesis as Ardawi (1988) found an increased glutamine synthetase activity in skeletal muscle in thermally injured rats, with an unchanged glutaminase activity compared to a control group.

Rennie et al., (1986) have suggested the presence of a sodium-dependent carrier for glutamine in muscle which could explain the large efflux from muscle found in trauma and ARF. Rennie et al., (1986) provided evidence that the intracellular glutamine concentration is related to the rate of protein synthesis in muscle. They suggested that the improvement in nitrogen balance seen with BCAA administration largely results from re-filling of the intracellular glutamine pool by non-competetive inhibition of glutamine efflux by BCAA.

The provision of exogenous glutamine might support the metabolic demands of the GI tract and prevent depletion of the intracellular pool, but glutamine is unstable in solution, hydrolysing to ammonia and pyrollidonecarboxylate which may be toxic. However, stable glutamine-containing dipeptides can be infused and these are rapidly hydrolysed to the constituent amino acids (Fürst et al., 1988; Neuhauser-Berthold et al., 1988; Albers et al., 1988). Fürst et al.

(1988) and Stehle et al. (1989) gave catabolic patients a glutamine-alanine dipeptide solution or isonitrogenous isocaloric TPN. The muscle glutamine pool was maintained in the dipeptide group, whilst it was reduced in the control group and nitrogen balance was better in the dipeptide group relative to the control group, supporting data from Rennie et al. (1986). Thus, in ARF, where patients are similarly catabolic, protein depleted and probably glutamine depleted judging from the low plasma glutamine, there may be benefit in infusing glutamine-containing dipeptides.

Aromatic Amino Acids

Phenylalanine, an essential amino acid, is initially catabolised to tyrosine via the enzyme phenylalanine hydroxylase (Fig. 28), is consistently elevated in plasma in injured patients and this results in a raised plasma phenylalanine:tyrosine ratio (Hoover-Plow et al., 1980; Askanazi et al., 1980b, 1980c). It is an index of skeletal muscle catabolism and is mainly catabolised by liver (Ayling et al., 1975). However, increased concentrations of phenylalanine may not indicate altered hepatic handling of phenylalanine following uncomplicated injury. In uncomplicated trauma patients, tyrosine (the phenylalanine metabolite) appearance was normal after a phenylalanine load (Herndon et al., 1978) which indicated that phenylalanine hydroxylase activity was normal and the rate of hepatic uptake of phenylalanine was increased following injury (Wilmore et al., 1980).

In chronic renal failure an elevated phenylalanine:tyrosine ratio is found in plasma with a normal phenylalanine concentration and low tyrosine concentration. This has also been found by Novarini et al. (1987) in ARF patients without trauma. Renal tissue produces large amounts of tyrosine from phenylalanine via the enzyme phenylalanine hydroxylase (Tizianello et al., 1980) and therefore the abnormal ratio may derive from reduced renal function, but in ARF hepatic degradation of tyrosine is increased (Fröhlich et al., 1977). Also, in CRF, the conversion of phenylalanine to tyrosine is reduced (Pickford et al., 1973; Kopple et al., 1972; Giordano et al., 1968; Jones et al., 1978) and this has been attributed to the inhibition of phenylalanine hydroxylase by uraemic toxins (Young and Parsons, 1973).

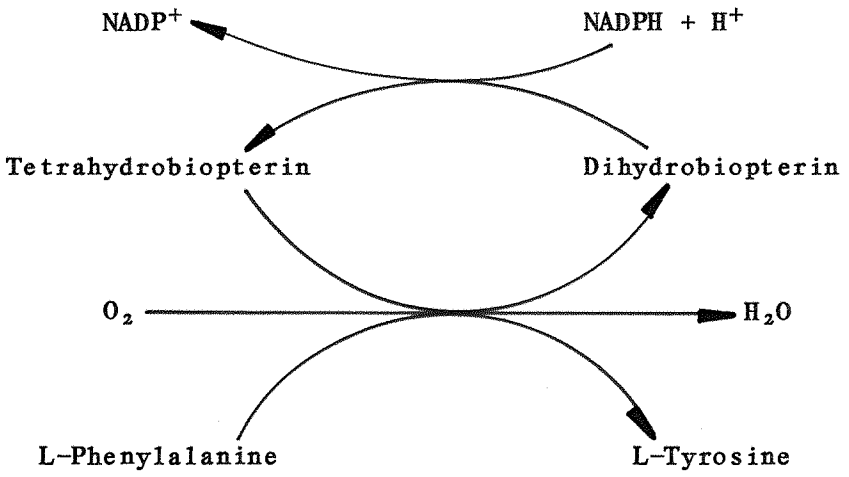


Fig. 28. The Phenylalanine Hydroxylase Reaction.

Studies in chronically uraemic rats show a direct relationship between protein intake and phenylalanine hydroxylase activity (Wang et al., 1975) and this is also suggested by studies in normal man (Jones et al., 1978). The low tyrosine may also be due to the increased activity of cytosol tyrosine aminotransferase as this is induced in the liver of acutely uraemic rats (Sapico et al., 1974) and could explain the increased hepatic degradation found by Fröhlich et al. (1977). Insulin (Sorimachi and Yasimura, 1981) and glucagon (Dickson et al., 1981) also increase this enzyme activity and both are increased in uraemia and the activity of this enzyme is also related to an increased urea synthesis rate (Sapico et al., 1974).

The altered plasma phenylalanine:tyrosine ratio found in this study would appear to be related to the affect of hypercatabolism and/or trauma as plasma phenylalanine concentration was raised whilst tyrosine was normal, and the loss of both these amino acids correlated with CRI (Table 17, Chapter 3, page 126). It is possible that the large amounts of phenylalanine released from skeletal muscle and hence a higher tyrosine appearance led to the normal tyrosine concentration found in these patients, obscuring any effect of phenylalanine hydroxylase inhibition, tyrosine aminotransferase induction or reduced renal production of tyrosine.

Histidine

Histidine is an amino acid that is considered to be essential for the new-born infant (Holt and Snyderman, 1965) and Kopple and Swendseid (1975) have suggested that it may also be essential for the normal adult. The kidney releases some histidine into the systemic circulation and this may help to explain the low histidine concentrations found in uraemia (Fukuda and Kopple, 1979). It has also been suggested that a competitive interference between histidine and arginine may affect the metabolism of this amino acid (Giordano *et al.*, 1975). Kobayashi *et al.* (1982) found an unaltered fractional hepatic extraction of histidine in ARF rats compared with sham-operated controls, supporting observations in direct hepatic perfusion studies in ARF rats (Lacy, 1970). Consequently, if the supply of histidine was reduced (e.g. impaired synthesis in the kidney) but the hepatic extraction remained constant the free histidine pool size would be reduced leading to a low plasma concentration, as found in this study. However, histidine was supplied in the TPN and liquid feeds, suggesting that either there were insufficient precursors available for synthesis, histidine synthesis was impaired or requirements were higher than the amount supplied.

Sulphur Amino Acids

Cystathionine, cyst(e)ine and taurine are all amino acids involved in the transulphuration pathway of methionine, an EAA. Cystathionine is formed from methionine by several steps, cysteine being produced from cystathionine by the action of the enzyme gamma-cystathionase. Taurine is formed from cysteine by further enzyme steps. Most enzymes for this pathway are found primarily in the liver (Fig. 29).

CRF patients treated with CAPD, HD and low protein diet are reported to have low muscle intracellular taurine concentration in spite of normal or elevated plasma and muscle methionine concentrations, with raised plasma cyst(e)ine, suggesting that the transulphuration pathway is inhibited (Garcia *et al.*, 1988). Raised

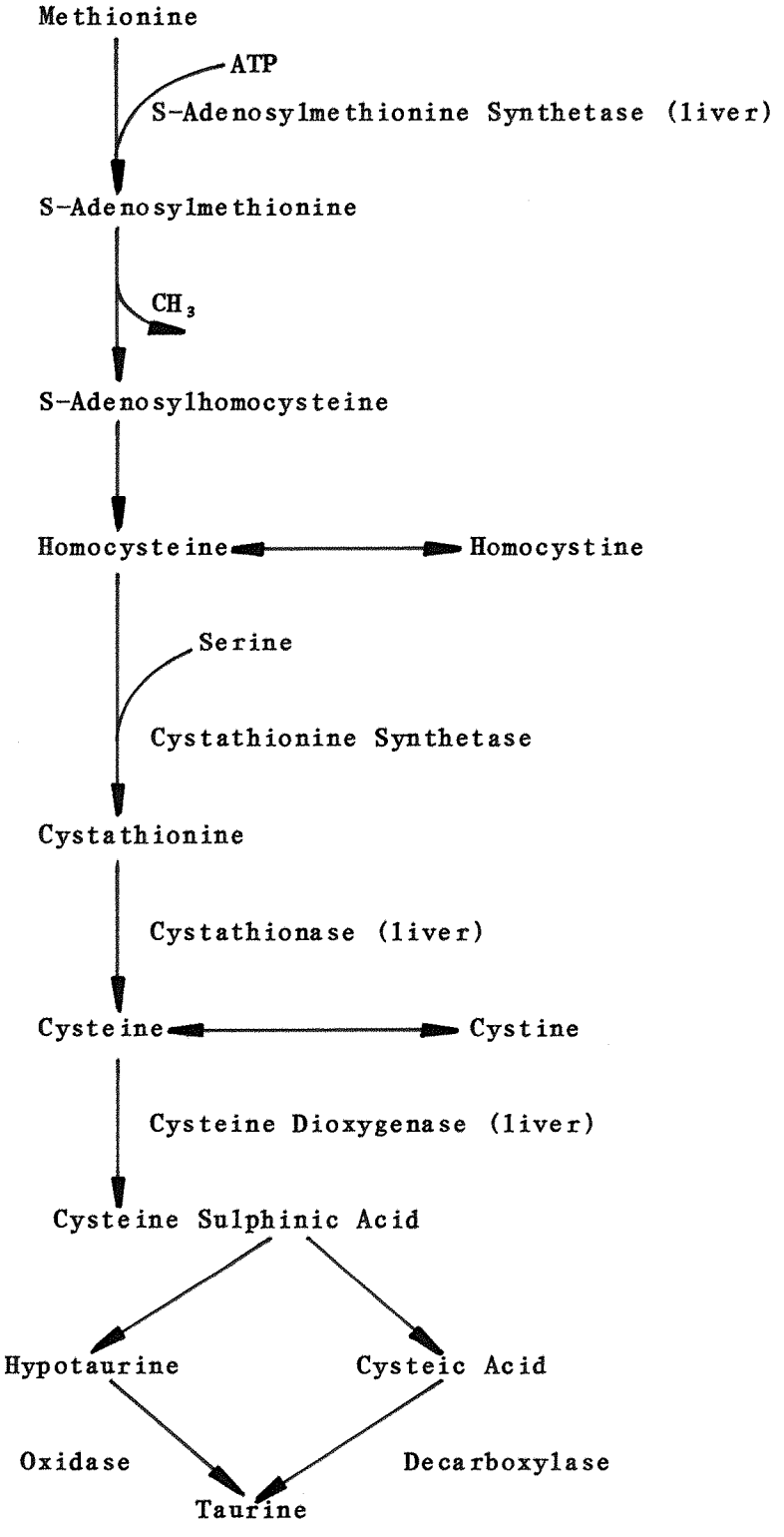


Fig. 29. The Transulphuration Pathway.

concentrations of cysteinsulphinic acid in CAPD plasma with undetectable concentrations in CAPD muscle and normal plasma have also been found (Garcia et al., 1988). Methionine in muscle and plasma in these CAPD patients was normal but taurine in CAPD muscle was reduced by 50%. They concluded that low taurine in the presence of high cysteinsulphinic acid suggested that cysteinsulphinic acid decarboxylase, the key enzyme for the conversion of cysteinsulphinic acid to taurine via hypotaurine, is inhibited.

Koide et al. (1988) found raised concentrations of cystathionine in a mixed group of 24 uraemic patients, containing both untreated and HD treated patients but this amino acid was not detected in their normal subjects. They also found that cystathionine synthase activity in rats with CRF was 1.7 times that in control rats. Cystathionase activity was the same in both control and CRF rats. They concluded that the raised plasma cystathionine in CRF could be attributed to increased cystathionine synthesis in the liver.

The normal plasma methionine concentration with increased levels of cystine and cystathionine with low plasma taurine found in this group of ARF patients is similar to the above findings in CRF patients. Therefore, although intracellular concentrations were not measured, it is likely that methionine metabolism is altered in a similar way to that in CRF patients.

4.14 Conclusions and Recommendations

In conclusion, it is clear that mortality rates remain at an unacceptably high level in ARF, although the use of early dialysis has had some impact on this. Also some nutritional studies have had encouraging results on the effect of nutrition on both mortality and rate of recovery of renal function, but so far these have been inconclusive and contradictory.

The data presented in this thesis shows that nitrogen requirements for the majority of patients treated by PD, CAVH or HD are in excess of 200 mg N kg^{-1} day^{-1}. The high nitrogen losses found in this study, relative to other studies, are probably a result of the collection and measurement of nitrogen in all excreta, demonstrating that these patients are more catabolic than has been suggested (Blackburn et al., 1978; Mirtallo and Fabri, 1984). As there is evidence from this study that ARF patients can utilise a nitrogen intake upto 200 mg N kg^{-1} day^{-1}, I therefore recommend that this nitrogen intake be given to reduce muscle wasting, impaired immune competence, wound healing and morbidity.

I would also recommend that TPN should be used for all patients to ensure that sufficient nitrogen and calories are given. The amino acid solution should have higher concentrations of threonine, serine, glycine, alanine, citrulline, valine, ornithine, lysine and arginine, with the addition of a glutamine dipeptide to correct some of the abnormalities of the amino acid profile found in ARF patients.

Also only biocompatible membranes should be used for CAVH and HD therapy, in order to minimise the catabolic effects of these treatments and the use of the Bistrian CRI is recommended to categorise patients.

Further controlled studies should be undertaken to determine the maximum nitrogen intake that can be given and if mortality rates can be improved. Also to determine whether the amino acid profile and nutritional status can be improved by the use of a supplemented amino acid solution and pharmaceutical measures such as the use of indomethacin (Arisi et al., 1988), anti-glucocorticoids (Schaefer et al., 1988b) and the addition of glutamine peptides to TPN solutions (Fürst et al., 1988).

APPENDIX I

Table 20. Means and standard deviations of various parameters measured.

Item	Mean	Standard Deviation	n
Nitrogen Intake ($mg\ N\ kg^{-1}\ day^{-1}$)	113.1	41.9	24
Non-Protein Energy Intake ($kcal\ kg^{-1}\ day^{-1}$)	21.5	7.1	24
Energy intake in patients who survived	20.6	6.6	16
Energy Intake in patients who died	23.3	8.2	8
3-Methylhistidine Loss ($\mu mol\ kg^{-1}\ day^{-1}$)	4.43	1.32	24
Plasma 3-MH change/day ($\mu mol/l$)	-0.35	2.9	23
Overall Urea Appearance Rate ($mg\ N\ kg^{-1}\ day^{-1}$)	161.8	40.4	24
UAR in patients subject to trauma	162.7	35.9	15
UAR in patients not subject to trauma	160.4	49.3	9
N Balance ($mg\ N\ kg^{-1}\ day^{-1}$)	-108.9	54.1	24
Non-urea N Loss ($mg\ N\ kg^{-1}\ day^{-1}$	61.3	26.1	24
Creatinine Appearance ($\mu mol\ kg^{-1}\ day^{-1}$)	86.8	29.9	24
Urate Appearance ($\mu mol\ kg^{-1}\ day^{-1}$)	50.5	19.9	23
Serum Urea:Creatinine Ratio	70.8	26.6	23
UAR:Creatinine Appearance Ratio	75.6	27.7	23
Serum Albumin excluding transfused patients (g/l)	28.4	3.4	9
Prealbumin in all patients (mg/l)	200.6	84.4	22
Prealbumin in patients not infused with blood or plasma protein	218.9	96.9	9
Blood Haemoglobin excluding transfused patients. (g/dl)	10.1	0.9	9

continued.

Table 20. Continued.

Item	Mean	Standard Deviation	n
MAC males (mm)	291.0	25.2	10
MAC females (mm)	289.6	30.5	5
SFT males (mm)	10.5	4.0	10
SFT females (mm)	15.3	4.6	5
MAMC males (mm)	257.9	19.1	10
MAMC females (mm)	241.6	35.8	5
Weight (kg)	76.7	11.8	24
Weight Mean Change/day (kg)	-0.32	0.6	24
Amino N loss in CAVH Effluent (mg N kg^{-1} day^{-1})	8.31	2.7	15
Amino N loss in HD Effluent	7.13	2.4	8

APPENDIX II

In this appendix the formulation of various solutions used in the treatment of the ARF patients and the amino acid analyses are presented.

Table 21. Standard Haemodialysis Solution.

Renalyte®, Macarthy Medical Ltd.

Concentrated Stock			After Dilution
Sodium	(mmol/l)	130.0	3.71 mmol/l
Calcium	(mmol/l)	1.55	44.3 µmol/l
Potassium	(mmol/l)	1.54	44.0 µmol/l
Magnesium	(mmol/l)	0.85	24.3 µmol/l
Chloride	(mmol/l)	96.3	2.75 mmol/l
Acetate	(mmol/l)	40.0	1.14 mmol/l
Dextrose	(g/l)	2.0	57.1 mg/l

Table 22. CAVH Replacement Solutions.

Acetate with Potassium Solution			Potassium-Free Solution	
Sodium	(mmol/l)	135		135
Potassium	(mmol/l)	3.0		0
Magnesium	(mmol/l)	0.75		0.75
Calcium	(mmol/l)	1.2		1.87
Phosphate	(mmol/l)	1.0		0
Bicarbonate (as Acetate)	(mmol/l)	34.0	Lactate	33.75
Chloride	(mmol/l)	111		106.5
Dextrose	(g/l)	1.36		1.65

Table 23. Dianeal® Peritoneal Dialysis Solutions.

		1.36% Dextrose Solution	6.36% Dextrose Solution
Sodium	(mmol/l)	132	142
Calcium	(mmol/l)	1.75	1.8
Magnesium	(mmol/l)	0.75	0.75
Chloride	(mmol/l)	102	101
Bicarbonate (as Lactate)	(mmol/l)	35	45

Table 24. Naso-Gastric Tube Feed Formulae.

Amino Acid (g/l)	Clinifeed ISO®	FAVOUR®	Nutranel®
L-Isoleucine	1.39	1.23	2.65
L-Leucine	3.2	2.96	4.3
L-Lysine	2.59	2.85	4.2
L-Methionine	0.8	0.72	0.8
L-Cystine	0.59	0.40	0.75
L-Phenylalanine	1.2	1.73	1.5
L-Tyrosine	1.2	1.41	1.1
L-Threonine	1.39	1.52	2.6
L-Tryptophan	0.51	0.35	0.75
L-Valine	1.71	1.41	2.2
L-Arginine	0.99	1.84	0.9
L-Histidine	0.59	1.23	0.55
L-Alanine	1.39	1.33	1.9
L-Aspartic Acid	2.8	3.55	4.2
L-Glutamic Acid	5.31	7.71	7.3
Glycine	0.59	1.01	0.65
L-Proline	2.0	2.96	2.2
L-Serine	1.39	1.92	1.5
Fat (g/l)	41.1	33.1	9.75
Carbohydrate (g/l)	130.7	140.0	186.2
Total Energy (kcal)	1000	1000	1000

Plus minerals, trace elements and vitamins.

Table 25. Intravenous Amino Acid Solutions

Amino Acid (g/l)	Vamin 9®	Vamin Glucose®	Vamin 14®	Vamin 18®	Aminofusin-L-Forte®
L-Alanine	3.0	3.0	12.0	16.0	12.0
L-Arginine	3.3	3.3	8.4	11.3	8.0
L-Aspartic Acid	4.1	4.1	2.5	3.4	0.0
L-Cysteine/Cystine	1.4	1.4	0.42	0.56	0.0
L-Glutamic Acid	9.0	9.0	4.2	5.6	18.0
Glycine	2.1	2.1	5.9	7.9	20.0
L-Histidine	2.4	2.4	5.1	6.8	2.0
L-Isoleucine	3.9	3.9	4.2	5.6	3.1
L-Leucine	5.3	5.3	5.9	7.9	4.4
L-Lysine	3.9	3.9	6.8	9.0	5.0
L-Methionine	1.9	1.9	4.2	5.6	4.2
L-Phenylalanine	5.5	5.5	5.9	7.9	4.4
L-Proline	8.1	8.1	5.1	6.8	14.0
L-Serine	7.5	7.5	3.4	4.5	0.0
L-Threonine	3.0	3.0	4.2	5.6	2.0
L-Tryptophan	1.0	1.0	1.4	1.9	0.9
L-Tyrosine	0.5	0.5	0.17	0.23	0.0
L-Valine	4.3	4.3	5.5	7.3	3.0
Total Amino Acids	70.2	70.2	85.0	114.0	101.0
Nitrogen (g/l)	9.4	9.4	13.5	18.0	15.2
Glucose (g/l)	0.0	100.0	0.0	0.0	0.0
Sodium (mmol/l)	50.0	50.0	100.0	0.0	40.0
Potassium (mmol/l)	20.0	20.0	50.0	0.0	30.0
Calcium (mmol/l)	2.5	2.5	5.0	0.0	0.0
Magnesium (mmol/l)	1.5	1.5	8.0	0.0	5.0
Chloride (mmol/l)	55.0	55.0	100.0	0.0	27.5
Sulphate (mmol/l)	0.0	0.0	8.0	0.0	0.0
Acetate (mmol/l)	0.0	0.0	135.0	0.0	10.0
Osmolarity (mosm/l)	700.0	1350.0	1145.0	1130.0	1050.0
pH	5.2	5.2	5.6	5.6	5.4
Energy (kcal)	250.0	650.0	350.0	460.0	400.0

Table 26. Intravenous Carbohydrate Solutions.

	Glucoplex® 1000	1600	Dextrose 5%	50%
Glucose	(g/l) 240.0	400.0	50	500
Sodium	(mmol/l) 50.0	50.0		
Potassium	(mmol/l) 30.0	30.0		
Magnesium	(mmol/l) 2.5	2.5		
Zinc	(µmol/l) 45.6	45.6		
Dihydrogen Phosphate	(mmol/l) 18.0	18.0		
Chloride	(mmol/l) 67.0	67.0		
Osmolality	(mosm/kg) 1750	2900		
Energy	(kcal/l) 1000	1600	200	2000

Table 27. Intravenous Lipid Suspensions.

Intralipid®	10%	20%
Fractionated Soya-bean oil (g/l)	100	200
Fractionated Egg Phospholipids (g/l)	12	12
Glycerol (g/l)	22	22
pH	7	7
Osmolality (mosmol/l)	300	350
Energy (kcal/l)	1100	2000

Table 28. Intravenous Electrolyte and Trace Element Solution (Addamel®).

Calcium	5.0 mmol per 10 ml ampoule.
Magnesium	1.5 mmol
Iron	50.0 µmol
Zinc	20.0 µmol
Manganese	40.0 µmol
Copper	5.0 µmol
Fluoride	50.0 µmol
Iodide	1.0 µmol
Chloride	13.3 mmol

Table 29. Intravenous Fat Soluble Vitamin Solution (Vitlipid®).

Retinol	750 µg
Calciferol	3 µg
Phytomenadione	150 µg
Fractionated Soya-bean oil	1 g
Fractionated Egg Phospholipids	120 mg
Glycerol	225 mg
Water to 10 ml.	

Table 30. Intravenous Water-soluble Vitamins (Solivito®).

Vitamin B_1	1.2 mg per vial (10 ml when reconstituted).
Vitamin B_2	1.8 mg
Nicotinamide	10.0 mg
Vitamin B_6	2.0 mg
Pantothenic acid	10.0 mg
Biotin	0.3 mg
Folic Acid	0.2 mg
Vitamin B_{12}	2.0 µg
Vitamin C	30.0 mg

Table 31. Sterilising Solutions.

1. 40% w/v Formalin solution diluted 1:20 with water.

2. Amuchina®. An electrolytic chloroxidiser in a hypertonic sodium chloride solution.

Available chlorine	11 g/l
Sodium Chloride	180 g/l
Hypochlorous acid	Trace

The above concentrate was diluted 1:20 with water.

3. Sodium Hypochlorite solution. Presept® disinfectant tablets (Surgikos Ltd.).

Sodium dichloroisocyanurate 2.5 g was dissolved in 1l water to provide 1400 ppm available chlorine.

Table 32. Protein Precipitating Solutions for Sample Pre-Treatment Prior to Amino Acid Analysis.

Reagent		Urine, CAVH and Standards Solution.	Plasma and IPD Effluent Solution	HD Effluent Solution
5-Sulphosalicylic Acid	(g)	6.0	10.0	23.0
Lithium Chloride Anhydrous	(g)	1.1	1.1	4.22
Lithium Hydroxide Monohydrate	(g)	0.9	0.6	3.44
25% Thiodiglycol Solution in water	(ml)	4.0	4.0	15.34
Phenol	(ml)	0.2	0.2	0.76
L-nor-Leucine	(µmol)	20.0	20.0	66.7
Distilled Water to		100 ml	100 ml	100 ml

Table 33. 1.5% Ninhydrin Reagent for Amino Acid Analysis.

Potassium Acetate	176.65 g
Sodium Acetate Anhydrous	49.22 g
Tri-potassium Citrate	2.40 g
Glacial Acetic Acid	60.0 ml
Ethanediol	400.0 ml
Ninhydrin	15.0 g
Brij 35 30% w/v Aqueous Solution	1.0 ml
Water to	1000 ml
pH	5.86

Table. 34. Eluting Buffers for Amino Acid Analysis.

		Acid	Basic
Citric Acid Monohydrate	(g)	21.0	9.85
Lithium Chloride Anhydrous	(g)	6.3	-
Thiodiglycol 25% w/v Aqueous solution	(ml)	2.5	-
Lithium Hydroxide	(g)	-	12.59
Boric Acid	(g)	-	8.8
Ethylene-Diamine-Tetra-Acetic Acid	(g)	-	0.8
Ascorbic Acid Solution (1 mol/1)	(ml)	12.0	12.0
Brij 35 30% w/v Aqueous solution	(ml)	1.0	1.0
pH		1.9	11.55

APPENDIX III

Method Validation Results

Table 35. Method Ranges and Quality Control Data.

Item	Top Limit of Linear Range	Quality Control Coefficient of Variation
HD Effluent		
Collector		2.4
Urea	90 mmol/l	1.5
Total Nitrogen	10 g/l	2.1
Creatinine	900 µmol/l	1.7
Uric Acid	2000 µmol/l	4.4
TBPA	470 mg/l	3.9
Amino Acids		
Aspartate	200 µmol/l	9.2
Threonine	500 µmol/l	6.3
Serine	500 µmol/l	5.4
Glutamate	700 µmol/l	5.2
Proline	1200 µmol/l	9.0
Glycine	700 µmol/l	6.9
Alanine	900 µmol/l	5.0
Valine	1000 µmol/l	4.1
Cystine	600 µmol/l	8.4
Methionine	1100 µmol/l	4.7
Isoleucine	1100 µmol/l	3.0
Leucine	1100 µmol/l	2.1
Tyrosine	900 µmol/l	2.8
Phenyl-alanine	900 µmol/l	3.9
Histidine	600 µmol/l	9.8
Lysine	700 µmol/l	9.0
Arginine	1200 µmol/l	7.0

Table 35. Continued.

Amino Acid	Quality Control Coefficient of Variation
Taurine	6.9
Asparagine	7.0
Glutamine	6.0
α-Aminoadipate	3.3
Citrulline	7.2
α-Aminobutyrate	6.3
Cystathionine	4.8
3-Methylhistidine	6.7
Tryptophan	6.6
Ornithine	7.7

Table 36. Total Nitrogen Recovery Results.

	Percent Recovery		
Item	Mean	S.D	n
Albumin	98.1	1.7	10
Lysine	99.3	1.2	10
Creatinine	99.4	0.9	10
Urate	99.1	1.8	14

Table 37. The Results of Recovery Studies.

					Effluents					
	Plasma		Urine		PD		HD		CAVH	
	n = 5		n = 7		n = 5		n = 7		n = 8	
Amino Acid	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Aspartate	102.5	7.3	108.6	8.0	*		91.0	3.0	94.2	5.7
Threonine	101.8	3.4	101.3	2.9	103.9	10.8	99.8	6.1	99.7	4.2
Serine	107.0	4.2	96.5	2.2	102.9	11.6	96.4	4.3	101.6	3.0
Glutamate	*110.8	8.3	97.4	7.4	100.1	8.3	101.3	1.6	99.7	3.0
Proline	98.4	2.0	106.9	7.0	97.6	3.2	109.3	5.7	96.6	2.9
Glycine	104.5	3.3	103.2	4.3	99.1	3.7	102.4	3.1	97.0	1.7
Alanine	102.5	1.4	101.8	2.2	100.8	5.1	102.0	2.9	100.3	1.6
Valine	102.4	4.8	102.2	5.2	100.8	3.3	101.2	2.3	98.0	1.3
Cystine	99.6	3.3	98.3	11.9	98.2	2.3	99.2	6.1	96.6	1.2
Methionine	103.2	8.4	103.6	6.9	106.5	5.7	104.4	2.6	95.8	4.2
Isoleucine	100.4	5.4	104.5	9.5	106.5	6.3	103.6	5.3	99.9	3.1
Leucine	101.9	4.0	108.9	9.1	102.5	5.6	101.1	3.4	98.1	1.3
Tyrosine	102.8	5.0	102.1	2.1	101.1	3.0	104.3	3.7	102.3	3.8
Phenyl-alanine	105.9	5.7	105.9	4.2	100.6	2.9	101.5	2.2	100.8	3.5
Histidine	102.6	3.8	*109.7	15.0	89.1	6.0	106.2	10.1	102.0	5.0
Lysine	101.5	2.6	106.2	5.8	100.5	3.6	105.5	5.0	100.2	4.0
Arginine	104.7	5.7	96.2	9.7	93.1	2.7	102.8	3.8	97.8	1.7
Taurine					105.4	3.6				
Asparagine					101.1	9.7				
Glutamine					98.3	5.6				
α-Aminoadipate					106.8	5.3				
Citrulline					103.7	6.1				
α-Aminobutyrate					106.6	6.2				
Cystathionine					105.9	4.6				
3-Methylhistidine					102.0	4.2				
Tryptophan					107.2	7.1				
Ornithine					101.5	5.2				

* Overlapping peaks causing inaccurate results.

Table 37. Continued.

			Effluents					
	Plasma		Urine		PD		HD		CAVH	
Item	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
							n = 9			
HD Effluent Collector							102.4	0.8		
			n = 8		n = 8		n = 8		n = 8	
Urea			98.1	3.1	100.6	2.5	97.6	4.6	100.7	2.6
			n = 8		n = 8		n = 8		n = 8	
Creatinine			101.1	2.2	100.1	4.1	100.9	2.5	102.2	3.6
			n = 6		n = 5		n = 6		n = 6	
Uric Acid			99.7	0.7	100.1	4.0	100.5	4.6	98.4	2.9
	n = 6									
TBPA	100.7	5.4								

Table 38. The Results of Membership of the External Quality Assurance of Quantitative Amino Acid Analyses Scheme operated by Dr. J.M. Rattenbury, Sheffield Children's Hospital.

Sample Code	1/85	1/86	2/86	3/86	4/86	5/86	6/86	1/87	2/87	3/87
Amino Acid				dSD						
Taurine					0.0	-0.9	0.2	0.0	0.1	
Aspartate	-4.4	-0.7		-2.2	-0.8	1.2		0.7		
Threonine	0.6	-0.4	-0.9	-0.8	-0.3	-1.9	0.2	-0.1	0.1	
Serine	-0.4	-1.0	-1.6	-1.2	-0.2	-1.7	0.3	-0.8	0.4	
Glutamate	0.8	0.1	-0.5	-0.2	0.1	-1.4	0.3	-0.3	-0.1	
Glutamine	0.4	-0.2	-0.6	-0.6	0.0	-1.1	0.0		-0.1	
Proline	-0.2			-1.2	-0.9	-1.6	0.5	-0.2	-0.2	
Glycine	0.6	-0.3	-1.3	-0.6	0.4	-2.6	0.2	-0.3	-0.1	
Alanine	1.0	-0.1	-0.8	-0.2	0.2	-1.3	1.2	-0.2	0.3	
Citrulline		-0.5	0.5	-0.5	1.2	-1.7	-0.3	0.0	0.3	
Valine	0.0	-0.2	-0.9	-0.3	0.3	-1.8	0.3	-0.1	-0.2	
Cystine								-0.2		
Methionine	-0.2	-0.4	0.4	-0.6	-0.1	-1.1	-0.6	-0.7	-0.5	
Isoleucine	0.4	-0.1	0.0	-0.3	0.6	-1.1	0.1	-1.4	0.4	
Leucine	0.4	0.2	-0.1	-0.4	0.6	-1.8	0.8	0.4	0.9	
Tyrosine	0.4	0.1	0.9	-0.7	1.2	-0.6	0.5	0.0	0.9	
Phenylalanine	0.0	-0.3	-0.2	-0.1	0.8	-1.8	0.3	-0.3	0.4	
Histidine	0.3	-0.6	-0.7	0.2	0.0	-1.1	0.2	-0.7	-0.2	
Ornithine	0.3	-0.6	-0.8	-0.5	-0.3	0.3	0.0	-0.7	0.8	
Lysine	0.0	-0.3	-0.8	-0.8	-0.3	-1.3	0.5	-0.4	-0.1	
Arginine	-0.5	0.0	-0.4	-0.6	-0.6	-1.4	0.8	-0.4	0.3	
RMS	0.84	0.42	0.80	0.79	0.52	1.49	0.49	0.45	0.42	
Rank for RMS	6/16	1/19	6/17	8/20	3/22	19/22	3/24	1/21	1/20	
Mean Bias(%)	-3.0	-5.5	-6.4	-12.8	0.1	-19.7	+5.0	-10.0	+4.2	
Rank for Mean Bias	5/16	9/19	9/17	14/20	1/22	20/22	10/24	11/21	7/20	

dSD = (result - mean) ÷ SD; SD = standard deviation

RMS = root mean square of dSD

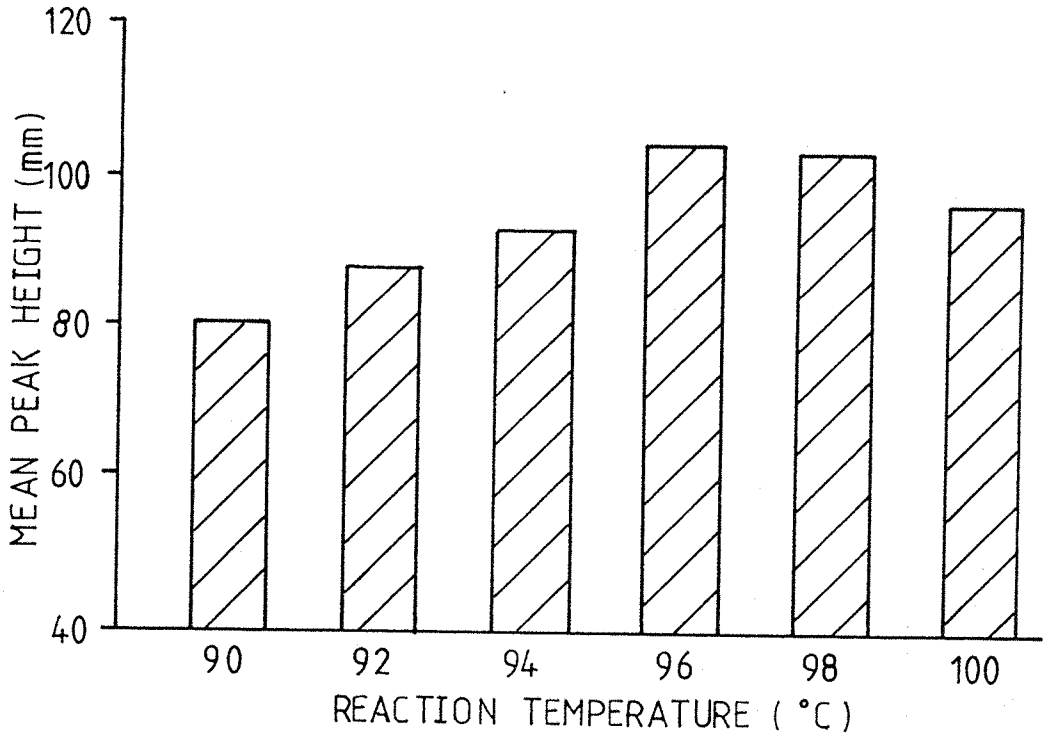


Fig. 30. THE EFFECT OF TEMPERATURE ON THE NINHYDRIN REACTION.

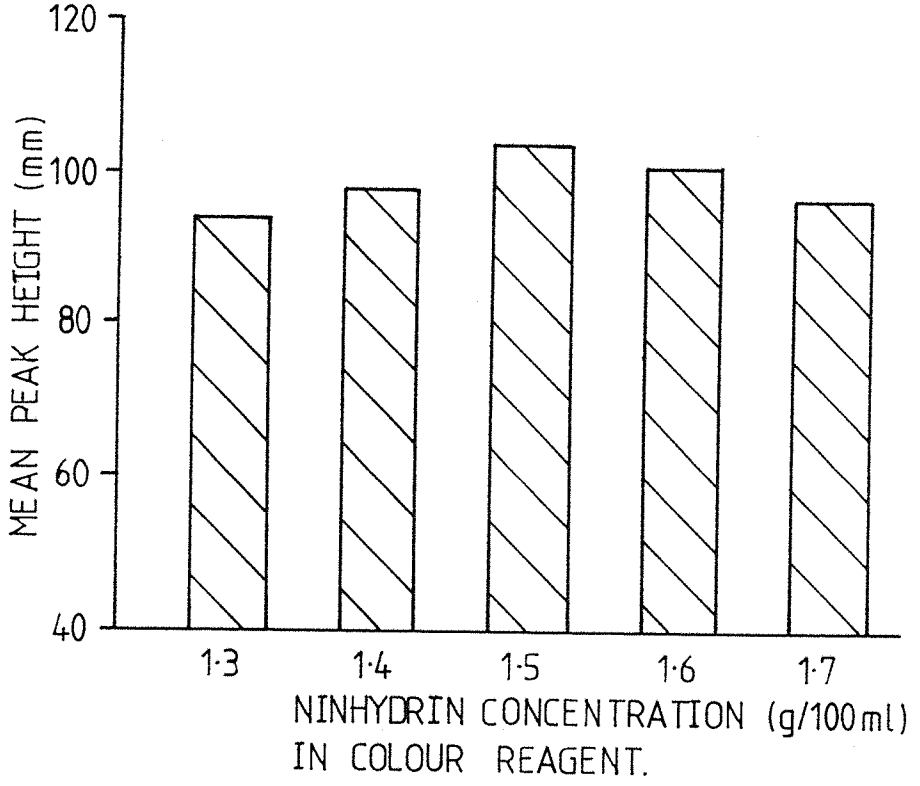


Fig. 31. THE EFFECT OF NINHYDRIN CONCENTRATION ON COLOUR DEVELOPMENT.

APPENDIX IV

Table 39. Enzyme Commission Numbers for the Enzymes Reported in this Thesis.

Enzyme	Enzyme Commission Number
5'-Nucleotidase	3.1.3.5
Adenosine deaminase	3.5.4.6
Alanine aminotransferase	2.6.1.2
Aldehyde dehydrogenase	1.2.1.5
Arginase	3.5.3.11
Argininosuccinase	4.3.2.1
Argininosuccinic acid synthase	6.3.4.5
Aspartate-oxaloacetate aminotransferase	2.6.1.1
ATPase	3.6.1.3
Branched-chain amino acid transferase	2.6.1.42
Carbamoyl phosphate synthase	6.3.4.16
Catalase	1.11.1.6
Choline kinase	2.7.1.32
Choline phosphotransferase	2.7.7.15
Creatine phosphokinase	2.7.3.2
Cystathionase	4.4.1.1
Cystathionine synthetase	4.2.1.22
Gamma cystathionase	4.4.1.1
Glucose-6-phosphatase	3.1.3.9
Glutamate dehydrogenase	1.4.1.2
Glutamine synthase	6.3.1.2
Glycogen synthetase	2.4.1.11
Guanase	3.5.4.3
Hypoxanthine-guanine phoshoribosyl transferase	2.4.2.8
Kynurenine aminotransferase	2.6.1.7
Kynurenine formylase	3.5.1.9
Leucine aminotransferase	2.6.1.6
Ornithine aminotransferase	2.6.1.13
Ornithine transcarbamoylase	2.1.3.3

Table 39. Continued.

Enzyme	Enzyme Commission Number
Peroxidase	1.11.1.7
Phenylalanine hydroxylase	1.14.16.1
Phosphocholine diacylglycerol transferase	2.7.8.2
Phosphocholine-cytidyl transferase	2.7.7.14
Phosphoenol pyruvate carboxykinase	4.1.1.32
Phosphofructokinase	2.7.1.11
Purine nucleoside phosphorylase	2.4.2.8
Pyruvate carboxylase	6.4.1.1
Serine hydroxymethyl transferase	2.1.1.1
S-adenosylmethionine synthetase	2.5.1.6
Tryptophan oxygenase	1.13.11.11
Tyrosine aminotransferase	2.6.1.5
Urease	3.5.1.5
Uricase	1.7.3.3
Xanthine oxidase	1.2.3.2

APPENDIX V

Table 40. Reference Ranges for Blood Components Reported in the Text.

Serum Urea	2.5 - 7.2	mmol/l
Serum Creatinine	57 - 105	μmol/l
Serum Potassium	3.5 - 5.0	mmol/l
Serum Albumin	37 - 49	g/l
Plasma TBPA	233 - 424	mg/l
Haemoglobin	11.5 - 16.5	g/dl
Leucocytes	4000 - 11000	cells/μl

Amino Acids - See Table 19, Chapter 2.

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